

**Table 3.2 Physical Properties of Thioglycosyl Donors**

Cpd.	mp (°C)	[ $\alpha$ ] <sub>b</sub> (CHCl <sub>3</sub> )	Formula	Elemental Analysis										
				Calculated (%)						Found (%)				
				C	H	N	S	C	H	N	S			
<b>32<sup>a</sup></b>	116.4-117.7	+46.8	C <sub>22</sub> H <sub>25</sub> NO <sub>5</sub> S	55.11	5.26	2.92	6.69	55.28	5.28	2.92	6.84			
<b>33<sup>a</sup></b>	145.7-146.2	+52.8	C <sub>26</sub> H <sub>25</sub> NO <sub>9</sub> S	59.19	4.78	2.66	6.08	59.29	5.00	2.69	5.87			
<b>34</b>	168.0-168.4	+40.6	C <sub>27</sub> H <sub>27</sub> NO <sub>9</sub> S	59.91	5.03	2.59	5.92	59.78	4.94	2.55	6.15			
<b>35</b>	140.2-140.5	+20.1	C <sub>22</sub> H <sub>25</sub> NO <sub>10</sub> S	58.16	4.88	2.51	5.75	58.08	4.94	2.47	5.64			
<b>36</b>	217.2-217.5	+42.7	C <sub>26</sub> H <sub>24</sub> N <sub>2</sub> O <sub>9</sub> S	53.06	4.11	4.76	5.45	53.17	4.25	4.95	5.28			
<b>37</b>	81.5-82.7	+36.0	C <sub>24</sub> H <sub>25</sub> N <sub>3</sub> O <sub>9</sub> S	54.23	4.74	7.91	6.03	53.99	4.97	7.81	6.12			

a: Known compounds and physical data agreed with reported values.

b: Solvent: CH<sub>2</sub>Cl<sub>2</sub>, c = 1.0 at room temperature.

The biological significance of  $\beta$ -1,4- linked oligomers of glucosamine (e.g., chitin) is well recognized.<sup>155</sup> Also, the hydroxyl group at the C-4 position of GlcN is known to be relatively unreactive.<sup>127b</sup> Therefore, the construction of this type of oligosaccharide is a challenging task and a test of the utility of a given method of glycosylation.<sup>156</sup> To this end, partially protected D-GlcN derivatives were prepared as indicated in scheme 3.5. The triol products of Zemplén de-O-acetylation of **32** and **36** were directly treated with  $\alpha,\alpha$ -dimethoxytoluene and p-TsOH in DMF Solvent (or acetonitrile), to give the benzylidene derivatives **38** and **39** in 85% and 90% yields, respectively. Compound **38** was benzylated using benzyl bromide and sodium hydride in N,N-dimethyl-formide, giving compound **40** in 85% yield. The 4,6-benzylidene acetal in **40** was opened by treatment with sodium cyanoborohydride and HCl-diethyl ether in tetrahydrofuran,<sup>157</sup> to give the 4-OH compound **41** in 83% yield. A possible mechanism for the reductive ring opening of a benzylidene acetal containing one primary and two secondary carbon atoms with a hydride and an acid was studied by Garegg *et al.*<sup>158</sup> for the sodium cyanoborohydride-hydrogen chloride reductions. The steric requirement of the electrophile H<sup>+</sup> is much smaller than that of Lewis acid and thus favors thermodynamic protonation of O-4 rather than O-6, giving mainly the 6-O-benzyl regioisomer. The regiochemistry of this ring opening reaction was confirmed by the <sup>13</sup>C-NMR spectrum, the C-4 signal showed a significant upfield shift from the  $\delta$  83.0 ppm in **40** to 74.8 ppm in **41**.<sup>159</sup>

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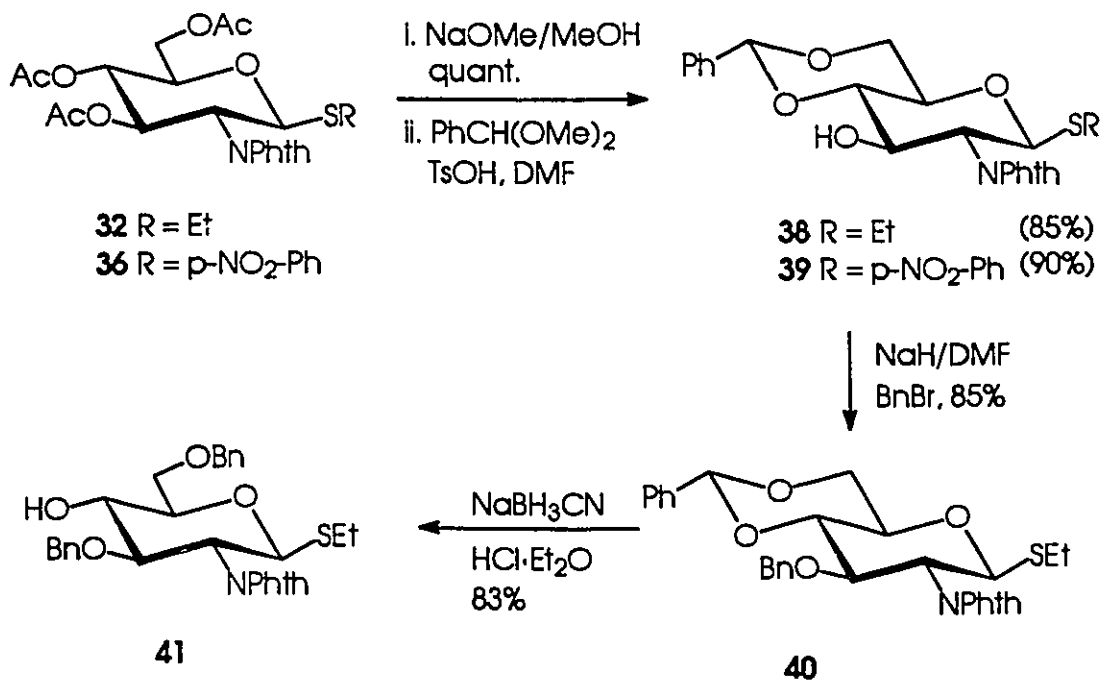
<sup>155</sup> Darvill, A.; Augaur, C.; Bergmann, C.; Carlson, R. W.; Cheong, J.-J.; Eberhard, S.; Hahn, M. G.; Ló, V.-M.; Marfà, V.; Meyer, B.; Mohnen, D.; O'Neil, M. A.; Spiro, M. D.; van Halbeek, H.; York, W. S.; Albersheim, P. *Glycobiology* **1992**, *2*, 181.

<sup>156</sup> Kuyama, H.; Nakahara, T.; Ogawa, T. *Carbohydr. Res.* **1993**, *243*, C1-C-7.

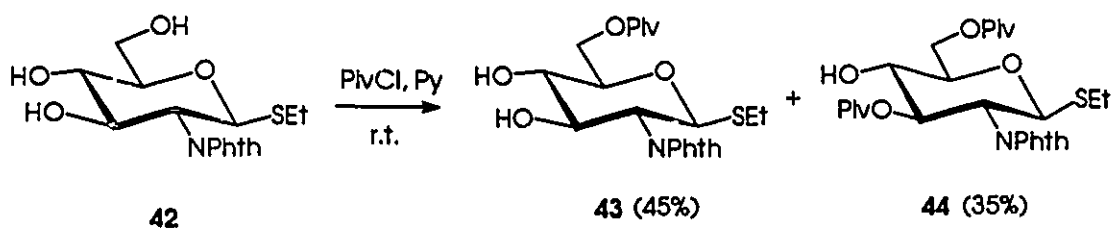
<sup>157</sup> (a) Nilsson, M.; Norberg, T. *J. Carbohydr. Chem.* **1990**, *9*, 1. (b) El-Sokkary, R. I.; Silwanis, B. A.; Nashed, M. A.; Paulsen, H. *Carbohydr. Res.* **1990**, *203*, 319.

<sup>158</sup> Garegg, P. J.; Hultberg, H.; Wallin, S. *Carbohydr. Res.* **1982**, *108*, 97.

<sup>159</sup> (a) Radic, L.; Kajtar-Fredy, M.; Corsano, S.; Standoli, L. *Tetrahedron Lett.* **1975**, 4278; (b) Tori, K.; Herata, T.; Koshitani, O.; Suga, T. *Tetrahedron Lett.* **1976**, 1311; (c) Yamasaki, K.; Kohda, H.; Kobayashi, T.; Kasai, R.; Tanaka, O. *Tetrahedron Lett.* **1976**, 1005; (d) Tori, K.; Seo, S.; Yoshimura, Y.; Nakamura, M.; Tomita, Y. *Tetrahedron Lett.* **1976**, 4167.



**Scheme 3.5** Synthesis of partially protected 2-deoxy 2-phthalimido-1-thio- $\beta$ -D-glucopyranosides



**Scheme 3.6** Pivaloylation of GlcN triol **42**

As an alternative, partially protected GlcN thioglycosyl acceptors could be prepared directly by pivaloylation of GlcN triol **42** (scheme 3.6). Treatment of triol **42**

with pivaloyl chloride (2.4 equiv.) in pyridine afforded, after a long reaction time (48 hours), 45% of monopivaloylated compound **43** and 35% dipivaloylated compound **44**.

### 3.3 Preparation of “Active and Latent” Galactopyranosyl Donors and Acceptors

D-Galactose is a constituent of complex glycosphingolipids and glycoproteins, where it plays an important role.<sup>160</sup> It is found as a terminal or subterminal building block in a variety of different linkages. In glycosphingolipids, galactose is part of the lactosyl ceramide core-structure. Terminal and subterminal  $\beta$ -(1 $\rightarrow$ 4) linkages to 2-acetamido-2-deoxy-D-glucopyranose (N-acetylglucosamine, GlcNAc) leads to the N-acetyllactosamine moiety, which is alone a very important building block in the synthesis of Le<sup>x</sup> and sialyl Le<sup>x</sup>. This topic will be discussed in detail in chapter 6 of this thesis. The GlcNAc  $\beta$ -(1 $\rightarrow$ 6) Gal is a core structure of the capsular polysaccharide (CPS) of *Haemophilus Pleuropneumonia* Serotype 1 which is recognized as an important bacterial virulence factor.<sup>161</sup>  $\beta$ -(1 $\rightarrow$ 6) linked D-galactopyranoside oligomers were found to be the optimal binding determinant of some monoclonal IgA's.<sup>162</sup>

Our “active-latent” glycosylation strategy for the synthesis of important oligosaccharides depends primarily on suitably protected active and latent thioglycosyl donors and acceptors. To this end,  $\beta$ -D-galactopyranosyl derivatives **48-77** with suitable protecting groups were prepared (Scheme 3.7).

Standard Zemplén deacetylation of peracetylated thioglycosides **7-9** gave intermediates **45-47** which, without further purification, were benzoylated using benzoyl chloride in pyridine to give per-O-benzoylated thioglycosides **48-50**.

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<sup>160</sup> Schaffer, R in Pigman, W.; Horton, D. (Eds.), *The Carbohydrates: Chemistry and Biochemistry*, 2nd ed., Vol. IA, p.69 Academic Press, New York, 1972.

<sup>161</sup> (a) Inzana, T. J. *Infect. Immun.* 1987, 55, 1573. (b) Inzana, T. J.; Mathison, B. *Infect. Immun.* 1987, 55, 1580.

<sup>162</sup> (a) Glaudemans, C. P. J.; Bhattacharjee, A. K.; Majula, B. N. *Molec. Immunol.* 1986, 23, 655. (b) Glaudemans, C. P. J. *Molec. Immunol.* 1987, 24, 371.

To demonstrate the effectiveness of active-latent glycosylation strategy, tetraol **47** was chosen as a key intermediate for further transformations, the facile experimental operation allowed the preparation of **47** in large scale without difficulty. It should be noted that the  $^1\text{H}$  NMR spectrum of **47** shows a multiplet pattern at 5.02 ppm for the H-1 signal instead of the expected doublet. A related phenomenon has been observed<sup>163</sup> in the disaccharide methyl N,N'-diacetyl- $\beta$ -chitobioside as arising from the long range coupling of H-2 and H-3. Benzoylation of **47** with benzyl bromide in N,N-dimethylformamide gave "armed-latent" glycosyl donor **52** in 81% yield.

The usual strategies for the preparation of primary 6-OH free galactosyl acceptor involved (1) temporary protection at 6-OH as trityl, tert-butyldimethylsilyl<sup>164</sup> (TBDMS), tert-butyldiphenylsilyl (TBDPS) or tosyl ether. (2) protection of the remaining hydroxyl groups and finally; (3) selective deprotection of O-6.

The most promising preparation of 6-OH free glycosyl acceptor appeared to be tert-butyldimethylsilyl (TBDMS) and tert-butyldiphenylsilyl (TBDPS) ethers which we chose accordingly. Both silyl chlorides (TBDMS-Cl and TBDPS-Cl) were capable of regioselective silylation of 1-thio-D-galactopyranoside using pyridine as solvent to give the 6-O-silyl derivatives **53** and **54** in 87% and 89% yield respectively. The remaining hydroxyl groups in **53** and **54** were protected as benzoyl, or pivaloyl esters to give bifunctional galactosides **57-60**. It should be mentioned that the protection of the remaining triols in **54** with the bulky pivaloyl groups encountered some difficulty. The pivaloylation reaction was very sluggish and was not finished after 3 days at room temperature, under a forcing condition, heating reaction mixture to 70 °C for 8 hours, gave the tripivaloylated product **60** in 53% and tetrapivaloylated derivative **51** in 36% yield respectively.

For the selective deprotection of the 6-hydroxyl group, two different reagents and procedures were examined. First, the widely used tetra-n-butylammonium fluoride<sup>165</sup>

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<sup>163</sup> Brisson, J.-R.; Carver, J. P. *J. Biol. Chem.* **1982**, *257*, 11207.

<sup>164</sup> Pelyvan, I.; Lindhorst, T.; Thiem, J. *Liebig Ann. Chem.* **1990**, 761.

<sup>165</sup> Nicolau, K. C.; Dolle, R. E.; Papahatjis, D. P.; Randall, J. L. *J. Am. Chem. Soc.* **1984**, *106*, 4189.

when reacted with galactoside derivatives **57**, **59** and **60** gave a complex mixture attributable to extensive O-acyl migration.<sup>166,167</sup> The most effective method<sup>168</sup> for selective de-O-silylation of galactopyranosides **57**, **59** and **60** was based on the use of 3% hydrogen chloride in methanol/ether (1:1, v/v), freshly prepared from acetyl chloride and methanol. Under these conditions, the cleavage of the silyl groups occurred smoothly at room temperature and the 6-O-deprotected galactopyranosides **63-65** were obtained almost quantitatively.

Attempts to prepare the "latent-armed" acceptor **66** using the method suggested by Ohri *et al.*<sup>169</sup> were not successful in our hands. Benzoylation of 6-O-silyl galactoside **54** led to concomitant cleavage of the 6-O-TBDMS group under basic conditions and gave perbenzylated galactopyranoside **52** as the major product. Therefore, the most stable para-nitrophenyl 6-O-trityl-2,3,4-tri-O-benzyl-1-thio- $\beta$ -D-galactopyranoside **61** was prepared accordingly. Thus, galactopyranoside **47** was treated with trityl chloride in pyridine<sup>170</sup> giving 6-O-tritylated galactopyranoside **51** which was directly benzylated to afford **61** in 86% yield (two steps). Subsequent detritylation of **61** using p-toluenesulfonic acid as a catalyst finished the preparation of "latent-armed" acceptor **66** in 95% yield.

One of the major breakthroughs in new strategies for oligosaccharide synthesis has been the use of "lightly protected" acceptors, i.e. acceptors where there are several OH groups unprotected, especially near the position at which one wishes the glycosidic bond to be formed. So far, total syntheses of gangliosides GM<sub>1b</sub><sup>171,172</sup> and GD<sub>1a</sub><sup>173</sup> have been reported using this strategy. The benefit of using lightly protected acceptors, which results mostly from the lack of additional steric impediments, is not unique to sialoside formation,

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<sup>166</sup> Dodd, G. M.; Golding, B. T.; Joannou, P. V. *J. Chem. Soc., Chem. Commun.* **1975**, 249.

<sup>167</sup> Franke, F.; Guthrie, R. D. *Aust. J. Chem.* **1978**, *31*, 1285.

<sup>168</sup> Hanessian, S.; Lavallée, P. *Can. J. Chem.* **1975**, *53*, 2975.

<sup>169</sup> Ohri, H.; Nishida, Y.; Itoh, H.; Meguro, H. *J. Org. Chem.* **1991**, *56*, 1726.

<sup>170</sup> Argawal, K. L.; Yamazaki, A.; Cashion, P. L.; Khorana, H. G. *Angew Chem. Int. Ed. Engl.* **1972**, *11*, 451.

<sup>171</sup> Sugimoto, M.; Fujikura, K.; Nunomura, H. *Tetrahedron Lett.* **1990**, *31*, 385.

<sup>172</sup> Prabhanjan, H.; Ishida, H.; Hasegawa, A. *Carbohydr. Res.* **1991**, *211*, C-1.

<sup>173</sup> Prabhanjan, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1991**, *222*, C-1.

but also to other complex oligosaccharide syntheses.<sup>174</sup> Our ongoing research project was to synthesize biologically important oligosaccharides, including the sialosides. To this end, "latent" lightly protected galactosides **70**, **71** were prepared.

A one-pot, two step procedure<sup>175</sup> for the preparation of para-nitrophenyl 3,4-O-isopropylidene-1-thio- $\beta$ -D-galactopyranoside **67** in high yield was used. Initially para-nitrophenyl-1-thio- $\beta$ -D-galactopyranoside **47** was transacetylated with 2,2-dimethoxypropane, catalyzed by *p*-toluenesulfonic acid, and then, through the mild selective hydrolysis of the cyclic acetal group, **67** was prepared.<sup>176</sup> Benzoylation of **67** followed deacetylation under acidic conditions gave "latent" 3,4-diol acceptor **70** in 90% yield (two steps). However, selective benzoylation at the 6-OH in **67** gave di- and mono-benzoylated product mixture. Under optimized reaction condition, i.e. using pyridine-dichloromethane (1:3 v/v) as solvent and low reaction temperatures (-70 °C), the desired 6-O benzoyl galactoside **69** was obtained in 63% yield together with dibenzoylated product **68** (18%) and recovered starting material **67** (10%). The structure of **68** and **69** was confirmed from their <sup>1</sup>H-NMR spectra. The H-2 signal ( $\delta$  = 3.76 ppm) of **69** was more shielded (upfield) compared to that of the H-2 signal ( $\delta$  = 5.38 ppm) of **68**. Finally, deacetylations of **68** and **69** were completed using *p*-toluenesulfonic acid as catalyst to give di- and mono-benzoylated glycosyl acceptors **70** and **71** in quantitatively yield.

It was recently reported<sup>177</sup> that a tosyl protected sugar could be directly coupled with 1-thioglycosyl donor to give biologically interesting sulfur linked oligosaccharides. To this end, a 6-tosyl protected galactoside **62** was prepared efficiently in 79% yield using one pot two-step procedure (tosylation and acetylation).

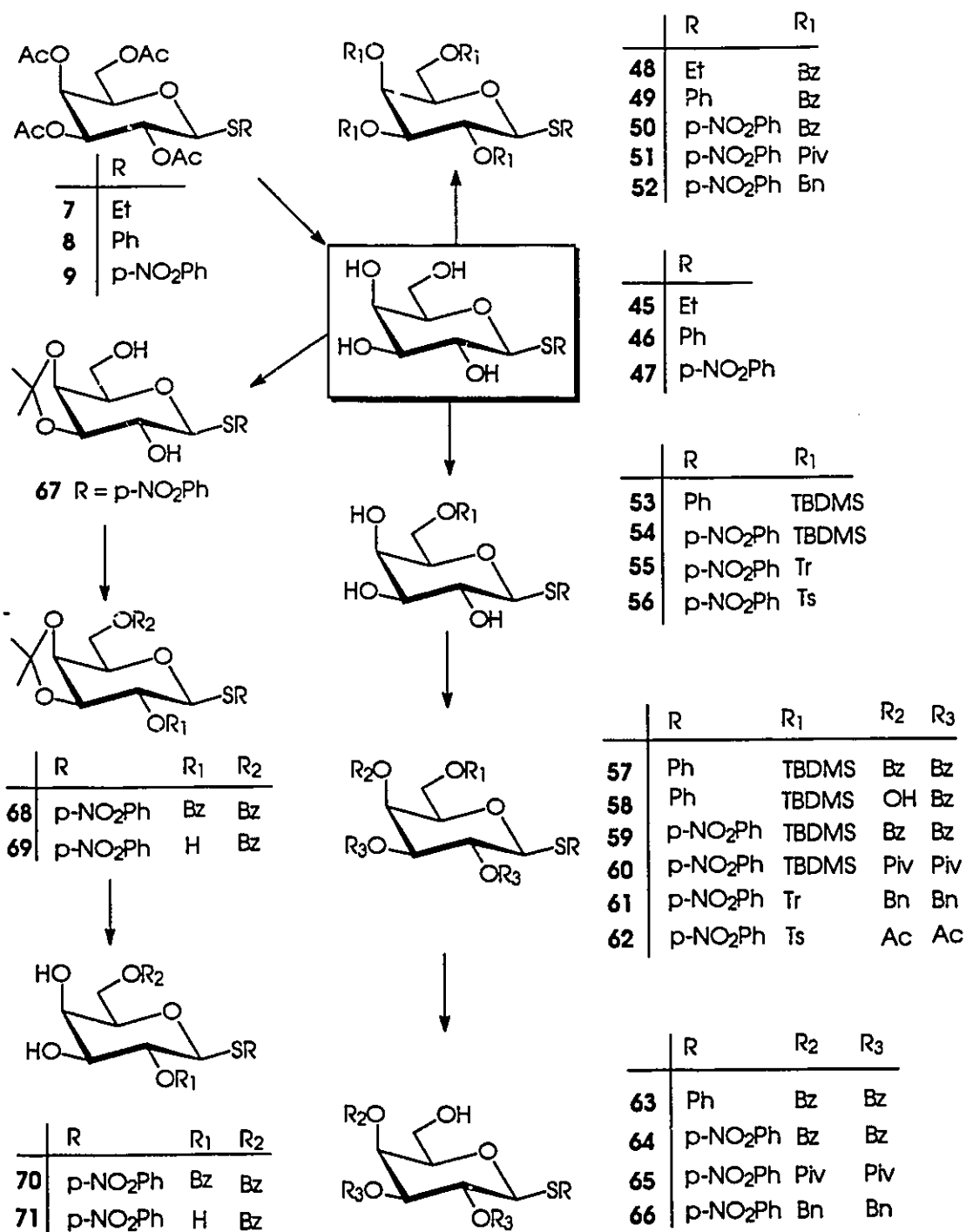
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<sup>174</sup> Saro, S.; Ito, Y. Ogawa, T. *Tetrahedron Lett.* 1988, 29, 5267.

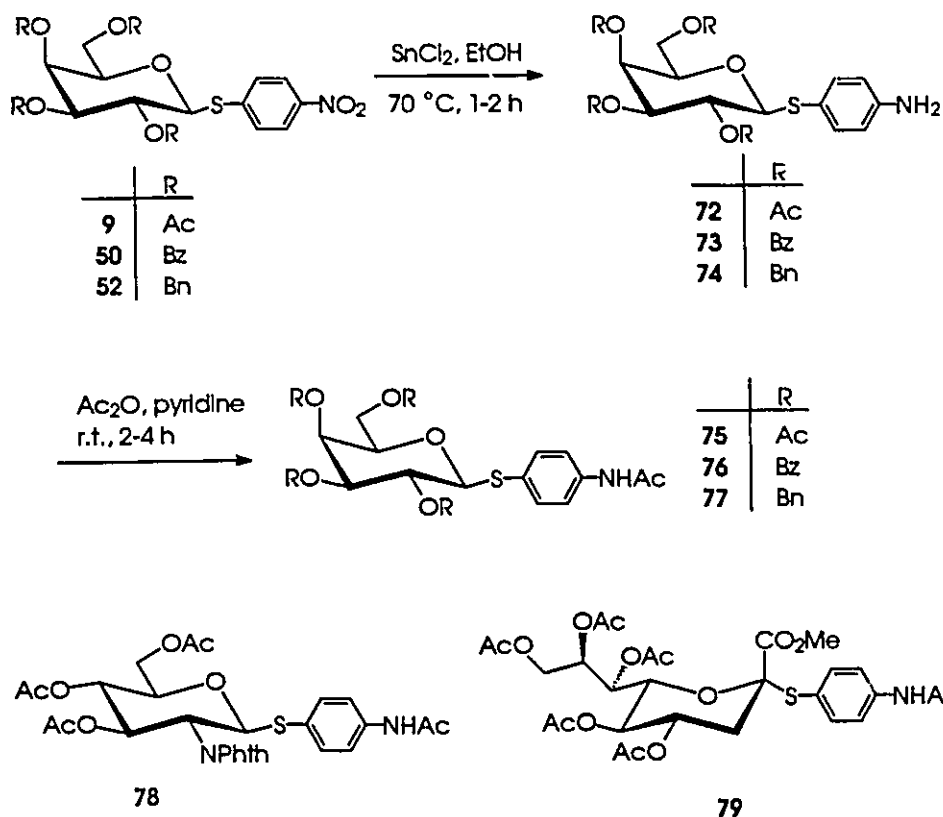
<sup>175</sup> Gatelani, G.; Colonna, F.; Marra, A. *Carbohydr. Res.* 1988, 182, 297.

<sup>176</sup> Barilli, P. L.; Berti, G.; Catalani, G.; Colonna, G.; Marra, A. *Tetrahedron Lett.*, 1986, 27, 2307.

<sup>177</sup> Hashimoto, H.; Shimada, K.; Horito, S. *Tetrahedron Lett.* 1993, 34, 4953.



**Scheme 3.7 Synthetic Transformations of Thiogalactopyranosides**



**Scheme 3.8 Transformation of “Latent” Thioglucosyl Donors Into “Active” Thioglucosyl Donors**

A very important aspect of our “active-latent” glycosylation strategy was to transform the “latent” glycosyl donors into an “active” form. An efficient method for the conversion of an electron withdrawing group (EWG) in aglycon moiety into an electron donating group (EDG) resulting in “active” glycosyl donors was desirable. Catalytic transfer hydrogenation with ammonium formate and 10% Pd-C,<sup>178</sup> was used successfully

<sup>178</sup> Ram, S.; Ehrenkauf, E. *Synthesis* 1988, 91.

in our lab for the reduction of O-para-nitrophenyl derivatives,<sup>180</sup> was unsuccessful when applied to the S-para-nitrophenyl glycosides. However, the nitro groups in thioglycosides were effectively reduced with tin(II) chloride in refluxing ethanol to give the corresponding aminophenyl thioglycosides (Scheme 3.8). The resulting aminophenyl thioglycosides could be directly acetylated with acetic anhydride in pyridine to provide the desired para-N-acetamidophenyl thioglycosides. The “active” donors 75-79 were prepared in 85-90% overall yield. These para-N-acetamidophenyl thioglycosides were successfully applied as “active” donors in our “active-latent” glycosylation strategy and this topic will be elaborated in the following section. It is worthy noting that the intermediate para-aminophenyl thioglycosides obtained were fairly stable and, whenever necessary, could be purified by silica gel column chromatography without oxidation.

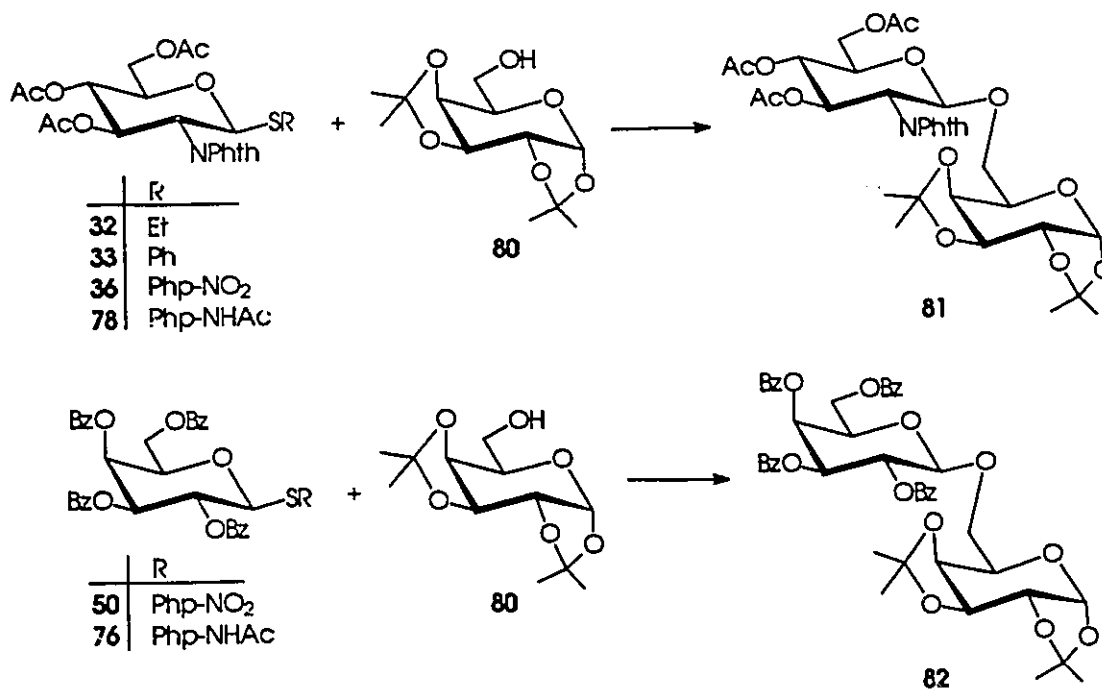
### 3.4 Synthesis of $\beta(1\rightarrow6)$ and $\beta(1\rightarrow3)$ Linked Oligosaccharides Using “Active-Latent” Glycosylation Strategy

The feasibility of the “active-latent” glycosylation strategy using the strong thiophilic N-iodosuccinimide/triflic acid (NIS/TfOH) promoter was first demonstrated by the glycosylation of terminal<sup>181</sup> glycosyl acceptor 1,2:3,4-di-O-isopropylidene- $\alpha$ -D-galactopyranose 80 with N-phthaloyl (Phth) protected 1-thio- $\beta$ -D-glucosamine (GluN) derivatives 32, 33, 36 and 78 as well as with thiogalactopyranosides 50 and 76 (scheme 3.9).

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<sup>180</sup> (a) Roy, R.; Tropper, F. D.; Romanowska, A.; Jain, R. K. Piskorz, C. F.; Matta, K. L. *Bioorg. Med. Chem. Lett.* 1992, 2, 911. (b) Roy, R.; Tropper, F. D.; Romanowska, A. *Bioconjugate Chem.* 1992, 3, 256.

<sup>181</sup> A terminal acceptor contains, in contrast with a non-terminal one, an anomeric group which cannot be activated *in situ* by a promoter.



**Scheme 3.9 Glycosylation of Terminal Acceptor 80**

We set out to test different reactivities of thioglycosyl donors. Glycosyl acceptor **80** was chosen as a model acceptor because of its intrinsic inertness under the glycosylation condition applied and thus excluded the possible cross coupling and hence simplifies the glycosylation reaction. Thioglycosides were activated with NIS/TfOH in the presence of the glycosyl acceptor and 4 Å molecular sieves (to ensure strict anhydrous conditions) in methylene chloride (CH<sub>2</sub>Cl<sub>2</sub>) at -30 °C leading to disaccharides in good to excellent yields, the reaction being completed usually in less than 30 minutes. Table 3.3 includes some of the examples of O-glycosides synthesized according to the above description and helps to illustrate the efficiency, applicability, and scope of this glycosylation strategy.

**Table 3.3 Glycosylation of 1,2:3,4-di-O-Isopropylidene  $\alpha$ -D-Galactose **80** with Donor **32**, **33**, **36**, **50**, **76** and **78****

Entry <sup>a</sup>	Donor	Promoter (equiv.)	Time	Yield <sup>b</sup> (%)
1	32	NIS/TfOH 1.8/0.2	10 min	92
2	32	DMTST 2.5	6 h	75
3	33	NIS/TfOH 1.8/0.6	25 min	77
4	36	NIS/TfOH 1.8/1.0	NR	-
5	78	NIS/TfOH 1.8/1.0	20 min	80
6	50	NIS/TfOH 1.8/1.0	NR	-
7	76	NIS/TfOH 1.8/1.0	15 min	94

<sup>a</sup> Reaction conditions: solvent, CH<sub>2</sub>Cl<sub>2</sub>; donor: acceptor = 1.2 :1; reactions were executed at -30 °C using NIS/TfOH as promoter and at r.t. using DMTST as promoter.

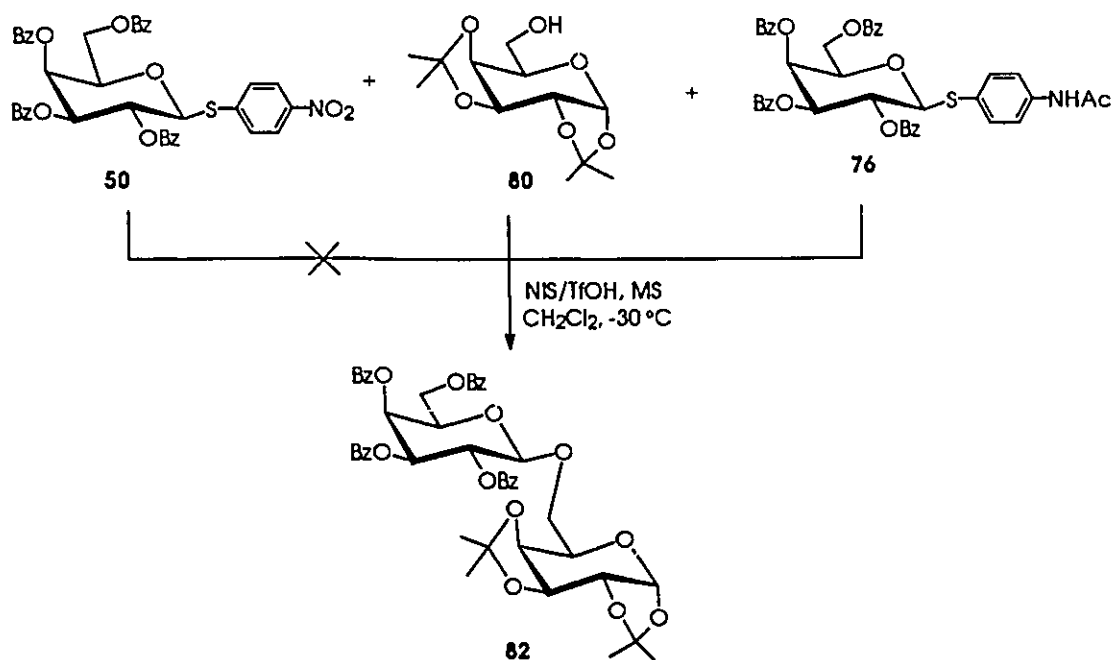
<sup>b</sup> Isolated yield.

Quite impressively, the coupling reaction between ethylthio glycoside **32** and acceptor **80** (table 3.3, entry 1) was finished in 10 min and provided disaccharide **81** in high yield (92%). As comparison, using DMTST as a promoter, the coupling reaction between the same donor **32** and acceptor **80** (entry 2) required longer reaction time (5h) to finish and afforded disaccharide **81** in 75% yield.

It is of interest to note that the glycosylation reaction between active phenyl thioglycosyl donor **33** and acceptor **80** was finished in 25 min (entry 3, 77%) while treatment of the “latent” (unreactive) para-nitrophenyl thioglycosyl donor **36** with the same acceptor **80** under the above conditions (entry 4) gave no detectable formation of the corresponding disaccharide **81**. However, transformation of the “latent” donor **36** into the active form **78** and glycosylation of **80** as above (entry 5) provided disaccharide **81** in 80%

yield. Similarly, treatment of the “latent” donor **78** with **80** (entry 6) gave no glycosylation product, while the glycosylation reaction between “active” donor **76** with **80** gave disaccharide **82** in an excellent yield (entry 7, 94%). The  $\beta$  configurations of the newly introduced anomeric centers of the disaccharides **81** and **82** were assigned from the  $^1\text{H}$  NMR spectra which showed doublets for H-1 at  $\delta$  5.42 ( $J_{1,2'} = 8.5$  Hz) and at 4.99 ppm ( $J_{1,2'} = 8.0$  Hz), respectively.

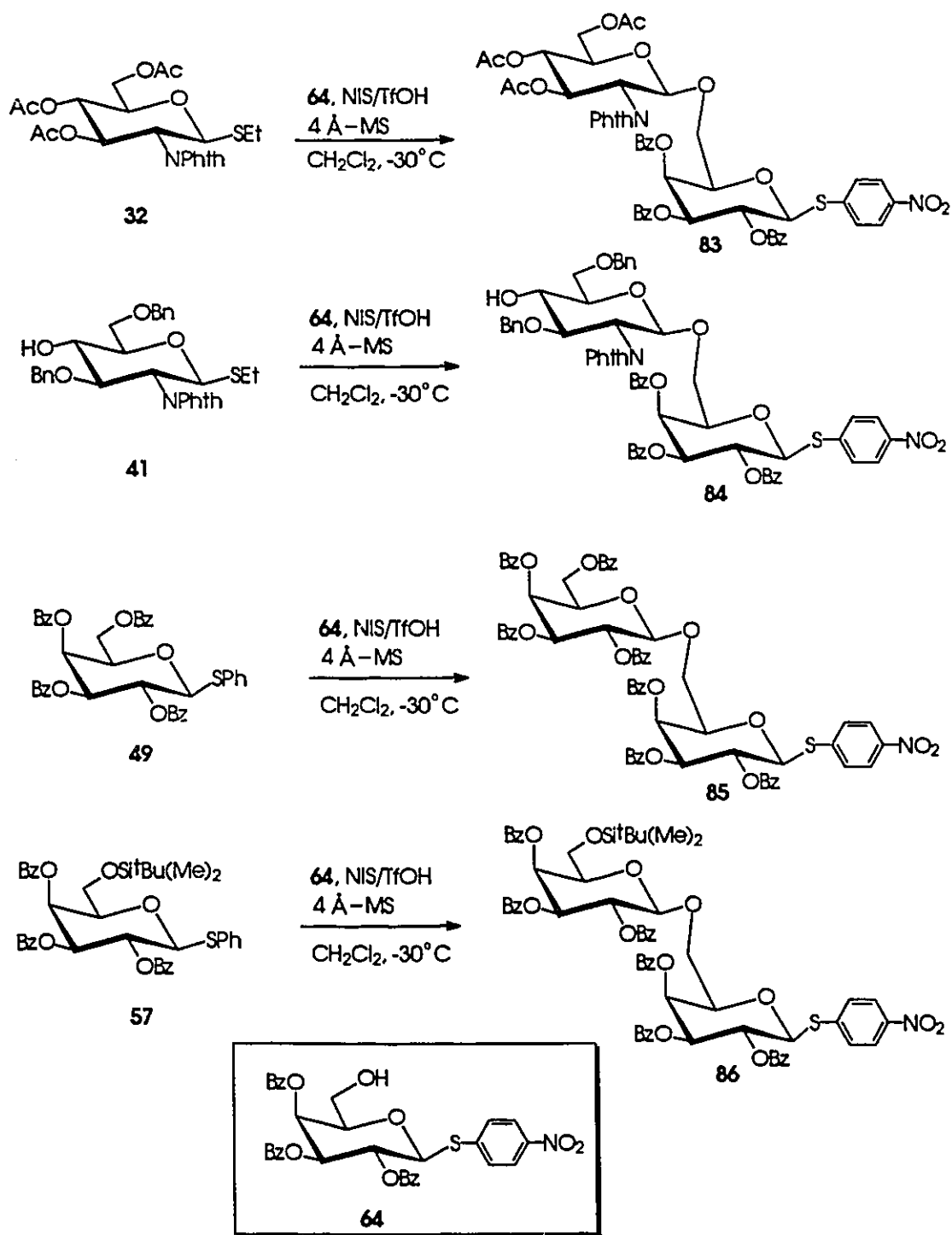
In order to further confirm the above finding, a qualitative experiment was performed (scheme 3.10). A mixture of “latent” donor **50** (0.5 eq) and “active” donor **78** (0.5 eq) were allowed to compete for the glycosyl acceptor **80** (1.0 eq) under the condition described above. The active donor **76** was consumed in 20 min while the “latent” donor **50** was found unreacted after 3 hrs.



**Scheme 3.10** Competitive Glycosylation Reaction Between a “Latent” Donor **50** and an “Active” Donor **76**

Encouraged by the observed different reactivities between the various ethylphenyl and para-nitrophenyl thioglycosides in the glycosylation reactions, we reasoned that selective activation of ethyl or phenyl thioglycosyl donors (active donors) in the presence of para-nitrophenyl thioglycosyl acceptors (latent acceptors) might be possible. To this end, the glycosylation reactions between “active” donors **32**, **41**, **49**, **53** and “latent” acceptor **64** using NIS/TfOH as promoter were examined (scheme 3.11).

Actually, all the *in situ* iodonium-ions promoted glycosylation of the “latent” acceptor **64** with “active” donors were essentially all completed in less than 30 min. No self coupling of **64** was observed under the glycosylation conditions. Some of the results are given in table 3.4. It should be noted that glycosyl donor **41** itself contains a free hydroxy group at C-4, however, the 4-OH group of glycoside **41** is known to be relatively unreactive, no  $\beta(1\rightarrow4)$  selfcoupling product was observed during the glycosylation reaction. The disaccharide **83** and **84** contained the core structure GlcNAc  $\beta$ -(1 $\rightarrow$ 6) Gal of the capsular polysaccharide (CPS) of a *Hæmophilus Pleuropneumonia* Serotype 1. Therefore disaccharide **83** and **84** could serve as building blocks for the synthesis of this biologically important macromolecule. Disaccharide **86** is very useful in blockwise synthesis of more complex oligosaccharide. It can serve as a potential donor as well as a potential acceptor. The conversion of the nitro group into the N-acetamido group allows disaccharide **86** to become an “active” donor. In addition, the eventual removal of 6-O silyl protecting group from the disaccharide **86** converts this disaccharide into a glycosyl acceptor.



**Scheme 3.11** Glycosylation of “latent-disarmed” acceptor **64** with “active-disarmed” donors **32**, **41**, **49** and **53**.

**Table 3.4 Results of Glycosylation of Latent Acceptor 64<sup>a</sup>**

Entry	Donor	Molar Ratio <sup>b</sup>	Time (min)	Product	Yield (%) <sup>c</sup>
1	32	1.2/1.0/2.0/0.2	15	83	78
2	41	1.1/1.0/2.0/0.2	10	84	89
3	49	1.2/1.0/2.0/0.6	20	85	81
4	53	1.0/1.0/2.0/0.5	25	86	79
5	76	1.2/1.0/2.0/0.6	15	85	87

<sup>a</sup> Reaction conditions: solvent, CH<sub>2</sub>Cl<sub>2</sub>; all the reactions were carried out at -30 °C.

<sup>b</sup> Molar ratios of donor/acceptor/NIS/TfOH.

<sup>c</sup> Isolated yield.

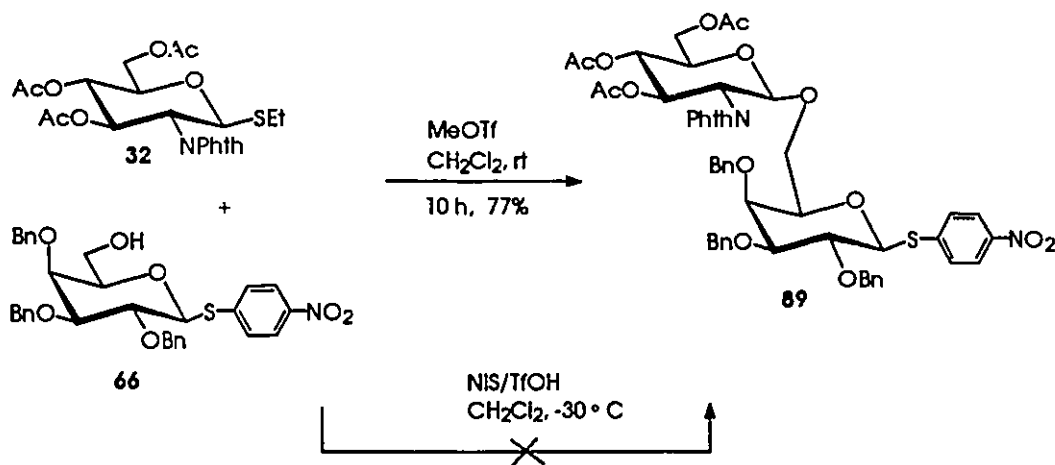
Selective activation of a “disarmed-active” donor in the presence of an “armed-latent” glycosyl acceptor was examined next (scheme 3.12). The first attempt to glycosylate “armed-latent” 66 acceptor with “disarmed-latent” donor 32 using NIS/TfOH as a promoter led to an intractable mixture of products. A possible explanation for this result is that the electron-donating effect by the benzyl group (armed) at C-2 minimized the effect of the electron withdrawing 4-nitrophenylthio group at the anomeric center. As a result, the “latent” acceptor was not really latent in the presence of the powerful activation system (NIS/TfOH). Formation of 1,6-anhydro derivative<sup>182</sup> as well as self coupling<sup>183</sup> was possible. However, we found that the “armed-latent” acceptor 66 was glycosylated smoothly in methylene chloride at room temperature with “disarmed-active” donor 32 using the weaker thiophilic methyl triflate (MeOTf) promoter, to give

<sup>182</sup> Sliedregt, L. A. J. M.; Zegelaar-Jaarsveld, K.; van der Marel, G. A.; van Boom, J. H. *Synlett*. 1993, 335.

<sup>183</sup> Self-coupling means that under certain glycosylation conditions, a non-terminal acceptor could be activated by the activating agent and coupled itself to form selfcoupled products, dimer, trimer, tetramer and so on.

disaccharide **89** in 77% yield. The configuration of the newly introduced anomeric center was expected to be  $\beta$  due to the presence of the phthalimido group in the glycosyl donor, which favor the formation of  $\beta$ -1,2-*trans* stereochemistry. Indeed, the  $^1\text{H-NMR}$  spectrum of **89** showed the anomeric H-1' signal as a doublet at  $\delta$  5.43 ppm ( $J_{1',2'} = 7.9$  Hz).

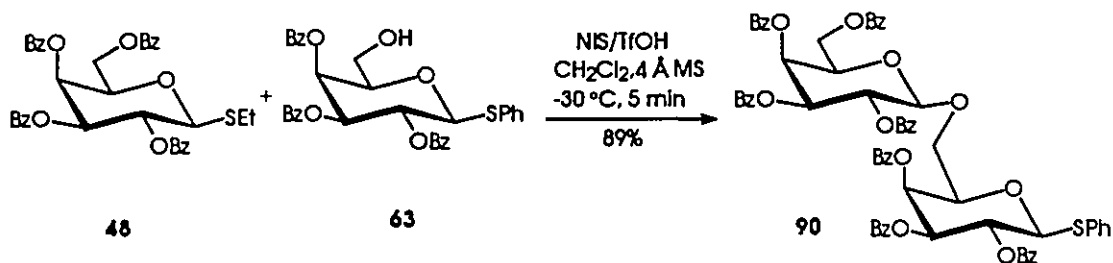
Disaccharide **89** could be directly used as a glycosyl donor using a more powerful promoter (such as NIS/TfOH) without manipulating the anomeric center or by a simple two step reaction (reduction and acetylation) to transform the 4-nitrophenylthio group into the more reactive 4-acetylamidophenylthio group. Furthermore, it is also well-known that the presence of a non-participating 2-ether function in the donor favors the formation of 1,2-*cis* linked glycosides. On the basis of this knowledge, together with the availability of effective thiophilic promoters, it is to be expected that the "armed" disaccharide **89** could be a valuable synthon for the synthesis of the capsular polysaccharide (CPS) of *Haemophilus Pleuropneumonia* Serotype 1 which contains GlcNAc $\beta$ (1 $\rightarrow$ 6)Gal  $\alpha$  phosphate repeating unit.



**Scheme 3.12** Glycosylation of "latent-armed" acceptor with "active-disarmed" donor

The selective activation of ethyl thioglycoside **48** in the presence of phenyl thioglycoside acceptor **63** was examined next. This coupling reaction (Scheme 3.13) was

complete within 5 min and gave disaccharide **90** in 89% yield. This observation, *i.e.* the preferential activation of ethyl thioglycosides over that of phenyl thioglycosides, was also corroborated by recent results reported by Zhang *et al.*<sup>184</sup> upon methyl triflate promoted glycosylation reactions.



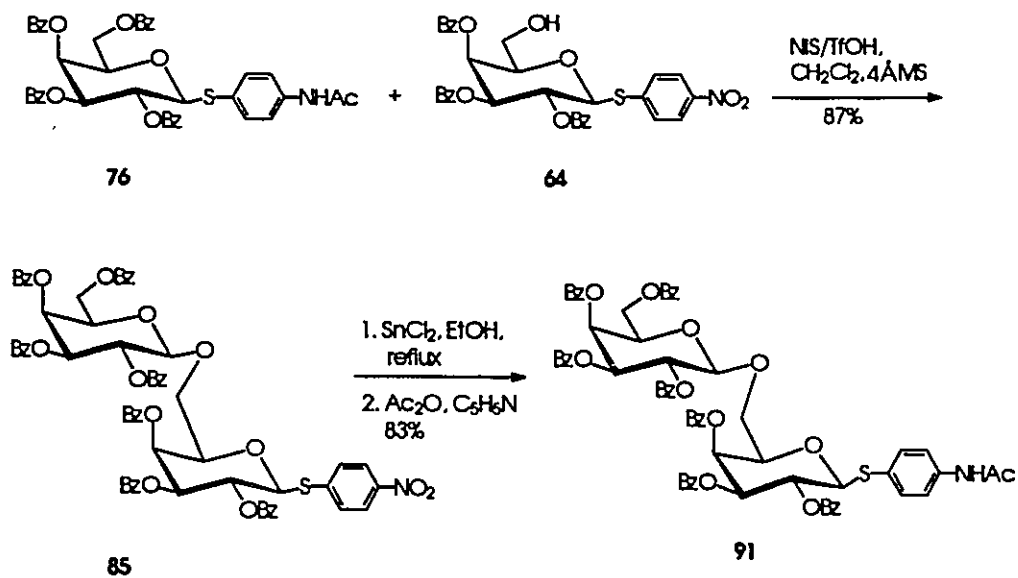
**Scheme 3.13** Selective Activation of Ethyl Thioglycosyl Donor over Phenyl Thioglycosyl Acceptor

The versatility of the “active-latent” glycosylation strategy in oligosaccharide synthesis was further illustrated by the synthesis of the trisaccharide  $\beta$ -D-Galp-(1-6)- $\beta$ -D-Galp-(1-6)- $\beta$ -D-Galp **92**. “Active” glycosyl donor **76**, prepared from the “latent” donor **73** (see section 3.3) was selectively activated in the presence of “latent” acceptor **64** using NIS/TfOH as promoter to give disaccharide **85** in 83 % yield. Again, the reactivity of the “latent” disaccharide **85** could be “turned on” by means of reduction (SnCl<sub>2</sub>, EtOH, reflux) and acetylation (Ac<sub>2</sub>O, pyridine) to give “active” form **91** (scheme 3.14).

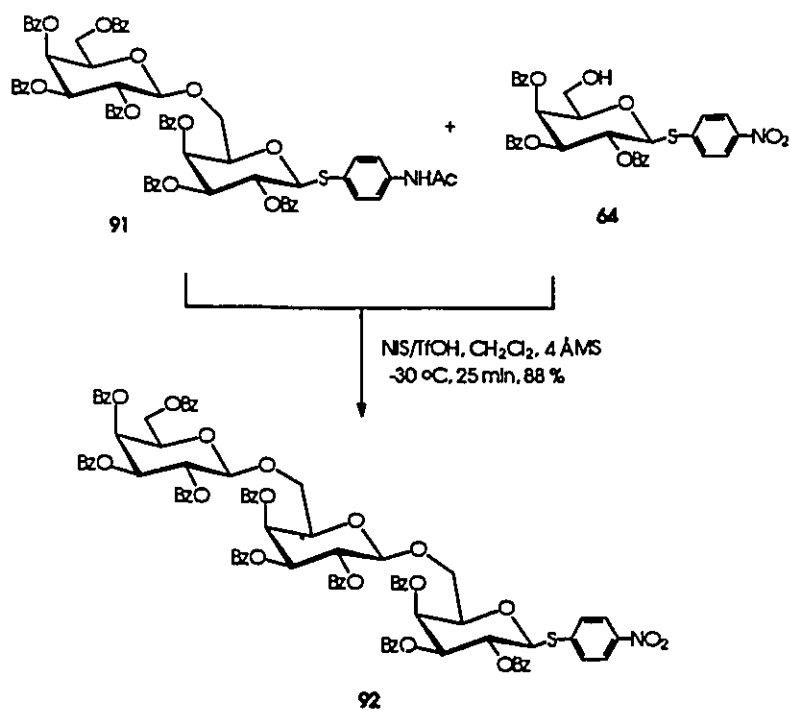
Successful glycosylation of “latent” glycosyl acceptor **64** with “active” disaccharide donor **91** was achieved in methylene chloride using NIS/TfOH as the activator to provide trisaccharide **92** in 88% yield. (Scheme 3.15). It is worthwhile mentioning that “latent” trisaccharide **92** could again be easily transformed to an “active”

<sup>184</sup> Zhang, Y.-M.; Brodzky, A.; Sinay, P.; Saint-Marcoux, G.; Perly, B. *Tetrahedron Asymmetry* 1995, 6, 1195.

form by the method described above and can serve as a new glycosyl donor in further chain elongation.

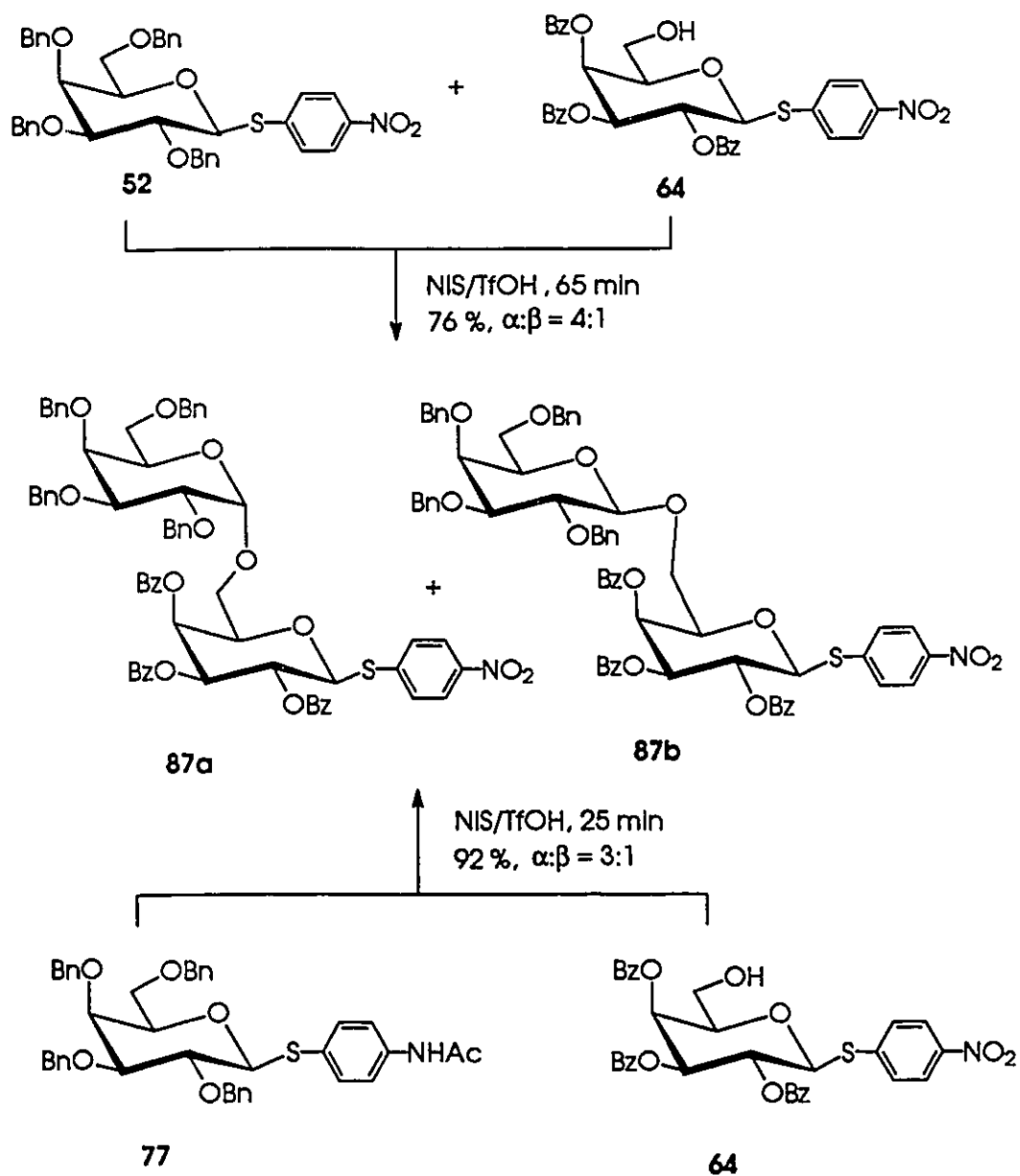


**Scheme 3.14** Synthesis of “Active” Galβ1→6Galβ Disaccharide



**Scheme 3.15** Synthesis of the Trisaccharide β-D-Galp-(1-6)-β-D-Galp-(1-6)-β-D-Galp

The next step was to examine the glycosylation reactions of “disarmed-latent” acceptor **64** with “armed-latent” **52** and “armed-active” **77** glycosyl donors (scheme 3.16).



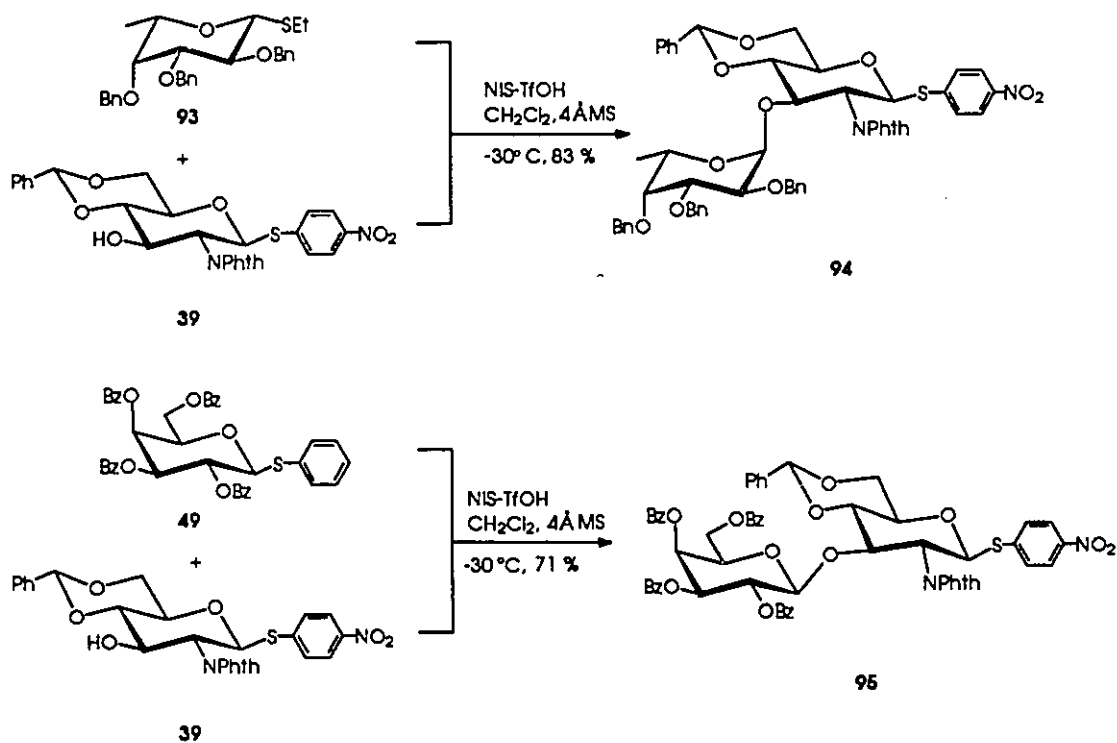
**Scheme 3.16** Glycosylation of “Disarmed-Latent” Acceptor with “Armed-Active” and “Armed-Latent” Donors

There are several important points worth of addressing. First, as expected, the glycosylation reaction was faster for the more reactive "armed-active" donor **77** (25 min) than for the "armed-latent" donor **52** (65 min) under the same glycosylation conditions. This provided an additional example for the higher reactivity of a 4-acetamidophenyl thioglycoside (active) over that of a 4-nitrophenylthio counterpart. Secondly, the  $\alpha$ -stereoselectivities of both glycosylation reactions were due to the non-participating nature of the C-2 ether group, though there was a slightly better  $\alpha$ -stereoselectivity using glycosyl donor **52** (4:1,  $\alpha$ : $\beta$ ) over that of glycosyl donor **77** (3:1,  $\alpha$ : $\beta$ ). Thirdly, not only "armed-active" glycosyl donor **77** but also "armed-latent" glycosyl donor **52** could be chemoselectively activated in the presence of "disarmed-latent" acceptor **64** using NIS/TfOH as thiophilic promoter. Disaccharides **87a** and **87b** were obtained as  $\alpha$ / $\beta$  mixture in 92% and 76% yield respectively and no self-condensation product was detected in either sequence. The  $\alpha$ / $\beta$  mixture could be successfully separated by silica gel column chromatography using 1% t-butanol in dichloromethane as eluent. The stereochemistry of the newly generated anomeric center was confirmed from the  $^1\text{H}$  NMR spectra which showed a doublet at  $\delta$  4.79 ppm ( $J_{1,2'} = 3.7$  Hz) for the  $\alpha$  anomer **87a** and at  $\delta$  4.38 ppm ( $J_{1,2'} = 7.7$  Hz) for the  $\beta$  anomer **87b**. Finally, the glycosylation reaction between the "armed-latent" glycosyl donor **52** and "disarmed-latent" acceptor **64** provided the first example for the coupling two so-called "latent" glycosides using powerful thiophilic promoter (NIS/TfOH). As a comparison, an earlier study from van Boom's group revealed that "disarmed" ethyl thioglycosyl acceptor could be condensed chemoselectively in the presence of the weak thiophilic promoter *sym*-collidine perchlorate (IDCP) with "armed" ethyl thioglycosyl donor.<sup>185</sup>

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<sup>185</sup> Veeneman, G. H. Van Leeuwen, S. H.; Van Boom, J. G. *Tetrahedron Lett.* 1990, 31, 1331.

To further demonstrate the generality of “latent-active” glycosyl strategy in oligosaccharide synthesis, the more difficult  $\beta(1\rightarrow3)$  glycosylation was examined next (scheme 3.17).



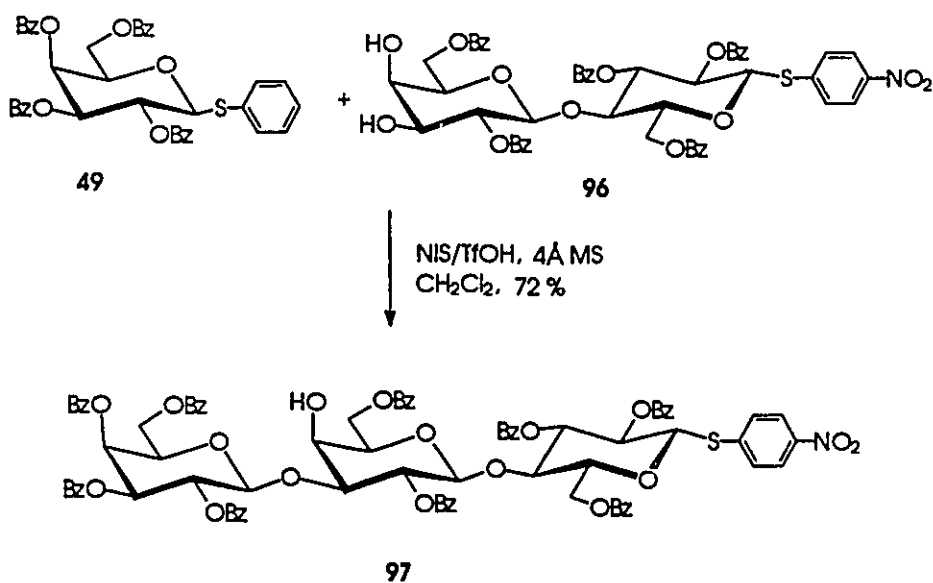
**Scheme 3.17** Preparation of Synthons for  $\text{Le}^x$  and  $\text{Le}^a$

Glycosylation of “latent-disarmed” acceptor **39** with ethyl 1-thio- $\beta$ -L-fucopyranoside<sup>186</sup> **93** at  $-30^\circ\text{C}$  in dichloromethane with 4Å MS, promoted by NIS/TfOH, gave the thermodynamically stable  $\alpha$ -(1 $\rightarrow$ 3) disaccharide **94** in 83 % yield. Under similar glycosylation conditions, glycosylation of “latent-disarmed” acceptor **39** with “active-armed”  $\beta$ -D-galactopyranoside **49** afforded  $\beta$ -(1 $\rightarrow$ 3) disaccharide **95** in 71% yield. The stereochemistry of the newly introduced anomeric centers of disaccharides **94** and **95** were

<sup>186</sup> Lönn, H. *Carbohydr. Res.* 1985, 139, 105

determined by  $^1\text{H}$  NMR spectra which showed doublets for H-1' at  $\delta$  4.79 ppm ( $J_{1',2'} = 1.2$  Hz) and at  $\delta$  4.93 ppm ( $J_{1',2'} = 8.0$  Hz) respectively. It is also worth mentioning that disaccharides **94** and **95** were versatile synthons for  $\text{Le}^a$  and  $\text{Le}^x$  respectively.

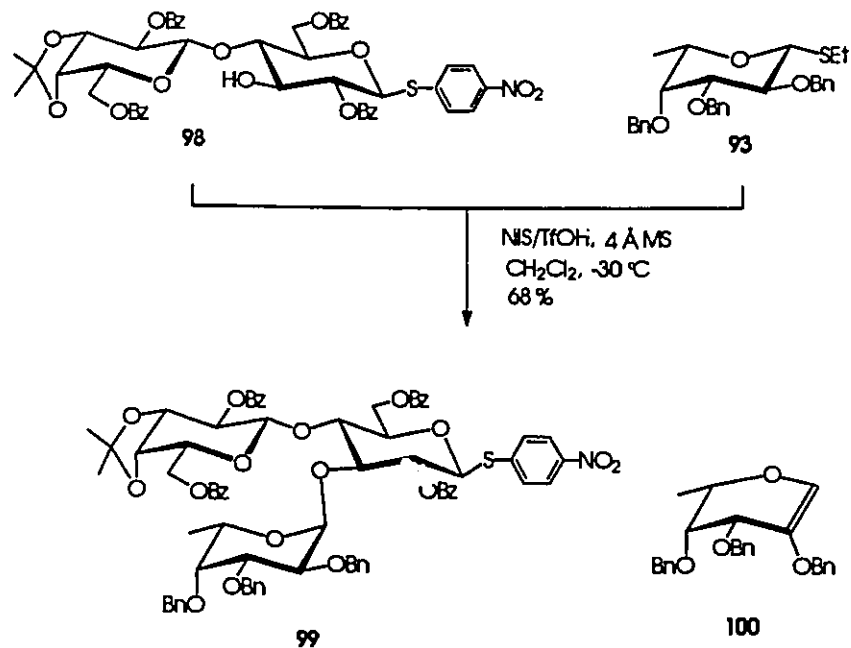
Condensation of phenyl 1-thio-D-galactopyranoside **49** with the diol **96**, readily obtained<sup>187</sup> from 4-nitrophenyl 3',4'-O-isopropylidene-2,3,6,2',6'-penta-O-benzoyl-1-thio- $\beta$ -D-lactopyranoside by hydrolysis, gave  $\beta(1\rightarrow3)$  linked trisaccharide **97** in 72% yield. The newly introduced  $\beta$ -anomeric center in **97** was assigned from the  $^1\text{H}$  NMR spectrum which showed a doublet for H-1'' at  $\delta$  4.89 ppm ( $J_{1'',2''} = 8.0$  Hz). The  $^1\text{H}$ -NMR spectrum of **97** was less informative in confirming the regiochemistry of the newly introduced glycosidic linkage of **97**. However, the regioselectivity could be confirmed from the  $^{13}\text{C}$  NMR spectrum which showed a deshielded signal for C-3' at  $\delta$  81.1 ppm ( $\Delta\delta = +8.0$  ppm) of trisaccharide **97** compared to  $\delta$  73.1 ppm for C-3' of the starting material **96**.



**Scheme 3.18 Selective Glycosylation of Diol 96**

<sup>187</sup> The preparation of this compound will be discussed in chapter 4 of this thesis.

The versatility of the “active-latent” glycosylation strategy in oligosaccharide synthesis was further demonstrated by the synthesis of a Le<sup>x</sup> analogue (scheme 3.19). The acceptor **98** was glycosylated with L-fucosyl donor **93** to give trisaccharide **99** in 68% yield after silica gel column chromatography. The relatively low glycosylation yield could be explained by the concomitant formation of the dehydro product **100**. This phenomenon is not uncommon when a very active donor and a relatively hindered glycosyl acceptor are used in a glycosylation reaction. Again the  $\alpha$  configuration of the newly introduced anomeric center for **99** was confirmed by <sup>1</sup>H NMR spectrum which showed a doublet for H-1'' at  $\delta$  5.33 ppm ( $J_{1'',2''} = 3.8$  Hz).



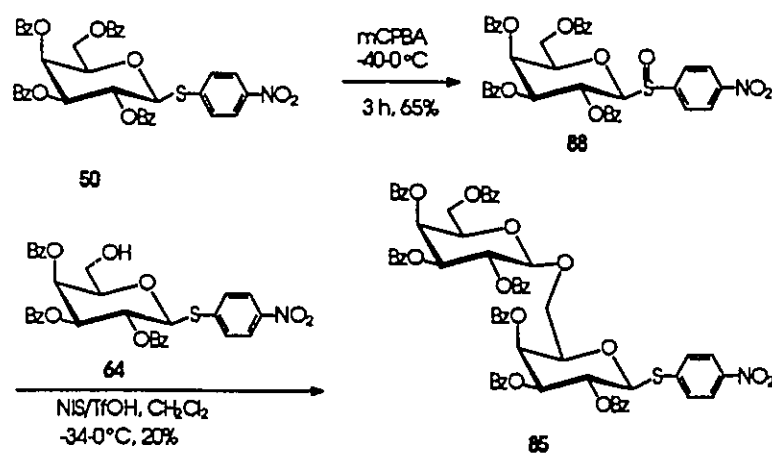
**Scheme 3.19** Synthesis of Le<sup>x</sup> Analogue

The application of thioglycosides gained a new impetus by the finding of Kahne *et al.*<sup>188</sup> Kahne and coworker realized that phenylsulfenyl glycosyl donors which are readily

<sup>188</sup> Kahne, D.; Walker, S.; Cheng, Y.; Van Boom, J. H.; Engen, D. *J. Am. Chem. Soc.* 1989, 111, 6881.

accessible by oxidation (mCPBA) of phenyl thioglycosides can be effectively glycosylated using triflic anhydride as activator. It was revealed by the same group<sup>189</sup> that the glycosylation of phenyl thioglycosides with phenylsulfenyl glycoside occurs selectively under the influence of triflic acid (TfOH) and in the presence of scavenger methyl propiolate (MP). The latter finding, together with the fact that the reactivity of phenylsulfenyl donors can be regulated by the introduction of EWG or EDG groups at the para position of the phenyl ring (reactivity order: OMe >H >NO<sub>2</sub>), enabled Kahne *et al.*<sup>54</sup> to assemble a precursor of the cyclamycin trisaccharide in a one-step synthesis.

To widen the scope of para-nitrophenyl thioglycosides in oligosaccharide synthesis, we also explored the possibility to activate the para-nitrophenyl thioglycoside by means of the oxidation method used by Kahne *et al.*<sup>184</sup> Our first attempt to condense perbenzoylated 4-nitrophenylsulfenyl glycoside **88**, prepared in 65% yield by oxidation of **50**<sup>190</sup> with mCPBA, with para-nitrophenyl thioglycosyl acceptor **64** using Tf<sub>2</sub>O as promoter at -78 °C failed. No trace of coupling product **85** could be detected. However, using NIS/TfOH as a promoter, disaccharide **85** was obtained in 20% yield (see Scheme



**Scheme 3.20 Glycosylation of 4-Nitrophenylsulfenyl Glycoside **88** with Latent Acceptor **64****

<sup>189</sup> Raghavan, S.; Kahne, D. *J. Am. Chem. Soc.* **1993**, *115*, 1580.

<sup>190</sup> Blanc-Muesser, M.; Defaye, J.; Driguez, H. *Carbohydr. Res.* **1978**, *67*, 305.

3.20). This preliminary result and our previous results revealed that to activate the “disarmed-latent” donor 50 using oxidation method was less effective than that using the reduction-acetylation method used previously.

### 3.5 Conclusion

In conclusion, the “active-latent” glycosylation strategy discussed above offers the possibility to prepare complex oligosaccharides by a highly convergent reiterative approach and extends the “armed-disarmed” glycosylation strategy described by Fraser-Reid *et al.*<sup>44</sup>

Fully or partially protected para-nitrophenyl thioglycosides proved to be very versatile reagents. The potential usefulness of the “latent” nature of nitro group was nicely illustrated by chemoselective glycosylation of partially benzoylated para-nitrophenyl thioglycosides with “active” thioglycosyl donors using NIS/TfOH as promoter. It was also established that a partially benzoylated para-nitrophenyl thioglycoside (“disarmed-latent” acceptor) could be condensed chemoselectively with a fully benzoylated para-nitrophenyl thioglycoside (“armed-latent” donor) under the influence of NIS/TfOH. Therefore, the reactivity of a para-nitrophenyl thioglycosides toward glycosylation reaction could be regulated by the protecting group at C-2 (acyl type vs ether type) or can simply be “turned on” by transforming their electron withdrawing thioaryl substituents into electron donating group. A simple reduction and acetylation could effectively transform “latent” para-nitrophenyl thioglycosides into the corresponding “active” para-acetamidophenyl glycosides. The oxidation method (mCPBA) to convert para-nitrophenyl thioglycosides into the corresponding phenylsulfinyl glycosyl donors had limited value, at least in the case of disarmed donors. The effectiveness of this method needs further exploration.

The results discussed in this chapter clearly indicate that NIS-TfOH is a valuable promoter system for the chemoselective glycosylation of “latent” acceptor with “active”

The results discussed in this chapter clearly indicate that NIS-TfOH is a valuable promoter system for the chemoselective glycosylation of “latent” acceptor with “active” thioglycosyl donors. Furthermore, this study revealed that the amount of triflic acid used was crucial for the selective activation of “active” thioglycosides over relatively “latent” thioglycosides.

The combination of “active-latent” and “armed-disarmed” glycosidation methodology made it possible to manipulate the reactivity of both the glycosyl donors and acceptors by means of changing the electron withdrawing or electron donating ability of the protecting groups at the anomeric center (active-latent) and at the C-2 position (armed-disarmed). The versatile chemistry allowed us to prepare complex oligosaccharides in a highly convergent manner. This strategy will be advantageous for the synthesis of still more complex oligosaccharides. Actually, this “active-latent” glycosylation strategy has been successfully applied to the synthesis of sialosides such as GM<sub>3</sub>, this will be discussed in the following chapter.

## 3.6 Experimental

### 3.6.1 Synthesis of 2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranosides using Lewis acid catalyzed method

#### Ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside **32**

Titanium tetrachloride ( $\text{TiCl}_4$ ) (1.5 mL) was added to a stirred mixture of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranose **31**<sup>190</sup> (5 g, 10.47 mmol), ethane-thiol (1.6 mL, 21.63 mmol), and ground molecular sieves (4 Å, 7.5 g) in methylene chloride (50 mL) at 0 °C (ice bath). After 1 hour at room temperature, the mixture was filtered through a layer of Celite, washed with ice-cold 1 M sulfuric acid, aqueous sodium bicarbonate and water, dried ( $\text{Na}_2\text{SO}_4$ ), and concentrated. The residue was crystallized from ether-hexane to give **32** as needles (3.7 g) in 74% yield: m.p. 116.5-117.6 °C;  $[\alpha]_{\text{D}} +46.8^\circ$  (C = 1.0,  $\text{CHCl}_3$ ); Lit.<sup>43</sup> m.p. 118-119 °C;  $[\alpha]_{\text{D}} +44^\circ$  (C = 1.0,  $\text{CH}_2\text{Cl}_2$ ); CI-MS (ether) gave m/z (ion, relative intensity): 479.8 ( $[\text{M}+1]^+$ , 2.8%), 417.9 ( $[\text{M}+1\text{-EtSH}]^+$ , 28.4%), 358.0 ( $[\text{M}+1\text{-SPhOMe-AcOH}]^+$ , 62.5%), 297.8 ( $[\text{M}+1\text{-SEt-2 (AcOH)}]^+$ , 100%);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 7.86-7.69 (m, 4H, Phth), 5.80 (dd, 1H,  $J_{3,4} = 9.2$  Hz, H-3), 5.45 (d, 1H,  $J_{1,2} = 10.6$  Hz, H-1), 5.15 (dd, 1H,  $J_{4,5} = 10.2$  Hz, H-4), 4.25 (dd, 1H,  $J_{2,3} = 10.3$  Hz, H-2), 4.30 (dd, 1H,  $J_{6a,6b} = 12.4$  Hz, H-6a), 4.14 (dd, 1H,  $J_{5,6b} = 2.3$  Hz, H-6b), 3.86 (ddd, 1H,  $H_{5,6a} = 4.7$  Hz, H-5), 2.65 (m, 2H,  $\text{SCH}_2$ ), 2.08, 2.00, 1.83 (s, 9H, 3xOAc), 1.18 (t, 3H,  $J = 7.4$  Hz,  $\text{SCH}_2\text{CH}_3$ );  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 170.6, 170.0, 169.4 (3xOAc), 167.7, 167.1 (C=O, Phth.), 134.3 (2C, C-ortho), 131.5, 131.1 (C-ipso), 123.7 (2C, C-meta), 81.1 (C-1), 75.9 (C-5), 71.5 (C-3), 68.8 (C-4), 62.2 (C-6), 53.6 (C-2), 24.3 ( $\text{SCH}_2$ ), 20.8, 20.6, 20.4 (3xMe, Ac), 14.9 ( $\text{SCH}_2\text{CH}_3$ ).

<sup>190</sup> Baker, B. R.; Joseph, J. P.; Schaub, R. E.; Eillams, J. H. *J. Org. Chem.* 1954, 19, 1786

### General procedure for the preparation of compounds 33-36:

Tin (IV) chloride (1 mL) was added to a stirred mixture of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranose **31** (1 equiv), arylthiol (2 equiv.) and ground molecular sieves (4 Å) in dichloromethane (30 mL) at 0 °C. The mixture was stirred under nitrogen at room temperature for 4-6 hours. The mixture was filtered through a layer of celite, washed with ice-cold 1M sulfuric acid, aqueous sodium bicarbonate, and water. The organic phase was dried with sodium sulfate, evaporated under reduced pressure and coevaporated with toluene three times. The residue was purified by silica gel column chromatography or directly crystallized from ether-hexane.

### Phenyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside **33**

The residue was crystallized from ether-hexane to afford pure crystalline **33** in 73% yield: m.p. 145.7-146.2 °C;  $[\alpha]_D +52.8^\circ$  (C = 1.0, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 418 ([M-SPh]<sup>+</sup>, 21.9%), 358 ([M-SPh-AcOH]<sup>+</sup>, 47.6%), 298 ([M-SPh-2(AcOH)]<sup>+</sup>, 100%), 240 ([M-SPh-AcOH-2(AcO)]<sup>+</sup>, 62.1%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm), 7.81-7.73 (m, 4H, phth), 7.71-7.25 (m, 5H, phenyl), 5.78 (dd, 1H, J<sub>3,4</sub> = 9.2 Hz, H-3), 5.70 (d, 1H, J<sub>1,2</sub> = 10.5 Hz, H-1), 5.12 (dd, 1H, J<sub>4,5</sub> = 10.2 Hz, H-4), 4.33 (dd, 1H, J<sub>2,3</sub> = 10.2 Hz, H-2), 4.27 (dd, 1H, J<sub>6a,6b</sub> = 12.3 Hz, H-6a), 4.19 (dd, 1H, H-6b), 3.89 (ddd, 1H, J<sub>5,6a</sub> = 5.0, J<sub>5,6b</sub> = 2.5 Hz, H-5), 2.06, 2.01, 1.82 (s, 3xOAc); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 170.6, 170.0, 169.4 (3s, 9H, OAc), 168.4, 167.6 (Phth), 134.4, 134.3, 133.2, 130.9, 128.8, 128.4, 123.7 (C-aromatic), 83.0 (C-1), 75.8 (C-5), 71.6 (C-3), 68.6 (C-4), 62.2 (C-6), 53.5 (C-2), 20.7, 20.6, 20.3 (3xAc).

### Para-Methylphenyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside **34**

Compound **34** was crystallized from methylene chloride-diethyl ether-hexane and gave white needles in 74% yield: m.p. 168.0-168.4 °C;  $[\alpha]_D +40.6^\circ$  (C = 1.0, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 482 ([M-AcO]<sup>+</sup>, 1.5%), 418 ([M-SPhMe]<sup>+</sup>, 25.8%), 358 ([M-SPh-SPhMe-AcOH]<sup>+</sup>, 66.6%), 298 ([M-SPhMe-2(AcOH)]<sup>+</sup>, 100%),

240 ([M-SPhMe-AcOH-2(AcO)]<sup>+</sup>, 71.0%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 7.87-7.71(m, 4H, Phth), 7.3 1-7.02 (m, 4H, Phenyl), 5.75 (dd, 1H, J<sub>2,3</sub> = 10.2, J<sub>3,4</sub> = 9.2 Hz, H-3), 5.62 (d, 1H, J<sub>1,2</sub> = 10.5 Hz, H-1), 5.09 (dd, 1H, J<sub>3,4</sub> = 9.2, J<sub>4,5</sub> = 10.2 Hz, H-4), 4.29 (dd, 1H, J<sub>1,2</sub> = 10.5, J<sub>2,3</sub> = 10.2 Hz, H-2), 4.30-4.13 (m, 2H, H-6a, H-6b), 3.85 (ddd, 1H, J<sub>4,5</sub> = 10.2, J<sub>5,6</sub> = 4.9, J<sub>5,6b</sub> = 2.5 Hz, H-5), 2.30 (s, 3H, SPh-Me), 2.08, 1.99, 1.81 (3s, 9H, OAc); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 170.5, 170.0, 169.4 (3xOAc), 168.4, 167.7 (Phthalimido), 138.7, 134.3, 134.2, 133.9, 129.6, 126.9, 123.6 (C-aromatic), 83.1 (C-1), 75.8 (C-5), 71.6 (C-3), 68.7 (C-4), 62.1(C-6), 53.6 (C-2), 21.1, 20.7, 20.5, 20.3 (3xAc, Me).

**Para-Methoxyphenyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside 35**

Compound 35 was crystallized from diethyl ether in 73 % yield: m.p. 140.2-140.5 °C; [α]<sub>D</sub> +20.1° (C = 1.0, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 558 ([M+1]<sup>+</sup>, 0.3%), 418 ([M+1-SPhOMe]<sup>+</sup>, 22.4%), 358 ([M+1-SPhOMe-AcOH]<sup>+</sup>, 62.5%), 298 ([M+1-SPhOMe-2 (AcOH)]<sup>+</sup>, 100%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 7.88-7.71 (m, 4H, Phth.), 7.35-6.76 (m, 4H, Ph), 5.73 (dd, 1H, J<sub>3,4</sub> = 9.7 Hz, H-3), 5.55 (d, 1H, J<sub>1,2</sub> = 10.4 Hz, H-1), 5.07 (dd, 1H, J<sub>4,5</sub> = 9.7 Hz, H-4), 4.25 (dd, 1H, J<sub>2,3</sub> = 9.8 Hz, H-2), 4.29-4.13 (m, 2H, H-6a, H-6b), 3.83 (ddd, 1H, J<sub>4,5</sub> = 9.7, H<sub>5,6a</sub> = 4.5, H<sub>5,6b</sub> = 2.7 Hz, H-5), 3.77 (s, 3H, OMe), 2.07, 1.98, 1.80 (3s, 9H, OAc); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 170.6, 170.1, 169.4 (3xOAc), 167.8, 167.0 (C=O, Phth), 136.5 - 114.4 (12C, C-aromatic), 83.0 (C-1), 75.8 (C-5), 71.7 (C-3), 68.6 (C-4), 62.1 (C-6), 55.3 (OMe), 53.5 (C-2), 20.8, 20.6, 20.4 (AcO).

**Para-Nitrophenyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside 36**

Compound 36 was crystallized from methylene chloride-diethyl ether. Compound 36 (1.09 g) was obtained in 91% yield: m.p. 217.2-217.5 °C; [α]<sub>D</sub> +65.7° (c = 0.68, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 420 ([M+1-SPhNO<sub>2</sub>]<sup>+</sup>, 2.3%), 358 ([M+1-HSPhNO<sub>2</sub>-AcOH]<sup>+</sup>, 11.3%), 298 ([M+1-HSPhNO<sub>2</sub>-2AcOH]<sup>+</sup>, 79.9%); IR

(thin film,  $\nu_{\text{cm}^{-1}}$ ): 1749, 1719, 1580, 1518, 1379, 1342, 1230, 1077, 1039, 911, 750, 720;  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.11-7.48 (m, 8H, aromatic H), 5.85 (d, 1H,  $J_{1,2} = 10.6$  Hz, H-1), 5.81 (dd, 1H,  $J_{3,4} = 9.2$  Hz, H-3), 5.13 (dd, 1H,  $J_{4,5} = 10.2$  Hz, H-4), 4.39 (dd, 1H,  $J_{2,3} = 10.2$  Hz, H-2), 4.29 (dd, 1H,  $J_{6a,6b} = 12.4$  Hz, H-6a), 4.20 (1H, H-6b), 3.98 (ddd, 1H,  $H_{5,6a} = 5.2$ ,  $H_{5,6b} = 2.3$  Hz, H-5), 2.10, 2.02, 1.82 (3s, 9H, OAc);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 170.4, 170.0, 169.4 (3xOAc), 168.4, 167.7 (phthalimido), 147.0, 141.1, 134.6, 131.2, 123.8 (C-aromatic), 81.9 (C-1), 76.2 (C-5), 71.3 (C-3), 68.4 (C-4), 62.1 (C-6), 53.3 (C-2), 20.9, 20.6, 20.4 (3xAc).

**Imidazolin-2-yl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside 37**

Tin (IV) chloride (275  $\mu\text{L}$ ) was added to a stirred mixture of **31** (700 mg, 1.47 mmol), 2-mercapto-1-methyl-imidazole (335 mg, 2.94 mmol) and ground molecular sieves (1.0 g, 4  $\text{\AA}$ ) in dichloromethane (10 mL) at 0  $^\circ\text{C}$ . The solution was then allowed to attain room temperature and stirred overnight and filtered through a pad of celite, washed with ice-cold 1M sulfuric acid, aqueous sodium bicarbonate, and water. The organic phase was dried with sodium sulfate, evaporated under reduced pressure, and coevaporated with toluene three times. crude product was purified by chromatography using ethyl acetate-hexane (1:1) as eluent to give pure **37** (281 mg) in 51% yield: m.p. 81.5-82.7  $^\circ\text{C}$ ;  $[\alpha]_D^{+36.0}$  ( $c = 0.68$ ,  $\text{CHCl}_3$ ); (+)FAB-MS (glycerol) gave  $m/z$  (ion, relative intensity): 532.2 ( $[\text{M}+1]^+$ , 18.4%); IR (thin film,  $\nu_{\text{cm}^{-1}}$ ): 2953, 1748, 1719, 1460, 1379, 1232, 1078, 1041, 913, 726;  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.11-8.07 (m, 2H, Ph-meta), 7.85-7.81 (m, 2H, Phth-meta), 7.77-7.72 (m, 2H, Phth-ortho), 7.86-7.68 (m, 4H, Phth), 7.06 (d, 1H,  $J = 1.2$  Hz, imidazoline), 6.96 (d, 1H, imidazoline), 5.80 (d, 1H,  $J_{1,2} = 10.6$  Hz, H-1), 5.74 (dd, 1H,  $J_{3,4} = 9.2$  Hz, H-3), 5.09 (dd, 1H,  $J_{4,5} = 9.2$  Hz, H-4), 4.34 (dd, 1H,  $J_{2,3} = 10.2$  Hz, H-2), 4.21 (dd, 1H,  $J_{5,6a} = 4.8$ ,  $J_{6a,6b} = 12.5$  Hz, H-6a), 4.11 (1H,  $J_{5,6b} = 2.5$  Hz, H-6b), 3.4 (ddd, 1H, H-5), 3.65 (s, 3H, NHAc), 2.04, 1.98, 1.81 (3s, 9H, OAc);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 173.6, 170.4, 169.9 (3xOAc), 167.6, 166.9 (C=O, Phth), 135.2-123.7 (10C, Phth).

imidazoline), 83.0 (C-1), 75.9 (C-5), 71.4 (C-3), 68.4 (C-4), 61.9 (C-6), 53.7 (C-2), 34.2 (NHAc), 20.7, 20.6, 20.3 (OAc).

### 3.6.2 Synthesis of partially protected 2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranosides

#### Ethyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside 38

To a suspension of ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside **32** (3.0 g, 6.26 mmol) in methanol (100 mL), sodium methoxide in methanol (0.3 mL, 1.0 M) was added. After 2 hours, acetic acid (1 mL) was added to the solution. The solution was concentrated, toluene was distilled from the residue. The crude product was then dissolved in N,N-dimethylformamide (20 mL) containing  $\alpha,\alpha$ -dimethoxytoluene (1.8 mL). *p*-Toluenesulfonic acid (180 mg, 0.18 eq) was added. The mixture was stirred at 50 °C for 1 hour, cooled and partitioned between aqueous sodium bicarbonate and toluene-ethyl ether (3:1). The organic layer was washed with water, dried ( $\text{Na}_2\text{SO}_4$ ), treated with silica gel for 5 min, filtered and concentrated. Recrystallisation from ethyl ether-dichloromethane-hexane gave **38** (2.35 mg) as prisms in 85% yield: m.p. 167.8-168.4 °C;  $[\alpha]_{\text{D}} -10.1^\circ$  ( $c = 1.1$ ,  $\text{CHCl}_3$ ); IR (thin film,  $\nu_{\text{cm}^{-1}}$ ): 3468, 2874, 1774, 1711, 1612, 1468, 1384, 1092, 1043, 989, 920, 873, 760, 721, 700; CI-MS (ether) gave  $m/z$  (ion, relative intensity): 442 ( $[\text{M}+1]^+$ , 32.9%), 380 ( $[\text{M}+1\text{-EtSH}]^+$ , 100%), 362 ( $[\text{M}+1\text{-EtSH-H}_2\text{O}]^+$ , 79.7%), 336 ( $[\text{M}+1\text{-PhCHO}]^+$ , 27.0%), 274 ( $[\text{M}+1\text{-PhCHO-EtSH}]^+$ , 100%);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 7.86-7.34 (m, 9H, Aromatic), 5.56 (s, 1H, PhCH), 5.40 (d, 1H,  $J_{1,2} = 10.5$  Hz, H-1), 4.66 (dd, 1H,  $J_{3,4} = 8.8$  Hz, H-3), 4.39 (dd,  $J_{6a,6b} = 10.1$  Hz, H-6a), 4.32 (dd, 1H,  $J_{2,3} = 10.1$  Hz, H-2), 3.80 (1H, H-6b), 3.70 (ddd,  $J_{5,6a} = 4.4$ ,  $J_{5,6b} = 9.9$  Hz, H-5), 3.61 (dd,  $J_{4,5} = 9.0$  Hz, H-4), 2.74-2.61 (m, 2H,  $\text{SCH}_2$ ), 1.70 (br., 1H, OH), 1.18 (t, 3H,  $J = 7.5$  Hz, Me);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 168.2, 167.7 (C=O Phthalimido), 136.9-123.3 (Aromatic), 101.9 (PhCH), 82.1 (C-4), 81.3 (C-1), 70.3 (C-5), 69.5 (C-3), 68.6 (C-6), 55.4 (C-2), 24.1 ( $\text{SCH}_2$ ), 14.8 ( $\text{CH}_3$ ).

**Para-Nitrophenyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside 39**

To a suspension of para-nitrophenyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **36** (4.0 g, 6.99 mmol) in methanol (100 mL), sodium methoxide in methanol (0.3 mL, 1.0 M) was added. After stirring for 2 hours at room temperature, the reaction was quenched with H<sup>+</sup> resin (Dowex 50w-X8) and then filtered. The filtrate was evaporated under reduced pressure. Toluene was added and distilled from the residue. The crude product was then dissolved in dry acetonitrile (20 mL) containing α,α-dimethoxytoluene (3.0 mL). p-TsOH (180 mg, 0.18 eq) was added. The mixture was stirred for 50 min at room temperature. TLC (4% ethanol in benzene) showed complete conversion of starting material **34** (R<sub>f</sub> = 1.7) to product **39** (R<sub>f</sub> = 0.48). The reaction mixture was filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using 2% methanol in methylene chloride as eluent to afford **39** (4.15 g) in 90% yield: m.p. 150-152 °C; [α]<sub>D</sub> +72° (c = 1.0, CHCl<sub>3</sub>); IR (thin film, ν<sub>cm-1</sub>): 3466, 1712, 1581, 1516, 1471, 1384, 1341, 1091, 873, 721; CI-MS (ether) gave m/z (ion, relative intensity): 534.8 ([M]<sup>+</sup>, 31.6%), 379.9 ([M-HSPhNO<sub>2</sub>]<sup>+</sup>, 71.9%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 8.12-7.29 (m, 9H, Aromatic), 5.80 (d, 1H, J<sub>1,2</sub> = 10.5 Hz, H-1), 5.55 (s, 1H, PhCH), 4.64 (dd, 1H, J<sub>3,4</sub> = 8.8 Hz, H-3), 4.38 (dd, 1H, H-6a), 4.36 (dd, 1H, J<sub>2,3</sub> = 9.9 Hz, H-2), 3.85-3.74 (m, 2H, H-5, H-6b), 3.60 (dd, J<sub>4,5</sub> = 8.9 Hz, H-4), 2.68 (br, 1H, OH); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 168.2, 167.4 (C=O Phth), 146.7-123.5 (12C, Aromatic), 101.9 (PhCH), 82.7 (C-4), 81.6 (C-1), 70.5 (C-5), 69.4 (C-3), 68.4 (C-6), 55.9 (C-2).

**Ethyl 3-O-benzyl-4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside 40**

To a solution of ethyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **32** (1.0 g, 2.3 mmol) in dry N,N-dimethylformamide (10 mL) was added grounded molecular sieve (1.0 g, 4 Å), sodium hydride (2.2 g, 50% in mineral oil, washed three time with hexane, 4.1 mmol) and the mixture was stirred for 15 min. To this mixture

was added benzyl bromide (0.5 mL, 0.41 mol) at 0 °C (ice bath) and the reaction mixture was stirred at room temperature for 6 hours. The mixture was quenched with ice-water and extracted with toluene-ethyl ether (3:1). The organic phase was washed with saturated sodium bicarbonate, water, dried over sodium sulfate and concentrated. The residue was purified by chromatography on silica gel using ethyl acetate-hexane 1:5 as eluent to afford **40** (1.03 g) in 85% yield. The product was crystallized from ethyl ether-hexane to give needles: m.p. 95.7-96.5 °C;  $[\alpha]_D +62^\circ$  (c = 0.95, CHCl<sub>3</sub>); Lit.<sup>43</sup>  $[\alpha]_D +66^\circ$  (c = 1.0, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 532 ([M]<sup>+</sup>, 17.7%), 470 ([M-EtSH]<sup>+</sup>, 60.4%), 426 ([M-PhCHO]<sup>+</sup>, 41.6%), 364 ([M-EtSH-PhCHO]<sup>+</sup>, 96.1%), 362 ([M-EtSH-PhCH<sub>2</sub>OH]<sup>+</sup>, 100%); IR (thin film)  $\nu$ : 3032, 2893, 1774, 1714, 1612, 1496, 1457, 1380, 1261, 1172, 1087, 1002, 917, 874, 793, 751, 721, 700, 660 cm<sup>-1</sup>; <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 7.87-6.83 (m, 14H, aromatic), 5.62 (s, 1H, PhCH), 5.33 (d, 1H, J<sub>1,2</sub> = 10.4 Hz, H-1), 4.64 (AB pattern, 2H, J<sub>a,b</sub> = 12.3 Hz), 4.42 (dd, 1H, J<sub>3,4</sub> = 10.2 Hz, H-3), 4.41 (dd, J<sub>6a,6b</sub> = 10.1 Hz, H-6a), 4.28 (dd, 1H, J<sub>2,3</sub> = 10.0 Hz, H-2), 3.83 (dd, 1H, H-6b), 3.80 (dd, 1H, J<sub>4,5</sub> = 10.0 Hz, H-4), 3.69 (ddd, J<sub>5,6a</sub> = 4.9, J<sub>5,6b</sub> = 4.4 Hz, H-5), 2.63 (m, 2H, SCH<sub>2</sub>), 1.15 (t, 3H, CH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 167.6, 167.3 (C=O Phth), 137.8-123.3 (Aromatic), 101.3 (PhCH), 83.0 (C-4), 81.7 (C-1), 75.4 (C-3), 74.1 (CH<sub>2</sub>), 70.4 (C-5), 68.7 (C-6), 54.6 (C-2), 24.0 (SCH<sub>2</sub>), 14.8 (CH<sub>3</sub>, ethyl).

#### **Ethyl 3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside 41**

Diethyl ether saturated with hydrogen chloride was added dropwise, at 0 °C (ice bath) to a stirred mixture of compound **40** (1.0 g, 1.88 mmol), sodium cyanoborohydride (710 mg, 11.28 mmol, 6 eq) and molecular sieves (1.2 g, 3 Å) in tetrahydrofuran (30 mL) until the mixture was acidic (as determined with pH paper and gas evolution). After the reaction mixture was stirred at 0 °C for 15 min, TLC showed a complete reaction. The reaction mixture was then poured into ice-water, and the product was extracted with dichloromethane. The extracts were washed with saturated sodium bicarbonate, dried over sodium sulfate and concentrated *in vacuo*. The crude product was purified by column

chromatography on silica gel (ethyl acetate-hexane, 2:5) to afford **37** (825 mg) in 82% yield. The product was jelly-like and solidified in one week: m.p. 109.3-110.5 °C;  $[\alpha]_D^{+40}$  (c = 0.85, CHCl<sub>3</sub>); lit.<sup>157a</sup> m.p. 110 °C;  $[\alpha]_D^{+43}$  (c = 1.5, CHCl<sub>3</sub>); M.S. (pos FAB) (rel intensity) (M/Z): 534 ([M]<sup>+</sup>, 1.2%), 472 ([M-EtSH]<sup>+</sup>, 7.3%), 364 ([M-EtSH-PhCH<sub>2</sub>OH]<sup>+</sup>, 3.4%); IR (thin film) v: 3468, 2903, 1774, 1711, 1611, 1496, 1453, 1386, 1081, 874, 721, 700 cm<sup>-1</sup>; <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 7.81-6.91 (m, 14H, aromatic), 5.26 (d, 1H, J<sub>1,2</sub>=10.2 Hz, H-1), 4.76-4.51 (m, 4H, 2xOCH<sub>2</sub>Ph), 4.25 (dd, 1H, J<sub>3,4</sub> = 8.2 Hz, H-3), 4.21(dd, 1H, J<sub>2,3</sub>=10.1 Hz, H-2), 3.82 (dd, 1H, J<sub>6a,6b</sub> = 10.1 Hz, H-6a), 3.80 (dd, 1H, J<sub>4,5</sub> = 9.5 Hz, H-4), 3.76 (dd, 1H, H-6b), 3.67 (ddd, 1H, J<sub>5,6a</sub> = 4.8, J<sub>5,6b</sub> = 5.2 Hz, H-5), 3.00 (br., 4-OH), 2.68-2.54 (m, 2H, SCH<sub>2</sub>), 1.15 (t, 3H, CH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 168.1, 167.5 (C=O Phth), 138.1-123.3 (Aromatic), 81.2 (C-1), 79.6 (C-3), 77.7 (C-5), 74.5 (OCH<sub>2</sub>Ph), 74.8 (C-4), 73.8 (OCH<sub>2</sub>Ph), 70.8 (C-6), 54.4 (C-2), 24.0 (SCH<sub>2</sub>), 14.9 (CH<sub>3</sub>).

#### **Ethyl 2-deoxy-2-phthalimido-3,6-di-O-pivaloyl-1-thio-β-D-glucopyranoside 44**

To a suspension of ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **32** (1.5 g, 4.17 mmol) in methanol (60 mL) was added 1M sodium methoxide in methanol (200 μL). After 35 min, TLC (MeOH/CH<sub>2</sub>Cl<sub>2</sub>, 1:9 v/v) showed that the Zemplén deacetylation was completed. The solution was neutralized with H<sup>+</sup> resin (Dowex 50w-X8), filtered and concentrated under reduced pressure. Toluene was added and distilled from the residue. The product was then dissolved in dry pyridine (20 mL) and cooled to 0 °C followed by the addition of pivaloyl chloride (1.21 mL, 10 mmol). The mixture was allowed to attain room temperature and the stirring was continued for 48 hours. The reaction mixture was then quenched with ice water and extracted with methylene chloride (20 mL x2). The extracts were washed with sodium bicarbonate, water and saturated sodium chloride, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under reduced pressure. The residue syrup was purified by column chromatography on silica gel using 10-50% gradient of ethyl acetate in hexane, to afford dipivaloyled product **44** (R<sub>f</sub> = 0.80, ethyl acetate/hexane 1:1) (760 mg) in 35% yield and monopivaloyled product **43** (R<sub>f</sub> =

0.33) (822 mg) in 45% yield. Compound 44 was recrystallised from ether-hexane gave prisms: m.p. 123.8-124.6 °C;  $[\alpha]_D -12.2^\circ$  (c = 1.0, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 552.0 ([M+1]<sup>+</sup>, 8.3%), 459.9 ([M+1-EtSH]<sup>+</sup>, 39.9%), 357.9 ([M+1-EtSH-(CH<sub>3</sub>)<sub>3</sub>C(O)OH]<sup>+</sup>, 100%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 7.83 (m, 2H, H-ortho, Phth), 7.73 (m, 2H, H-meta, Phth), 5.64 (dd, 1H, J<sub>3,4</sub> = 9.0 Hz, H-3), 5.45 (d, 1H, J<sub>1,2</sub> = 10.5 Hz, H-1), 4.44 (dd, J<sub>6a,6b</sub> = 12.1 Hz, H-6a), 4.38 (dd, 1H, J<sub>5,6b</sub> = 4.9 Hz, H-6b), 4.31 (dd, 1H, J<sub>2,3</sub> = 10.4 Hz, H-2), 3.76 (ddd, 1H, J<sub>5,6a</sub> = 2.7 Hz, H-5), 3.57 (dd, J<sub>4,5</sub> = 9.7 Hz, H-4), 2.60 (m, 2H, SCH<sub>2</sub>CH<sub>3</sub>), 1.23 (s, 9H, tBu), 1.22 (t, 3H, J = 7.4 Hz, SCH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 179.1, 178.8 (C=O, Piv), 167.67, 167.3 (C=O, Phth), 134.3-123.5 (C-aromatic, Phth), 80.8 (C-1), 78.4 (C-5), 73.9 (C-3), 70.3 (C-4), 63.4 (C-6), 53.4 (C-2), 38.9, 38.7 (C(O)C(CH<sub>3</sub>)<sub>3</sub>), 27.2 (3C, tBu), 26.7 (3C, tBu), 24.1 (SCH<sub>2</sub>), 15.0 (CH<sub>3</sub>).

Anal. Calcd for (C<sub>26</sub>H<sub>35</sub>O<sub>8</sub>NS): C, 59.86; H, 6.76; N, 2.69; S, 6.15. Found: C, 60.00; H, 6.80; N, 2.51; S, 6.10.

#### **Ethyl 2-deoxy-2-phthalimido-6-O-pivaloyl-1-thio-β-D-glucopyranoside 43**

Compound 43 had: m.p. 69.4-71.2 °C;  $[\alpha]_D -18.9^\circ$  (c = 1.0, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 437.8 ([M]<sup>+</sup>, 2.7%), 375.9 ([M-EtSH]<sup>+</sup>, 100%), 357.9 ([M-EtSH-H<sub>2</sub>O]<sup>+</sup>, 18.2%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 7.82 (m, 2H, H-ortho, Phth), 7.72 (m, 2H, H-meta, Phth), 5.30 (d, 1H, J<sub>1,2</sub> = 10.4 Hz, H-1), 4.46 (dd, J<sub>6a,6b</sub> = 12.2 Hz, H-6a), 4.36 (dd, 1H, J<sub>3,4</sub> = 8.7 Hz, H-3), 4.32 (dd, 1H, J<sub>5,6b</sub> = 2.3 Hz, H-6b), 4.16 (dd, 1H, J<sub>2,3</sub> = 10.4 Hz, H-2), 3.62 (ddd, 1H, J<sub>5,6a</sub> = 4.7 Hz, H-5), 3.32 (dd, J<sub>4,5</sub> = 9.8 Hz, H-4), 2.63 (m, 2H, SCH<sub>2</sub>CH<sub>3</sub>), 1.21 (s, 9H, tBu), 1.19 (t, 3H, J = 7.5 Hz, SCH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 179.3 (C=O, Piv), 168.2, 168.1 (C=O, Phth), 136.1-123.4 (C-aromatic, Phth), 81.0 (C-1), 77.9 (C-5), 72.3 (C-3), 71.7 (C-4), 63.7 (C-6), 55.4 (C-2), 38.8 (C(O)C(CH<sub>3</sub>)<sub>3</sub>), 27.1 (3C, tBu), 24.0 (SCH<sub>2</sub>), 15.0 (CH<sub>3</sub>).

Anal. Calcd for (C<sub>21</sub>H<sub>27</sub>O<sub>7</sub>NS): C, 57.64; H, 6.22; N, 3.19; S, 7.32. Found: C, 57.13; H, 6.18; N, 2.91; S, 7.04.

### 3.6.3 Synthesis of “Active-Latent” $\beta$ -D-galactopyranosyl donors and acceptors

#### Phenyl 1-thio- $\beta$ -D-galactopyranoside 46

Compound **8** (2.0 g, 4.1 mmol) was deacetylated using the standard Zemplén deacetylation procedure. The resulting residue was purified by silica gel chromatography using methanol-chloroform (9:1, v/v) as eluent to afford **46** (1.17 g) in 96 % yield. The pure **46** was recrystallised from ethanol: m.p. 120.5-121.3 °C;(ethanol);  $[\alpha]_D -51.0^\circ$  (c = 1.15, CH<sub>3</sub>OH); IR (thin film,  $\nu_{cm-1}$ ): 3383 (br), 1345, 1056, 865, 745, 693; CI-MS (ether) gave m/z (ion, relative intensity): 273.3 ([M+1]<sup>+</sup>, 29.4%), 255.2 ([M+1-H<sub>2</sub>O]<sup>+</sup>, 79.4%), 163.3 ([M+1-PhSH]<sup>+</sup>, 99.7%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 7.63-7.55 (m, 2H, H-meta), 7.49-7.37 (m, 3H, H-ortho, H-para); 4.82 (d, 1H,  $J_{1,2} = 9.3$  Hz, H-1), 4.74-3.60 (m, 6H, H-2, 3, 4, 5, 6a, 6b); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 135.8 (C-ipso), 134.0 (2C, C-meta), 132.4 (2C, C-ortho), 130.8 (C-para), 91.1 (C-1), 81.9 (C-5), 77.0 (C-3), 72.2 (C-4), 71.7 (C-2), 63.9 (C-6).

#### Para-Nitrophenyl 1-thio- $\beta$ -D-galactopyranoside 47

To a suspension of **9** (10.0 g, 20.6 mmol) in methanol (100 mL) was added 1 M NaOMe (1.5 mL) (pH = 9-10). The mixture was stirred at room temperature for 50 min, TLC (1/10, MeOH/CHCl<sub>3</sub>) showed that the Zemplén deacetylation was finished. The solution was neutralized with H<sup>+</sup> resin (Dowex 50w-X8) and evaporated under reduced pressure to afford **47** (6.28 g) in 96% yield. The product was recrystallised from ethanol: m.p. 160.2-161.5 °C;  $[\alpha]_D -100.8^\circ$  (c = 1.0, CH<sub>3</sub>OH); CI-MS (ether) gave m/z (ion, relative intensity): 318 ([M+1]<sup>+</sup>, 1.1%), 163 ([M+1-SPhNO<sub>2</sub>]<sup>+</sup>, 38.3%); <sup>1</sup>H-NMR (D<sub>2</sub>O)  $\delta$  (ppm): 8.17 (d,  $J_{m,o} = 9.1$  Hz, H-meta), 7.63 (d, H-ortho), 5.02 (d, 1H,  $J_{1,2} = 9.7$  Hz, H-1), 4.06 (d, 1H,  $J_{3,4} = 2.0$  Hz, H-4), 3.92-3.69 (m, 5H, H-2, H-3, H-5, H-6a, H-6b); <sup>13</sup>C-NMR (D<sub>2</sub>O)  $\delta$  (ppm): 148.2 (C-para), 146.6 (C-ipso), 130.9 (C-meta), 126.6 (C-ortho), 88.6 (C-1), 81.6 (C-5), 76.4 (C-3), 71.5 (C-4), 71.1 (C-2), 63.4 (C-6).

### **Ethyl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-galactopyranoside 48**

Tin (IV) chloride (0.5 mL) was added to a stirred mixture of 1,2,3,4,6-penta-O-benzoyl-β-D-galactopyranoside (701 mg, 1 mmol), ethanethiol (0.5 mL, 6.75 mmol), and ground molecular sieve (4Å, 1 g) in methylene chloride (10 mL) at 0 °C (ice bath). After 1 hour at room temperature, the mixture was filtered through a layer of celite, washed with ice-cold 1 M sulfuric acid, aqueous sodium bicarbonate and water, dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate-hexane, 1:4) to afford **48** (531 mg) in 83% yield: m.p. 101-102 °C; [α]<sub>D</sub> +110.2° (c = 1.0, CHCl<sub>3</sub>); M.S.(C.I. ether) (M/Z): 640.9 ([M]<sup>+</sup>, 2.7%), 578.9 ([M-EtSH]<sup>+</sup>, 100%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 8.10-7.18 (20H, aromatic), 6.00 (dd, 1H, J<sub>4,5</sub> <1.0 Hz, H-4), 5.85 (dd, 1H, J<sub>2,3</sub> = 9.9 Hz, H-2), 5.60 (dd, 1H, J<sub>3,4</sub> = 3.3 Hz, H-3), 4.86 (d, 1H, J<sub>1,2</sub> = 9.7 Hz, H-1), 4.65 (m, 1H, H-6a), 4.59-4.30 (m, 2H, H-5, H-6b); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 165.1, 165.7, 165.5, 165.4 (4x C=O, Bz), 133.5-128.3 (C-aromatic), 88.2 (C-1), 82.9 (C-5), 81.1 (C-3), 77.9 (C-4), 70.2 (C-2), 63.5 (C-6).

Anal. Calcd for C<sub>36</sub>H<sub>32</sub>O<sub>9</sub>S (640.68): C, 67.49; H, 5.03. Found: C, 67.54; H, 5.01.

### **Phenyl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-galactopyranoside 49**

To a solution of 2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl bromide (6.65 g, 10.08 mmol.), TBAHS (5.1 g, 1.5 equiv.) and thiophenol (2.08 mL, 20.16 mmol, 2 equiv.) in ethyl acetate (100 mL) was added 1M aq Na<sub>2</sub>CO<sub>3</sub> (100 mL). The two phase reaction mixture was stirred vigorously at room temperature for 50 min. The mixture was diluted with ethyl acetate (100 mL) and the organic layer was washed with saturated sodium bicarbonate, water (3x50 mL), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under reduced pressure. The residue was purified from silica gel chromatography (ethyl acetate-hexane 1:3) to give **49** (6.64 g) in 96% yield: [α]<sub>D</sub> +92.2° (c = 1.0, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 688.8 ([M]<sup>+</sup>, 1.9%), 579.0 ([M-SPh]<sup>+</sup>, 95%); IR (thin film, ν<sub>cm-1</sub>): 3065, 2960, 1727, 1600, 1451, 1268, 1100, 1069, 1027, 709; <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 8.05-7.10 (25H, aromatic), 6.00 (dd, 1H, J<sub>4,5</sub> <1.0 Hz, H-4), 5.77 (dd, 1H, J<sub>2,3</sub> = 9.9 Hz, H-2), 5.60 (dd, 1H, J<sub>3,4</sub> = 3.2 Hz, H-3), 5.04 (d, 1H, J<sub>1,2</sub> = 9.7 Hz, H-1), 4.64 (m,

1H, H-6a), 4.48-4.41 (m, 2H, H-5, H-6b); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 166.0, 165.4, 165.3, 165.1 (4xC=O, Bz), 138.9-123.2 (C-aromatic), 85.8 (C-1), 75.1 (C-5), 72.9 (C-3), 67.3 (C-2), 67.8 (C-4), 62.5 (C-6).

#### **Para-Nitrophenyl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-galactopyranoside 50**

Para-nitrophenyl 1-thio-β-D-galactopyranoside **47** (0.5 g, 1.6 mmol) was dissolved in dry pyridine (8 mL). Benzoyl chloride (1.1 mL, 9.6 mmol) and a catalytic amount of 4-dimethylaminopyridine were added. The solution was stirred at 0 °C for 1 hour, and was then allowed to attain to room temperature for an additional 3 hours. TLC (ether, 100%) showed that the reaction was complete. The reaction mixture was then quenched with ice water and extracted with methylene chloride (10 mL x2). The extracts were washed with sodium bicarbonate, water and saturated sodium chloride. The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, concentrated under reduced pressure and coevaporated with toluene (2x20 mL). The product was crystallized from diethyl ether-hexane mixture (10 mL, 1:1) to afford **50** (1.03 g) in 89% yield as needles: m.p. 157-159 °C; [α]<sub>D</sub> +76.5° (c = 1.0, CHCl<sub>3</sub>); IR (thin film, ν<sub>cm<sup>-1</sup></sub>): 3067, 2966, 1727, 1600, 1582, 1518, 1452, 1342, 1269, 1099, 1069, 910, 852, 711; CI-MS (ether) gave m/z (ion, relative intensity): 733.7 ([M]<sup>+</sup>, 3.3%), 579.2 ([M-HSPhNO<sub>2</sub>]<sup>+</sup>, 100%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 8.03-7.19 (m, 24H, aromatic), 6.03 (dd, 1H, J<sub>4,5</sub> < 1.0 Hz, H-4), 5.82 (dd, 1H, J<sub>2,3</sub> = 9.9 Hz, H-2), 5.66 (dd, 1H, J<sub>3,4</sub> = 3.3 Hz, H-3), 5.17 (d, 1H, J<sub>1,2</sub> = 9.9 Hz, H-1), 4.64 (dd, 1H, H<sub>5,6a</sub> = 7.2, J<sub>6a,6b</sub> = 11.3 Hz, H-6a), 4.53 (dd, J<sub>5,6b</sub> = 4.5 Hz, H-6b), 4.47 (m, 1H, H-5); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 165.9, 165.3(2C), 165.1 (C=O, 4xBz), 146.9 (C-para), 141.5 (C-ipso), 133.9 - 123.7 (28C, aromatic), 84.6 (C-1), 75.6 (C-5), 72.5 (C-3), 68.2 (C-4), 67.5 (C-2), 62.6 (C-6).

Anal. Calcd for C<sub>40</sub>H<sub>31</sub>O<sub>11</sub>NS (733.75): C, 65.48; H, 4.26; N, 1.91; S, 4.37. Found: C, 65.61; H, 4.46; N, 1.92; S, 4.20.

**Para-Nitrophenyl 6-O-t-butyldimethylsilyl-2,3,4-tri-O-pivaloyl-1-thio-β-D-galactopyranoside 60**

To a solution of **47** (1.2 g, 3.79 mmol) in dry pyridine (10 mL) was added t-butyldimethylsilyl chloride (0.742 g, 4.92 mmol) at 0°C. The reaction mixture was then allowed to attain room temperature and stirred for 3 hours. TLC (8% methanol in dichloromethane) showed a clear conversion of starting material **47** to 6-O silylated intermediate. The reaction mixture was cooled to 0 °C again and pivaloyl chloride (1.86 mL, 15 mmol) was added dropwise to the reaction mixture. The stirring was continued for additional 72 hours. TLC (Hexane-EtOAc, 4:1, v/v) showed that reaction was still not complete. The reaction temperature was raised to 70°C for additional 8 hours. The reaction mixture was then quenched with ice water and extracted with methylene chloride (20 mL x2). The extracts were washed with sodium bicarbonate, water and saturated sodium chloride, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under reduced pressure. Column chromatography on silica gel (ethyl acetate-hexane 1:8 v/v) of the residue gave tripivaloylated product **60** (1.32 g) in 53% yield (R<sub>f</sub> = 0.47, EtOAc-hexane 1:8 v/v) and tetrapivaloylated product **51** (860 mg) in 36% yield (R<sub>f</sub> = 0.32).

Compound **60** had: m.p. 121.5-123.4 °C; (ether-hexane); [α]<sub>D</sub> -22.0° (c = 1.0, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 683.9 ([M]<sup>+</sup>, 2.8%), 529.1 ([M-HSPHNO<sub>2</sub>]<sup>+</sup>, 100%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 8.13 (d, J<sub>m,o</sub> = 9.0 Hz, H-meta), 7.61 (d, H-ortho), 5.49 (dd, 1H, J<sub>4,5</sub> = 0.9 Hz, H-4), 5.26 (dd, 1H, J<sub>2,3</sub> = 9.9 Hz, H-2), 5.14 (dd, 1H, J<sub>3,4</sub> = 3.0 Hz, H-3), 4.81 (d, 1H, J<sub>1,2</sub> = 9.6 Hz, H-1), 3.86 (ddd, 1H, J<sub>5,6a</sub> = 6.3 Hz, H-5), 3.72 (dd, 1H, J<sub>6a,6b</sub> = 10.0 Hz, H-6a), 3.56 (dd, J<sub>5,6b</sub> = 6.7 Hz, H-6b), 1.18, 1.15, 1.06 (3s, 27 H, 3xtBu, Piv), 0.85 (s, 9H, tBu), 0.02, 0.01 (2s, 6H, ≡SiCH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 177.0, 176.4 (2C) (C=O, Piva), 146.8 (C-para), 141.8 (C-ipso), 131.3 (2C, C-meta), 123.7 (C-ortho), 84.5 (C-1), 76.5 (C-5), 72.1 (C-3), 66.9 (C-2), 66.5 (C-4), 61.0 (C-6), 38.9, 39.7 (2C), (3xC(O)C(CH<sub>3</sub>)<sub>3</sub>), 27.00 (9C, 3xtBu, Piv), 25.7 [3C, SiC(CH<sub>3</sub>)<sub>3</sub>], 18.1 (SiC(CH<sub>3</sub>)<sub>3</sub>), -5.6, -5.5 [2C, Si(CH<sub>3</sub>)<sub>2</sub>].

Anal. Calcd for C<sub>33</sub>H<sub>53</sub>O<sub>10</sub>NSSi (683.31): C, 57.95; H, 7.82; N, 2.05; S, 4.68. Found: C, 57.87; H, 7.96; N, 2.00; S, 5.03.

### Para-Nitrophenyl 2,3,4,6-tetra-O-pivaloyl-1-thio-β-D-galactopyranoside 51

Compound 51 had: m.p. 132.5-133.4 °C; (CH<sub>2</sub>Cl<sub>2</sub>); [α]<sub>D</sub><sup>-16.6°</sup> (c = 1.0, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 530.9 ([M]<sup>+</sup>, 1.2%), 489.9 ([M-HSPhNO<sub>2</sub>]<sup>+</sup>, 100%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 8.14 (d, J<sub>m,o</sub> = 9.0 Hz, H-meta), 7.63 (d, H-ortho), 5.49 (dd, 1H, J<sub>4,5</sub> = 2.2 Hz, H-4), 5.23 (dd, 1H, J<sub>2,3</sub> = 9.3 Hz, H-2), 5.14 (dd, 1H, J<sub>3,4</sub> = 2.9 Hz, H-3), 4.83 (d, 1H, J<sub>1,2</sub> = 9.9 Hz, H-1), 4.18 (dd, 1H, J<sub>6a,6b</sub> = 8.9 Hz, H-6a), 4.08 (ddd, 1H, H<sub>5,6a</sub> = 5.4 Hz, H-5), 4.01 (dd, J<sub>5,6b</sub> = 4.2 Hz, H-6b), 1.17, 1.16, 1.15, 1.06 (4s, 36 H, 4xtBu, Piva); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 177.6, 176.9, 176.5, 176.3 (4xC=O, Piva), 147.0 (C-para), 141.2 (C-ipso), 131.8 (2C, C-meta), 123.6 (C-ortho), 84.3 (C-1), 75.0 (C-5), 71.7 (C-3), 66.8 (C-2), 66.2 (C-4), 61.3 (C-6), 38.9, 38.6 (3C), (4xC(O)C(CH<sub>3</sub>)<sub>3</sub>), 26.9 (12C, 3xtBu, Piv).

### Para-Nitrophenyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-galactopyranoside 52

To para-nitrophenyl 1-thio-β-D-galactopyranoside 47 (634 mg, 2.0 mmol) in dry N,N-dimethyl formamide (5 mL) were added a sodium hydride dispersion (50% in oil, 768 mg, 16 mmol) and then benzyl bromide (1.46 mL, 12 mmol) at 0 °C. The mixture was allowed to attain room temperature and stirred for 4 hours. Methanol (1 mL) was added to destroy excess benzyl bromide, diluted with toluene-ether (3:1 v/v) 100 mL, and washed with 5% HCl solution (2x50 mL), dried, and concentrated under reduced pressure. The residue was recrystallised from ether-hexane (20 mL, 1:1 v/v) to give the desired product (1.0 g) and the mother liquors were concentrated and subject to column chromatography on silica gel (ethyl acetate-hexane 1:2) to give a second portion of product (90 mg) in a total yield (1.09 g) 81%: m.p. 99-100 °C; [α]<sub>D</sub><sup>-34.0°</sup> (c = 1.0, CHCl<sub>3</sub>); IR (KBr, ν<sub>cm<sup>-1</sup></sub>): 3063, 3030, 2887, 1595, 1579, 1514, 1544, 1343, 1091, 910, 851, 740, 699; CI-MS (ether) gave m/z (ion, relative intensity): 525.0 ([M+1-HSPhNO<sub>2</sub>]<sup>+</sup>, 0.8%); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 7.86 (d, J<sub>m,o</sub> = 9.0 Hz, H-meta), 7.58 (d, H-ortho), 7.35-7.26 (m, 22H, aromatic), 4.77-4.43 (m, 8H, 4xOCH<sub>2</sub>), 4.73 (d, 1H, J<sub>1,2</sub> = 9.5 Hz, H-1), 4.01 (d 1H, J<sub>4,5</sub> < 1.0 Hz, H-4), 4.00 (dd, 1H, J<sub>2,3</sub> = 9.0 Hz, H-2), 3.67-3.61 (4H, H-3, H-5, H6a, H-6b); <sup>13</sup>C-NMR (125.8 MHz, CDCl<sub>3</sub>) δ (ppm): 165.1 (C=O, 4xBz,

145.9 (C-para), 144.4 (C-ipso), 138.3 - 123.8 (28C, aromatic), 84.7 (C-1), 84.2 (C-5), 84.0 (C-3), 76.7 (C-4), 75.8 (OCH<sub>2</sub>), 74.7 (OCH<sub>2</sub>), 73.7 (OCH<sub>2</sub>), 73.3 (C-2), 72.7 (OCH<sub>2</sub>), 68.7 (C-6).

Anal. Calcd for C<sub>40</sub>H<sub>39</sub>NO<sub>7</sub>S (677.82): C, 70.88; H, 5.80; N, 2.07; S, 4.73. Found: C, 71.07; H, 6.00; N, 2.07; S, 4.76.

#### Phenyl 6-O-t-butyldimethylsilyl-1-thio-β-D-galactopyranoside 53

Phenyl 1-thio-β-D-galactopyranoside **46** (0.5 g, 1.84 mmol) in dry pyridine (8 mL) was added t-butyldimethylsilyl chloride (332 mg, 2.21 mmol) at 0 °C (ice bath). The reaction mixture was stirred at room temperature for 3 hours. TLC (methanol-chloroform 1:12) indicated that the starting material (R<sub>f</sub> = 0.09) was converted to 6-O-silylated product (R<sub>f</sub> = 0.35). The reaction mixture was then concentrated under reduced pressure and coevaporated with toluene. The residue was purified by radial silica gel chromatography on Chromatotron (2 mm plate) using 6% methanol-methylene chloride as eluent to give compound **53** (629 mg) in 87% yield: [α]<sub>D</sub> -24.3° (c = 0.5, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 387.0 ([M]<sup>+</sup>, 75.0%); 277.0 ([M-PhSH]<sup>+</sup>, 100%); IR (thin film, ν<sub>cm<sup>-1</sup></sub>) 3394 (br), 2930, 2856, 1608, 1472, 1454, 1081, 841, 743, 693; <sup>1</sup>H-NMR(CDCl<sub>3</sub>) δ (ppm): 7.55-7.23 (m, 5H, Ph), 4.49 (d, 1H, J<sub>1,2</sub> = 8.2 Hz, H-1), 4.07-3.48 (m, 6H, H-2, 3, 4, 5, 6a, 6b), 3.05 (br, 3H, OH), 0.88 (s, 9H, SiCMe<sub>3</sub>), 0.08, 0.06 (s, 6H, SiCH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 147.6-125.8 (6C, aromatic), 88.8 (C-1), 78.7 (C-5), 69.8 (C-3), 69.7 (C-4), 69.5 (C-2), 63.1 (C-6), 25.9 (3C, SiCMe<sub>3</sub>), 18.2 (C(CH<sub>3</sub>)<sub>3</sub>), -5.3, -5.4 [2C, Si(Me)<sub>2</sub>].

#### Para-Nitrophenyl 6-O-t-butyldimethylsilyl-1-thio-β-D-galactopyranoside 54

To a solution of para-nitrophenyl 1-thio-β-D-galactopyranoside **47** (2.0 g, 6.30 mmol) in dry pyridine (60 mL) was added t-butyldimethylsilyl chloride (1.07 g, 7.10 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 3.5 hours. TLC (methanol-chloroform 1:10) indicated that the starting material (R<sub>f</sub> = 0.11) was converted to 6-O-silylated product **54** (R<sub>f</sub> = 0.40). The reaction mixture was concentrated under

reduced pressure and coevaporated with toluene to give crude **54** which was purified by radial chromatography on silica gel with ethyl acetate-hexane (2:1, v/v) as eluent to give pure **54** (2.42 g) in 89% yield: m.p. 161.7-162.9 °C;  $[\alpha]_D -91.3^\circ$  (c = 0.9, CH<sub>3</sub>OH); IR (KBr,  $\nu_{\text{cm}^{-1}}$ ): 3509, 2928, 1578, 1516, 1473, 1340, 1252, 1090, 855, 740; CI-MS (ether) gave m/z (ion, relative intensity): 431.9 ([M]<sup>+</sup>, 66.3%), 277.0 ([M-HSPhNO<sub>2</sub>]<sup>+</sup>, 95.5%); <sup>1</sup>H-NMR (CHCl<sub>3</sub>)  $\delta$  (ppm): 8.12 (d,  $J_{\text{m,o}} = 9.0$  Hz, H-meta), 7.61 (d, H-ortho), 4.67 (d, 1H,  $J_{1,2} = 9.7$  Hz, H-1), 4.06 (d, 1H,  $J_{3,4} = 2.5$ ,  $J_{4,5} < 1.0$  Hz, H-4), 3.95-3.56 (m, 5H, H-2, H-3, H-5, H-6a, H-6b), 0.88 (s, 9H, SiCMe<sub>3</sub>), 0.07, 0.06 (s, 6H, SiCH<sub>3</sub>).

**Phenyl 2,3,4-tri-O-benzoyl-6-O-t-butyltrimethylsilyl-1-thio- $\beta$ -D-galactopyranoside**  
**57**

To a solution of phenyl 6-O-t-butyltrimethylsilyl-1-thio- $\beta$ -D-galactopyranoside **53** (600 mg, 1.56 mmol) in dry pyridine (5 mL) was added benzoyl chloride (667  $\mu$ L, 5.69 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 3.5 hours. The reaction mixture was then poured onto ice and extracted with chloroform. The extracts were collected and washed with saturated sodium bicarbonate and brine. The solution was dried over sodium sulfate and evaporated to dryness under reduced pressure. The residue was purified by radial chromatography on Chromatotron (2 mm plate) using ethyl acetate-hexane (1:3 v/v) as eluent to give 2,3,4-tri-O-benzoylated product **57** (925 mg) in 85% yield and 2,3-di-O-benzoylated byproduct **58** (47 mg) in 5% yield. Compound **57** could be crystallized from ethanol: m.p. 86.3-88.2 °C;  $[\alpha]_D +103^\circ$  (c = 1.0, CHCl<sub>3</sub>); IR (neat,  $\nu_{\text{cm}^{-1}}$ ): 3065, 2941, 2857, 1729, 1602, 1452, 1316, 1269, 1102, 842, 709; M.S.(C.I. ether) (M/Z): 699.2 ([M]<sup>+</sup>, 5.3%), 588.9 ([M-HSPh]<sup>+</sup>, 100%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 7.97-7.16 (m 20H, aromatic), 5.94 (dd, 1H,  $J_{4,5} = 0.8$  Hz, H-4), 5.69 (dd, 1H,  $J_{2,3} = 9.9$  Hz, H-2), 5.56 (dd, 1H,  $J_{3,4} = 3.1$  Hz, H-3), 4.98 (d, 1H,  $J_{1,2} = 9.5$  Hz, H-1), 4.06 (ddd, 1H,  $J_{5,6a} = 6.0$ ,  $J_{5,6b} = 7.4$  Hz, H-5), 3.86 (dd, 1H,  $J_{6a,6b} = 10.3$  Hz, H-6a), 3.77 (dd, 1H, H-6b), 0.83 (s, 9H, t-Butyl), -0.02, -0.07 (2s, 2xSiMe); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 165.5, 165.2, 165.1 (3xC=O, Bz), 133.9-128.2 (24C, aromatic), 85.8 (C-1), 78.0 (C-5), 73.4 (C-3),

68.2 (C-2), 68.1 (C-4), 61.1 (C-6), 25.8 (3C, SiCMe<sub>3</sub>), 18.2 (CMe<sub>3</sub>), -5.5, -5.6 [2C, Si(Me)<sub>2</sub>].

Anal. Calcd for C<sub>39</sub>H<sub>41</sub>O<sub>10</sub>NSSi (698.88): C, 67.02; H, 6.06; S, 4.59. Found: C, 66.78; H, 6.11; S, 4.61.

#### **Phenyl 2,3-di-O-benzoyl-6-O-t-butyltrimethylsilyl-1-thio-β-D-galactopyranoside 58**

Compound **58** had: m.p. 60.9-63.2 °C; [α]<sub>D</sub>+80° (c = 1.1, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 594.9 ([M]<sup>+</sup>, 4.4%), 484.9 ([M-HSPH]<sup>+</sup>, 96.4%); IR (neat, ν<sub>cm</sub><sup>-1</sup>): 3478, 3065, 2941, 2857, 1720, 1602, 1460, 1273, 1100, 842, 710; <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 7.98-7.18 (m, 15H, aromatic), 5.86 (dd, 1H, J<sub>2,3</sub> = 9.9 Hz, H-2), 5.36 (dd, 1H, J<sub>3,4</sub> = 2.8 Hz, H-3), 4.99 (d, 1H, J<sub>1,2</sub> = 9.9 Hz, H-1), 4.66 (dd, 1H, J<sub>4,5</sub> = 0.9 Hz, H-4), 4.06-3.95 (m, 2H, H-5, H-6a), 3.78 (dd, 1H, H-6b), 3.45 (br, 1H, OH), 0.92 (s, 9H, t-Butyl), 0.12, 0.11 (2s, 2xSiMe); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 165.9, 165.26 (2xC=O, Bz), 133.3-127.9 (18C, aromatic), 86.7 (C-1), 78.0 (C-5), 75.9 (C-3), 68.4, 68.1 (C-2, C-4), 63.5 (C-6), 25.9 (3C, SiCMe<sub>3</sub>), 18.3 (CMe<sub>3</sub>), -5.4 [2C, Si(Me)<sub>2</sub>].

Anal. Calcd for C<sub>39</sub>H<sub>41</sub>O<sub>10</sub>NSSi (594.77): C, 64.62; H, 6.44; S, 5.39. Found: C, 64.51; H, 6.54; S, 4.98.

#### **Para-nitrophenyl 2,3,4-tri-O-benzoyl-6-O-t-butyltrimethylsilyl-1-thio-β-D-galactopyranoside 59**

To a solution of 6-O-t-butyltrimethylsilyl-β-D-galactopyranoside **54** (2.72 g, 6.3 mmol) in dry pyridine (60 mL) was added benzoyl chloride (2.7 mL) at 0 °C. The reaction mixture was stirred at room temperature under nitrogen for 5 hours. TLC (Ethyl Acetate-Hexane 3:5) indicated that the reaction was complete. The mixture was then poured onto ice and extracted with chloroform (50 mL x3). The extracts were collected and washed with saturated sodium bicarbonate, saturated sodium chloride and water. The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>) filtered and evaporated under reduced pressure. The residue was crystallized from ethanol to afford **59** (4.03 g) in 86% yield: m.p. 159.2-160.5 °C; [α]<sub>D</sub>+84.4° (c = 1.0, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 474 (

$[M+1]^+$ , 7.1%), 589 ( $[M+1-SPhNO_2]^+$ , 49.0%), 298 ( $[M-SPh-2(AcOH)]^+$ , 100%). 240 ( $[M-SPh-AcOH-2(AcO)]^+$ , 62.1%);  $^1H$ -NMR ( $CDCl_3$ )  $\delta$  (ppm): 8.20-7.18 (m 19H, Aromatic), 5.98 (dd, 1H,  $J_{4,5} = 0.9$  Hz, H-4), 5.76 (dd, 1H,  $J_{2,3} = 9.9$  Hz, H-2), 5.63 (dd, 1H,  $J_{3,4} = 3.3$  Hz, H-3), 5.23 (d, 1H,  $J_{1,2} = 9.8$  Hz, H-1), 4.12 (ddd, 1H,  $J_{5,6a} = 6.8$ ,  $J_{5,6b} = 6.2$  Hz, H-5), 3.88 (dd, 1H,  $J_{6a,6b} = 10.2$  Hz, H-6a), 3.77 (dd, 1H, H-6b), 0.84 (s, 9H, t-Butyl), -0.01, -0.05 (2s, 2xSiMe);  $^{13}C$ -NMR ( $CDCl_3$ )  $\delta$  (ppm): 165.4, 165.2, 165.1 (C=O), 141.8-123.8 (C-Aromatic), 84.7 (C-1), 78.5 (C-5), 72.9 (C-3), 67.9 (C-2), 67.8 (C-4), 61.2 (C-6), 25.7 (3c, 3xMe), 18.2 (C(CH<sub>3</sub>)<sub>3</sub>), -5.5, -5.6 [2C, Si(Me)<sub>2</sub>].

Anal. Calcd for (C<sub>39</sub>H<sub>41</sub>O<sub>10</sub>NSSi): C, 62.96; H, 5.56; N, 1.88; S, 4.31. Found: C, 63.20; H, 5.54; N, 1.91; S, 4.52.

#### **Para-Nitrophenyl 2,3,4-tri-O-benzyl-6-O-trityl-1-thio- $\beta$ -D-galactopyranoside 61**

Trityl chloride (1.05 g, 3.78 mmol) was added to a solution of p-nitrophenyl-1-thio- $\beta$ -D-galactopyranoside **47** (1 g, 3.15 mmol) in pyridine (15 mL). The mixture was stirred at room temperature overnight. TLC (MeOH/CHCl<sub>3</sub>, 1:10) showed clear and complete conversion of starting material ( $R_f = 0.05$ ) to intermediate 6-O-trityl-1-thio- $\beta$ -D-galactopyranoside ( $R_f = 0.39$ ). The solution was concentrated under reduced pressure. Sodium hydride (50% in oil, 680 mg, 14.18 mmol) was slowly added to a solution of the residue and benzyl bromide (1.69 mL, 14.18 mmol) in dry N,N-dimethyl formamide (18 mL) at 0 °C. The mixture was allowed to attain room temperature, and after 15 hours methanol (5 mL) was added to destroy excess benzyl bromide. After 45 min the solution was neutralized with acetic acid, diluted with dichloromethane (60 mL), washed with water (2x50 mL), dried, and concentrated. Column chromatography of the residue on silica gel (ethyl acetate-hexane 1:5) gave **61** (2.24 g) in 86% yield: m.p. 136.4-137.3 °C;  $[\alpha]_D^{25} +3.5^\circ$  (c = 0.97, CHCl<sub>3</sub>); M.S. (Pos FAB) (relative intensity) M/Z: 830.27 ( $[M+1]^+$ , 2%), 570.18 ( $[M-Ph_3COH]^+$  12%); I.R.(thin film,  $\nu_{cm^{-1}}$ ): 3050, 2890, 1585, 1514, 1451, 1339, 1085, 852, 763, 700;  $^1H$ -NMR ( $CDCl_3$ )  $\delta$  (ppm): 7.83-7.80 (m, 2H, Ph-meta,  $J_{o,m} = 9.0$  Hz), 7.56-7.53 (m, 2H, Ph-ortho,  $J_{o,m} = 9.0$  Hz), 7.39-7.10 (m, 30 H, aromatic), 4.84 (AB pattern,  $J_{ab} = 11.0$  Hz, H-a), 4.73-4.62 (m, 4H, 2xPhCH<sub>2</sub>), 4.65 (d, 1H,  $J_{1,2} = 9.5$  Hz,

H-1), 4.50 (AB pattern,  $J = 11.0$  Hz, H-b), 3.96 (dd, 1H,  $J_{1,2} = 9.5$ ,  $J_{2,3} = 9.5$  Hz, H-2), 3.89 (d, 1H,  $J_{3,4} = 2.6$ ,  $J_{4,5} < 1.0$  Hz, H-4), 3.56 (dd, 1H,  $J_{3,4} = 2.6$  Hz, H-3), 3.53-3.20 (m, 3H, H-5, H-6a, H-6b);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 145.7-123.7 (42C, C-aromatic), 85.5 (C-1), 83.9 (C-3), 77.8 (C-5), 76.6 (C-2), 75.7 ( $\text{PhCH}_2$ ), 74.4 ( $\text{PhCH}_2$ ), 73.5 (C-4), 72.8 ( $\text{PhCH}_2$ ), 62.8 (C-6).

**Para-Nitrophenyl 2,3,4-tri-O-acetyl-6-O-p-toluenesulfonyl-1-thio- $\beta$ -D-galactopyranoside 62**

To a solution of 4-nitrophenyl 1-thio- $\beta$ -D-galactopyranoside **47** (0.4 g, 1.26 mmol) dissolved in dry pyridine (15 mL) was added p-toluenesulfonyl chloride (0.34g, 1.76 mmol). After the reaction mixture was stirred at room temperature for 2.5 hours., TLC (5% methanol in methylene chloride) showed complete conversion of the starting material ( $R_f = 0.18$ ) to 6-tosylated derivative ( $R_f = 0.48$ ). To this reaction mixture was added acetic anhydride (3 mL), stirring was continued for additional 4 hours. The solvent was evaporated under reduced pressure and the residue was purified by radial chromatography on silica gel Chromatotron (2 mm plate) using ethyl acetate-hexane (2:5, v/v) as eluent to provide **62** (512 mg) in 68% yield: m.p. 93.7-95.4 °C;  $[\alpha]_D -23.5^\circ$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ); (+)FAB-MS (glycerol) gave  $m/z$  (ion, relative intensity): 597.1 ( $[\text{M}+1]^+$ , 1.6%), 443.1 ( $[\text{M-SPhNO}_2]^+$ , 99.4%);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.09 (d,  $J_{m,o} = 9.0$  Hz, 2H, H-meta of  $\text{SPhNO}_2$ ), 7.74 (d,  $J_{m,o} = 8.2$  Hz, 2H, H-meta of tosyl), 7.54 (d, 2H, H-ortho of  $\text{SPhNO}_2$ ), 7.32 (d, 2H, H-ortho of tosyl), 5.43 (dd, 1H,  $J_{4,5} = 1.0$  Hz, H-4), 5.24 (dd, 1H,  $J_{2,3} = 9.9$  Hz, H-2), 5.06 (dd, 1H,  $J_{3,4} = 3.3$  Hz, H-3), 4.84 (d, 1H,  $J_{1,2} = 9.9$  Hz, H-1), 4.15-4.04 (m, 3H, H-5, H-6a, H-6b), 2.42 (s, 3H, Me), 2.06 (2C), 1.95 (3x OAc);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 170.5, 170.3, 170.0 (C=O, 3xOAc), 147.0-128.5 (12C, Aromatic), 85.0 (C-1), 75.1 (C-5), 72.0 (C-3), 67.7 (C-4), 67.5 (C-2), 67.2 (C-6), 22.1 ( $\text{PhCH}_3$ ), 21.2, 21.0 (2C) (3xOAc).

Anal. Calcd for  $\text{C}_{25}\text{H}_{27}\text{NO}_{14}\text{S}_2$  (597.61): C, 50.25; H, 4.55; N, 2.34. Found: C, 50.06; H, 4.61; N, 2.41.

### General Procedure for the Selective De-O-silylation of Monosaccharides

Acetyl chloride (1 mL) was added dropwise to methanol (20 mL) and the hydrogen chloride solution obtained was cooled to 20 °C. A solution of the silyl derivatives **57**, **59**, **60** (1 mmol) in ethyl ether (20 mL) was added. The reaction mixture was stirred at room temperature for 30 min (TLC solvent EtOAc/hexane 2:5, v/v). The reaction mixture was neutralized with Amberlite IR-45 (OH<sup>-</sup>), concentrated and the crude residue was purified by silica gel column chromatography using ethyl acetate-hexane (1:5, v/v) as an eluent. Thus, compounds **63-65** were obtained.

### Phenyl 2,3,4-tri-O-benzoyl-1-thio-β-D-galactopyranoside **63**

Compound **63** (567 mg) was obtained in 97% yield:  $[\alpha]_D +123.2^\circ$  (c = 1.0, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 584.8 (M<sup>+</sup>, 1.5%), 474.8 ([M-HSPh]<sup>+</sup>, 13.5%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 7.99-7.09 (m 20H, 4 Ph), 5.80 (d, 1H, J<sub>4,5</sub> < 1.0 Hz, H-4), 5.77 (dd, 1H, J<sub>2,3</sub> = 9.9 Hz, H-2), 5.56 (dd, 1H, J<sub>3,4</sub> = 3.2 Hz, H-3), 5.00 (d, 1H, J<sub>1,2</sub> = 9.9 Hz, H-1), 4.07 (ddd, 1H, H<sub>5,6a</sub> = 6.6, H<sub>5,6b</sub> = 6.5 Hz, H-5), 3.88 (dd, 1H, J<sub>6a,6b</sub> = 11.9 Hz, H-6a), 3.65 (dd, 1H, H-6b), 2.50 (br, 1H, 6-OH); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 166.4, 165.5, 165.2 (C=O, Bz), 134.1-128.3 (C-aromatic), 85.5 (C-1), 77.9 (C-5), 73.2 (C-3), 68.9 (C-2), 68.0 (C-4), 60.8 (C-6).

Anal. Calcd for C<sub>33</sub>H<sub>28</sub>O<sub>8</sub>S (584.61): C, 67.79; H, 4.83. Found: C, 67.39; H, 4.76.

### Para-Nitrophenyl 2,3,4-tri-O-benzoyl-1-thio-β-D-galactopyranoside **64**

Compound **64** (2.82 g) was obtained in 91% yield: m.p. 106.5-107.7 °C;  $[\alpha]_D +69.9^\circ$  (c = 1.0, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 630 (M<sup>+</sup>, 8.5%), 612 ([M-H<sub>2</sub>O]<sup>+</sup>, 4.1%), 504 ([M-PhCO<sub>2</sub>H]<sup>+</sup>, 4.8%), 475 ([M-HSPhNO<sub>2</sub>]<sup>+</sup>, 100%), 353 ([M-PhCO<sub>2</sub>H-HSPhNO<sub>2</sub>]<sup>+</sup>, 27.4%), 233 ([M-HSPhNO<sub>2</sub>-2xPhCO<sub>2</sub>]<sup>+</sup>, 100%), 215 ([M-HSPhNO<sub>2</sub>-2xPhCO<sub>2</sub>-H<sub>2</sub>O]<sup>+</sup>, 24.6%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 8.17-7.18 (m 19H, aromatic), 5.87 (d, 1H, J<sub>4,5</sub> < 1.0 Hz, H-4), 5.86 (dd, 1H, J<sub>2,3</sub> = 9.9 Hz, H-2), 5.64 (dd,

1H,  $J_{3,4} = 3.3$  Hz, H-3), 5.14 (d, 1H,  $J_{1,2} = 9.9$  Hz, H-1), 4.17 (ddd, 1H,  $J_{5,6a} = 6.8$ ,  $J_{5,6b} = 6.2$  Hz, H-5), 3.88 (dd, 1H,  $J_{6a,6b} = 12.0$  Hz, H-6a), 3.65 (dd, 1H, H-6b), 2.05 (br., 1H, 6-OH);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm): 166.5, 165.4, 165.1 (C=O), 147.1-123.8 (C-aromatic), 84.5 (C-1), 78.3 (C-5), 72.8 (C-3), 68.8 (C-2), 67.6 (C-4), 60.6 (C-6).

#### Para-Nitrophenyl 2,3,4-tri-O-pivaloyl-1-thio- $\beta$ -D-galactopyranoside 65

Compound 65 (337 mg) was obtained in 98% yield:  $[\alpha]_{\text{D}} -11.6^\circ$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ); CI-MS (ether) gave  $m/z$  (ion, relative intensity): 569.7 ( $[\text{M}]^+$ , 1.1%), 414.9 ( $[\text{M}-\text{HSPHNO}_2]^+$ , 100%);  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.12 (d,  $J_{\text{m,o}} = 9.0$  Hz, H-meta), 7.61 (d, H-ortho), 5.38 (d, 1H,  $J_{4,5} < 1.0$  Hz, H-4), 5.29 (dd, 1H,  $J_{2,3} = 9.9$  Hz, H-2), 5.15 (dd, 1H,  $J_{3,4} = 3.0$  Hz, H-3), 4.83 (d, 1H,  $J_{1,2} = 9.6$  Hz, H-1), 3.90 (dd, 1H,  $J_{5,6a} = 6.8$  Hz, H-5), 3.71 (dd, 1H,  $J_{6a,6b} = 11.7$  Hz, H-6a), 3.45 (dd,  $J_{5,6b} = 6.2$  Hz, H-6b), 2.15 (br, 1H, OH), 1.18, 1.16, 1.07 (3s, 27 H, 3xtBu, Piv.);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm): 177.7, 177.2, 176.5 (C=O, Piv), 146.9 (C-para), 141.3 (C-ipso), 131.6 (C-meta), 123.7 (C-ortho), 84.2 (C-1), 78.1 (C-5), 72.0 (C-3), 67.5 (C-2), 66.3 (C-4), 60.6 (C-6), 39.0, 38.7 (2C, (3xC(O)C(CH<sub>3</sub>)<sub>3</sub>), 27.0 (9C, 3xtBu, Piv).

#### Para-Nitrophenyl 2,3,4-tri-O-benzyl-1-thio- $\beta$ -D-galactopyranoside 66

To a solution of 4-nitrophenyl 2,3,4-tri-O-benzyl-6-O-trityl-1-thio- $\beta$ -D-galactopyranoside 61 (2.0 g, 2.41 mmol) in  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  (30 mL, 1:1 v/v) was added *p*-toluenesulfonic acid (20 mg, 0.12 mmol). The mixture was stirred at room temperature for 30 min. TLC (ethyl acetate/hexane 3:7) indicated complete conversion of the starting material ( $R_f = 0.52$ ) to product ( $R_f = 0.15$ ). Triethyl amine (1 mL) was added the reaction mixture which was stirred for additional 15 min. The reaction mixture was evaporated under reduced pressure and coevaporated with toluene (10 mL x2). The residue was then purified by column chromatography using ethyl acetate-hexane (2:7 in v/v) as eluent. Compound 66 (1.34 g) was obtained in 95% yield: m.p. 129.4-130.3  $^\circ\text{C}$ ;  $[\alpha]_{\text{D}} -29.5^\circ$  ( $C = 0.78$ ,  $\text{CHCl}_3$ ); CI-MS (ether) gave  $m/z$  (ion, relative intensity): 570.3 ( $[\text{M}-\text{H}_2\text{O}]^+$ , 1.1%), 433.2 ( $[\text{M}-\text{HSPHNO}_2]^+$ , 15.2%), 325 ( $[\text{M}-\text{HSPHNO}_2-\text{BnOH}]^+$ , 13.1%), 219 ( $[\text{M}$ -

HSPhNO<sub>2</sub>-2xPhCH<sub>2</sub>OJ<sup>+</sup>, 32.8%); I.R.(thin film,  $\nu_{\text{cm}^{-1}}$ ): 3448 (br), 3031, 2890, 1585, 1513, 1453, 1340, 1083, 852, 739, 698; <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 7.96-7.91 (m, 2H,  $J_{\text{om}} = 9.0$  Hz Ph-meta, ), 7.60-7.55 (m, 2H,  $J_{\text{om}} = 9.0$  Hz Ph-ortho), 7.41-7.23 (m, 15H, aromatic), 5.00 (AB pattern,  $J_{\text{ab}} = 11.2$  Hz, H-a), 4.77-4.68 (m, 4H, 2xPhCH<sub>2</sub>), 4.72 (d, 1H,  $J_{1,2} = 8.9$  Hz, H-1), 4.61 (AB pattern,  $J_{\text{ab}} = 11.2$  Hz, H-b), 4.01 (dd, 1H,  $J_{2,3} = 9.5$  Hz, H-2), 3.91 (d 1H,  $J_{4,5} < 1.0$  Hz, H-4), 3.63 (dd, 1H,  $J_{3,4}=2.6$  Hz, H-3), 3.88-3.51 (m, 3H, H-5, H-6a, H-6b), 1.59 (br, 1H, OH); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 145.9-123.9 (24C, C-aromatic), 85.6 (C-1), 84.1 (C-3), 79.1 (C-5), 76.7 (C-2), 75.8 (PhCH<sub>2</sub>), 74.5 (PhCH<sub>2</sub>), 73.12 (C-4), 72.9 (PhCH<sub>2</sub>), 62.1(C-6).

Anal. Calcd for (C<sub>33</sub>H<sub>33</sub>O<sub>7</sub>NS): C, 67.44; H, 5.66; N, 2.38; S, 5.46. found: C, 67.18; H, 5.79; N, 2.35; S, 5.61.

#### **Para-Nitrophenyl 3,4-O-isopropylidene-1-thio- $\beta$ -D-galactopyranoside 67**

To a solution of para-nitrophenyl 1-thio- $\beta$ -D-galactopyranoside **47** (2.0 g, 6.3 mmol.) in 2,2-dimethoxypropane (50 mL) was added p-toluenesulfonic acid (61 mg, 0.325 mmol). The mixture was stirred for 2.5 hours at room temperature under nitrogen, triethylamine (0.45 mL, 3.25 mmol) was then added. After stirring for 15 min, TLC showed that one major ( $R_f = 0.68$ ) and two minor products ( $R_f = 0.84$ ,  $R_f = 0.33$ ) were produced. The reaction mixture was concentrated under reduced pressure. The residue in 10:1 MeOH-water (50 mL) was boiled under reflux until TLC (EtOAc, 100%) showed the complete disappearance of the fast-moving product ( $R_f = 0.82$ ) (4.5 hours). The mixture was concentrated. The product mixture was separated by silica gel column chromatography using EtOAc/Hexane (3:5 v/v) as eluent to afford **67a** (2.25 g) in 81% yield and **67b** (240 mg) in 11%yield.

Compound **67a** had: m.p. 127-128 °C;  $[\alpha]_D -62.1^\circ$  (c = 1.0, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 358.0 ([M+1]<sup>+</sup>, 7.9%), 202.9 ([M+1-SPhNO<sub>2</sub>]<sup>+</sup>, 100%); IR (KBr,  $\nu_{\text{cm}^{-1}}$ ): 3400 (br), 3104, 2989, 2936, 1726, 1587, 1517, 1479, 1356, 1231, 1070, 965, 849, 739; <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 8.15 (d, 2H,  $J_{\text{mo}} = 9.0$  Hz, H-meta), 7.58 (d, 2H, H-ortho), 4.68 (d, 1H,  $J_{1,2} = 10.1$  Hz, H-1), 4.24 (dd, 1H,  $J_{4,5} = 1.9$

Hz, H-4), 4.14 (dd, 1H,  $J_{3,4} = 5.8$  Hz, H-3), 4.00-3.95 (m, 2H, H-5, H-6a), 3.84 (m, 1H, H-6b), 3.70 (dd, 1H,  $J_{2,\text{OH}} = 2.9$ ,  $J_{2,3} = 6.8$  Hz, H-2), 2.53 (d, 1H,  $J_{2,\text{OH}} = 3.0$  Hz, 2-OH), 2.12 (dd, 1H, 6-OH), 1.47 and 1.35 (2s, 6H,  $\text{Me}_2\text{C}$ );  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm): 146.4 (C-para), 143.0 (C-ipso), 129.6 (C-meta), 124.0 (C-ortho), 110.7 ( $\text{CMe}_2$ ), 86.0 (C-1), 79.2 (C-3), 77.1 (C-5), 73.7 (C-4), 71.5 (C-2), 62.4 (C-6), 28.0 and 26.1 ( $\text{CMe}_2$ ).

Anal. Calcd for ( $\text{C}_{15}\text{H}_{19}\text{O}_7\text{NS}$ ): C, 50.41; H, 5.36; N, 3.92; S, 8.97. Found: C, 50.03; H, 5.32; N, 3.78; S, 8.68.

Compound **67b** had:  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.11 (d,  $J_{\text{m,o}} = 9.0$  Hz, H-meta), 7.55 (d, H-ortho), 4.60 (d, 1H,  $J_{1,2} = 9.5$  Hz, H-1), 4.22 (dd, 1H,  $J_{4,5} = 1.1$  Hz, H-4), 4.10-3.92 (AB system, 2H, H-6a, H-6b), 3.78 (dd, 1H,  $J_{2,3} = 9.1$  Hz, H-2), 3.60 (dd, 1H,  $J_{3,4} = 5.8$  Hz, H-3), 3.48 (m, 1H, H-5), 1.45 and 1.34 (2s, 6H,  $\text{Me}_2\text{C}$ ).

#### **Para-Nitrophenyl 2,6-di-O-benzoyl-3,4-O-isopropylidene-1-thio- $\beta$ -D-galactopyranoside 68**

Para-Nitrophenyl 3,4-O-isopropylidene-1-thio- $\beta$ -D-galactopyranoside **67** (2.4 g, 6.72 mmol) in dry pyridine (15 mL) was cooled 0 °C and benzoyl chloride (4.7 mL, 40.32 mmol) was added in one portion. The mixture was allowed to reach room temperature. The stirring was continued overnight. TLC (Toluene-Ethyl Acetate 2:1) indicated that the starting material ( $R_f = 0.21$ ) was completely converted to product ( $R_f = 0.57$ ). The mixture was then poured onto ice water, extracted with methylene chloride (2x25 mL). The extracts were collected and washed with saturated  $\text{NaHCO}_3$ , saturated  $\text{NaCl}$  solution and dried ( $\text{Na}_2\text{SO}_4$ ), filtered and evaporated under reduced pressure. The residue was recrystallised from ether-hexane (1:3, v/v) to afford the first portion of the product **68** (3.05 g) as a crystalline compound. The mother solution was concentrated and chromatographed on silica gel (eluent: ethyl acetate-hexane, 1:2, v/v) to afford the second portion of the product **68** (0.38 g). The total yield of **68** was 3.43 g (90%): m.p. 204-205 °C;  $[\alpha]_{\text{D}} -11.5^\circ$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ),  $[\alpha]_{\text{Hg}}^{436} -94.0^\circ$  ( $C = 1.0$ ,  $\text{CHCl}_3$ ); IR (KBr,  $\nu_{\text{cm}^{-1}}$ ): 2992, 1721, 1596, 1515, 1450, 1381, 1341, 1266, 1093, 1036, 876, 844, 707;  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.06-7.39 (m, 14H, aromatic), 5.38 (dd, 1H,  $J_{2,3} = 9.6$  Hz, H-2), 4.96 (d, 1H,  $J_{1,2}$

= 9.6 Hz, H-1), 4.76 (dd, 1H,  $J_{6a,6b} = 12.0$  Hz, H-6a), 4.60 (dd, 1H,  $J_{5,6b} = 8.4$  Hz, H-6b), 4.45 (dd, 1H,  $J_{3,4} = 5.6$  Hz, H-3), 4.39 (dd, 1H,  $J_{4,5} = 1.9$  Hz, H-4), 4.32 (ddd, 1H,  $J_{5,6a} = 3.3$  Hz, H-5), 1.55, 1.30 (2s,  $\text{CMe}_2$ );  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 166.2, 165.3 (C=O, Bz), 146.1 (C-para), 143.9 (C-ipso), 133.7-123.3 (16C, aromatic), 111.4 ( $\text{CMe}_2$ ), 83.9 (C-1), 77.8 (C-3), 74.6 (C-5), 73.4 (C-4), 71.4 (C-2), 64.0 (C-6), 27.6 and 26.3 ( $\text{CMe}_2$ ).

Anal. Calcd for ( $\text{C}_{29}\text{H}_{27}\text{O}_9\text{NS}$ ): C, 61.59; H, 4.81; N, 2.48; S, 5.67. Found: C, 61.90; H, 4.97; N, 2.52; S, 5.76.

### Para-Nitrophenyl 6-O-benzoyl-3,4-O-isopropylidene-1-thio- $\beta$ -D-galactopyranoside 69

Para-Nitrophenyl 3,4-O-isopropylidene-1-thio- $\beta$ -D-galactopyranoside **67** (0.8 g, 2.23 mmol) in dry pyridine (2 mL) and methylene chloride (5 mL) was cooled to  $-70$  °C and benzoyl chloride (0.31 mL) in methylene chloride (4 mL) was added slowly. The mixture was stirred at  $-70$  °C for 30 min. The mixture was then poured onto ice, extracted with methylene chloride (2x10 mL). The extracts were collected and washed with saturated  $\text{NaHCO}_3$ , saturated  $\text{NaCl}$  solution and dried ( $\text{Na}_2\text{SO}_4$ ), filtered and evaporated under reduce pressure. The residue was chromatographed on silica gel (ethyl acetate-hexane, 3:7) to afford 6-O-benzoylated product **69** (642 mg) in 63% yield: m.p. 98-99 °C,  $[\alpha]_D -107^\circ$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ); CI-MS (ether) gave  $m/z$  (ion, relative intensity): 461.9 ( $[\text{M}]^+$ , 3.9%), 306..9 ( $[\text{M-HSPhNO}_2]^+$ , 100%); IR (KBr,  $\nu_{\text{cm}^{-1}}$ ): 3434, 2989, 2930, 1719, 1586, 1514, 1479, 1380, 1340, 1270, 1220, 1190, 1030, 873, 852, 750, 713;  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.05-7.42 (m 9H, aromatic), 4.70 (dd, 1H,  $J_{5,6a} = 3.6$ ,  $J_{6a,6b} = 12.0$  Hz, H-6a), 4.68 (d, 1H,  $J_{1,2} = 10.0$  Hz, H-1), 4.58 (dd, 1H,  $J_{5,6b} = 8.4$  Hz, H-6b), 4.31 (dd, 1H,  $J_{3,4} = 5.6$ ,  $J_{4,5} = 2.2$  Hz, H-4), 4.27-4.12 (m, 2H, H-3, H-5), 3.76 (m, 1H, H-2), 2.60 (d, 1H,  $J = 2.8$  Hz, OH), 1.52, 1.37 (2s, 6H,  $\text{CMe}_2$ );  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 167.0 (C=O, Bz), 147.1 (C-para), 143.9 (C-ipso), 134.4-124.6 (10C, aromatic), 111.7 ( $\text{CMe}_2$ ), 86.8 (C-1), 80.0 (C-3), 75.4 (C-5), 74.1 (C-4), 72.6 (C-2), 64.7 (C-6), 28.8 and 26.97 ( $\text{CMe}_2$ ).

Anal. Calcd for ( $\text{C}_{22}\text{H}_{23}\text{O}_8\text{NS}$ ): C, 57.26; H, 5.02; N, 3.04; S, 6.95. Found: C, 57.06; H, 4.83; N, 2.87; S, 6.77.

### Para-Nitrophenyl 2,6-di-O-benzoyl-1-thio- $\beta$ -D-galactopyranoside 70

Compound **68** (3 g, 5.32 mmol) was suspended in methanol (100 mL) and a catalytic amount of p-TsOH (0.5 g) was added. The reaction mixture was stirred at 40 °C overnight, after which time TLC (EtOAc/Hexane 1:1) showed complete conversion of starting material to product. To the reaction mixture was added triethylamine (100 mL). The solution was evaporated under reduced pressure to afford a yellow mass. The crude product was purified with silica gel chromatography using CHCl<sub>3</sub>-MeOH (9:1) as eluent to give **70** (2.74 g) in 97% yield: m.p. 182-183 °C; [ $\alpha$ ]<sub>D</sub> -44.0° (c = 1.0, DMSO); IR (KBr,  $\nu_{\text{cm}^{-1}}$ ): 2992, 1721, 1596, 1515, 1450, 1381, 1341, 1266, 1093, 1036, 876, 844, 707; CI-MS (ether) gave m/z (ion, relative intensity): 526.0 ([M+1]<sup>+</sup>, 0.9%), 507.9 ([M+1-H<sub>2</sub>O]<sup>+</sup>, 1.7%), 370.9 ([M+1-HSPhNO<sub>2</sub>]<sup>+</sup>, 100%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 7.76-7.28 (m 14H, aromatic), 5.21 (dd, 1H, J<sub>2,3</sub> = 9.5 Hz, H-2), 4.84 (d, 1H, J<sub>1,2</sub> = 10.1 Hz, H-1), 4.37-3.08 (m, 7H, H-3 - H-6, 2xOH); <sup>13</sup>C-NMR (DMSO)  $\delta$  (ppm): 166.6, 166.4 (C=O, Bz), 146.7 (C-para), 145.8 (C-ipso), 134.2-124.5 (16C, aromatic), 84.4 (C-1), 77.8 (C-3), 73.3 (C-5), 71.8 (C-4), 70.2 (C-2), 65.4 (C-6).

Anal. Calcd for (C<sub>26</sub>H<sub>23</sub>O<sub>9</sub>NS): C, 59.42; H, 4.41; N, 2.66; S, 6.10. Found: C, 59.73; H, 4.25; N, 2.47; S, 6.30.

### Para-Nitrophenyl 6-O-benzoyl-1-thio- $\beta$ -D-galactopyranoside 71

Para-nitrophenyl 6-O-benzoyl-3,4-O-isopropylidene-1-thio- $\beta$ -D-galactopyranoside **69** (0.493 g, 1.07 mmol) was suspended in methanol (25 mL) and a catalytic amount of p-TsOH was added. The solution was reflux for 5 hours. To the reaction solution was added triethylamine (1 mL). The solution was evaporated under reduced pressure to afford a yellow mass. The crude product was purified by silica gel chromatography (eluent: CHCl<sub>3</sub>:MeOH, 8:1) to provided **71** (440 mg) in 98% yield: m.p. 205-206 °C; [ $\alpha$ ]<sub>D</sub> -49.0° (c = 1.0, DMSO); IR (KBr,  $\nu_{\text{cm}^{-1}}$ ): 3359 (br), 3095, 2919, 2848, 1733, 1585, 1577, 1515, 1451, 1342, 1283, 1133, 1074, 853, 811, 742, 707; (+)FAB-MS (glycerol) gave m/z (ion,

relative intensity): 421.2 ( $[M]^+$ , 1.2%), 273.1 ( $[M+HSPHNO_2]^+$ , 10%);  $^1H$ -NMR (500 MHz, Acetone- $d_6$ )  $\delta$  (ppm): 8.07-7.52 (m, 9H, aromatic), 5.06 (d, 1H,  $J_{1,2} = 10.1$  Hz, H-1), 4.60 (dd, 1H,  $J_{5,6a} = 8.3$ ,  $J_{6a,6b} = 11.6$  Hz, H-6a), 4.53 (dd, 1H,  $J_{5,6b} = 3.7$  Hz, H-6b), 4.23 (m, 1H, H-5), 4.11 (d, 1H,  $J_{4,5} = 0.9$  Hz, H-4), 3.83-3.72 (m, 2H, H-2, H-3);  $^{13}C$ -NMR (DMSO)  $\delta$  (ppm): 165.5 (C=O, Bz), 146.3 (C-para), 144.6 (C-ipso), 133.5-123.6 (10C, aromatic), 84.9 (C-1), 76.3 (C-3), 74.2 (C-5), 69.0 (C-4), 68.8 (C-2), 64.9 (C-6).

Anal. Calcd for ( $C_{19}H_{19}O_8NS$ ): C, 54.15; H, 4.55; N, 3.32; S, 7.61. Found: C, 54.25; H, 4.57; N, 3.22; S, 7.86.

### 3.5.3 Conversion of Latent Glycosyl Donors to Active Glycosyl Donors by Reduction and Acetylation

#### Para-Acetamidophenyl 2,3,4,6-tetra-O-acetyl -1-thio- $\beta$ -D-galactopyranoside 75

Para-Nitrophenyl 2,3,4,6-tetra-O-acetyl-1-thio- $\beta$ -D-galactopyranoside 9 (250 mg, 0.515 mmol) was dissolved in absolute ethanol (20 mL) to which was added Tin(II) chloride dihydrate ( $SnCl_2 \cdot 2H_2O$ ) (280 mg, 2.57 mmol). The reaction mixture was stirred at 70 °C for 2 hours. TLC (2% ethanol in dichloromethane) indicated clear transformation of nitro compound 9 ( $R_f = 0.34$ ) into amino intermediate ( $R_f = 0.28$ , ninhydrin positive). The reaction mixture was then cooled and poured onto ice-water and the final pH of the solution adjusted to 7-8 with  $NaHCO_3$ . The resulting mixture was extracted with EtOAc (40x3 mL) which was successively washed with sat.  $NaHCO_3$  and water. The organic phase was dried ( $Na_2SO_4$ ) and concentrated under reduced pressure. The resulting crude 4-aminophenyl thioglycoside was immediately treated with pyridine (4 mL) and acetic anhydride (2 mL) at room temperature overnight. The solution was concentrated under reduced pressure and coevaporated with toluene. The residue was purified by silica gel chromatography using EtOAc/Hexane (1:1-3:1, v/v) as eluent to provided 75 (207 mg) in 81% yield: m.p. 135-136 °C;  $[\alpha]_D -6.4^\circ$  ( $c = 0.96$ ,  $CHCl_3$ ); CI-MS (ether) gave  $m/z$  (ion, relative intensity): 497.8 ( $[M+1]^+$ , 86.9%), 331.9 ( $[M+1-SPhNHAc]^+$ , 20.8%);  $^1H$ -NMR ( $CDCl_3$ )  $\delta$  (ppm): 7.46 (m, 4H, Ph), 7.21(br, 1H, NH), 5.38 (dd, 1H,  $J_{4,5} = 0.8$  Hz, H-4),

5.18 (dd, 1H,  $J_{2,3} = 9.9$  Hz, H-2), 5.01 (dd, 1H,  $J_{3,4} = 3.3$  Hz, H-3), 4.61 (d, 1H,  $J_{1,2} = 9.9$  Hz, H-1), 4.16 (dd, 1H,  $J_{6a,6b} = 11.4$  Hz, H-6a), 4.10 (dd,  $J_{5,6b} = 6.2$  Hz, H-6b), 3.87 (ddd, 1H,  $J_{5,6a} = 7.0$  Hz, H-5), 2.13, 2.06, 2.05, 1.96 (s, OAc);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 170.3, 170.1, 170.0, 169.4, 168.4 (5x $\text{C}=\text{O}$ , OAc), 138.3 (C-*ipso*), 134.1 (C-*ortho*), 126.7 (C-*para*), 119.1 (C-*meta*), 86.7 (C-1), 74.3 (C-5), 71.9 (C-3), 67.2 (C-2), 67.1 (C-4), 61.5 (C-6), 24.5 (NHAc), 20.8, 20.6, 20.6, 20.5 (OAc).

Anal. Calcd for  $\text{C}_{20}\text{H}_{27}\text{O}_{10}\text{NS}$  (497.51): C, 53.11; H, 5.47; N, 2.82; S, 6.44. Found: C, 53.75; H, 5.53; N, 2.77; S, 6.26.

### Para-Aminophenyl 2,3,4,6-tetra-O-benzoyl-1-thio- $\beta$ -D-galactopyranoside 73

Para-nitrophenyl 2,3,4,6-tetra-O-benzoyl-1-thio- $\beta$ -D-galactopyranoside 50 (300 mg, 0.408 mmol) was suspended in absolute ethanol (20 mL) to which was added Tin(II) chloride dihydrate ( $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ ) (460 mg, 2.04 mmol). The reaction mixture was stirred at 70 °C for 2 hours. TLC (ether 100%) indicated that a clear nitro compound ( $R_f = 0.60$ ) to amino product ( $R_f = 0.30$ , ninhydrin positive). The reaction mixture was then cooled and poured onto ice-water and the final pH of the solution adjusted to 7-8 with  $\text{NaHCO}_3$ . The resulting mixture was extracted with EtOAc (20x3 mL) which was successively washed with sat.  $\text{NaHCO}_3$  and water. The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated under reduced pressure. The resulting crude product was purified on silicon gel chromatography with ether-hexane (1:1 in v/v) as eluent to afford pure 73 (239 mg) in 83% yield: m.p. 98-99 °C;  $[\alpha]_D^{25} +26^\circ$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ); IR (thin film,  $\nu_{\text{cm}^{-1}}$ ): 3474, 3381, 3066, 2970, 1724, 1622, 1600, 1495, 1451, 1315, 1271, 1098, 909, 826, 712; CI-MS (ether) gave  $m/z$  (ion, relative intensity): 704.1 ( $[\text{M}+1]^+$ , 12.3%), 579.2 ( $[\text{M}+1\text{-HSPhNH}_2]^+$ , 100%);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.03-7.18 (m, 24H, aromatic), 6.79 (d, 2H,  $J = 8.3$  Hz,  $\text{NH}_2$ ), 5.97 (dd, 1H,  $J_{4,5} < 1.0$  Hz, H-4), 5.72 (dd, 1H,  $J_{2,3} = 9.9$  Hz, H-2), 5.57 (dd, 1H,  $J_{3,4} = 3.2$  Hz, H-3), 4.89 (d, 1H,  $J_{1,2} = 9.9$  Hz, H-1), 4.63 (m, 1H, H-5), 4.41-4.34 (m, 2H, H-a, H-6b);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 166.0, 165.5, 165.3, 165.1 ( $\text{C}=\text{O}$ , 4xBz), 146.7 (C-*para*), 137.3 (C-*ipso*), 133.4 - 115.4 (28C, aromatic), 85.8 (C-1), 74.8 (C-5), 73.1 (C-3), 68.2 (C-4), 67.8 (C-2), 62.3 (C-6).

Anal. Calcd for  $C_{40}H_{33}O_9NS$  (703.76): C, 68.27; H, 4.73; N, 1.99; S, 4.55. Found: C, 67.98; H, 4.10; N, 2.00; S, 4.20.

#### **Para-Acetamidophenyl 2,3,4,6-tetra-O-benzoyl-1-thio- $\beta$ -D-galactopyranoside 76**

Para-aminophenyl 2,3,4,6-tetra-O-benzoyl-1-thio- $\beta$ -D-galactopyranoside **73** (560 mg, 0.8 mmol) was dissolved dry pyridine (4 mL) and acetic anhydride (2 mL) was added. The reaction mixture was stirred at room temperature for 2 hours. The solution was concentrated under reduced pressure and co-evaporated with toluene (2x10 mL). The residue was purified by silica gel chromatography using EtOAc/Hexane (1:1, v/v) as eluent to afford pure **76** (502 mg) in 85% yield: m.p. 119-121 °C;  $[\alpha]_D +91^\circ$  (c = 0.96,  $CHCl_3$ ); IR (thin film,  $\nu_{cm^{-1}}$ ): 3344, 3065, 2970, 1716, 1594, 1522, 1452, 1396, 1269, 1178, 1102, 1070, 909, 834, 711; CI-MS (ether) gave m/z (ion, relative intensity): 745.8 ( $[M]^+$ , 1.7%), 578.9 ( $[M+1-HSPhNHAc]^+$ , 4.5%);  $^1H$ -NMR ( $CDCl_3$ )  $\delta$  (ppm): 8.02-7.18 (m, 24H, aromatic), 5.98 (dd, 1H,  $J_{4,5} < 1.0$  Hz, H-4), 5.72 (dd, 1H,  $J_{2,3} = 9.9$  Hz, H-2), 5.57 (dd, 1H,  $J_{3,4} = 2.7$  Hz, H-3), 4.95 (d, 1H,  $J_{1,2} = 9.8$  Hz, H-1), 4.64 (dd, 1H,  $J_{5,6a} = 6.0$ ,  $J_{6a,6b} = 10.5$  Hz, H-6a), 4.41-4.34 (m, 2H, H-5, H-6b), 1.83 (s, 3H, Me);  $^{13}C$ -NMR ( $CDCl_3$ )  $\delta$  (ppm): 168.2 (NHAc), 166.0, 165.4, 165.4, 165.1 (C=O, 4xBz), 138.7-119.6 (30C, aromatic), 86.0 (C-1), 75.0 (C-5), 72.9 (C-3), 68.3 (C-4), 67.8 (C-2), 62.4 (C-6).

Anal. Calcd for  $C_{42}H_{35}O_{10}NS$  (745.80): C, 67.64; H, 4.73; N, 1.88; S, 4.30. Found: C, 67.57; H, 5.00; N, 1.86; S, 4.17.

#### **Para-Aminophenyl 2,3,4,6-tetra-O-benzyl-1-thio- $\beta$ -D-galactopyranoside 74**

Para-nitrophenyl 2,3,4,6-tetra-O-benzyl-1-thio- $\beta$ -D-galactopyranoside **52** (660 mg, 0.97 mmol) was dissolved in absolute ethanol (8 mL) to which was added Tin (II) chloride dihydrate ( $SnCl_2 \cdot 2H_2O$ ) (1.31 g, 5.81 mmol). The reaction mixture was stirred at 70 °C for 2.5 hours. The reaction mixture was then cooled and poured onto ice-water and the final pH of the solution adjusted to pH  $\approx$  7 with sodium bicarbonate. The resulting mixture was extracted with ethyl acetate (3x20 mL). The extracts were washed successively with sat. sodium bicarbonate and water. The organic phase dried ( $Na_2SO_4$ )

and concentrated under reduced pressure. The resulting crude 4-aminophenylthio glycoside was purified by column chromatography on silica gel (ether-hexane 2:1) to give pure **74** (500 mg) in 80% yield:  $[\alpha]_D -4.0^\circ$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ),  $[\alpha]_{365(\text{Hg})} -11.0^\circ$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ); IR (KBr,  $\nu_{\text{cm}^{-1}}$ ): 3464, 3371, 3062, 3029, 2883, 1261, 1598, 1496, 1454, 1360, 1283, 1094, 825, 739, 698; CI-MS (ether) gave  $m/z$  (ion, relative intensity): 647.9 ( $[\text{M}]^+$ , 45.3%), 540.0 ( $[\text{M}-\text{OBn}]^+$ , 13.9%);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 7.42-7.24 (m, 22H, aromatic), 6.53 (d, 2H,  $J = 8.5$  Hz,  $\text{NH}_2$ ), 4.95-4.41 (m, 8H, 4x $\text{OCH}_2$ ), 4.47 (d, 1H,  $J_{1,2} = 9.7$  Hz, H-1), 3.94 (d 1H,  $J_{3,4} = 2.4$ ,  $J_{4,5} < 1.0$  Hz, H-4), 3.83 (dd, 1H,  $J_{2,3} = 9.1$  Hz, H-2), 3.63-3.53 (4H, H-3, H-5, H6a, H-6b);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 145.8-115.6 (30C, aromatic), 88.7 (C-1), 84.2 (C-5), 77.4 (C-3), 77.1 (C-4), 75.5 ( $\text{OCH}_2$ ), 74.3 ( $\text{OCH}_2$ ), 73.5 (2C,  $\text{OCH}_2$ , C-2), 72.6 ( $\text{OCH}_2$ ), 68.7 (C-6).

Anal. Calcd for  $\text{C}_{40}\text{H}_{41}\text{NO}_5\text{S}$  (647.82.): C, 74.16; H, 6.38; N, 2.16; S, 4.95. Found: C, 74.17; H, 6.40; N, 2.16; S, 4.74.

#### **Para-Acetamidophenyl 2,3,4,6-tetra-O-benzyl-1-thio- $\beta$ -D-galactopyranoside **77****

Para-aminophenyl 2,3,4,6-tetra-O-benzyl-1-thio- $\beta$ -D-galactopyranoside **74** (280 mg, 0.40 mmol) was dissolved in pyridine (2.5 mL) and acetic anhydride (5 mL) was added and the solution was stirred for 2 hours at room temperature. TLC (EtOAc-Hexane 2:1) showed a clear conversion of starting material ( $R_f = 0.52$ ) to product ( $R_f = 0.23$ ). The solution was evaporated under reduced pressure and coevaporated with toluene (5 mLx2). The residue was purified by silica gel chromatography (ether, 100%) to afford **77** (294.4 mg) in 91% yield as white solid: m.p. 146-148  $^\circ\text{C}$ ;  $[\alpha]_D -4.0^\circ$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ),  $[\alpha]_{365(\text{Hg})} -19.0^\circ$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ); IR (KBr,  $\nu_{\text{cm}^{-1}}$ ): 3300, 3031, 2881, 1673, 1592, 1527, 1495, 1454, 1396, 1313, 1091, 829, 737, 698; CI-MS (ether) gave  $m/z$  (ion, relative intensity): 689.9 ( $[\text{M}]^+$ , 1.8%), 540 ( $[\text{M}-\text{SPhNHAc}]^+$ , 1.6%);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 7.54-7.25 (m, 22H, aromatic), 7.04 (s, 1H, NH), 4.95-4.36 (m, 8H, 4xAB pattern, 4x $\text{OCH}_2$ ), 4.55 (d, 1H,  $J_{1,2} = 9.7$  Hz, H-1), 3.95 (d, 1H,  $J_{3,4} = 2.4$ ,  $J_{4,5} < 1.0$  Hz, H-4), 3.86 (dd, 1H,  $J_{2,3} = 9.4$  Hz, H-2), 3.63-3.55 (4H, H-3, H-5, H-6a, H-6b), 1.78 (s, 3H, Me);  $^{13}\text{C}$ -

NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 168.1 (C=O, NHAc), 138.7-119.9 (30C, aromatic), 84.5 (C-1), 84.09 (C-5), 77.2 (C-4), 75.6 (C-OCH<sub>2</sub>), 74.4 (OCH<sub>2</sub>), 73.5 (OCH<sub>2</sub>), 73.5 (C-2), 72.6 (OCH<sub>2</sub>), 68.7 (C-6), 24.6 (NHAc).

Anal. Calcd for C<sub>42</sub>H<sub>43</sub>NO<sub>6</sub>S (689.87): C, 73.12; H, 6.28; N, 2.03; S, 4.65. Found: C, 72.86; H, 6.56; N, 2.03; S, 4.49.

### **Para-Acetamidophenyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranoside 78**

Para-Nitrophenyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranoside **36** (1 g, 1.75 mmol) was dissolved in absolute ethanol (30 mL) to which was added Tin(II) chloride dihydrate (SnCl<sub>2</sub>·2H<sub>2</sub>O) (1.84 g, 5.25 mmol). The reaction mixture was stirred at 70 °C for 2.5 hours. TLC (ethyl acetate-hexane 1:1) indicated a clear and complete transformation of nitro compound (R<sub>f</sub> = 0.51) to amino intermediate (R<sub>f</sub> = 0.18). The reaction mixture was then cooled and poured onto ice-water and the final pH of the solution adjusted to 7-8 with NaHCO<sub>3</sub>. The resulting mixture was extracted with ethyl acetate (40x3 mL) which was successively washed with sat. sodium bicarbonate and water. The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under reduced pressure. The resulting crude p-aminophenyl thio glycoside was treated with pyridine (4 mL) and acetic anhydride (2 mL) at room temperature overnight. The solution was evaporated under reduced pressure using toluene co-evaporation. The crude residue was purified by silica gel column chromatography using ethyl acetate/hexane (3:5, v/v) as eluent to give **78** (864 mg) in 93% yield: m.p. 184.7-185.6 °C; [ $\alpha$ ]<sub>D</sub> +25.1° (c = 1.0, CHCl<sub>3</sub>); IR (KBr,  $\nu_{\text{cm}^{-1}}$ ): 3328, 1748, 1718, 1592, 1525, 1495, 1380, 1234, 1039, 912, 726; (+)FAB-MS (glycerol) gave m/z (ion, relative intensity): 532.2 ([M+1]<sup>+</sup>, 18.4%), 585.2 ([M+1]<sup>+</sup>, 5.9%); 418.1 ([M-HSPhNHAc]<sup>+</sup>, 1.2%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 7.86-7.72 (m, 4H, Phth), 7.41-7.30 (m, 4H, SPhNHAc), 5.74 (dd, 1H, J<sub>3,4</sub> = 9.2 Hz, H-3), 5.59 (d, 1H, J<sub>1,2</sub> = 10.5 Hz, H-1), 5.08 (dd, 1H, J<sub>4,5</sub> = 10.1 Hz, H-4), 4.26 (dd, 1H, J<sub>2,3</sub> = 10.3 Hz, H-2), 4.25-4.12 (m, 2H, H-6a, H-6b), 3.84 (ddd, 1H, J<sub>4,5</sub> = 10.2 Hz, J<sub>5,6a</sub> = 2.6 Hz, J<sub>5,6b</sub> = 5.0 Hz, H-5), 2.15, 2.08, 1.99, 1.80 (4s, OAc, NHAc), 1.62 (br, 1H, NH); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 170.7,

170.1, 169.4, 168.5 (C=O, OAc, NHAc), 167.8, 167.0 (C=O, Phth), 138.7-119.9 (12C, aromatic), 83.1 (C-1), 75.9 (C-5), 71.6 (C-3), 68.6 (C-4), 62.1 (C-6), 53.5 (C-2), 24.6, 20.8, 20.6, 20.4 (OAc, NHAc).

Anal. Calcd for  $C_{28}H_{28}N_2O_{10}S$  (584.58): C, 57.53; H, 4.83; N, 4.79; S, 5.48. Found: C: 57.48; H, 4.76; N, 4.86; S, 5.58.

**Para-N-Acetamidophenyl (Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosid) onate 79**

To a solution of methyl (para-nitrophenyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosid) onate (800 mg, 1.27 mmol) in absolute ethanol (40 mL) was added Tin (II) chloride dihydrate ( $SnCl_2 \cdot H_2O$ ) (1.44 g, 6.37 mmol). The reaction mixture was stirred at 70 °C for 3 hours. TLC (4% ethanol in dichloromethane) indicated the transformation to be completed. The reaction mixture was then cooled and poured onto ice-water and the final pH was adjust to 7-8 with  $NaHCO_3$ . The resulting mixture was extracted with EtOAc (120 mL) which was successively washed with saturated  $NaHCO_3$  and water. The organic phase was dried over  $MgSO_4$  and evaporated under reduced pressure. The resulting crude p-aminophenylthio glycoside was immediately treated overnight with pyridine (8 mL) and acetic anhydride (4 mL) at room temperature. The solution was thoroughly evaporated under reduced pressure using toluene evaporation. The crude residue was purified by silica gel chromatography using 3% methanol in dichloromethane as eluent. After pooling and evaporating the desired fractions, compound **79** (705 mg) was obtained in 87% overall yield: m.p. 97-98 °C; (EtOAc);  $[\alpha]_D^{25} +33.9^\circ$  (c = 1.86,  $CHCl_3$ ); I.R.(thin film,  $\nu_{cm^{-1}}$ ): 3302, 3099, 3016, 1742, 1668, 1591, 1529, 1494, 1437, 1371, 1233, 1129, 1041, 954, 835, 756; CI-MS (ether) gave m/z (ion, relative intensity): 641 ( $[M]^+$ , 87.5%), 581 ( $[M-AcOH]^+$ , 100%), 521 ( $[M-2xAcOH]^+$ , 3.1%), 476 ( $[M-HSPhNHAc]^+$ , 56.2%);  $^1H$ -NMR ( $CDCl_3$ )  $\delta$  (ppm): 7.81(s, br, 1H, NH), 7.49 (d, 2H,  $J_{o,m} = 8.7$  Hz, H-ortho), 7.39 (d, 2H, H-meta), 5.43 (d, 1H,  $J_{5,N} = 9.3$ , NH), 5.27 (dd, 1H,  $J_{7,8} = 7.2$  Hz, H-7), 5.22 (dd, 1H,  $J_{8,9a} = 2.5$  Hz, H-8), 4.81 (ddd, 1H,  $J_{4,5} = 10.2$  Hz, H-4), 4.36 (dd, 1H,  $J_{9a,9b} = 12.5$ , H-9a), 4.16 (dd, 1H,  $J_{8,9b} = 5.3$

Hz, H-9b), 4.01 (dd, 1H,  $J_{5,6} = 10.1$  Hz, H-5), 3.84 (dd, 1H,  $J_{6,7} = 1.6$  Hz, H-6), 3.55 (s, 3H, OCH<sub>3</sub>), 2.75 (dd, 1H,  $J_{3c,4} = 4.6$  Hz, H-3e), 2.05 (dd, 1H,  $J_{3a,4} = 11.8$ ,  $J_{3a,3c} = 12.8$  Hz, H-3a), 2.14, 2.10, 2.03, 2.00 (4xOAc), 1.98, 1.83 (2xNHAc); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 170.8, 170.7, 170.4, 170.2, 170.0, 169.0, 167.8 (C=O, C-1, NHAc, OAc), 87.5 (C-2), 74.6, 70.0, 69.7, 67.6 (C-4, C-6, C-7, C-8), 61.9 (C-9), 52.7 (CH<sub>3</sub>O), 49.0 (C-5), 37.9 (C-3), 24.5, 23.0, 21.4 (3C), 20.8 (CH<sub>3</sub>, NHAc, OAc).

Anal. Calcd for (C<sub>28</sub>H<sub>36</sub>N<sub>2</sub>O<sub>13</sub>S)H<sub>2</sub>O: (658.66): C, 51.06; H, 5.81; N, 4.25. Found: C: 51.41; H, 5.75; N, 4.29.

### 3.6.4 Application of “Active-Latent” Glycosylation Methodology in the Synthesis of (1→6) and (1→3) Linked Oligosaccharides

#### O-(3,4,6-tri-O-Acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose **81**

Method A: Ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **32** (132 mg, 0.28 mmol) and 1,2:3,4-di-O-isopropylidene α-D-galactopyranose **80** (60 mg, 0.23 mmol) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (2 mL) under nitrogen. Pulverized molecular sieves (4 Å, 150 mg) was added and the solution was stirred under nitrogen for 1 hour. Then the reaction mixture was cooled -30 °C and N-iodosuccinimide (128 mg, 0.46 mmol) and trifluoromethanesulfonic acid (4 μL) were then added. The reaction was essentially complete (as judged by TLC) after 15 min. The mixture was then diluted with dichloromethane (10 mL) and filtered through celite. The filtrate was washed with 10% aqueous sodium thiosulfate (10 mL), saturated aqueous sodium bicarbonate (2x10 mL) and brine (10 mL). The solution was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to a foam that was flash chromatographed on silica gel (2% EtOH/CH<sub>2</sub>Cl<sub>2</sub>) to give disaccharide **81** (156 mg) in 92% yield.

Method B: The coupling reaction between **33** (66 mg, 0.144 mmol) and **80** (30 mg, 0.12 mmol) was carried out at room temperature in dichloromethane using DMTST (2.5 eq) as

promoter. The reaction was finished in 5 hours as judged by TLC (4% ethanol in benzene). After purification, disaccharide **81** was obtained in 75% yield.

Method C: The coupling reaction between **33** (66 mg, 0.144 mmol) and **80** (30 mg, 0.12 mmol) was carried out essentially as in method A, but using trifluoromethanesulfonic acid (0.6 eq). The reaction was finished in 25 min and after similar work-up to afford disaccharide **81** in 77% yield.

Method D: Attempted coupling reaction between **36** (1.2 eq) and **80** (1.0 eq) was carried out in similar condition except using trifluoromethanesulfonic acid (1.0 equiv.). The reaction was run at -30 °C for 5 hours, no coupling product **81** was detected.

Method E: The coupling reaction between **78** (72 mg, 0.144 mmol) and **80** (30 mg, 0.12 mmol) was carried out essentially as in method A but using trifluoromethanesulfonic acid (1.0 eq), reaction was generally finished in 20 min and after similar work-up afforded disaccharide **81** in 80% yield.

Compound **81** had: Rf = 0.33 (4% ethanol in benzene); m.p. 215.8-216.9 °C;  $[\alpha]_D^{25}$  = 21.2° (c = 1.0, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 578.3 ([M+1]<sup>+</sup>, 63.1 %); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 7.80 (m, 2H, Phth), 7.67 (m, 2H, Phth), 5.82 (dd, 1H, J<sub>3,4'</sub> = 9.1 Hz, H-3'), 5.42 (d, 1H, J<sub>1',2'</sub> = 8.5 Hz, H-1'), 5.14 (dd, 1H, J<sub>4,5'</sub> = 10.2 Hz, H-4'), 5.07 (d, 1H, J<sub>1,2</sub> = 5.1 Hz, H-1), 4.37 (dd, 1H, J<sub>3,4</sub> = 7.9 Hz, H-3), 4.32 (dd, 1H, J<sub>6a',6b'</sub> = 12.4 Hz, H-6a'), 4.28 (dd, 1H, J<sub>2',3</sub> = 10.6 Hz, H-2'), 4.13 (dd, 1H, J<sub>5,6b'</sub> = 2.4 Hz, H-6b'), 4.06 (dd, 1H, J<sub>2,3</sub> = 2.4 Hz, H-2), 3.96 (dd, 1H, J<sub>4,5</sub> = 1.2 Hz, H-4), 3.93 (m, 2H, H-6a, H-6b), 3.86 (ddd, 1H, J<sub>5',6a'</sub> = 4.6 Hz, H-5'), 3.67 (m, 1H, H-5), 2.09, 2.00, 1.84 (3s, 9 H, 3xOAc), 1.37 (s, 3H, Me), 1.21 (s, 3H, Me), 0.99 (s, 6H, Mex<sub>2</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 170.7, 170.1, 169.5, (C=O, 3xOAc), 109.2 (C-O-C), 107.9 (C-O-C), 99.3 (C-1'), 95.8 (C-1), 71.5 (C-5'), 70.8 (C-4), 70.6 (C-3'), 70.6 (C-3), 70.1 (C-2), 69.3 (C-6), 69.0 (C-4'), 67.4 (C-5), 62.0 (C-6'), 54.5 (C-2'), 25.8, 25.3, 24.6, 24.2 (4C, 2xCMe), 20.7, 20.6, 20.4 (3xOAc).

Anal. Calcd for C<sub>32</sub>H<sub>39</sub>NO<sub>15</sub> (677.64): C, 56.68; H, 5.80; N, 2.06. Found: C, 56.69; H, 5.98; N, 1.88.

**O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-(1→6)-1,2:3,4-di-O-isopropylidene-α-D-galactopyranoside 82**

**Method A:** Para-acetamidophenyl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-galactopyranoside **76** (100 mg, 0.134 mmol) and 1,2:3,4-di-O-isopropylidene α-D-galactose **80** (29 mg, 0.11 mmol) were dissolved in dry dichloromethane (2 mL) under nitrogen and pulverized molecular sieves (4Å, 100 mg) was added. The mixture was stirred at room temperature for 3 hours. The mixture was cooled to -30°C and was added N-iodosuccinimide (46 mg, 0.20 mmol) and trifluoromethanesulfonic acid (13 μL) was added. The reaction was essentially complete (as judged by TLC) after 10 min. The mixture was then diluted with dichloromethane (10 mL) and filtered through celite. The filtrate was washed with 10% aqueous sodium thiosulfate (10 mL), and saturated aqueous sodium bicarbonate (2x10 mL), and brine (10 mL). The solution was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to a foam that was flash chromatographyed on silica gel (ethyl acetate/hexane, 3:1) to afford the titled disaccharide (87 mg) in 94% yield.

**Method B:** The reaction between **76** and **80** was carried out essentially as in method A, no detected coupling product after 5 hours at -30 °C could be observed.

Compound **82** had: m.p. 87-89°C; [α]<sub>D</sub> +50° (c = 1.0, CHCl<sub>3</sub>); IR (KBr ν<sub>cm<sup>-1</sup></sub>): 2051, 1729, 1602, 1452, 1266, 1111, 1069, 1027, 711; <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 8.09-7.19 (m, 20H, Aromatic), 5.92 (dd, 2H, J<sub>4,5</sub> = 0.9 Hz H-4'), 5.79 (dd, 1H, J<sub>2,3'</sub> = 10.4 Hz, H-2'), 5.58 (dd, 1H, J<sub>3',4'</sub> = 3.4 Hz, H-3'), 5.39 (d, 1H, J<sub>1,2</sub> = 9.8 Hz, H-1), 4.99 (d, 1H, J<sub>1',2'</sub> = 8.0 Hz, H-1'), 4.65 (dd, 1H, J<sub>5',6a'</sub> = 6.4, J<sub>6a',6b'</sub> = 11.0 Hz, H-6a'), 4.40 (dd, 1H, J<sub>5',6b'</sub> = 6.8 Hz, H-6b'), 4.39 (dd, 1H, J<sub>3,4</sub> = 7.9 Hz, H-3), 4.32 (m, 1H, H-5'), 4.20 (dd, 1H, J<sub>2,3</sub> = 2.4 Hz, H-2), 4.09 (dd, 1H, J<sub>4,5</sub> = 1.5 Hz, H-4), 5.58 (dd, 1H, J<sub>3,4</sub> = 3.4 Hz, H-3), 4.64 (dd, 1H, J<sub>2,3</sub> = 9.8 Hz, H-2), 4.03 (m, 1H, H-5), 3.88 (m, H-6a, H-6b), 1.37, 1.22, 1.19, 1.17 (4s, 12H, 2xCM<sub>2</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 166.0, 165.5, 165.3 (C=O), 147.0-124.4 (30C, Aromatic), 109.2 (C-O-C), 108.4 (C-O-C), 101.7 (C-1), 96.1 (C-1'), 71.5, 70.9, 70.5, 70.3, 69.6, 68.1, 67.4, 62.0 (C-2 - C-5, C-2' - C-5'), 68.4 (C-6), 63.0 (C-6).

Anal. Calcd for C<sub>46</sub>H<sub>46</sub>NO<sub>15</sub> (838.864): C, 65.86; H, 5.53. Found: C, 65.89; H, 5.58.

**Para-nitrophenyl O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-1-thio-β-D-galactopyranoside 83**

Ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **32** (110 mg, 0.21 mmol) and para-nitrophenyl 2,3,4-tri-O-benzoyl-1-thio-β-D-galactopyranoside **64** (107 mg, 0.17 mmol) were dissolved in dry dichloromethane (2 mL) under nitrogen and pulverized molecular sieves (4 Å, 200 mg) was added. The mixture was stirred at room temperature for 3 h, cooled to -30°C and NIS (57 mg, 0.34 mmol) and TfOH (3.0 μL) was added. The reaction was essentially complete (as judged by TLC) after 15 min. The reaction mixture was then diluted with dichloromethane (10 mL) and filtered through a layer of celite. The filtrate was washed with 10% aqueous sodium thiosulfate (10 mL), saturated aqueous sodium bicarbonate (2x10 mL) and brine (10 mL). The solution was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to a foam that was flashchromatographed on silica gel (3:2, ethyl acetate/hexane) to give disaccharide **83** (137 mg) in 78% yield: m.p. 135.1-136.3 °C; [α]<sub>D</sub> +81.2° (c = 1.2, CHCl<sub>3</sub>); (+)FAB-MS (glycerol) gave m/z (ion, relative intensity): 892.3 ([M-HSPHNO<sub>2</sub>]<sup>+</sup>, 5.7%), 612.1 ([M-3,4,6-O-tri-acetyl-2-phthalimido-2-deoxy-Glc]<sup>+</sup>, 12.5%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 8.20-7.15 (m, 19H, Aromatic), 5.79 (d, 1H, H-4), 5.68 (dd, 1H, J<sub>3,4</sub> = 9.1 Hz, H-3'), 5.64 (dd, 1H, J<sub>2,3</sub> = 9.8 Hz, H-2), 5.52 (dd, 1H, J<sub>3,4</sub> = 3.2 Hz, H-3), 5.46 (d, 1H, J<sub>1,2</sub> = 8.5 Hz, H-1'), 5.14 (dd, 1H, J<sub>4,5</sub> = 10.1 Hz, H-4'), 5.01 (d, 1H, J<sub>1,2</sub> = 9.8 Hz, H-1), 4.33 (dd, 1H, J<sub>2,3</sub> = 9.8 Hz, H-2'), 4.23 (m, 2H, H-6b, H-5'), 4.21 (dd, 1H, J<sub>5,6a</sub> = 2.3, J<sub>6a,6b</sub> = 12.3 Hz, H-6a), 3.94 (dd, 1H, J<sub>5,6a</sub> = 2.4, J<sub>6a,6b</sub> = 11.2 Hz, H-6a'), 3.79 (dd, 1H, J<sub>5,6b</sub> = 7.4 Hz, H-6b'), 3.74 (m, 1H, H-5), 2.08, 2.00, 1.85 (3xAc); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 170.6, 170.1, 169.4 (3C, C=O, Ac), 155.2, 165.1, 165.0 (3C, C=O, Bz), 147.0-123.6 (30C, Aromatic), 98.2 (C-1'), 84.2 (C-1), 77.1 (C-5'), 72.5 (C-3), 72.1 (C-5), 70.7 (C-3'), 68.7 (2C, C-4', C-6'), 68.5 (C-4), 67.4 (C-2), 61.7 (C-6), 54.4 (C-2'), 20.7, 20.5, 20.4 (3xAc).

Anal. Calcd for C<sub>53</sub>H<sub>46</sub>NO<sub>19</sub>S (1046.99): C, 60.80; H, 4.43; N, 2.70; S, 3.06. Found: C, 60.60; H, 4.61; N, 2.58; S, 3.07.

**Para-Nitrophenyl O-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-1-thio-β-D-galactopyranoside 84**

Ethyl 3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **41** (70 mg, 0.13 mmol) and para-nitrophenyl 2,3,4-tri-O-benzoyl-1-thio-β-D-galactopyranoside **64** (69 mg, 0.11 mmol) were dissolved in dry dichloromethane (2 mL) under nitrogen and pulverized molecular sieves (4 Å, 120 mg) were added. The mixture was stirred at room temperature for 3 hours then cooled to -30°C. NIS (49.5 mg, 0.22 mmol) and TfOH (2 μL). The reaction was essentially complete (as judged by TLC) after 10 min. The mixture was then diluted with dichloromethane (10 mL) and filtered through celite. The filtrate was washed with 10% aqueous sodium thiosulfate (10 mL), saturated aqueous sodium bicarbonate (2x10 mL) and brine (10 mL). The solution was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to a foam that was flash chromatographed on silica gel (1:3, Ethyl acetate/hexane) to isolate disaccharide **84** (116 mg) in 89% yield: m.p. 103.4-105.2 °C; [α]<sub>D</sub> +71.3° (c = 1.3, CHCl<sub>3</sub>); IR (Thin film, ν<sub>cm<sup>-1</sup></sub>): 3480, 3064, 3030, 2917, 1775, 1721, 1599, 1582, 1518, 1545, 1390, 1341, 1270, 1083, 1069, 1027, 853, 751, 711; <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 8.16- 6.91 (m, 19H, Aromatic), 5.76 (d, 1H, J<sub>4,5</sub> < 1.0 Hz, H-4), 5.59 (dd, 1H, J<sub>2,3</sub> = 9.8 Hz, H-2), 5.47 (dd, 1H, J<sub>3,4</sub> = 3.2 Hz, H-3), 5.22 (d, 1H, J<sub>1',2'</sub> = 7.9 Hz, H-1'), 4.90 (d, 1H, J<sub>1,2</sub> = 9.9 Hz, H-1), 4.72 - 4.49 (AB, A'B', 4H, 2xOCH<sub>2</sub>), 4.19 - 4.13 (m, 3H, H-2', H-3', H-5'), 3.88 (dd, 1H, J<sub>5',6a'</sub> = 3.2, J<sub>6a',6b'</sub> = 12.2 Hz, H-6a'), 3.81 (dd, J<sub>5,6a</sub> = 7.8, J<sub>5,6b</sub> = 9.5 Hz, H-5), 3.73 -3.68 (m, 3H, H-6a, H-6b, H-6b'), 3.57 (dd, 1H, J<sub>3',4'</sub> = 4.9, J<sub>4',5'</sub> = 9.6 Hz, H-4'); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 165.2, 165.1, 165.1 (3C, C=O, Bz), 147.1-123.3 (42 C, Aromatic), 98.6 (C-1'), 84.4 (C-1), 78.5 (C-5'), 77.2 (C-3'), 74.4 (C-5), 74.3 (OCH<sub>2</sub>), 73.8 (OCH<sub>2</sub>), 73.5 (C-4'), 70.6 (C-6), 68.5 (C-4), 68.3 (C-6'), 67.5 (C-2).

Anal. Calcd for C<sub>61</sub>H<sub>52</sub>O<sub>19</sub>N<sub>2</sub>S (1101.15): C, 66.53; H, 4.76; N, 2.55; S, 2.91.  
Found: C, 66.74; H, 5.00; N, 2.49; S, 3.06.

**Para-Nitrophenyl O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-1-thio-β-D-galactopyranoside 85**

Method A: Phenyl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-galactopyranoside **49** (100 mg, 0.145 mmol) and para-nitrophenyl 2,3,4-tri-O-benzoyl-1-thio-β-D-galactopyranoside **64** (76 mg, 0.12 mmol) were dissolved in dry dichloromethane (2 mL) under nitrogen and pulverized molecular sieves (4 Å, 150 mg) were added. The mixture was stirred at room temperature for 3 hours then cooled to -30°C. NIS (54 mg, 0.24 mmol) and TfOH (6.4 μL) were then added. The reaction was essentially complete (as judged by TLC) after 20 min. The mixture was then diluted with dichloromethane (10 mL) and filtered through celite. The filtrate was washed with 10% aqueous sodium thiosulfate (10 mL), saturated aqueous sodium bicarbonate (2x10 mL) and brine (10 mL). The solution was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to a foam that was flash chromatographed on silica gel (3:5, ethyl acetate/hexane) to give disaccharide **85** (118 mg) in 81% yield.

Method B: The coupling reaction between para-acetamidophenyl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-galactopyranoside **77** (40 mg, 0.053 mmol) and thioglycoside **64** (27.5 mg, 0.044 mmol) was carried out essentially as in method A, except that TfOH (1.0 equiv.) was used. The glycosylation reaction was generally finished in 25 min and after similar work-up and purification to give disaccharide **85** (46.2 mg) in 87% yield.

Compound **85** had: m.p. 143.4-145.9 °C; [α]<sub>D</sub> +108° (c = 1.0, CHCl<sub>3</sub>); IR (thin film, ν<sub>cm<sup>-1</sup></sub>): 1729, 1600, 1518, 1451, 1342, 1286, 1093, 853, 710; (+)FAB-MS (glycerol) gave m/z (ion, relative intensity): 1208.2 ([M+1]<sup>+</sup>, 1.2%), 1053.3 ([M+1-HSPhNO<sub>2</sub>]<sup>+</sup>, 6.6%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 8.12-7.19 (m, 39H, Aromatic), 5.95 (d, 2H, H-4, H-4'), 5.82 (dd, 1H, J<sub>2,3</sub> = 10.5 Hz, H-2'), 5.74 (dd, 1H, J<sub>2,3</sub> = 9.8 Hz, H-2), 5.58 (dd, 1H, J<sub>3,4</sub> = 3.3 Hz, H-3), 5.56 (dd, 1H, J<sub>3,4</sub> = 3.3 Hz, H-3'), 5.00 (d, 1H, J<sub>1,2</sub> = 9.8 Hz, H-1), 4.91 (d, 1H, J<sub>1,2</sub> = 8.0 Hz, H-1'), 4.48-4.27 (m, 4H, H-5, H-5', H-6a', H-6b'), 4.16 (dd,

$^1\text{H}$ ,  $J_{5,6a} = 4.2$ ,  $J_{6a,6b} = 10.5$  Hz, H-6a), 3.92 (dd, 1H,  $J_{5,6b} = 7.5$  Hz, H-6b);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm): 165.9, 165.5 (2C), 165.2, 165.1 (2C), 168.1 (8x C=O), 146.9-123.8 (48C, Aromatic), 101.4 (C-1'), 84.7 (C-1), 77.1 (C-5), 72.54 (C-3), 71.7 (C-3'), 71.4 (C-5'), 69.9, 69.6 (C-2'), 68.5, 68.0 (C-4, C-4'), 68.1 (C-6), 67.5 (C-2), 61.8 (C-6').

Anal. Calcd for  $\text{C}_{67}\text{H}_{53}\text{NO}_{19}\text{S}$  (1208.16): C, 66.60; H, 4.42; N, 1.16. Found: C, 66.32; H, 4.47; N, 1.28.

**Para-Nitrophenyl O-(2,3,4-tri-O-benzoyl-6-tert-butyldimethylsilyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-benzoyl-1-thio- $\beta$ -D-galactopyranoside 86**

Phenyl 2,3,4-tri-O-benzoyl-6-tert-butyldimethylsilyl-1-thio- $\beta$ -D-galactopyranoside **53** (60 mg, 0.086 mmol) and para-nitrophenyl 2,3,4-tri-O-benzoyl-1-thio- $\beta$ -D-galactopyranoside **64** (45 mg, 0.072 mmol) were dissolved in dry dichloromethane (2 mL) under nitrogen and pulverized molecular sieves (4 Å, 100 mg) was added. The mixture was stirred at room temperature for 3 hours and cooled to  $-30$  °C. NIS (35 mg, 0.157 mmol) and TfOH (3.2  $\mu\text{L}$ ) were then added. The reaction was essentially complete (as judged by TLC) after 20 min. The mixture was then diluted with dichloromethane (25 mL) and filtered through celite. The filtrate was washed with 10% aqueous sodium thiosulfate (10 mL), saturated aqueous sodium bicarbonate (2x10 mL) and brine (10 mL). The solution was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated to a foam that was flash chromatographed on silica gel (0.5% ethanol in dichloromethane) to afford desired disaccharide **86** (70 mg) in 82% yield: m.p. 109.5-111.9°C;  $[\alpha]_{\text{D}} +128.2^\circ$  ( $c = 1.1$ ,  $\text{CHCl}_3$ ); IR (thin film,  $\nu_{\text{cm}}$ ): 3067, 2939, 2856, 1731, 1601, 1515, 1451, 1281, 1101, 1070, 844, 752, 709; (+)FAB-MS (glycerol) gave  $m/z$  (ion, relative intensity): 1194.5 ( $[\text{M}]^+$ , 0.5%), 1137.5 ( $[\text{M-tBu}]^+$ , 2.5%), 1096.4 ( $[\text{M-PhCO}_2\text{H}]^+$ , 0.9%), 1074.5 ( $[\text{M-HSitBuMe}_2]^+$ , 0.3%), 1039.5 ( $[\text{M-HSPhNO}_2]^+$ , 0.4%);  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.12-7.19 (m, 34 H, Aromatic), 5.94 (d, 1H,  $J_{4,5} < 1.0$  Hz, H-4), 5.89 (d, 1H,  $J_{4',5'} < 1.0$  Hz, H-4'), 5.74 (dd, 1H,  $J_{2,3} = 10.4$  Hz, H-2'), 5.73 (dd, 1H,  $J_{2,3} = 9.8$  Hz, H-2), 5.56 (dd, 1H,  $J_{3,4} = 3.3$  Hz, H-3), 5.44 (dd, 1H,  $J_{3,4'} = 3.4$  Hz, H-3'), 4.96 (d, 1H,  $J_{1,2} = 9.9$  Hz, H-1), 4.83 (d, 1H,  $J_{1,2'} = 7.9$  Hz, H-1'),

4.30 (ddd, 1H,  $J_{5,6a} = 4.2$ ,  $J_{5,6b} = 7.4$  Hz, H-5), 4.14 (dd, 1H,  $J_{6a,6b} = 10.6$  Hz, H-6a), 3.94 (ddd, 1H,  $J_{5',6a'} = 5.8$ ,  $J_{5',6b'} = 8.0$  Hz, H-5'), 3.88 (dd, 1H, H-6b), 3.76 (dd, 1H,  $J_{6a',6b'} = 9.9$  Hz, H-6a'), 3.60 (dd, 1H, H-6b), 0.77 (s, SiC(CH<sub>3</sub>)<sub>3</sub>), -0.04, -0.17 (s, 2x SiCH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 165.6, 165.4, 165.3, 165.3, 168.2, 168.1 (6 x C=O), 147.0-123.8 (42C, Aromatic C), 101.5 (C-1'), 84.8 (C-1), 77.3 (C-5), 74.2 (C-5'), 72.6 (C-3), 72.0 (C-3'), 70.0 (C-2), 68.6 (C-4), 68.1 (C-6), 67.65 (C-4'), 67.6 (C-2'), 60.5 (C-6'), 25.7 (3x Me<sub>3</sub>), 25.4 (SiC), 18.1 (SiCH<sub>3</sub>).

**Para-nitrophenyl O-(2,3,4,6-tetra-O-benzyl-α or (β)-D-galactopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-1-thio-β-D-galactopyranoside 87**

**Method A:** Para-nitrophenyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-galactopyranoside **52** (68 mg, 0.1 mmol) and para-nitrophenyl 2,3,4-tri-O-benzoyl-1-thio-β-D-galactopyranoside **64** (63 mg, 0.1 mmol) were dissolved in dry dichloromethane (2 mL) under nitrogen and pulverized molecular sieves (4 Å, 100 mg) was added. The mixture was stirred at room temperature for 3 hours and cooled to -30 °C. NIS (56.2 mg, 0.25 mmol) and TfOH (12.6 μL) were then added. The coupling reaction was complete (as judged by TLC) after 65 min. The mixture was then diluted with dichloromethane (10 mL) and filtered through a layer of celite. The filtrate was washed with 10% aqueous sodium thiosulfate (10 mL), saturated aqueous sodium bicarbonate (2x10 mL) and brine (10 mL). The solution was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to a syrup that was chromatographed on silica gel (ethyl acetate-hexane, 1:4) to isolate disaccharides **87** (87.9 mg) in 76% as a mixture of α/β (≈ 4/1) anomers as judged by <sup>1</sup>H NMR spectrum.

**Method B:** The coupling reaction between para-acetamidophenyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-galactopyranoside **77** (33 mg, 0.048 mmol) and thioglycoside **64** (25 mg, 0.040 mmol) was carried out essentially as in method A, but using trifluoromethanesulfonic acid (1.2 equiv.). The glycosylation reaction was generally finished in 25 min and after similar work-up and purification procedure to give disaccharide (42 mg) in 92% yield as a mixture of α/β (≈ 3/1) anomers as judged by <sup>1</sup>H NMR spectrum. The

anomer mixture was then successfully separated by radial chromatography on a silica gel Chromatotron (1 mm plate) using 1 % tBuOH in methylene chloride as eluent.

Compound **87a** had:  $[\alpha]_D +62.0^\circ$  ( $c = 1.0$ ,  $\text{CHCl}_3$ );  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 8.13-7.20 (m, 39H, Aromatic), 5.94 (d, 1H,  $J_{4,5} < 1.0$  Hz, H-4), 5.73 (dd, 1H,  $J_{2,3} = 9.9$  Hz, H-2), 5.59 (dd, 1H,  $J_{3,4} = 3.4$  Hz, H-3), 5.00 (d, 1H,  $J_{1,2} = 9.9$  Hz, H-1), 4.90-4.64 (m, 6H, 3xOCH<sub>2</sub>), 4.79 (d, 1H,  $J_{1',2'} = 3.7$  Hz, H-1'), 4.38-4.32 (m, 3H, H-5, OCH<sub>2</sub>), 4.02 (dd, 1H,  $J_{2',3'} = 10.5$  Hz, H-2'), 3.99 (m, 1H, H-5'), 3.89 (d, 1H,  $J_{4',5'} < 1.0$  Hz, H-4'), 3.87 (dd, 1H,  $J_{3',4'} < 1.0$  Hz, H-3'), 3.86 (dd, 1H,  $J_{5,6a} = 6.1$ ,  $J_{6a,6b} = 11.0$  Hz, H-6a), 3.73 (dd, 1H,  $J_{5,6b} = 7.3$  Hz, H-6b), 3.50 (dd, 1H,  $J_{5',6a'} = 6.3$ ,  $J_{6a',6b'} = 9.3$  Hz, H-6a'), 3.44 (dd, 1H,  $J_{5',6b'} = 6.5$  Hz, H-6b');  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 165.4, 165.3, 165.1 (3xC=O), 147.0-123.7 (48C, Aromatic), 98.8 (C-1'), 84.3 (C-1), 79.0 (C-3'), 76.5 (C-5), 74.9 (C-5'), 74.8 (C-4'), 73.7 (OCH<sub>2</sub>), 73.4 (OCH<sub>2</sub>), 73.2 (2C, 2xOCH<sub>2</sub>), 72.7 (C-3), 69.9 (C-2'), 69.2 (C-6'), 68.7 (C-4), 67.7 (C-2), 67.4 (C-6).

Anal. Calcd for  $\text{C}_{67}\text{H}_{62}\text{NO}_{15}\text{S}$  (1152.287): C, 69.84; H, 5.34; N, 1.22; S, 2.78. Found: C, 70.00; H, 5.59; N, 1.09; S, 2.86.

Compound **87b** had:  $[\alpha]_D +70.2^\circ$  ( $c = 1.2$ ,  $\text{CHCl}_3$ );  $^1\text{H-NMR}$   $\delta$  (500 MHz,  $\text{CDCl}_3$ ): 8.13-7.20 (m, 39H, Aromatic), 5.90 (d, 1H,  $J_{4,5} < 1.0$  Hz, H-4), 5.79 (dd, 1H,  $J_{2,3} = 9.9$  Hz, H-2), 5.58 (dd, 1H,  $J_{3,4} = 3.4$  Hz, H-3), 5.13 (d, 1H,  $J_{1,2} = 9.9$  Hz, H-1), 5.00-4.64 (m, 6H, 3xOCH<sub>2</sub>), 4.38 (d, 1H,  $J_{1',2'} = 7.7$  Hz, H-1'), 4.40-4.33 (m, 3H, H-5', OCH<sub>2</sub>), 3.98 (dd, 1H,  $J_{5,6a} = 3.4$ ,  $J_{6a,6b} = 11.4$  Hz, H-6a), 3.92 (dd, 1H,  $J_{5,6b} = 8.0$  Hz, H-6b), 3.86 (d, 1H,  $J_{4',5'} < 1.0$  Hz, H-4'), 3.85 (dd, 1H,  $J_{2',3'} = 9.8$  Hz, H-2'), 3.79-3.49 (m, 2H, H-5', H-6a'), 3.48 (dd, 1H,  $J_{3',4'} = 3.0$  Hz, H-3'), 3.39 (dd, 1H,  $J_{5',6b'} = 9.3$ ,  $J_{6a',6b'} = 11.9$  Hz, H-6b');  $^{13}\text{C-NMR}$   $\delta$  (125.8 MHz,  $\text{CDCl}_3$ ): 165.4 (1C, C=O), 165.2 (2C, 2xC=O), 146.7-123.9 (48C, Aromatic), 104.6 (C-1'), 84.2 (C-1), 82.2 (C-5'), 79.6 (C-2'), 77.6 (C-5), 75.4 (OCH<sub>2</sub>), 74.4 (OCH<sub>2</sub>), 73.5 (2C, OCH<sub>2</sub>, C-3'), 73.2 (OCH<sub>2</sub>), 73.2 (C-4'), 72.8 (C-3), 69.3 (C-6), 68.8 (2C, C-4, C-6'), 67.7 (C-2).

**Para-Nitrophenylsulfenyl 1,3,4,6-Tetra-O-benzoyl- $\beta$ -D-galactopyranoside 88**

Para-nitrophenyl 2,3,4,6-tetra-O-benzoyl-1-thio- $\beta$ -D-galactopyranoside **50** (150 mg, 0.204 mmol) was dissolved in dry dichloromethane (15 mL). The solution was cooled to -40 °C and a solution of m-chloroperoxybenzoic acid (67 mg, 0.388 mmol) in dichloromethane (7 mL) was added. The reaction was kept at -40 °C for 15 min and then allowed to attain temperature to 0 °C. After 45 min, added additional mCPBA (7 mg) and stirred at 0 °C for additional 2 hours. TLC (ether-hexane 3:1) showed a clear conversion starting material ( $R_f$  = 0.43) to product ( $R_f$  = 0.19). The mixture was diluted with methylene chloride (15 mL) and washed with saturated sodium bicarbonate and water, dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated under reduced pressure. The residue was purified by silica gel chromatography using ether-hexane (2:1, v/v) as eluent to give titled product (103 mg) in 65% yield:  $[\alpha]_D^{+21}$  (c = 1.0,  $\text{CHCl}_3$ );  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.27-7.26 (m, 24H, aromatic), 6.11 (dd, 1H,  $J_{2,3}$  = 10.0 Hz, H-2), 5.94 (dd, 1H,  $J_{4,5}$  < 1.0 Hz, H-4), 5.66 (dd, 1H,  $J_{3,4}$  = 3.2 Hz, H-3), 4.73 (d, 1H,  $J_{1,2}$  = 9.8 Hz, H-1), 4.55 (dd, 1H,  $J_{5,6a}$  = 8.7,  $J_{6a,6b}$  = 12.7 Hz, H-6a), 4.37-4.28 (m, 2H, H-5, H-6b); FAB-MS (pos.) Calcd. for  $\text{C}_{40}\text{H}_{31}\text{NO}_{11}\text{S}$ : 733.7, Found: 733.6.

**Para-Nitrophenyl O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-benzyl-1-thio- $\beta$ -D-galactopyranoside 89**

Ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside **32** (70 mg, 0.145 mmol) and para-nitrophenyl 2,3,4-tri-O-benzyl-1-thio- $\beta$ -D-galactopyranoside **66** (102 mg, 0.174 mmol) were dissolved in dry dichloromethane (2 mL). To this mixture was added pulverized molecular sieves (4 Å, 150 mg). After stirring at room temperature for 3 hours, the reaction mixture was added methyl trifluoromethanesulfonate (196  $\mu\text{L}$ , 1.74 mmol) and stirred for additional 10 hours. The mixture was then diluted with dichloromethane (10 mL) and filtered through celite. The filtrate was washed saturated aqueous sodium bicarbonate (2x10 mL) and brine (10 mL). The solution was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated to a foam that was chromatographed on silica gel (2:5, Ethyl acetate/hexane) to afford disaccharide **89** (113 mg) in 77% yield:

m.p. 84.3-85.9 °C;  $[\alpha]_D -3.5^\circ$  (c = 1.0, CHCl<sub>3</sub>); IR (thin film,  $\nu_{\text{cm}^{-1}}$ ): 3029, 2904, 1749, 1718, 1579, 1515, 1454, 1387, 1339, 1231, 1083, 1043, 905, 853, 750, 699; (+)FAB-MS (glycerol) gave m/z (ion, relative intensity): 850.4 ([M-HSPhNO<sub>2</sub>]<sup>+</sup>, 0.7%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 7.93-7.22 (m, 23H, Aromatic), 5.67 (dd, 1H,  $J_{3,4} = 9.2$  Hz H-3'), 5.43 (d, 1H,  $J_{1,2} = 8.7$  Hz, H-1'), 5.16 (dd, 1H,  $J_{4,5} = 10.1$  Hz, H-4'), 4.90-4.60 (m, 6H, 3xOCH<sub>2</sub>), 4.62(d, 1H,  $J_{1,2} = 9.5$  Hz, H-1), 4.30 (dd, 1H,  $J_{2,3} = 10.6$  Hz, H-2'), 4.27 (dd, 1H,  $J_{5,6a} = 4.4$ ,  $J_{6a,6b} = 12.5$  Hz, H-6a'), 4.07(dd, 1H,  $J_{5,6b} = 7.4$  Hz, H-6b'), 3.90 (dd, 1H,  $J_{2,3} = 9.1$  Hz, H-2), 3.88 (dd, 1H,  $J_{5,6a} = 3.0$ ,  $J_{6a,6b} = 10.2$  Hz, H-6a), 3.82 (d, 1H,  $J_{4,5} < 1.0$  Hz, H-4), 3.70 (m, 1H, H-5'), 3.53-3.45 (m, 2H, H-3, H-5), 2.03, 2.71, 1.84 (3s, 3xAc); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 170.6, 170.0, 169.4 (3C, C=O, Ac), 145.9-123.6 (30C, Aromatic), 97.7 (C-1'), 85.4 (C-1), 853.8 (C-5), 77.6 (C-3), 76.3 (C-2), 75.6, 74.6, 72.5 (3xOCH<sub>2</sub>), 73.1 (C-4), 71.9 (C-5'), 70.7 (C-3'), 68.6 (C-4'), 67.9 (C-6), 61.7 (C-6'), 54.6 (C-2'). 20.7, 20.5, 20.4 (3xAc).

**Phenyl O-(2,3,4,6-tetra-O-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-benzoyl-1-thio- $\beta$ -D-galactopyranoside 90**

Ethyl 2,3,4,6-tetra-O-benzoyl-1-thio- $\beta$ -D-galactopyranoside **48** (92 mg, 0.14 mmol) and phenyl 2,3,4-tri-O-benzoyl-1-thio- $\beta$ -D-galactopyranoside **63** (70 mg, 0.12 mmol) were dissolved in dry dichloromethane (2 mL) under nitrogen and pulverized molecular sieves (4 Å, 150 mg) was added. The mixture was stirred at room temperature for 3 hours and cooled to -30 °C. N-Iodosuccinimide (46 mg, 0.24 mmol) and triflic acid (2.1  $\mu$ L) were then added. The reaction was essentially complete (as judged by TLC) after 5 min. The mixture was then diluted with dichloromethane (10 mL) and filtered through celite. The filtrate was washed with 10% aqueous sodium thiosulfate (10 mL), saturated aqueous sodium bicarbonate (2x10 mL) and brine (10 mL). The solution was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to a foam that was flash chromatographed on silica gel (3:7, ethyl acetate/hexane) to isolate disaccharide **90** (124 mg) in 89% yield: m.p. 87.5-89.4 °C;  $[\alpha]_D +54.3^\circ$  (c = 1.2, CHCl<sub>3</sub>); IR (thin film,  $\nu_{\text{cm}^{-1}}$ ): 3065, 2934, 1726, 1602, 1451, 1315,

1268, 1177, 1105, 1069, 1027, 754, 709; (+)FAB-MS (glycerol) gave m/z (ion, relative intensity): 1163.3 ( $[M]^+$ , 2.3%), 1053.4 ( $[M+1-HSPhNO_2]^+$ , 5.4%);  $^1H$ -NMR  $\delta$  ( $CDCl_3$ ): 8.11-7.20 (m, 40H, Aromatic), 6.00 (d, 1H,  $J_{4,5}$ , < 1.0 Hz H-4), 5.89 (d, 1H, H-4'), 5.73 (dd, 1H,  $J_{2,3}$  = 9.8 Hz, H-2), 5.58 (dd, 1H,  $J_{3,4}$  = 3.0 Hz, H-3), 5.57 (H-3'), 5.42 (H-2'), 5.32 (H-1'), 5.02 (d, 1H,  $J_{1,2}$  = 9.8 Hz, H-1), 4.56-4.48 (m, H-5', H-6a'), 4.32 (dd, 1H,  $J_{5',6b'}$  = 3.3,  $J_{6a',6b'}$  = 12.2 Hz, H-6b'), 4.30 (m, 1H, H-5), 3.96 (dd, 1H,  $J_{5,6a}$  = 7.3,  $J_{6a,6b}$  = 9.6 Hz, H-6a), 3.80 (dd, 1H,  $J_{5,6b}$  = 5.8 Hz, H-6b);  $^{13}C$ -NMR ( $CDCl_3$ )  $\delta$  (ppm): 165.9, 165.8, 165.7, 165.4 (2C), 165.3, 165.1 (C=O, Bz), 134.3-128.1 (48C, Aromatic), 106.0 (C-1'), 85.7 (C-1), 82.1 (C-2'), 81.7 (C-5'), 77.4 (C-3'), 76.0 (C-5), 73.2 (C-3), 70.4 (C-4'), 68.2 (C-4), 67.9 (C-2), 65.1 (C-6), 63.7 (C-6').

**Para-Acetamidophenyl O-(2,3,4,6-tetra-O-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-benzoyl-1-thio- $\beta$ -D-galactopyranoside 91**

To a suspension of **85** (140 mg, 0.116 mmol) in absolute ethanol (5 mL) was added tin(II) chloride dehydrate (132 mg, 0.580 mmol). The mixture was heated to 70 °C and stirred for 1 hour. The solution was then cooled, poured into ice-water (50 mL) and the pH was adjusted to 7-8 with sodium bicarbonate. Dichloromethane (50 mL) was added and the organic phase was washed with water (2x25 mL). The dried organic phase (sodium sulfate) was filtered and evaporated. The crude product was then dissolved in pyridine (3 mL) and acetyl anhydride (2 mL) and stirred for 2 hours. The mixture was concentrated and the product was purified by silica gel column chromatography to afford **91** (118 mg) in 83% yield: m.p. 125.4-127.1 °C;  $[\alpha]_D^{25} +79.1^\circ$  (c = 1.0,  $CHCl_3$ ); IR (thin film,  $\nu_{cm^{-1}}$ ): 3359, 3065, 2968, 1726, 1594, 1520, 1493, 1451, 1269, 1070, 910, 837, 802, 710, 684; (+)FAB-MS (glycerol) gave m/z (ion, relative intensity): 1221.6 ( $[M+1]^+$ , 0.4%), 1098.4 ( $[M-PhCOOH]^+$ , 0.3%), 1054.5 ( $[M+1-HSPhNHAc]^+$ , 1.0%), 1053.4 ( $[M-HSPhNHAc]^+$ , 1.6%);  $^1H$ -NMR ( $CDCl_3$ )  $\delta$  (ppm): 8.27-7.18 (m, 39H, Aromatic), 5.89 (d, 1H, H-4), 5.85 (d, 1H, H-4'), 5.69 (dd, 1H,  $J_{2,3}$  = 9.8 Hz, H-2), 5.68 (dd, 1H,  $J_{2,3}$  = 9.8 Hz, H-2'), 5.51 (dd, 1H,  $J_{3,4}$  = 3.5 Hz, H-3'), 5.47 (dd, 1H,  $J_{3,4}$  = 3.4 Hz, H-3), 4.90 (d,

1H,  $J_{1,2} = 10.1$  Hz, H-1'), 4.89 (d, 1H,  $J_{1,2} = 10.0$  Hz, H-1), 4.47 (dd, 1H,  $J_{5,6a} = 6.6$ ,  $J_{6a,6b} = 11.4$  Hz, H-6a'), 4.27 (dd, 1H,  $J_{5,6b} = 7.6$  Hz, H-6b'), 4.12-4.09 (m, 2H, H-5, H-5'), 4.02 (dd, 1H,  $J_{5,6a} = 6.8$ ,  $J_{6a,6b} = 12.5$  Hz, H-6a), 3.97 (dd, 1H,  $J_{5,6b} = 3.5$  Hz, H-6b), 2.12 (s, 3H, NHAc);  $^{13}\text{C-NMR}$  ( $\text{CHCl}_3$ )  $\delta$  (ppm): 168.4, 166.0, 165.6, 165.4, 168.3, 165.3, 165.2 (8C, C=O), 138.9-120.1 (48C, Aromatic), 100.6 (C-1'), 85.9 (C-1), 78.2 (C-5'), 72.8 (C-3), 71.8 (C-3'), 71.3 (C-5), 69.9, 68.1 (2C, C-2, C-2'), 69.0 (C-4'), 68.0 (C-4), 67.5 (C-6'), 61.9 (C-6), 24.5 (1C, NHAc).

Anal. Calcd for  $\text{C}_{69}\text{H}_{57}\text{NO}_{18}\text{S}$ : C, 67.91; H, 4.71; N, 1.15. Found: C, 67.79; H, 4.89; N, 1.26.

**Para-Nitrophenyl O-(2,3,4,6-tetra-O-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 6)-(2,3,4-tri-O-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 6)-O-2,3,6-tri-O-benzoyl-1-thio- $\beta$ -D-glucopyranoside 92**

Disaccharide 91 (35 mg, 0.029 mmol) and para-nitrophenyl 2,3,4-tri-O-benzoyl-1-thio- $\beta$ -D-galactopyranoside 64 (15 mg, 0.024 mmol) were dissolved in dry dichloromethane (2 mL) under nitrogen and pulverized molecular sieves (4 Å, 50 mg) were added. The mixture was stirred at room temperature for 3 hours and was then cooled to -30 °C. N-Iodosuccinimide (5.4 mg, 0.048 mmol) and triflic acid (2.1  $\mu\text{L}$ ) were then added. The reaction was essentially complete (as judged by TLC) after 25 min. The mixture was then diluted with dichloromethane (10 mL) and filtered through celite. The filtrate was washed with 10% aqueous sodium thiosulfate (10 mL), saturated aqueous sodium bicarbonate (2x10 mL) and brine (10 mL). The solution was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated to a foam that was chromatographed on silica gel (1.5% ethanol in dichloromethane) to afford trisaccharides 37 (35.5 mg) in 88% yield: m.p. 133.2-144.3 °C;  $[\alpha]_{\text{D}} +166.7^\circ$  ( $c = 0.75$ ,  $\text{CHCl}_3$ ); IR (thin film,  $\nu_{\text{cm}^{-1}}$ ): 3066, 2926, 2855, 1730, 1601, 1583, 1519, 1451, 1342, 1315, 1268, 1176, 1099, 1070, 1028, 853, 751, 709; (+)FAB-MS (glycerol) gave  $m/z$  (ion, relative intensity): 1682.9 ( $[\text{M}]^+$ , 1.9%), 1528.8 ( $[\text{M-HSPhNO}_2]^+$ , 0.7%);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.10-7.17 (m, 54H, Aromatic), 5.92 (dd,

1H,  $J_{4,5} < 1.0$  Hz, H-4), 5.90 (dd,  $J_{4',5'} < 1.0$  Hz, H-4'), 5.89 (dd, 1H,  $J_{4'',5''} < 1.0$  Hz, H-4''), 5.72 (dd, 1H,  $J_{2',3'} = 10.3$  Hz, H-2'), 5.71 (dd, 1H,  $J_{2,3} = 9.9$  Hz, H-2), 5.69 (dd, 1H,  $J_{2'',3''} = 10.3$  Hz, H-2''), 5.60 (dd, 1H,  $J_{3,4} = 3.3$  Hz, H-3), 5.55 (dd, 1H,  $J_{3',4'} = 3.4$  Hz, H-3'), 5.48 (dd, 1H,  $J_{3'',4''} = 3.4$  Hz, H-3''), 5.00 (d, 1H,  $J_{1,2} = 9.9$  Hz, H-1), 4.76 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'), 4.72 (d, 1H,  $J_{1'',2''} = 7.8$  Hz, H-1''), 4.76 (dd, 1H,  $J_{5'',6a''} = 6.2$ ,  $J_{6a'',6b''} = 11.1$  Hz, H-6a''), 4.26 (m, 1H, H-5), 4.18 (m, 1H, H-5''), 4.10 (dd, 1H,  $J_{5'',6b''} = 6.7$  Hz, H-6b''), 4.09 (m, 1H, H-5'). 4.05 (dd, 1H,  $J_{5,6a} = 4.5$ ,  $J_{6a,6b} = 10.5$  Hz, H-6a), 3.97 (dd, 1H,  $J_{5',6a'} = 6.2$ ,  $J_{6a',6b'} = 10.6$  Hz, H-6a'), 3.83 (dd, 1H,  $J_{5,6b} = 7.5$  Hz, H-6b), 3.70 (dd, 1H,  $J_{5'',6b''} = 6.2$  Hz, H-6b'');  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 165.9, 165.6, 165.5, 165.4, 165.2 (2C), 165.2, 165.1, 165.1 (C=O, Bz), 147.0 -123.8 (66C, Aromatic), 101.4 (C-1'), 100.0 (C-1''), 84.8 (C-1), 77.0 (C-5), 72.9 (C-3), 72.7 (C-5'), 71.7 (C-3''), 71.6 (C-3'), 75.4 (C-5''), 69.9 (2C, C-2', C-2''), 69.8 (C-4), 68.6 (2c, C-4', C-4''), 68.1 (C-6), 67.9 (C-2), 67.7 (C-6'), 61.6 (C-6'').

Anal. Calcd for  $\text{C}_{94}\text{H}_{75}\text{NO}_{27}\text{S}$  (1682.611): C, 67.09; H, 4.50; N, 0.83. Found: C, 67.25; H, 4.35; N, 0.94.

**Para-Nitrophenyl O-(2,3,4-tri-O-benzyl- $\alpha$ -L-fucopyranosyl)-(1 $\rightarrow$ 3)-4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside 94**

To a solution of ethyl 2,3,4-tri-O-benzyl-1-thio- $\beta$ -L-fucopyranoside **93** (100 mg, 0.22 mmol) and para-nitrophenyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside **39** (96 mg, 0.18 mmol) in dry dichloromethane (2 mL) was added powdered molecular sieves 4 Å (200 mg) under nitrogen. The mixture was stirred at room temperature for 3 hours and then cooled to  $-30^\circ\text{C}$ . N-Iodosuccinimide (72.5 mg, 0.32 mmol) and triflic acid (3.2  $\mu\text{L}$ ) were then added. The reaction was essentially complete (as judged by TLC) in 5 min. The mixture was then diluted with dichloromethane (10 mL) and filtered through celite. The filtrate was washed with 10% aqueous sodium thiosulfate (10 mL), saturated aqueous sodium bicarbonate (2x10 mL) and brine (10 mL). The solution was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated. The residue was purified on radial silica gel

chromatography (1 mm plate) using ethyl acetate/hexane (1:5) as eluent to give disaccharide **94** (124 mg) in 72% yield: m.p. 91.4-93.1 °C;  $[\alpha]_D +33.6^\circ$  (c = 1.2, CHCl<sub>3</sub>); IR (thin film,  $\nu_{\text{cm}^{-1}}$ ): 3063, 3030, 2928, 2872, 1776, 1715, 1597, 1581, 1517, 1496, 1453, 1387, 1340, 1095, 1053, 847, 750, 720, 699, 647; (+)FAB-MS (glycerol) gave m/z (ion, relative intensity): 952.4 ([M+1]<sup>+</sup>, 1.0%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 8.13-6.98 (m, 24H, Aromatic), 5.89 (d, 1H,  $J_{1,2} = 10.6$  Hz, H-1), 5.56 (s, 1H, PhCH), 4.79 (d, 1H,  $J_{1,2} = 1.2$  Hz, H-1'), 4.77 (AB pattern 1H, H-A of OCH<sub>2</sub>), 4.69 (dd, 1H,  $J_{3,4} = 9.7$  Hz, H-3), 4.50-4.21 (m, 6H, H2, H-6a, 2xOCH<sub>2</sub>), 4.02 (tert, 1H,  $J_{5,6} = 6.5$  Hz H-5'), 3.84-3.67 (m, 6H, H-6b, H-B of OCH<sub>2</sub>, H-5, H-4', H-2', H-4), 3.46 (d, 1H,  $J_{3,4} < 1.0$  Hz, H-3'). 0.86 (d, 3H, H-6'); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 168.3, 167.4 (2C, 2xC=O, Phth.), 146.8-123.4 (48C, Aromatic), 101.2 (PhCH=), 99.7 (C-1'), 82.7 (C-1), 81.7 (C-5), 79.6 (C-4'), 77.8 (C-3'), 76.4 (C-3), 75.5 (C-2'), 74.7, 73.0, 72.8 (3C, 3xOCH<sub>2</sub>), 70.7 (C-4), 68.4 (C-6), 67.4 (C-5'), 54.3 (C-2), 16.4 (C-6').

**Para-Nitrophenyl O-(2,3,4,6-tetra-O-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 3)-4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside **95****

To a solution of phenyl 2,3,4,6-tetra-O-benzoyl-1-thio- $\beta$ -D-galactopyranoside **49** (100 mg, 0.145 mmol) and para-nitrophenyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside **39** (65 mg, 0.121 mmol) in dry dichloromethane (2 mL) was added powdered molecular sieves 4 Å (200 mg) under nitrogen and was stirred at room temperature for 3 hours. The mixture was cooled to -30 °C and N-iodosuccinimide (49 mg, 0.218 mmol) and triflic acid (6.5  $\mu$ L) were added. The reaction was essentially complete (as judged by TLC) in 25 min. The mixture was then diluted with dichloromethane (10 mL) and filtered through celite. The filtrate was washed with 10% aqueous sodium thiosulfate (10 mL), saturated aqueous sodium bicarbonate (2x10 mL) and brine (10 mL). The solution was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to a foam that was flash chromatographed on silica gel (1:5, ethyl acetate/Hexane) to afford disaccharide **95** (93 mg) in 71% yield: m.p. 119.7-121.5 °C;  $[\alpha]_D +112.3^\circ$  (c = 1.74, CHCl<sub>3</sub>); IR (neat):

3066, 3033, 2925, 2872, 1777, 1726, 1600, 1583, 1518, 1451, 1383, 1341, 11767, 1101, 1070, 1027, 852, 754, 711  $\text{cm}^{-1}$ ; (+)FAB-MS (glycerol) gave  $m/z$  (ion, relative intensity): 1113.4 ( $[\text{M}]^+$ , 0.7%), 1007.3  $[\text{M}-\text{PhCO}_2\text{H}]^+$ , 0.3%), 958.4 ( $[\text{M}-\text{HSPHNO}_2]^+$ , 0.3%);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.08-7.09 (m, 33H, Aromatic), 5.79 (d, 1H,  $J_{4',5'} < 1.0$  Hz, H-4'), 5.71 (d, 1H,  $J_{1,2} = 10.6$  Hz, H-1), 5.64 (s, 1H, PhCH), 5.50 (dd, 1H,  $J_{2,3'} = 10.3$  Hz, H-2'), 5.32 (dd, 1H,  $J_{3,4'} = 3.4$  Hz, H-3'), 4.93 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'), 4.88 (dd, 1H,  $J_{3,4} = 9.4$  Hz, H-3), 4.52 (dd, 1H,  $J_{2,3} = 10.0$  Hz, H-2), 4.42 (dd, 1H,  $J_{5,6a} = 2.5$ ,  $J_{6a,6b} = 8.2$  Hz, H-6a), 4.31 (dd, 1H,  $J_{5',6a'} = 5.4$ ,  $J_{6a',6b'} = 11.0$  Hz, H-6a'), 4.27 (dd, 1H,  $J_{5',6b'} = 8.3$  Hz, H-6b'), 4.00 (dd, 1H,  $J_{4,5} = 8.8$  Hz, H-4), 3.88-3.83 (m, 2H, H-5, H-6b), 3.76 (m 1H, H-5');  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 168.5, 167.6 (C=O, Phth.), 165.5, 165.4, 165.4, 164.6 (C=O, PhCO<sub>2</sub>), 146.8-123.9 (42C, Aromatic), 102.0 (PhCH), 100.5 (C-1'), 82.8 (C-1), 76.7 (C-3), 71.7 (C-3'), 70.9 (C-5), 70.7 (C-5'), 70.2 (C-2'), 68.5 (C-6), 67.5 (C-4'), 61.0 (C-6'), 53.8 (C-2).

**Para-Nitrophenyl O-(2,3,4,6-tetra-O-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 3)-(2,6-di-O-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-O-2,3,6-tri-O-benzoyl-1-thio- $\beta$ -D-glucopyranoside 97**

Phenyl 2,3,4,6-tetra-O-benzoyl-1-thio- $\beta$ -D-galactopyranoside **49** (48 mg, 0.07 mmol) and para-nitrophenyl 2,2',3,6,6'-penta-O-acetyl-1-thio- $\beta$ -D-lactopyranoside **96** (500 mg, 0.05 mmol) were dissolved in dry dichloromethane (2 mL) under nitrogen and pulverized molecular sieves (4 Å) were added. The mixture was stirred at room temperature for 3 hours and cooled to  $-30^\circ\text{C}$ . N-Iodosuccinimide (28 mg, 0.125 mmol) and TfOH (2.8  $\mu\text{L}$ ) were added. After reaction mixture was stirred at  $-30^\circ\text{C}$  for 25 min, TLC indicated the completion of reaction. The reaction mixture was then diluted with dichloromethane (10 mL) and filtered through celite. The filtrate was washed with 10% aqueous sodium thiosulfate (10 mL), saturated aqueous sodium bicarbonate (2x10 mL) and brine (10 mL). The solution was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated to a foam that was chromatographed on silica gel (2% ethanol in dichloromethane) to afford trisaccharides **97**

(53.0 mg) in 72% yield: m.p. 115.3-117.2 °C;  $[\alpha]_D^{+70.2^\circ}$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ); IR (thin film,  $\nu_{\text{cm}^{-1}}$ ): 3066, 2931, 1727, 1601, 1583, 1452, 1341, 1268, 1177, 1111, 1070, 1028, 852, 753, 709; (+)FAB-MS (glycerol) gave  $m/z$  (ion, relative intensity): 1581.6 ( $[\text{M}+1]^+$ , 0.8%), 1425.8 ( $[\text{M}-\text{HSPHNO}_2]^+$ , 3.6%);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.03 - 7.00 (m, 49H, Aromatic), 5.88 (d, 1H,  $J_{4',5'} < 1.0$  Hz, H-4'), 5.72 (dd, 1H,  $J_{2',3'} = 7.9$  Hz, H-2''), 5.70 (dd, 1H,  $J_{3,4} = 9.2$  Hz, H-3), 5.44 (dd, 1H,  $J_{2',3'} = 9.8$  Hz, H-2'), 5.42 (dd, 1H,  $J_{3',4'} = 3.4$  Hz, H-3''), 5.37 (dd, 1H,  $J_{2,3} = 9.4$  Hz, H-2), 4.92 (d, 1H,  $J_{1,2} = 9.9$  Hz, H-1), 4.89 (d, 1H,  $J_{1',2''} = 8.0$  Hz, H-1''), 4.53 (d, 1H,  $J_{1,2'} = 8.0$  Hz, H-1'), 4.51 (dd, 1H,  $J_{5',6a'} = 7.3$ ,  $J_{6a',6b'} = 11.6$  Hz, H-6a''), 4.49 (dd, 1H,  $J_{5,6a} = 10.2$ ,  $J_{6a,6b} = 12.2$ , H-6a), 4.40 (dd, 1H,  $J_{5',6b'} = 5.5$  Hz, H-6b''), 4.35 (dd, 1H,  $J_{5,6b} = 5.8$  Hz, H-6b), 4.28 (m, 1H, H-5''), 4.06 (dd,  $J_{4',5'} < 1.0$  Hz, H-4'), 4.00 (dd, 1H,  $J_{4,5} = 9.8$  Hz, H-4), 3.98 (dd, 1H,  $J_{5',6a'} = 6.7$  Hz, H-6a'), 3.85 (dd, 1H,  $J_{3',4'} = 3.0$  Hz, H-3''), 3.83 (m, 1H, H-5), 3.64 (dd, 1H,  $J_{5',6b'} = 7.4$ ,  $J_{6a',6b'} = 11.5$  Hz, H-6b'), 3.54 (m, 1H, H-5');  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 165.9 (2C), 165.7, 165.5, 165.4 (2C), 165.1, 164.7, 164.2 (9x C=O), 146.8 -123.6 (60C, Aromatic), 101.8 (C-1''), 100.6 (C-1'), 84.1 (C-1), 81.1 (C-3'), 77.2 (C-5), 75.3 (C-4), 73.3 (C-3), 72.3(C-5'), 75.7 (C-5''), 71.3, 70.5, 70.1 (3C, C-2', C-2, C-3''), 69.2 (C-2''), 68.1 (C-4'), 67.7 (C-4''), 62.8, 62.5, 62.0 (3C, C-6, C-6', C-6'').

**Para-Nitrophenyl O-(2,3,4-tri-O-benzyl- $\alpha$ -L-fucopyranosyl)-(1 $\rightarrow$ 3)-O-(2,6-di-O-benzoyl-4-O-[(2,6-di-O-benzoyl-3,4-O-isopropylidene- $\beta$ -D-galactopyranosyl)]-(1 $\rightarrow$ 4)-1-thio- $\beta$ -D-glucopyranoside 99**

To a solution of para-nitrophenyl O-(2,6-di-O-benzoyl-3,4-O-isopropylidene- $\beta$ -D-galactopyranosyl)-2,6-di-O-benzoyl-1-thio- $\beta$ -D-glucopyranoside **98** (50 mg, 0.054 mmol) and ethyl 2,3,4-tri-O-benzyl-1-thio- $\beta$ -L-fucopyranoside **93** (31 mg, 0.065 mmol) in dry dichloromethane (3 mL) was added powdered molecular sieves (4 Å, 100 mg) and the mixture was stirred at room temperature for 3 hours. The mixture was then cooled to -30 °C and N-iodosuccinimide (22 mg, 0.097 mmol) and trifluoromethanesulfonic acid (0.94  $\mu\text{L}$ ) were added. After stirring at -30 °C for 15 min. TLC showed that the reaction was

finished. The reaction mixture was then diluted with dichloromethane (6 mL) and filtered through celite. The filtrate was washed with 10% aqueous sodium thiosulfate (8 mL), saturated aqueous sodium bicarbonate (2x8 mL) and brine (8 mL). The solution was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated to a foam that was purified by radial silica gel chromatography (1 mm plate) using ethyl acetate-hexane (1:3, v/v) as eluent. Pure **99** (49 mg) was obtained in 68% yield: MS (Electron Spray): 1374.2 ( $[\text{M}+\text{Na}]^+$ , 30%); IR (thin film,  $\nu_{\text{cm}^{-1}}$ ): 2924, 1601, 1458, 1372, 1274, 1115, 711;  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 8.06-7.08 (m, 39H, aromatic), 5.72 (dd, 1H,  $J_{3,4} = 8.5$  Hz, H-3), 5.33 (d, 1H  $J_{1',2''} = 3.8$  Hz, H-1''), 5.08 (dd,  $J_{2',3'}$  = 6.7 Hz, H-2'), 5.00 (d, 1H,  $J_{1,2} = 9.2$  Hz, H-1), 4.60 (d, 1H, H-1'), 4.58 (d, 1H,  $J_{5,6a} = 5.3$  Hz, H-6a), 4.38 (dd, 1H,  $J_{5,6b} = 6.2$ ,  $J_{6a,6b} = 12.0$  Hz, H-6b), 4.23 (dd, 1H,  $J_{3',4'}$  = 6.7 Hz, H-3'), 4.16 (dd, 1H,  $J_{2,3} = 8.8$  Hz, H-2), 4.03 (dd, 1H, H-4), 3.87 (dd, 1H,  $J_{2',3''} = 10.1$  Hz, H-2''), 1.49, 1.23 (2s, 6H, Me, isopropylidene), 1.10 (d, 3H,  $J_{5'',6''} = 6.4$  Hz, H-6'');  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 165.9, 165.7, 165.0, 164.8 (4xC=O, OBz), 143.3-123.7 (48C, aromatic), 110.9 (C-O-C), 100.2 (C-1'), 98.0 (C-1''), 85.0 (C-1), 79.0 (C-3''), 76.3 (4''), 75.5 (C-3'), 75.0 (C-5), 74.7 (C-3), 74.1 (C-4), 73.9 (2C,  $\text{CH}_2$ , C-2''), 73.1 (2C, C-2, C-2'), 73.1 (C-4'), 73.0 (2C, 2x $\text{CH}_2$ ), 75.4 (C-5'), 67.8 (C-5''), 62.9 (2C, C-6, C-6'), 27.4 (isopropylidene), 16.6 (C-6'').

## Chapter 4 Synthesis of Sialosides using “Active-Latent”

### Glycosylation Strategy

#### 4.1 Introduction

Sialic acid-containing glycoconjugates, especially gangliosides, play critical roles in numerous biological phenomena such as cell-cell adhesion,<sup>191</sup> malignancy,<sup>192</sup> and cell growth regulation.<sup>193</sup> They have also been identified as tumor associated antigens<sup>194</sup> and cell differentiation markers.<sup>195</sup> Ganglioside GM<sub>3</sub> (Figure 4.1) was first isolated from equine erythrocytes by Yamakawa's group in 1952.<sup>196</sup> It has been shown to serve as the precursor in the biosynthetic pathway<sup>197</sup> of many complex gangliosides and is known to modulate the epidermal growth factor (EGF) and the platelet-derived growth factor (PDGF) receptors.<sup>30</sup> GM<sub>3</sub> was also found to be expressed in abnormally high concentration in tumor cells.<sup>27</sup> Given the extreme difficulty of isolating homogeneous gangliosides from natural sources,<sup>198</sup> effective syntheses are desirable.

Glycosylation using sialic acid-derived donors has been recognized as a most challenging task in carbohydrate chemistry for the following reasons. First, the carboxylic acid function at C-2 electronically disfavors oxonium ion formation, an intermediate necessary for almost all known glycosidation reactions. Secondly, the carboxy group

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<sup>191</sup> Taki, T.; Hirabayashi, Y.; Ishikawa, H.; Ando, S.; Kon, K.; Tanaka, Y.; Matsumoto, M. *J. Biol. Chem.* 1986, 261, 3075.

<sup>192</sup> Hirabayashi, Y.; Hyogo, A.; Nakao, T.; Tsuchiya, K.; Suzuki, Y.; Matsumoto, M.; Kon, K.; Ando, S. *J. Biol. Chem.* 1990, 265, 8144.

<sup>193</sup> Matta, S. G.; Yorke, G.; Roisen, F. J. *Develop. Brain Res.* 1986, 27, 243.

<sup>194</sup> (a) Higashi, H.; Hirabayashi, Y.; Fukui, Y.; Kaiki, M.; Matsumoto, M.; Ueda, S.; Kato, S.; *Cancer Res.* 1985, 45, 3796. (b) Hakomori, S. *Annu. Rev. Immunol.* 1984, 2, 103.

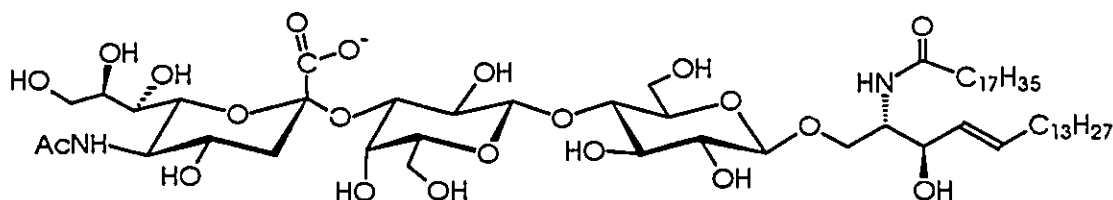
<sup>195</sup> (a) Ebel, F.; Schemit, E.; Peter-Katalinic, J.; Kniep, B.; Muhlradt, P. F. *Biochemistry* 1992, 31, 12190. (b) Feizi, T. *Nature* 1985, 314, 53.

<sup>196</sup> Yamakawa, T.; Suzuka, S. *J. Biochem. (Tokyo)*, 1952, 39, 383.

<sup>197</sup> Bouhours, D.; Bouhours, J.-F. *J. Biol. Chem.* 1991, 266, 12944.

<sup>198</sup> Ando, S.; Weki, H.; Kon, K.; Kishimoto, Y. In *Gangliosides and Modulation of Neuronal Functions*; Rahmann, H.; Ed.; Springer-Verlag: Berlin-Heidelberg, 1987, pp 167-177.

sterically restricts glycoside formation. Thirdly, the lack of a substituent at C-3 precludes possible anchimeric group participation. These combined factors reduce the reactivity of sialyl donors in glycosidation, disfavor glycoside formation and promote an elimination pathway to produce a 2,3-dehydro derivative. Despite these drawbacks, major advances in sialoside syntheses have been achieved in recent years.<sup>199,200</sup>



**Figure 4.1 Structure of GM<sub>3</sub>**

Ganglioside GM<sub>3</sub> has been used as a target for the evaluation of a variety of synthetic methodologies. This trisaccharide sequence was generally synthesized from lactosyl acceptor and sialyl donor building blocks and several synthetic methods have been reported (table 4.1). The first total synthesis of ganglioside GM<sub>3</sub> was achieved by Ogawa *et al.*<sup>201a</sup> Glycosylation of lactosyl acceptor with methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosyl chloride) onate 4.1 as a glycosyl donor, Hg(CN)<sub>2</sub>-HgBr<sub>2</sub> as promoter gave GM<sub>3</sub> trisaccharide as a mixture of α- and β- anomers (2:1) in 18% yield. When a 3-β-phenylthio stereocontroller was introduced into the C-3 position of the sialyl donor 4.2, the yield and stereoselectivity of GM<sub>3</sub> synthesized was greatly increased (78%, α anomer only).<sup>201b</sup> However, the introduction and removal of the auxiliary group necessitated additional steps. This therefore limit the efficiency of this approach. Using thioglycosides, Hasegawa and

<sup>199</sup> Okamoto, K.; Goto, T. *Tetrahydron* 1990, 46, 5835

<sup>200</sup> De Ninno, M. P.; *Synthesis*, 1991, 583.

<sup>201</sup> (a) Sugimoto, M.; Ogawa, T. *Glycoconjugate J.* 1985, 2, 5. (b) Sugimoto, M.; Ogawa, T. *Carbohydr. Res.* 1985, 135, C5.

coworkers<sup>202</sup> found DMTST to be an efficient glycosylation catalyst using thioglycoside often producing exclusively the  $\alpha$  anomers. The DMTST-catalyzed glycosylation reaction of methyl thioglycosyl donor **4.3** and lactosyl acceptor **4.7** afforded 47% yield of GM<sub>3</sub>. Schmidt's group<sup>203</sup> has demonstrated the utility of sialyl dialkyl phosphites as glycosyl donors. Glycosylation of lactosyl acceptor **4.6** with dialkyl phosphite glycosyl donor **4.4** using TMSOTf as catalyst gave GM<sub>3</sub> trisaccharide in 55% yield. Another successful example as shown from the work of Lönn and Stenvall,<sup>204</sup> the AgOTf-MSB catalyzed glycosidation of sialyl-S-glycosyl xanthate **4.5** and lactosyl acceptor **4.8** afforded GM<sub>3</sub> in 63% yield.

Though tremendous progress has been made in terms of glycosylation reactions between sialyl donors and lactosyl acceptors, all of those involving GM<sub>3</sub> trisaccharides (table 4.1) have the anomeric center of the glucosyl moiety blocked as O-ether groups. Hence, further transformations of GM<sub>3</sub> trisaccharide into biologically important glycoconjugates need deblocking reactions at the anomeric centers and transformations of the reducing trisaccharides into activated glycosyl donors such as 1-O-acyls, 1-halides or 1-trichloroacetimidates.<sup>205</sup> Activation at the anomeric position from directly blocked trisaccharide, is highly desirable. This could be achieved using our "active-latent" glycosylation strategy. The synthesis of GM<sub>3</sub> trisaccharide unit can be achieved by glycosylation reaction between an "active" glycosyl donor and a "latent" glycosyl acceptor in the presence of thiophilic promoter (NIS/TfOH). Both the "active" sialyl donors and the "latent" lactosyl acceptors could be prepared from the corresponding glycosyl halides under PTC conditions as we discussed in chapter 2. The synthetic strategy toward GM<sub>3</sub> and related gangliosides is illustrated in scheme 4.1.

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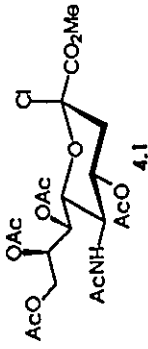
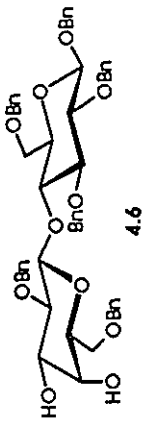
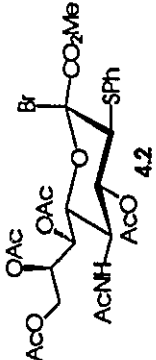
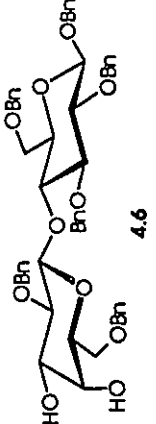
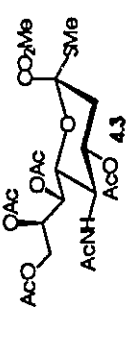
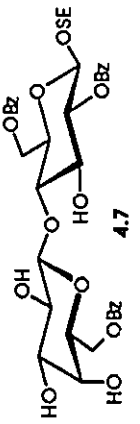
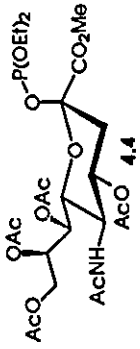
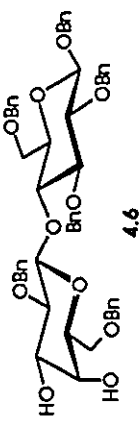
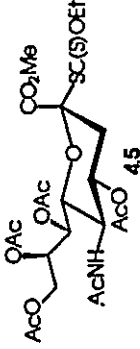
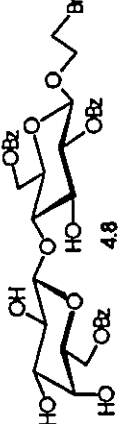
<sup>202</sup> Murase, T.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* 1988, 184, C1.

<sup>203</sup> Martin, T. J.; Brescello, R.; Toepfer, A.; Schmidt, R. R. *Glycoconjugate J.* 1993, 10, 16.

<sup>204</sup> Lönn, H.; Stenvall, K. *Tetrahedron Lett.* 1992, 33, 115.

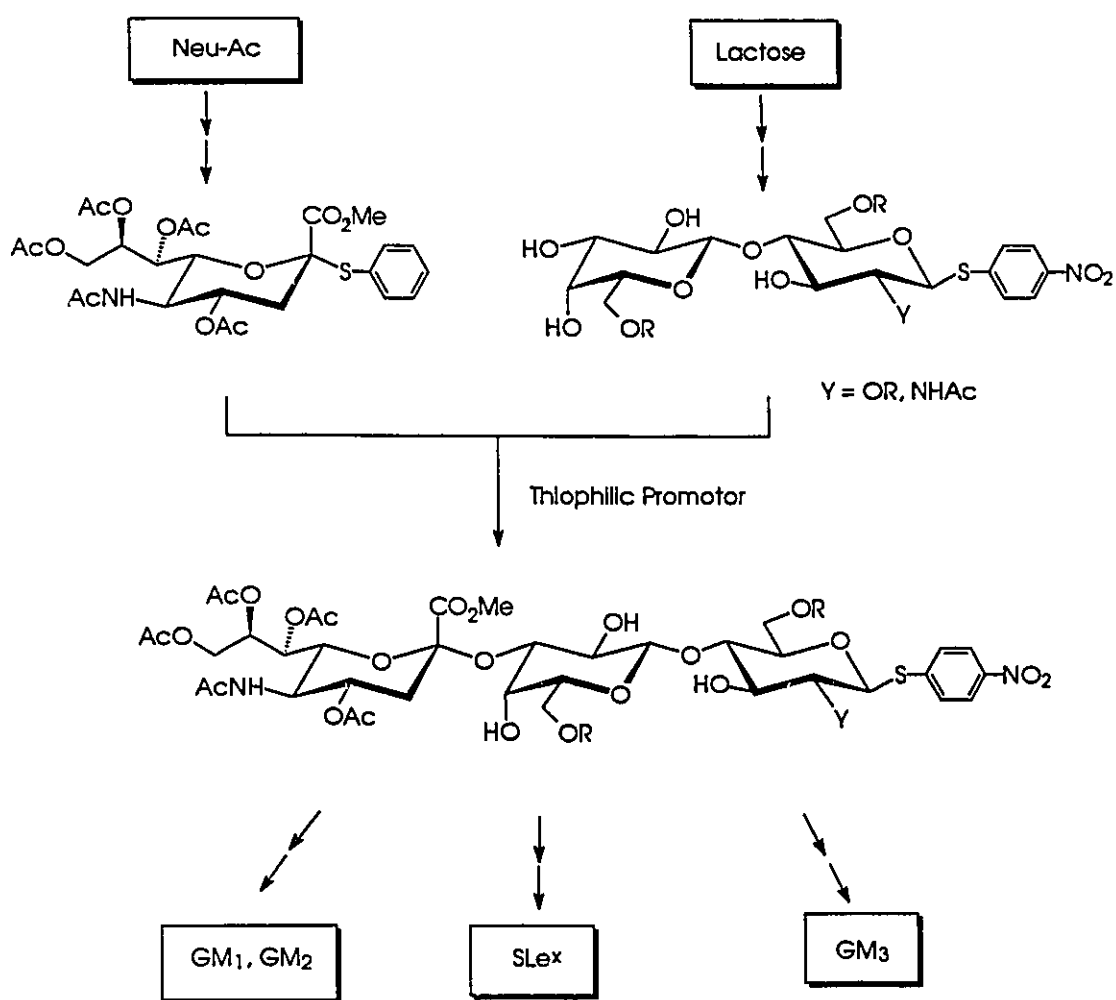
<sup>205</sup> Schmidt, R. R. *Pure Appl. Chem.* 1989, 61, 1257.

**Table 4.1. Synthesis of  $\alpha(2\rightarrow3)$  Sialosides**

Glycosyl donor	Glycosyl Acceptor	Reaction conditions	Trisaccharides yields (%)		Ref.
			$\alpha$ -linked	$\beta$ -linked	
 4.1	 4.6	Hg(CN) <sub>2</sub> -HgBr <sub>2</sub> , Cl(CH <sub>2</sub> ) <sub>2</sub> Cl,	12	6	201a
 4.2	 4.6	D:A = 1:1.6, Hg(CN) <sub>2</sub> -HgBr <sub>2</sub> , 20 °C, CCl <sub>4</sub>	78	b	201b
 4.3	 4.7	D:A = 2:1, DMTST -15 °C, CH <sub>3</sub> CN	47	b	202
 4.4	 4.6	D:A = 1:1.5, TMSOTf, -40 °C, CH <sub>3</sub> CN	55	b	203
 4.5	 4.8	D:A = 1:1:1, AgOTf/MSB, -60 °C °CH <sub>3</sub> CN/CH <sub>2</sub> Cl <sub>2</sub>	63	c	204

a. D, Donor; A, Acceptor; b, Not detected; c, not reported.

The remarkable character of the sialosides obtained from our "active-latent" glycosylation method is that these "latent" oligosaccharides can be transformed into an "active" donor by a mild reduction-acetylation method in almost quantitative yield. The new "active" oligosaccharide donors are very useful in further block synthesis of oligosaccharides and glycoconjugates. The S-linkage between the GM<sub>3</sub> moiety and aglycon instead of the usual O-linked forms seen in natural GM<sub>3</sub> increases the stability of GM<sub>3</sub> glycoconjugates toward the hydrolysis by glucohydrolases.

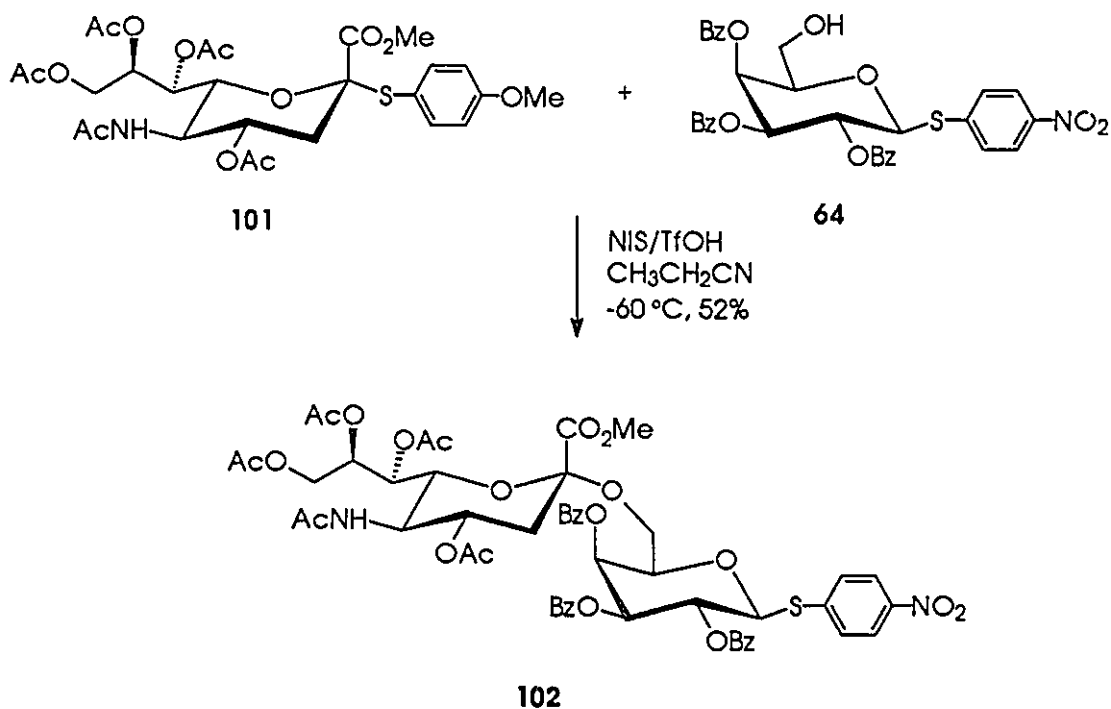


**Scheme 4.1** General Strategy for the Synthesis of GM<sub>3</sub> and Related Sialosides

## 4.2 Results and Discussion

### 4.2.1 Synthesis of Neu5Ac- $\alpha$ -(2 $\rightarrow$ 6)- $\beta$ -D-Galactoside

For the initial attempts to evaluate the feasibility of the “active-latent” glycosylation strategy toward the synthesis of GM<sub>3</sub> and GM<sub>3</sub>-related sialosides, we set out to synthesize Neu5Ac- $\alpha$ -(2 $\rightarrow$ 6)- $\beta$ -D-Gal disaccharide as a model reaction for studying suitable glycosylation conditions (Scheme 4.2).<sup>66</sup>



**Scheme 4.2** Synthesis of Neu5Ac- $\alpha$ -(2 $\rightarrow$ 6)- $\beta$ -D-Gal Disaccharide

The active sialosyl donor **101** was prepared from  $\beta$ -acetochloroneuramnic acid **4** and para-methoxythiophenol under PTC conditions described in Chapter 2. The latent glycosyl acceptor **64** has already been described in Chapter 3. In the glycosylation reactions, we chose propionitrile as solvent to form glycosyl nitrilium ions as

intermediates<sup>206</sup> in order to influence the configuration of the newly introduced anomeric center. To increase the  $\alpha$ -stereoselectivity and to reduce the amount of 2,3-elimination product of Neu5Ac 103,<sup>207</sup> a lower glycosylation reaction temperature was preferred.

Glycosylation of "active" (reactive) para-methoxyphenyl thiosialoside 101 with "latent" (temporary inactive) para-nitrophenyl 2,3,4-tri-O-benzoyl-1-thio- $\alpha$ -D-galactopyranoside 64 in propionitrile using N-iodosuccinimide (NIS) and triflic acid (TfOH) as promoter proceeded well at -60 °C. The resulting disaccharide 102 was obtained in good yield (52%) and  $\alpha$ -stereoselectivity. Elimination product of Neu5Ac 103 was obtained in 19% yield. Neither cross-coupling product, nor  $\beta$ -disaccharide were detected under these glycosylation conditions.

Although sialosides lack an anomeric proton, the anomeric configuration can be inferred on the basis of empirical rules. These rules include: (i)  $\alpha \delta H_{3_{eq}} > \beta \delta H_{3_{eq}}$ ;<sup>208</sup> (ii)  $\alpha \delta H_4 < \beta \delta H_4$ ;<sup>209</sup> (iii)  $\alpha J_{7,8} > \beta J_{7,8}$ .<sup>210</sup> Another NMR method for determination of the anomeric configuration was based on coupling constants. In the  ${}^2C_5$  chair conformation, the  ${}^3J_{C1,H3a}$  of the  $\alpha$ -anomer is larger than that of the  $\beta$ -anomer.<sup>211</sup> Accordingly, the  $\alpha$ -configuration of C-2' of disaccharide 102 was confirmed by  ${}^1H$ - and  ${}^{13}C$ -NMR spectra which showed H-3e at  $\delta$  2.46 ppm ( $J_{3e,4'} = 4.6$ ,  $J_{3e',3a} = 12.9$  Hz) and C-1' at  $\delta$  165.1 ppm ( ${}^3J_{C1,H3a} = 6.3$  Hz).

The high stereoselectivity of this glycosylation reaction can be explained by the effect of nitriles as participating solvents.<sup>208</sup> The general principle of solvent participation is shown in Scheme 4.3. A  $\beta$ -nitrilium ion intermediate was formed during the reaction

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<sup>206</sup> Schmidt, R. R.; Behrendt, M.; Toepfer, A. *Synlett*. 1990, 11, 694.

<sup>207</sup> Birberg, W.; Lönn, H. *Tetrahedron Lett.* 1991, 32, 7453.

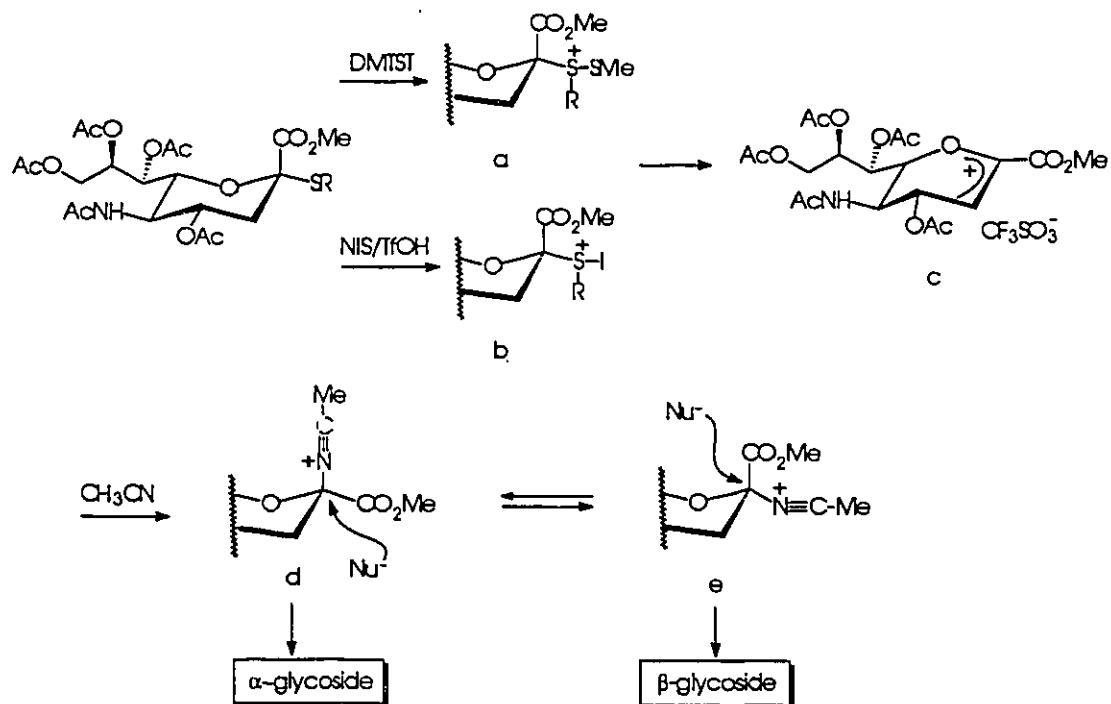
<sup>208</sup> Dabrowski, U.; Friebolin, H.; Brossmer, R.; Supp, M. *Tetrahedron Lett.* 1979, 48, 4637.

<sup>209</sup> Paulson, H.; Tietz, H. *Angew. Chem. Int. Ed. Engl.* 1982, 21, 927.

<sup>210</sup> van der Vleugel, D. J. M.; van Boom, J. H. Heeswijk, W. A. R.; Vliegenhart, J. F. G. *Carbohydr. Res.* 1982, 102, 121.

<sup>211</sup> (a) Hori, H.; Nakajima, T.; Nishida, Y.; Ohruji, H.; Meguro, H. *Tetrahedron Lett.* 1988, 29, 6317. (b) Czarmiecki, M. F.; Thornton, E. R. *J. Am. Chem. Soc.* 1977, 99, 8173.

performed in nitrile solvents at low temperature. The  $\beta$ -nitrilium ion can then undergo inverse displacement at anomeric center to form the  $\alpha$ -anomer.<sup>204,212</sup>



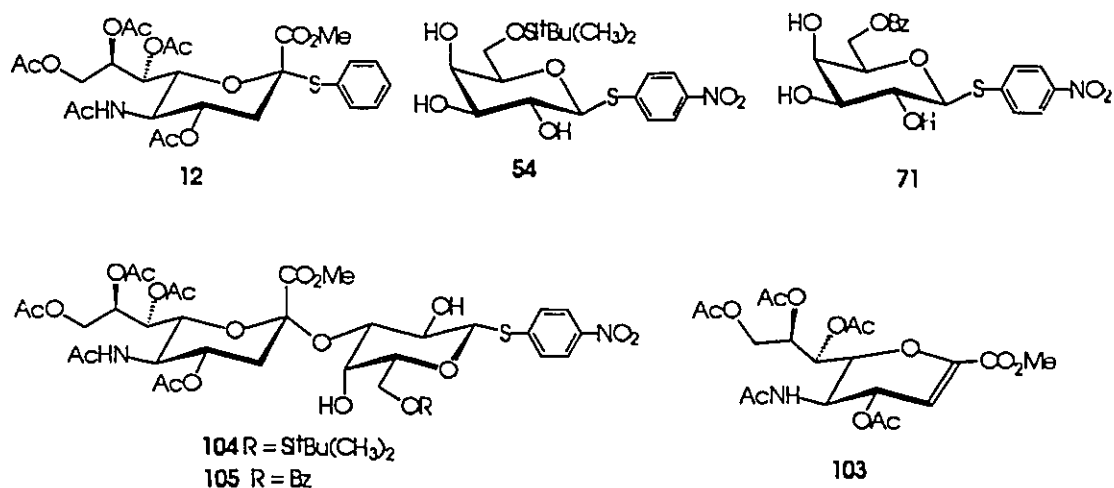
**Scheme 4.3** The Possible Mechanism of Thiophilic Promoters Mediated Sialylation

Encouraged by these results, we also tried the direct glycosylation of “latent” galactosyl acceptor **54** with “active” sialyl donor **12**. This was performed under the same conditions described above for **104**. Despite a large number of attempts, a complex reaction mixture was obtained, including 2,3-elimination product **103**. The crude NMR spectrum of the mixture did not show the presence of disaccharide **104**. The use of 6-O-benzoylated galactose **71** as the “latent” acceptor proved equally frustrating. These results could possibly be explained by the partial activation of glycosyl acceptors **54** and **71** by the thiophilic promoter. Since “latent” glycosyl acceptors **54** and **71** are not deactivated by benzoyl groups as in acceptor **64**, they might not be completely inert under these

<sup>212</sup> Kirchner, E.; Thiem, F.; Demick, R. *J. Carbohydr. Chem.* **1988**, *7*, 453.

glycosylation conditions. Self-couplings and partial decompositions of these acceptors might be possible.

Thus, further investigations need to be done to explain these results and design suitable derivatives for a direct condensation approach as described above.



**Scheme 4.4 Glycosylation of “Latent” Galactosyl Acceptors**

#### 4.2.2 Selectively Protected Lactose and Lactosamine Derivatives as Building Blocks for GM<sub>3</sub> Sialosides

The benefit using of lightly protected acceptors, which results mostly from the lack of additional steric impediments, was already recognized by several groups.<sup>203-206,213,214</sup> Our “active-latent” glycosylation strategy for the synthesis of the GM<sub>3</sub> trisaccharide and its analogues first required the preparation of lightly protected lactoside building blocks.

<sup>213</sup> Ito, Y.; Ogawa, T. *Tetrahedron* 1990, 46, 89.

<sup>214</sup> Kanie, O.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* 1988, 7, 501.

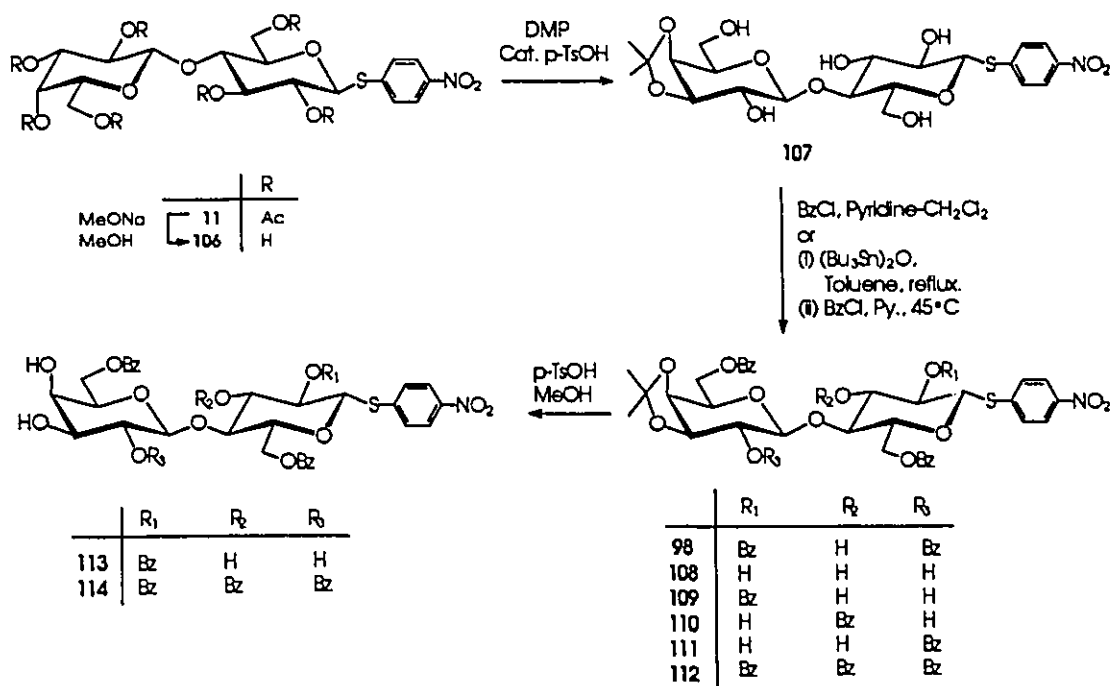
The starting material, para-nitrophenyl hepta-O-acetyl-1-thio- $\beta$ -D-lactoside **11**, was conveniently prepared on large scale using PTC methods well established in this group.<sup>55-61</sup> Catalytic deacetylation (NaOMe, MeOH) of **11** afforded quantitatively para-nitrophenyl 1-thio- $\beta$ -D-lactoside **106**. Treatment of **106** with 2,2-dimethoxypropane in the presence of *p*-toluene sulfonic acid gave para-nitrophenyl O-(3,4-O-isopropylidene- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1-thio- $\beta$ -D-glucopyranoside **107** in 80% yield. A sizable amount of isomeric and thermodynamically unstable 4',6'-O-isopropylidene acetal<sup>215</sup> was also detected in the reaction mixture by thin-layer chromatography (Rf 0.72, 8:1, chloroform-methanol) and could be converted to the thermodynamically stable 3',4'-O-isopropylidene acetal **107** by boiling the product mixture in methanol-water 10:1 for 2 hours. The position of the isopropylidene acetal in **107** was verified by NMR spectroscopy. The signal in the <sup>13</sup>C NMR spectrum at  $\delta$  27.6, 26.2 and 111.4 clearly indicated the presence of a five-membered isopropylidene structure fused to pyranoside ring.<sup>216</sup>

Sequential benzylation of **107** using benzoyl chloride (large excess) in pyridine and deacetylation under acidic conditions (cat. *p*-TsOH) gave para-nitrophenyl O-(2,6-di-O-benzoyl-1-thio- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzoyl- $\beta$ -D-glucopyranoside **124** were straightforward and efficient (Scheme 4.5). However, initial attempt of regioselective benzylation at 2-, 6-, 6'-OH groups of lactoside **107** gave mixtures of di-, tri- and tetra-benzoylated products. Despite a large number of attempts to improve the regioselectivity by changing the reaction temperatures and the pyridine-dichloromethane solvent system, no dramatical improvements were observed. Under optimized reaction conditions, *i.e.* using pyridine-dichloromethane (1:1 in v/v) as solvent and low reaction temperatures (-50°C), the desired 2,6,6'-tri-O-benzoylated derivative **69** was obtained in 31% yield together with four other partially benzoylated products (Table 4.2). In addition, the separation of the five partially benzoylated lactosides, especially three tri-O-benzoylated regioisomers **109-111**, was very difficult. Obviously, this synthetic route was not practical for preparative synthesis.

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<sup>215</sup> (a) Pozsgay, V.; Jennings, H. *Synthesis* 1990, 724. (b) Leontein, K.; Nilsson, M.; Norberg, T. *Carbohydr. Res.* 1985, 144, 231.

<sup>216</sup> Grant Buchannan, J.; Encarnacion Chacon-Fuertes, M.; Edgar, A. R.; Moorhouse, S. J.; Rawson, D. L.; Wightman, R. H. *Tetrahedron Lett.* 1980, 21, 1793.



### Scheme 4.5 Synthesis of Lactosyl Acceptors

Table 4.2. Selective Benzoylation of Lactoside 107

Reaction Conditions	Yields <sup>a</sup>				
	98	108	109	110	111
BzCl (4.5 equiv.), -50°C, Pyridine-CH <sub>2</sub> Cl <sub>2</sub> , (1:1)	19%	17%	31%	8%	12%
i) (Bu <sub>3</sub> Sn) <sub>2</sub> O, toluene, reflux. ii) BzCl, pyridine, 45 °C.	6%	3%	74%	-	-

<sup>a</sup> Isolated compounds.

Alternatively, regioselective benzylation of lactoside **107** via stannylene intermediate formation<sup>217</sup> proved to be very efficient. In fact, treatment of para-nitrophenyl 3,4-O-isopropylidene  $\beta$ -D-lactoside derivative **107** in boiling toluene with 6 equiv. of stannylating reagent  $(\text{Bu}_3\text{Sn})_2\text{O}$  for 23 h and subsequent treatment with benzoyl chloride (6 equiv.) for an additional 24 h at 45 °C afforded the desired para-nitrophenyl 2,6,6'-tri-O-benzoylated  $\beta$ -D-lactoside derivative **109** in 74% yield together with 6% of di-O-benzoylated derivative **108** and 3% of tetra-O-benzoylated derivative **98**. Very interestingly, under this stannylating reagent mediated regioselective benzylation reaction, the other two tri-O-benzoylated regioisomer **110** and **111** were not detected.

In the process of characterization of partially benzyolated derivatives **98**, **109-111** and **112**, we found that <sup>1</sup>H-NMR data (Table 4.3), particularly the data of H-2, H-3 and H-2', are very informative. Benzoyl groups showed a strong deshielding effect. A  $\Delta \delta = +1.5$  ppm downshifted C-H signal were usually observed at the benzyolated position compared with that of unbenzyolated counterpart. All the partially benzyolated compounds **98**, **108-111** and **112** showed downshifted H-6 and H-6' signals at  $\delta$  4.5-4.8 ppm, therefore the two benzyolated groups must be at positions -6 and -6' of **98**, **108-111**. Three tribenzyolated regioisomers **109**, **110** and **111** could be unambiguously assigned from the H-2, H-3 and H-2' signals in the <sup>1</sup>H-NMR spectra. As we see from table 4.3, 2, 6, 6'-tri-O-benzyolated regioisomer **109** showed a downshifted H-2 signal of its at  $\delta$  5.26 ppm, the 3, 6, 6'-tri-O-benzyolated regioisomer **110** showed a downshifted H-3 signal of its at  $\delta$  5.44 ppm and the 2', 6, 6'-tri-O-benzyolated regioisomer **111** showed its H-2' signal downshifted at  $\delta$  5.33 ppm. Tetra-O-benzyolated compound **98** showed two shifted downfield signals at  $\delta$  5.19 and 5.35 for H-2 and H-2' respectively. From these regioselective benzyolation experiments, we found that the order of benzyolation of the five hydroxyl groups of 3',4'-O-isopropylidene- $\beta$ -D-lactoside **107** derivatives is HO-6 ~ HO-6' > HO-2 > HO-2' > HO-3. In the mass spectra, all three regioisomers **109**, **110** and **111** showed common positive-ion at ,  $m/z$  832.3 due to  $(M+1)^+$ ,

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<sup>217</sup> (a) Ogawa, T.; Matsui, M. *Tetrahedron*, 1981, 37, 2363. (b) David, S.; Hanessian, S. *Tetrahedron*, 1985, 41, 643.

while di- and tetra-O-benzoylated lactosides **108** and **98** showed (M+1)<sup>+</sup> peaks at m/z 728.3 and 936.3 respectively.

**Table 4.3 Selected <sup>1</sup>H-NMR Data of Partially Benzoylated Lactosides<sup>a</sup>**

Compound	<sup>1</sup> H Chemical shifts, δ (coupling constants, Hz) <sup>b</sup>					
	H-1	H-2	H-3	H-1'	H-2'	H-3'
<b>98</b>	4.87	5.19	4.02	4.65	5.35	4.41
	(10.0)	(9.2)	(8.4)	(8.2)	(7.8)	(5.3)
<b>109</b>	4.95	<u>5.26</u>	3.99	4.33	3.95	4.14
	(10.1)	(9.5)	(8.5)	(8.3)	(7.5)	(5.4)
<b>110</b>	4.92	3.71	<u>5.44</u>	4.30	3.50	4.02
	(10.0)	(8.9)	(8.9)	(8.0)	(7.8)	(5.6)
<b>111</b>	4.70	3.42	3.70	4.63	<u>5.33</u>	4.41
	(9.8)	(8.4)	(8.7)	(7.9)	(7.2)	(5.4)
<b>112</b>	4.97	5.41	5.72	4.62	5.31	3.73
	(9.9)	(9.7)	(9.2)	(7.8)	(7.7)	(3.4)

a: Recorded on a Bruker AMX-500 MHz spectrometer at ambient temperature.

b: Assignment are based on COSY and TOCSY experiments. Chemical shifts are referenced to internal CHCl<sub>3</sub> at 7.24 ppm and are given in ppm and spin-spin coupling constants appear in parentheses.

The last step in the preparation of lightly protected lactosyl acceptors consists in the deprotection of the isopropylidene groups. The isopropylidene groups in tribenzoylated lactoside **109** and tetrabenzoylated lactoside **112** could be removed quantitatively by refluxing in methanol in the presence of *p*-toluenesulfonic acid as catalyst. This procedure gave lightly protected lactosyl acceptors **113** and **114**. The 2',3',4'-OH free on lactosyl acceptor **113** allow selective 3'-O-α-sialylation with sialyl donor **12** to produce GM<sub>3</sub> trisaccharide.

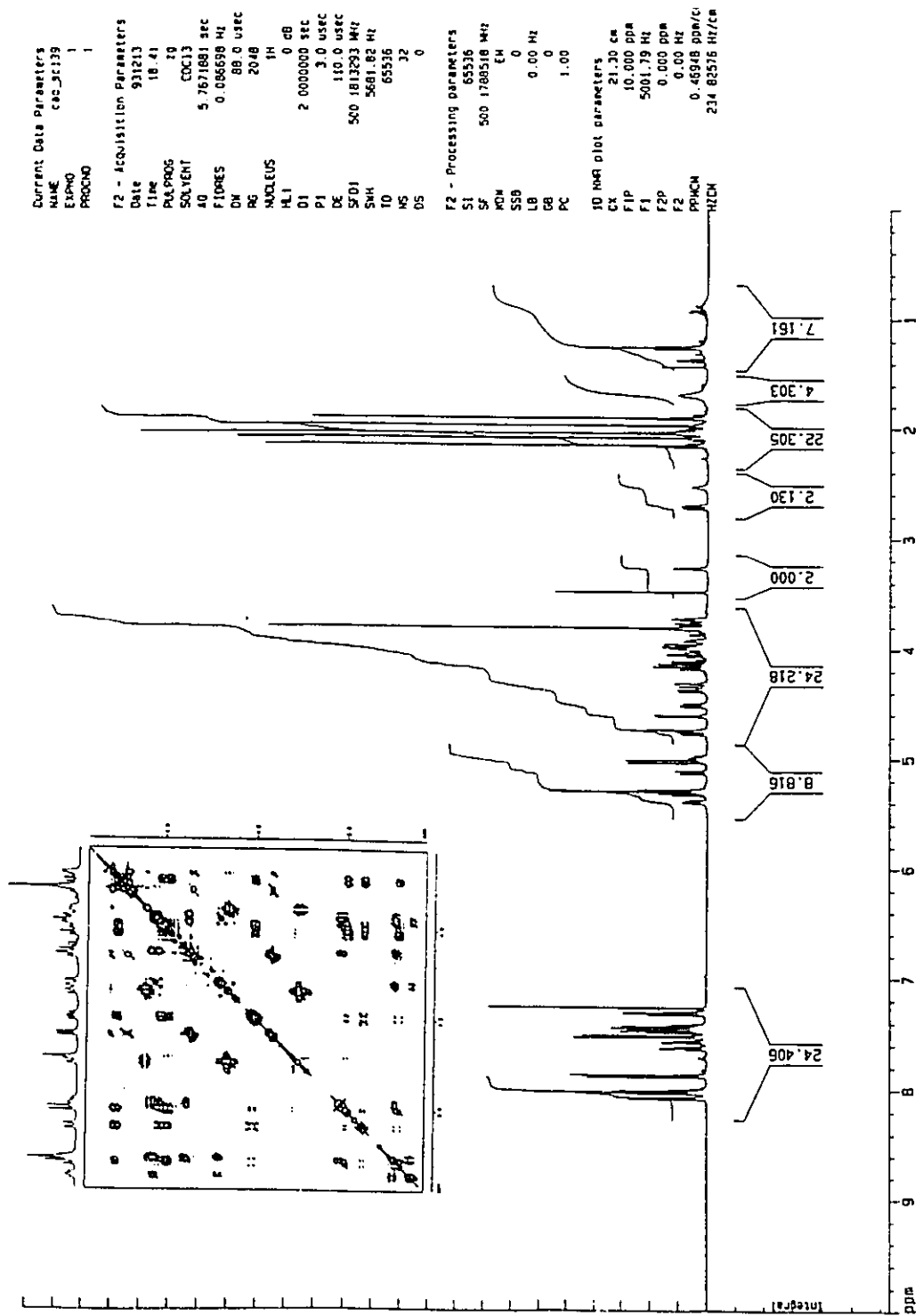
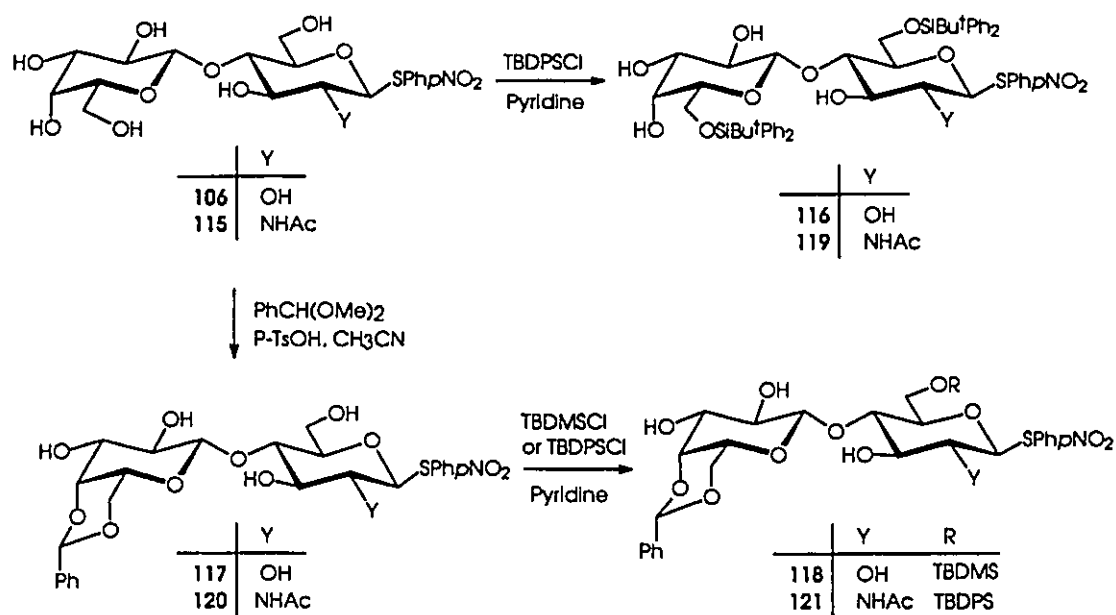


Figure 4.2a <sup>1</sup>H-NMR and <sup>1</sup>H-<sup>1</sup>H TOCSY Spectra (500 MHz, CDCl<sub>3</sub>) of GM<sub>3</sub> Trisaccharide 122



Tetrabenzoylated lactosyl acceptor **98** was suitable for fucosylation at position-3' to produce the corresponding Le<sup>x</sup> analogue **99** as described in section 3.4.



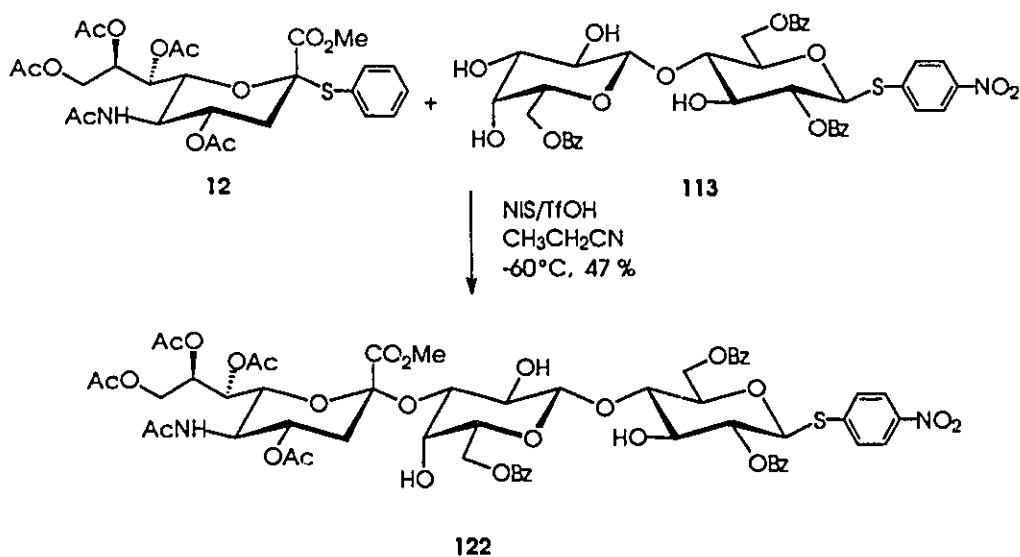
**Scheme 4.6 Selective Protection of Lactoside 106 and Lactosaminide 115**

Alternatively, two other very concise synthetic routes outlined in Scheme 4.6 were devised to prepare lactosyl building blocks. In the first approach, para-nitrophenyl thiolactoside **106** and N-acetyllactosamine derivative **115** were used as starting compounds. In the first approach, treatment of **106** and **115** with tert-butyldiphenylsilyl chloride (TBDPSCI) in dry pyridine at room temperature for 6-8 hours afforded 6,6'-di-O-silylated derivatives **116** and **119**. It is worthy to mention that evaporation of dry pyridine from the starting materials 2-3 times prior to the addition of the silylating reagent (TBDPSCI) resulted in significant improvement of yields (~90%) of silylated lactoside derivatives **116** and **119**.

In the second approach, treatment of lactoside derivatives **106** and **115** with benzaldehyde dimethylacetal in dry acetonitril using *p*-toluenesulfonic acid as catalyst gave the 4',6'-O-benzylidene derivatives **117** and **120** in 93% and 89% yield respectively.

#### 4.2.3 Regio- and $\alpha$ -Stereo-selective Glycosylation of Sialic Acid-Synthesis GM<sub>3</sub>-trisaccharide and GM<sub>3</sub> analogs

Having prepared the key glycosyl acceptors, a crucial coupling of thiosialoside **12** (1.5 equivalents) with glycosyl donor **113** was performed in the presence of the N-iodosuccinimide (NIS) and triflic acid (TfOH) in dry propionitrile at -60°C to give the expected  $\alpha$ -glycoside **122** of Neu5Ac in 47% yield (Scheme 4.7).



**Scheme 4.7** Synthesis of GM<sub>3</sub> Trisaccharide

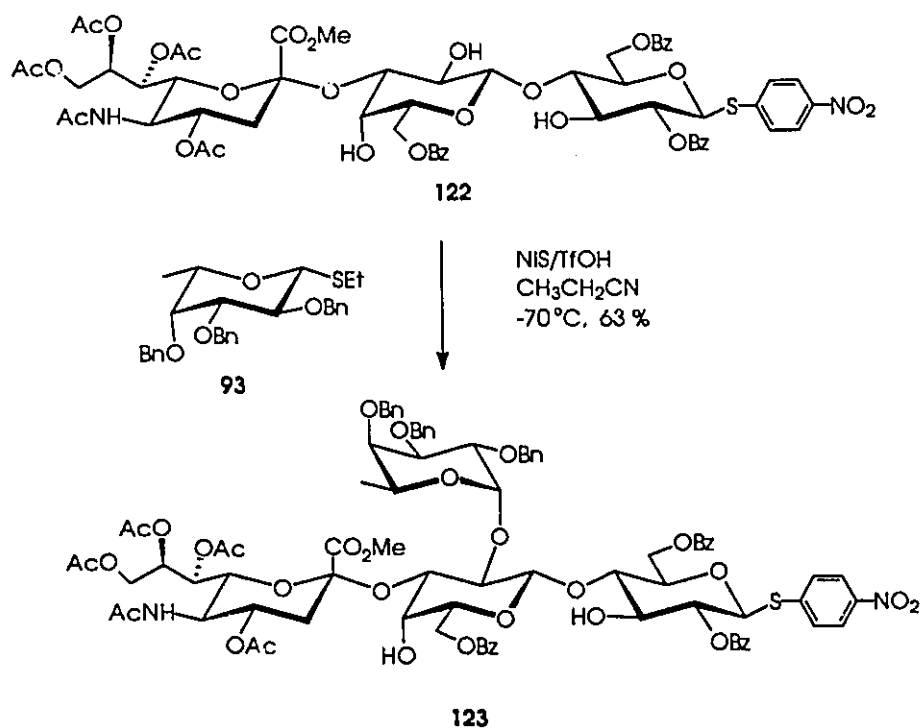
Again, the 2,3-elimination product of Neu5Ac (**103**) was isolated in 15% yield as a by-product of the reaction. Neither self-coupling product of acceptor **113** nor  $\beta$ -trisaccharide were detected under this glycosylation conditions. The reaction showed

remarkable  $\alpha$ -stereoselectivity in accordance with previous experiments from section 4.2.1. Similar results were obtained using acetonitrile and methylene chloride mixture as solvent. Molecular sieves (4 Å) were added to ensure strict anhydrous and neutral conditions.<sup>145</sup> It should be noted that the amount of triflic acid required for the glycosylation reaction depended on both the intrinsic reactivity of the glycosyl donors and the amount of molecular sieves added to the glycosylation reactions. Due to electron withdrawing character<sup>221</sup> of the carboxylic acid function at C-2, the “active” thiophenyl donor **12** became “less-active” compared to other phenyl thioglycosyl donors that were discussed in Chapter 3. Careful control of the amount of triflic acid used in the sialylation reaction and the selective activation of the phenyl thiosialyl donor in the presence of para-nitrophenyl thiolactosyl acceptor were crucial for the successful synthesis of the GM<sub>3</sub> trisaccharide unit.

The structure of **122** was assigned as follows. The  $\alpha$ -configuration at C-2 in **122** was readily assigned from the <sup>1</sup>H-NMR spectrum which contained a deshielded doublet for H-3e at  $\delta$  2.69 ppm ( $J_{3e'',4''} = 4.6$ ,  $J_{3e'',3a''} = 12.9$  Hz) and from the <sup>13</sup>C-NMR spectrum at  $\delta$  168.1 ppm (C-1'',  $J_{C-1'',H-3ax''} = 4.2$  Hz). The regioselectivity for the newly introduced interglycosidic linkage in **122** was determined by its <sup>1</sup>H-NMR spectrum. The H-3' signal in **122** was shifted downfield from 3.66 to 4.16 ppm upon sialylation and the anomeric carbon (C''-2) of sialic acid was shifted downfield from 87.5 to 97.5 ppm by substituting the anomeric sulfur atom to oxygen respectively. The mass spectrum of **122** gave M<sup>+</sup> peak at 1265.3.

Further derivatization of **122** was considered in order to prepare sialyl Le<sup>x</sup> as shown in Scheme 4.8. The trisaccharide acceptor **122** was first coupled with methyl 2,3,4-tri-O-benzyl-1-thio- $\beta$ -L-fucopyranoside<sup>43</sup> **93** in the presence of NIS/TfOH in dichloromethane at -70 °C to give sialylated tetrasaccharide **123** in 63% yield together with recovered trisaccharide acceptor **122** (17%) and a minor unidentified regioisomer (less than 10% as judged by TLC. This result was in accordance with our previous observations (Section 4.3.2) for the reactivity of the 2'-OH over that of 3-OH in  $\beta$ -D-lactoside.

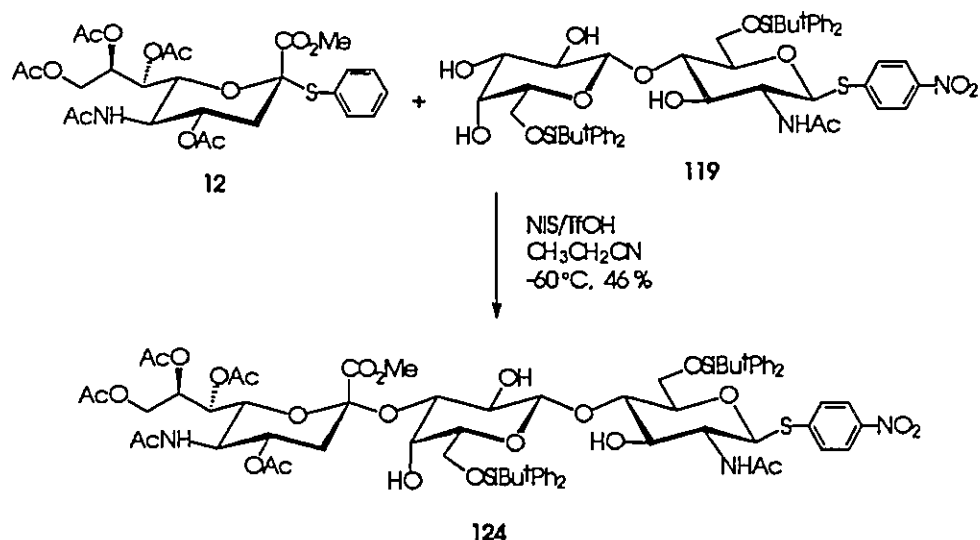
The  $\alpha$ -configuration of the newly introduced anomeric center of **123** was determined from the  $^1\text{H-NMR}$  spectrum which showed a signal for H-1 (fucosyl moiety) at  $\delta$  5.58 ppm ( $^3J_{1,2} = 4.1$  Hz). The regiochemistry of the newly introduced interglycosidic linkage in **122** was determined by its  $^{13}\text{C-NMR}$  spectrum. The C-2' signal in **123** was shifted downfield from 68.7 to 74.3 ppm upon fucosylation. The mass spectrum of **123** gave a  $M^+$  peak at 1681.3 and  $[\text{M-HSPhNO}_2]^+$  peak at 1425.3 respectively.



**Scheme 4.8** Fucosylation of GM<sub>3</sub>-Synthesis Sialyl Le<sup>x</sup> Regioisomer

The glycosylation of **119** with phenyl thiosialoside **12** (1.7 equivalents) in propionitrile for 50 min at -60 °C in the presence of NIS/TfOH (2:1 equivalent relative to acceptor) gave the expected  $\alpha$ -sialoside **124** in 46% yield, together with the 2,3-dehydro derivative **103** isolated in 17% yield as a by-product (Scheme 4.9). No  $\beta$ -glycoside of

Neu5Ac was isolated. The observed chemical shifts and coupling constants ( $\delta$  2.69 ppm,  $J_{3a,3c}$  12.0 Hz,  $J_{3c,4}$  4.5 Hz,  $\delta$  4.92 ppm,  $J_{4',5'}$  10.0 Hz;  $\delta$  5.27 ppm,  $J_{7',8'}$  8.7 Hz) for H-3e, H-4', and H-7' in the Neu5Ac moiety are characteristic of  $\alpha$  sialoside linkage. Other  $^1\text{H}$ -NMR data (see experimental section) are consistent with the structure 124.



**Scheme 4.9** Synthesis of Neu5Ac $\alpha$ (2,3) $\beta$ Gal(1,4) $\beta$ GlcNAc Trisaccharide

### 4.3 Conclusion

In conclusion, our “active-latent” glycosylation strategy was successfully extended to the synthesis of NeuAc-containing glycosides. Selective activation of “active”  $\alpha$ -thiosialosides of Neu5Ac over “latent” para-nitrophenyl thioglycosyl acceptors in the presence of NIS/TfOH promoter was possible. The facile regio- and  $\alpha$ -stereo-selective glycosidation of Neu5Ac with several glycosyl acceptors was achieved using thiophilic promoter in propionitrile under kinetically controlled conditions. By using the “active-latent” thioglycosylation strategy, GM<sub>3</sub> trisaccharide and GM<sub>3</sub> related sialosides have been synthesized in good yield.

A striking merit of the sialosides prepared using the “active-latent” strategy was that the “latent” glycosides could be directly activated through the simple two-step conversion of the anomeric EWG (NO<sub>2</sub>) to EDG (NHAc) as discussed in the third chapter. The versatile chemistry used to make them is very useful in the building of more complex oligosaccharides and glycoconjugates. Furthermore, the versatile transformations of the *p*-nitrophenylthio groups into *p*-acrylamidophenylthio groups and the usefulness of the *p*-acrylamidophenylthio groups for the synthesis of glycopolymers can serve other applications. The latter topic will be elaborated in the next chapter.

## 4.4 Experimental

### **Para-Nitrophenyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosylate)-(2 $\rightarrow$ 6)-2,3,4-tri-O-benzoyl-1-thio- $\alpha$ -D-galactopyranoside 102**

To a solution of para-methoxyphenyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosid) onate **101** (58.5 mg, 95.4  $\mu$ mol), glycosyl acceptor **64** (50 mg, 79.5  $\mu$ mol) in dry propionitrile (3 mL) was added molecular sieves (4 Å, 100 mg). The mixture was stirred 4 hours at room temperature and then cooled to -60°C. N-Iodosuccinimide (43 mg, 191  $\mu$ mol) and trifluoromethanesulfonic acid (5.9  $\mu$ L, 66.8  $\mu$ mol) were then added. The solution was stirred for 45 min at -60°C. The progress of the reaction was monitored by TLC using a mixture of benzene/acetone (3:2, v/v) as eluent. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and filtered through a pad of Celite. The filtrate was successively washed with 10% aqueous thiosulfate, saturated aqueous sodium bicarbonate and brine. The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography using 3% ethanol in dichloromethane as eluent. Disaccharide **102** was obtained (44.8 mg) in 52% yield: m.p. 117.4-118.5 °C; [ $\alpha$ ]<sub>D</sub> +45.2° (c = 1.1, CHCl<sub>3</sub>); FAB-MS (glycerol) gave m/z (ion, relative intensity): 1086.2 ([M]<sup>+</sup>, 0.3 %),

948.3 ([M-SPhNO<sub>2</sub>]<sup>+</sup>, 2.4 %), 612.1 ([M-Neu5Ac]<sup>+</sup>, 2.1 %), 474.2 ([M-Gal]<sup>+</sup>, 11.9 %); IR (thin film): 3331, 3067, 2946, 2854, 1739, 1674, 1590, 1520, 1451, 1370, 1249, 1095, 1038, 908, 854, 754, 710 cm<sup>-1</sup>; <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 8.16-7.19 (m, 19H, aromatic H), 6.06 (d, 1H, J<sub>4,5</sub> < 1.0 Hz, H-4), 5.80 (d, 1H, J<sub>3,4</sub> = 3.2 Hz, H-3), 5.7 (dd, 1H, J<sub>2,3</sub> = 10.7 Hz, H-2), 5.55 (ddd, 1H, J<sub>8',9a'</sub> = 2.6, J<sub>8',9b'</sub> = 4.9 Hz, H-8'), 5.41 (d, 1H, J<sub>1,2</sub> = 9.7 Hz, H-1), 5.25 (dd, 1H, J<sub>7',8'</sub> = 9.4 Hz, H-7'), 5.14 (d, 1H, J = 9.7 Hz, NH), 4.80 (ddd, 1H, J<sub>4',5'</sub> = 10.2 Hz, H-4'), 4.50 (dd, 1H, J<sub>5',6a</sub> = 6.1, J<sub>5',6b</sub> = 8.4 Hz, H-5), 4.44 (dd, 1H, J<sub>9a',9b</sub> = 12.3 Hz, H-9a), 4.12 (dd, 1H, J<sub>6',7'</sub> = 1.9 Hz, H-6'), 4.07 (dd, 1H, J<sub>5',6'</sub> = 10.6 Hz, H-5'), 4.04 (dd, 1H, H-9b), 3.85 (dd, 1H, J<sub>6a,6b</sub> = 11.0 Hz, H-6a), 3.68 (dd, 1H, H-6b), 3.57 (s, 3H, OMe), 2.46 (dd, 1H, J<sub>3a,3c</sub> = 12.9, J<sub>3c,4'</sub> = 4.6 Hz, H-3c), 1.96 (dd, 1H, J<sub>3a,4'</sub> = 11.0 Hz, H-3a), 2.24, 2.17, 1.99, 1.91, 1.87 (5s, 5x Ac); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 171.0, 170.9, 170.8, 170.2, 170.1, 167.8, 165.3, 165.2, 165.1 (C=O), 146.64 (C-para), 142.6 (C-ipso), 133.5-123.8 (aromatic C), 99.5 (C-2'), 83.9 (C-1), 75.8 (C-5), 72.8 (C-6'), 72.6 (C-3), 68.6 (C-4'), 67.9 (C-2, C-4), 67.8 (C-8'), 67.3 (C-7'), 63.4 (C-9'), 63.0 (C-6), 52.9 (OCH<sub>3</sub>), 49.3 (C-5'), 37.8 (C-3'), 23.2, 21.2, 20.8 (5xAc).

Anal. Calcd for C<sub>53</sub>H<sub>34</sub>N<sub>2</sub>O<sub>21</sub>S (1087.05): C, 58.56; H, 5.01; N, 2.58. Found: C, 58.15; H, 4.96; N, 2.42.

#### Para-Nitrophenyl O-(β-D-galactopyranosyl)-(1→4)-1-thio-β-D-glucopyranoside 106

Para-nitrophenyl O-(2,3,4,6-tera-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-2,3,6-tri-O-acetyl-1-thio-β-D-glucopyranoside 11 (15 g, 2.8 mmol) was suspended in 150 mL methanol to which was added 1.25 mL of sodium methoxide (1M). The reaction mixture was stirred at room temperature for 1.5 hours, after which time solution turned clear. TLC (methanol/dichloromethane 3:5) showed that a clean and complete conversion of the starting material (R<sub>f</sub> = 0.95) to product 106 (R<sub>f</sub> = 0.15) had occurred. The reaction mixture was concentrated under reduced pressure to 50 mL. Crystalline product precipitated out as fine needles, which were filtered to provide 106 as a first crop product (6.7 g). The filtrate was concentrated and a second crop of product (1.8 g) was obtained. Total 8.5 g of 106 was obtained in quantitative yield: m.p. 222-224 °C; [α]<sub>D</sub> -56.0° (c =

1.0, DMSO); IR (Thin film,  $\nu_{\text{cm}^{-1}}$ ): 3318, 2907, 1595, 1579, 1511, 1459, 1376, 1342, 1131, 1079, 854, 739; FAB-MS (glycerol) gave  $m/z$  (ion, relative intensity): 480.3 ( $[M+1]^+$ , 3.6%), 325.1 ( $[M+1-\text{HSPhNO}_2]^+$  6.7%);  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )  $\delta$  (ppm): 8.20 (d, 2H,  $J_{\text{o,m}} = 9.1$  Hz, H-meta), 7.66 (d, 1H, H-ortho), 5.10 (d, 1H,  $J_{1,2} = 9.9$  Hz, H-1), 4.47 (d, 1H,  $J_{1',2'} = 7.5$  Hz, H-1'), 4.38-3.35 (m, 12H, H-2-H-6b, H-2'-H-6b');  $^{13}\text{C-NMR}$  (DMSO)  $\delta$  (ppm): 145.7 (C-ipso), 143.2 (C-para), 126.8 (C-meta), 124.6 (C-ortho), 103.8 (C-1'), 84.6 (C-1), 79.9 (C-4), 78.9 (C-3), 76.3 (C-5), 75.6 (C-5'), 73.3 (C-2'), 72.3 (C-3'), 70.6 (C-2), 68.2 (C-4'), 64.5 (C-6), 60.2(C-6').

Anal. Calcd for  $\text{C}_{18}\text{H}_{25}\text{O}_{15}\text{N}_1\text{S}_1$  (479.45): C, 45.08; H, 5.26; N, 2.92. Found: C, 44.75, H, 5.20; N, 2.60.

#### **Para-nitrophenyl O-(3,4-O-isopropylidene- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1-thio- $\beta$ -D-glucopyranoside 107**

Para-nitrophenyl thiolactopyranoside **106** (2.0 g, 4.17 mmol) was suspended in 2,2-dimethoxypropane (100 mL). A catalytic amount of *p*-toluenesulfonic acid was added and the reaction mixture was then heated under reflux for 15 minutes. TLC (chloroform-methanol 8:1) showed two products ( $R_f = 0.72$ ,  $R_f = 0.61$ ) and no starting material. Triethylamine was added until the pH becomes lightly basic. Evaporation of the solution gave a residue which was dissolved in methanol-water 10:1 (100 mL) and boiled under reflux until TLC showed complete disappearance of the compound of  $R_f = 0.72$  (2 hours). The mixture was concentrated and toluene (3x20 mL) was evaporated from the residue. The crude product was purified by silica gel chromatography (5% methanol/dichloromethane) affording **107** (1.73 g) in 80% yield: m.p. 175-176 °C;  $[\alpha]_{\text{D}} -32^\circ$  ( $c = 1.0$ , DMSO); IR (Thin film,  $\nu_{\text{cm}^{-1}}$ ): 3395 (br, OH), 2909, 1580, 1516, 1341, 1220, 1063, 853, 743; FAB-MS (glycerol) gave  $m/z$  (ion, relative intensity): 519.5 ( $[M+1]^+$ , 1.8 %);  $^1\text{H-NMR}$  (500 MHz, acetone- $d_6$ )  $\delta$  (ppm): 8.13-.731 (m, 14 H, Aromatic H), 8.14 (d, 2H,  $J_{\text{o,m}} = 9.0$  Hz, H-meta), 7.69 (d, 2H, H-ortho), 5.04 (d, 1H,  $J_{1,2} = 9.9$  Hz, H-1), 4.44 (d, 1H,  $J_{1',2'} = 8.2$  Hz, H-1'), 4.26-3.21 (m, 12 H, H-2 - H-6, H-2' - H-6');  $^{13}\text{C-NMR}$  (125.7 MHz, acetone- $d_6$ )  $\delta$  (ppm): 146.7 (C-para of  $\text{SPhNO}_2$ ), 146.4 (C-ipso of

SPhNO<sub>2</sub>), 129.5 (C-meta of SPhNO<sub>2</sub>), 124.5 (ortho of SPhNO<sub>2</sub>), 110.1 (O-C-O), 104.3 (C-1'), 86.2 (C-1), 81.8, 80.7, 80.6, 80.0, 75.0, 74.5, 74.1, 74.0, 62.3 (C-6'), 63.8 (C-6), 28.4, 26.5 (2xCH<sub>3</sub>); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>) δ (ppm): 146.8 (C-para of SPhNO<sub>2</sub>), 146.1 (C-ipso of SPhNO<sub>2</sub>), 128.0 (C-meta of SPhNO<sub>2</sub>), 124.9 (ortho of SPhNO<sub>2</sub>), 109.8 (O-C-O), 103.8 (C-1'), 85.7 (C-1), 80.8, 80.4, 79.9, 77.3, 74.5, 74.3, 73.6, 73.4, 61.6, 61.0, 29.3, 27.4 (2xCH<sub>3</sub>).

Anal. Calcd for C<sub>21</sub>H<sub>29</sub>O<sub>12</sub>NS·CH<sub>3</sub>OH (549.53): C, 47.91; H, 6.03; N, 2.54, S, 5.81. Found: C, 47.90; H, 5.68; N, 2.46; S, 5.81.

**Para-nitrophenyl O-(2,6-di-O-benzoyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-1-thio-β-D-glucopyranoside 112**

3', 4'-O-Isopropylidene-β-lactoside **107** (500 mg, 0.481 mmol) was dissolved in 10 mL of dry pyridine, the solution was cooled to 0°C and benzoyl chloride (844 μL) was added. The mixture was then allowed to reach room temperature and was stirred overnight. TLC (1:9, CH<sub>3</sub>OH-CH<sub>2</sub>Cl<sub>2</sub>) showed complete conversion of the starting material **107** (R<sub>f</sub> = 0.08) to product **112** (R<sub>f</sub> = 0.68). The reaction mixture was poured onto ice water and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x20 mL). The combined extracts were washed successively with saturated sodium bicarbonate, saturated sodium chloride and water. The solution was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to a foam that was chromatographed on silica gel (3:5, ethyl acetate/hexane, 5% ethanol in dichloromethane) to afford lactoside **112** (647 mg) in 92 % yield: m.p. 254-255 °C; [α]<sub>D</sub> +4.7° (c = 1.0, CHCl<sub>3</sub>); IR (Thin film, ν<sub>cm<sup>-1</sup></sub>): 3055, 2931, 1726, 1719, 1599, 1549, 1515, 1450, 1342, 1265, 1093, 842, 708; FAB-MS (glycerol) gave m/z (ion, relative intensity): 1040 ([M+1]<sup>+</sup>, 0.2%), 885.3 ([M+1-HSPhNO<sub>2</sub>]<sup>+</sup>, 1.9%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 8.05-7.35 (m, 29H, aromatic H), 5.76 (dd, 1H, J<sub>3,4</sub> = 8.8 Hz, H-3), 5.40 (dd, 1H, J<sub>2,3</sub> = 9.7 Hz, H-2), 5.13 (dd, 1H, J<sub>2',3'</sub> = 6.8 Hz, H-2'), 4.96 (d, 1H, J<sub>1,2</sub> = 9.7 Hz, H-1), 4.68 (dd, 1H, J<sub>5,6a</sub> = 1.6, J<sub>6a,6b</sub> = 11.8 Hz, H-6a), 4.61 (d, 1H, J<sub>1',2'</sub> = 7.7 Hz, H-1'), 4.46 (dd, 1H, J<sub>5,6b</sub> = 5.2 Hz, H-6b), 4.29-3.92 (m, 5H, H-3', H-4, H-4', H5', H-6a'), 3.88 (ddd, 1H, H-5), 3.73 (dd, 1H, J<sub>5',6b'</sub> = 7.1, J<sub>6a',6b'</sub>

= 11.2 Hz, H-6b'); <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm): 166.0, 165.7, 165.6, 165.1, 164.9 (5x C=O), 146.7 (C-para of SPhNO<sub>2</sub>), 142.2 (C-ipso of SPhNO<sub>2</sub>), 133.69-123.7 (24C, aromatic C), 110.9 (O-C-O), 100.9 (C-1'), 84.1 (C-1), 77.4 (C-5), 77.0 (C-4), 75.3 (C-3), 73.6 (C-2'), 73.4 (C-5'), 73.1 (C-3'), 71.4 (C-2), 70.2 (C-4'), 62.8 (C-6), 61.6 (C-6'), 27.6, 26.3 (2xMe).

**Para-nitrophenyl O-(2,6-di-O-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-1-thio-β-D glucopyranoside 114**

To a solution of lactoside **112** (500 mg, 0.481 mmol) dissolved in 15 mL of a 1:1 mixture of CH<sub>2</sub>Cl<sub>2</sub> and CH<sub>3</sub>OH was added p-toluenesulfonic acid (100 mg). The mixture was stirred at 40°C for 2 hours. TLC (5% t-BuOH in CH<sub>2</sub>Cl<sub>2</sub>, v/v) showed complete conversion of **112** (R<sub>f</sub> = 0.51) into lactoside **114** (R<sub>f</sub> = 0.45). The reaction mixture was neutralized with triethylamine and concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford **114** (440 mg) in 92% yield: m.p. 178-180 °C; [α]<sub>D</sub> +11.8° (c =1.0, CHCl<sub>3</sub>); IR (Thin film, ν<sub>cm<sup>-1</sup></sub>): 3446, 3066, 2932, 1728, 1741, 1600, 1582, 1517, 1451, 1341, 1316, 1270, 1178, 1097, 1028, 848, 742, 710; FAB-MS (glycerol) gave m/z (ion, relative intensity): 1000.3 ([M+1]<sup>+</sup>, 0.3%), 845.3 ([M+1-HSPhNO<sub>2</sub>]<sup>+</sup>, 3.9%), 724.3 ([845.3-PhCO<sub>2</sub>H]<sup>+</sup>, 1.0%); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 8.01-7.27 (m, 29H, Aromatic H), 5.72 (dd, 1H, J<sub>3,4</sub> = 9.2 Hz, H-3), 5.41 (dd, 1H, J<sub>2,3</sub> = 9.7 Hz, H-2), 5.31 (dd, 1H, J<sub>2',3'</sub> = 7.7 Hz, H-2'), 4.97 (d, 1H, J<sub>1,2</sub> = 9.9 Hz, H-1), 4.65 (dd, 1H, J<sub>6a,6b</sub> = 12.1 Hz, H-6a), 4.62 (d, 1H, J<sub>1',2'</sub> = 7.8 Hz, H-1'), 4.51 (dd, 1H, J<sub>5,6b</sub> = 5.9 Hz, H-6b), 4.05 (dd, 1H, J<sub>4,5</sub> = 9.8 Hz, H-4), 4.02 (dd, 1H, J<sub>6a',6b'</sub> = 11.3 Hz, H-6a'), 3.96 (dd, 1H, J<sub>5,6a</sub> = 1.8 Hz, H-5), 3.84 (dd, 1H, J<sub>4',5'</sub> < 1.0 Hz, H-4'), 3.73 (dd, 1H, J<sub>3',4'</sub> = 3.4 Hz, H-3'), 3.64 (dd, 1H, J<sub>5',6b'</sub> = 6.5 Hz, H-6b'), 3.65 (dd, 1H, J<sub>5',6a'</sub> = 6.4 Hz, H-5'); <sup>13</sup>C-NMR (125.8 MHz, CDCl<sub>3</sub>) δ (ppm): 166.4, 166.1, 165.7, 165.7, 165.2, (5x C=O), 146.8 (C-para of SPhNO<sub>2</sub>), 141.66 (C-ipso of SPhNO<sub>2</sub>), 133.7-123.7 (24C, aromatic C), 100.9 (C-1'), 84.1 (C-1), 77.4 (C-5), 76.1 (C-4), 73.8 (C-3), 73.7 (C-2'), 72.7 (C-5'), 72.6 (C-3'), 70.1 (C-2), 68.5 (C-4), 62.7 (C-6), 61.9 (C-6').

Anal. Calcd for C<sub>53</sub>H<sub>45</sub>O<sub>17</sub>N<sub>1</sub>S<sub>1</sub>: C, 63.66 ; H, 4.54; N, 1.40. Found: C,63.11; H, 4.68; N, 1.30.

### Regioselective Benzoylation

#### Method A

The 3',4'-O-isopropylidene β-D-lactoside derivative **107** (1.39 g, 2.68 mmol) was dissolved in 4:1 mixture (16 mL) of pyridine and CH<sub>2</sub>Cl<sub>2</sub>. The solution was cooled to -50 °C. To this cooled solution was slowly added 1.41 mL (4.5 equiv.) of benzoyl chloride dissolved in 9 mL CH<sub>2</sub>Cl<sub>2</sub>. The reaction mixture was kept stirring at -50°C for 1 hour. TLC (5:4 ethyl acetate/hexane) indicated that no starting material was left. Work up was done as standard procedure. The benzoylated products were separated by silica gel chromatography using 3:7 ethyl acetate/hexane and 3% ethanol in methylene chloride respectively. Five benzoylated products were separated as: 2, 2', 6, 6'-tetra-O-benzoylated lactoside **98** (456 mg) in 19 % yield, 6,6'-di-O-benzoylated lactoside **108** (325 mg) in 17 % yield, 2, 6, 6'-tri-O-benzoylated lactoside **109** (675 mg) in 31 % yield, 3, 6, 6'-tri-O-benzoylated lactoside **110** (173 mg) in 8 % yield, 2', 6, 6'-tri-O-benzoylated lactoside **111** (251 mg) in 12 % yield

#### Method B

The 3',4'-O-isopropylidene β-D-lactoside derivative **107** (900 mg, 1.73 mmol) was dissolved in 50 mL of dry toluene and 2.85 mL of (Bu<sub>3</sub>Sn)<sub>2</sub>O was added. The reaction mixture heated under reflux with stirring for 23 hours. The reaction mixture was cooled to room temperature. Benzoyl chloride (1.2 mL, 10.38 mmol, 6 eq) and pyridine (5 mL) were added and the reaction temperature was raised to 45°C with stirring for an additional 24 hours. TLC (8% acetone/chloroform) showed that the starting material (*R<sub>f</sub>* = 0.06) was completely consumed and compound **109** (*R<sub>f</sub>* = 0.34) was obtained as a major product. After usual work-up, the crude mixture of products was chromatographed on silica gel (1:12 acetone/chloroform) to afford compound **109** (1.07 g), **98** (155 mg), and **108** (38 mg) in 74 %, 6 %, and 3 % yield respectively.

**Para-Nitrophenyl O-(2,6-di-O-benzoyl-3,4-O-isopropylidene- $\beta$ -D-galactopyranosyl)-  
(1 $\rightarrow$ 4)-2,6-di-O-benzoyl-1-thio- $\beta$ -D-glucopyranoside 98**

Compound 98 had: m.p. 233-234 °C;  $[\alpha]_D = -14.1^\circ$  (c = 1.0, CHCl<sub>3</sub>); IR (Thin film,  $\nu_{\text{cm}^{-1}}$ ): 3457, 3067, 2987, 1725, 1600, 1516, 1451, 1341, 1268, 1110, 1070, 853, 709; FAB-MS (glycerol) gave m/e (ion, relative intensity): 936.3 ([M+1]<sup>+</sup>, 1.8%), 781.3 ([M+1-HSPhNO<sub>2</sub>]<sup>+</sup>, 3.3%); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 8.09-7.23 (m, 24 H, Aromatic H), 5.35 (dd, 1H,  $J_{2',3'} = 7.8$  Hz, H-2'), 5.19 (dd, 1H,  $J_{2,3} = 9.2$  Hz, H-2), 4.87 (d, 1H,  $J_{1,2} = 10.0$  Hz, H-1), 4.85 (dd, 1H,  $J_{5',6a'} = 2.5$ ,  $J_{6a',6b'} = 12.5$  Hz, H-6a'), 4.65 (d, 1H,  $J_{1',2'} = 8.2$  Hz, H-1'), 4.43 (dd, 1H,  $J_{5,6a} = 1.8$ ,  $J_{6a,6b} = 11.9$  Hz, H-6a), 4.42 (m, 1H, H-6b'), 4.41 (dd, 1H,  $J_{3',4'} = 5.3$  Hz, H-3'), 4.29-4.25 (m, 2H, H-4', H-5'), 4.22 (dd, 1H,  $J_{5,6b} = 5.3$  Hz, H-6b), 4.02 (dd, 1H,  $J_{3,4} = 8.4$  Hz, H-3), 3.80 (ddd, 1H,  $J_{5,6b} = 5.4$  Hz, H-5), 3.65 (dd, 1H,  $J_{4,5} = 9.8$  Hz, H-4); <sup>13</sup>C-NMR (125.7 MHz)  $\delta$  (CDCl<sub>3</sub>): 165.5, 165.4, 165.3, (3x C=O), 146.7 (C-para of SPhNO<sub>2</sub>), 141.7 (C-ipso of SPhNO<sub>2</sub>), 133.7- 123.55) (30C, aromatic), 111.4 (O-C-O), 101.6 (C-1'), 83.9 (C-1), 82.1 (C-4), 76.2 (C-3'), 74.7 (C-5), 73.4 (C-3), 73.1 (C-2'), 72.3 (C-4', C-5'), 71.2 (C-2), 63.6 (C-6'), 62.5 (C-6), 27.6, 26.7 (2xCH<sub>3</sub>).

**Para-Nitrophenyl O-(6-O-benzoyl-3,4-O-isopropylidene- $\beta$ -D-galactopyranosyl)-  
(1 $\rightarrow$ 4)-6-O-benzoyl-1-thio- $\beta$ -D-glucopyranoside 108**

Compound 108 had m.p. 157-158 °C;  $[\alpha]_D -33.7^\circ$  (c = 0.86, CHCl<sub>3</sub>); IR (Thin film,  $\nu_{\text{cm}^{-1}}$ ): 3444 (br, OH), 3068, 2987, 2919, 1717, 1598, 1600, 1581, 1515, 1451, 1340, 1275, 1165, 1113, 1072, 1032, 853, 754, 713; FAB-MS (glycerol) gave m/z (ion, relative intensity): 728.27 ([M+1]<sup>+</sup>, 4.8 %), 573.27 ([M+1-HSPhNO<sub>2</sub>]<sup>+</sup>, 6.1 %); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 8.13-7.31 (m, 14 H, aromatic H), 4.98 (dd, 1H,  $J_{5,6a} = 0.8$ ,  $J_{6a',6b'} = 12.7$  Hz, H-6a'), 4.78 (d, 1H,  $J_{1,2} = 9.7$  Hz, H-1), 4.74 (dd, 1H,  $J_{5,6b} = 2.8$  Hz, H-6a), 4.34 (d, 1H,  $J_{1',2'} = 8.4$  Hz, H-1'), 4.56-3.45 (m, 8H, H-2 - H-6, H-2' - H-6'), 1.57, 1.38 (s, 6H, 2XCH<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 166.5, 166.4 (CO, Bz), 146.3 (C-para of SPhNO<sub>2</sub>),

143.1 (C-ipso of SPhNO<sub>2</sub>), 134.8-123.8 (16C, aromatic C), 111.0 (O-C-O), 103.3 (C-1'), 85.3 (C-1), 80.6 (C-4), 78.9 (C-5), 77.3 (C-3), 77.1 (C-5'), 76.6 (C-3'), 73.2 (C-2), 72.9 (C-2'), 72.0 (C-4'), 71.9 (C-6'), 63.8 (C-6), 28.1, 26.2 (2xCH<sub>3</sub>).

**Para-Nitrophenyl O-(6-O-benzoyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-  
(1→4)-2,6-di-O-benzoyl-1-thio-β-D-glucopyranoside 109**

Compound **109** had m.p. 234-235 °C;  $[\alpha]_D +4.3^\circ$ ,  $[\alpha]_{Hg} -34.0^\circ$  (c = 1.0, CHCl<sub>3</sub>); IR (Thin film,  $\nu_{cm^{-1}}$ ): 3400, 2932, 1721, 1509, 1516, 1451, 1341, 1270, 1113, 1071, 853, 711; FAB-MS (glycerol) gave m/z (ion, relative intensity): 832.30 ([M+1]<sup>+</sup>, 4.6 %), 677.21 ([M+1-HSPhNO<sub>2</sub>]<sup>+</sup>, 10.4 %); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 7.88-7.23 (m, 19H, Aromatic H), 5.26 (dd, 1H,  $J_{2,3} = 9.5$  Hz, H-2), 4.99 (dd, 1H,  $J_{5,6a} = 1.5$ ,  $J_{6a,6b} = 12.1$  Hz, H-6a), 4.95 (d, 1H,  $J_{1,2} = 10.1$  Hz, H-1), 4.78 (dd, 1H,  $J_{5',6a'} = 2.4$ ,  $J_{6a',6b'} = 12.2$  Hz, H-6a'), 4.44 (dd, 1H,  $J_{5,6b} = 6.3$  Hz, H-6b), 4.37 (dd, 1H,  $J_{5',6b'} = 6.3$  Hz, H-6b'), 4.33 (d, 1H,  $J_{1',2'} = 8.3$  Hz, H-1'), 4.31-4.15 (m 2H, H-4', H-5'), 4.14 (dd, 1H,  $J_{3',4'} = 5.4$  Hz, H-3'), 3.99 (dd, 1H,  $J_{3,4} = 8.5$  Hz, H-3), 3.87 (ddd, 1H,  $J_{4,5} = 9.9$  Hz, H-5), 3.95 (dd, 1H,  $J_{2',3'} = 7.5$  Hz, H-2'), 3.58 (dd, 1H,  $J_{4,5} = 9.9$  Hz, H-4); <sup>13</sup>C-NMR (125.7 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 167.2 (2C), 166.0 (3x C=O), 147.3 (C-para of SPhNO<sub>2</sub>), 143.2 (C-ipso of SPhNO<sub>2</sub>), 134.6 - 124.3 (24C, aromatic C), 111.6 (O-C-O), 104.3 (C-1'), 84.8 (C-1), 82.9 (C-4), 79.7 (C-3'), 78.2 (C-5), 75.6 (C-3), 74.0 (C-5'), 74.0 (C-2'), 72.9 (C-4'), 71.9 (C-2), 64.5 (C-6), 64.4 (C-6'), 28.8, 26.9 (2xCH<sub>3</sub>).

Anal. Calcd for C<sub>42</sub>H<sub>41</sub>O<sub>15</sub>N<sub>1</sub>S<sub>1</sub> (831.85): C, 60.64; H, 4.97; N, 1.68; S, 3.86. Found: C, 60.88; H, 5.37; N, 1.54; S, 4.02.

**Para-Nitrophenyl O-(6-O-benzoyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-  
(1→4)-3,6-di-O-benzoyl-1-thio-β-D-glucopyranoside 110**

Compound **110** had m.p. 189.3-191.2 °C;  $[\alpha]_D -54.9^\circ$  (c = 0.7, CHCl<sub>3</sub>); IR (Thin film,  $\nu_{cm^{-1}}$ ): 3454 (br, OH), 3067, 2986, 2929, 1718 (C=O), 1599, 1581 (NO<sub>2</sub>), 1452 (NO<sub>2</sub>), 1341, 1273, 1117, 1070, 853, 712; FAB-MS (glycerol) gave m/z (ion, relative

intensity): 832.23 ([M+1]<sup>+</sup>, 11.0 %), 677.20 ([M+1-HSPhNO<sub>2</sub>]<sup>+</sup>, 12.9 %); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 8.04-7.24 (m, 19H, aromatic H), 5.44 (dd, 1H, J<sub>3,4</sub> = 8.9 Hz, H-3), 4.92 (d, 1H, J<sub>1,2</sub> = 10.0 Hz, H-1), 4.92 (dd, 1H, J<sub>5,6a</sub> < 1 Hz, H-6a), 4.53 (dd, 1H, J<sub>5,6b</sub> = 5.3, H<sub>6a,6b</sub> = 11.9 Hz, H-6b), 4.30 (d, 1H, J<sub>1',2'</sub> = 8.0 Hz, H-1'), 4.18 (dd, 1H, J<sub>5',6a'</sub> = 4.5, J<sub>6a',6b'</sub> = 10.7 Hz, H-6a'), 4.13-4.03 (m, 3H, H-4, H-4', H-5), 4.02 (dd, 1H, J<sub>3',4'</sub> = 5.6 Hz, H-3'), 3.88 (dd, 1H, J<sub>5',6b'</sub> = 7.4 Hz, H-6b'), 3.84 (m, 1H, H-5'), 3.71 (dd, 1H, J<sub>2,3</sub> = 8.9 Hz, H-2), 3.50 (dd, 1H, J<sub>2',3'</sub> = 7.8 Hz, H-2'), 1.36, 1.24 (s, 6H, 2xCH<sub>3</sub>); <sup>13</sup>C-NMR (125.7 MHz, CDCl<sub>3</sub>) δ (ppm): 167.0, 166.0, 165.9, (3x C=O), 147.2 (C-para of SPhNO<sub>2</sub>), 142.5 (C-ipso of SPhNO<sub>2</sub>), 134.3 - 124.3 (24C, aromatic C), 110.4 (O-C-O), 102.1 (C-1'), 86.3 (C-1), 78.7 (C-4), 77.4, 77.1, 74.6, 73.5, 73.1, 71.2, 71.2, (C-2, C-3, C-5, C-2'-C-5'), 71.2 (C-2), 63.1 (C-6'), 63.0 (C-6), 27.9, 26.2 (2xCH<sub>3</sub>).

**Para-Nitrophenyl O-(2,6-di-O-benzoyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-(1→4)-6-O-benzoyl-1-thio-β-D-glucopyranoside 111**

Compound 111 had m.p. 103.5-105.2 °C; [α]<sub>D</sub> -31.3° (c = 0.98, CHCl<sub>3</sub>); IR (Thin film, ν<sub>cm<sup>-1</sup></sub>): 3464 (br, OH), 3066, 2932, 1728, 1714, 1600, 1582, 1517, 1451, 1341, 1270, 1178, 1097, 848, 710; FAB-MS (glycerol) gave m/z (ion, relative intensity): 832.30 ([M+1]<sup>+</sup>, 1.0 %), 677.21 ([M+1-HSPhNO<sub>2</sub>]<sup>+</sup>, 1.4 %); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 8.10-7.24 (m, 19H, aromatic H), 5.33 (dd, 1H, J<sub>2',3'</sub> = 7.2 Hz, H-2'), 4.81 (dd, 1H, J<sub>5,6a</sub> = 3.1, J<sub>6a',6b'</sub> = 12.1 Hz, H-6a'), 4.70 (d, 1H, J<sub>1,2</sub> = 9.8 Hz, H-1), 4.63 (d, 1H, J<sub>1',2'</sub> = 7.9 Hz, H-1'), 4.54 (dd, 1H, J<sub>5',6b'</sub> = 8.5, H<sub>6a',6b'</sub> = 12.1 Hz, H-6b'), 4.41 (dd, 1H, J<sub>3',4'</sub> = 5.4 Hz, H-3'), 4.33 (dd, 1H, J<sub>5,6a</sub> = 2.0 Hz, H-6a), 4.32-4.28 (m, 2H, H-4', H-5'), 4.24 (dd, 1H, J<sub>5,6b</sub> = 6.2, J<sub>6a,6b</sub> = 11.9 Hz, H-6b), 3.72 (m, 1H, H-5'), 3.70 (dd, 1H, J<sub>3,4</sub> = 8.7 Hz, H-3), 3.46 (dd, 1H, J<sub>4,5</sub> = 9.6 Hz, H-4), 3.42 (dd, 1H, J<sub>2,3</sub> = 8.4 Hz, H-2), 1.62, 1.34 (s, 6H, 2xCH<sub>3</sub>); <sup>13</sup>C-NMR (125.7 MHz, CDCl<sub>3</sub>) δ (ppm): 166.3, 165.5, 165.3 (3x C=O), 146.4 (C-para of SPhNO<sub>2</sub>), 142.7 (C-ipso of SPhNO<sub>2</sub>), 133.5-123.6 (24C, aromatic C), 111.3 (O-C-O), 101.4 (C-1'), 85.2 (C-1), 81.2 (C-4), 76.8 (C-3'), 76.4 (C-5), 76.2 (C-3), 73.3 (C-5'), 72.9 (C-2'), 71.9 (C-2), 71.8 (C-4'), 63.6 (C-6'), 62.8 (C-6), 27.6, 26.2 (2xCH<sub>3</sub>).

**Para-nitrophenyl O-(6-O-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-6-O-benzoyl-1-thio- $\beta$ -D-glucopyranoside 113**

Compound 109 (1.0 g, 1.2 mmol) was suspended in 50 mL of methanol and a catalytic amount of p-TsOH (0.2 g) was added. The reaction mixture was stirred at 40 °C overnight, after which time TLC (CH<sub>2</sub>Cl<sub>2</sub>/EtOAc, 7/3) showed complete conversion of starting material 109 (R<sub>f</sub> = 0.88) to product 113 (R<sub>f</sub> = 0.24). The reaction mixture was cooled and neutralized with triethylamine and evaporated under reduced pressure. The residue was purified by silica gel chromatography to give crystalline compound 113 (893.2 mg) in 94% yield: m.p. 226.2-228.5 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> -34.3° (c = 1.0, CHCl<sub>3</sub>); IR (Thin film,  $\nu_{\text{cm}^{-1}}$ ): 3436, 2894, 1718, 1590, 1581, 1516, 1452, 1341, 1273, 1177, 1082, 850, 742, 721; FAB-MS (glycerol) gave m/z (ion, relative intensity): 1583.6 ([2xMe]<sup>+</sup>), 792.2 ([M+1]<sup>+</sup>, 7.7%), 637.2 ([M+1-HSPhNO<sub>2</sub>]<sup>+</sup>, 11.4 %); <sup>1</sup>H-NMR (500 MHz, Acetone-D<sub>6</sub>)  $\delta$  (ppm): 7.95-7.19 (m, 29H, aromatic H), 5.52 (d, 1H, J<sub>1,2</sub> = 10.2 Hz, H-1), 5.32 (dd, 1H, J<sub>2,3</sub> = 9.1 Hz, H-2), 4.98 (dd, 1H, J<sub>5,6a</sub> = 1.9, J<sub>6a,6b</sub> = 12.0 Hz, H-6a), 4.73 (dd, 1H, J<sub>5',6a'</sub> = 3.2, J<sub>6a',6b'</sub> = 11.9 Hz, H-6a'), 4.65 (d, 1H, J<sub>1',2'</sub> = 7.8 Hz, H-1'), 4.64 (dd, 1H, J<sub>5,6b</sub> = 7.1 Hz, H-6b), 4.39 (dd, 1H, J<sub>5',6b'</sub> = 6.5 Hz, H-6b'), 4.28 (dd, 1H, J<sub>3,4</sub> = 9.6 Hz, H-3), 4.16-4.11 (m, 2H, H-5, H-5'), 4.10 (dd, 1H, J<sub>4',5'</sub> < 1.0 Hz, H-4'), 3.95 (dd, 1H, J<sub>2',3'</sub> = 9.5 Hz, H-2'), 3.74 (dd, 1H, J<sub>4,5</sub> = 8.2 Hz, H-4), 3.66 (dd, 1H, J<sub>3',4'</sub> = 3.3 Hz, H-3'); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 7.90-7.19 (m, 29H, aromatic H), 5.23 (dd, 1H, J<sub>2,3</sub> = 9.1 Hz, H-2), 5.02 (d, 1H, J<sub>1,2</sub> = 10.1 Hz, H-1), 4.98 (dd, 1H, J<sub>5,6a</sub> < 1.0, J<sub>6a,6b</sub> = 10.8 Hz, H-6a), 4.66 (dd, 1H, J<sub>5',6a'</sub> = 3.4, J<sub>6a',6b'</sub> = 11.9 Hz, H-6a'), 4.39 (d, 1H, J<sub>1',2'</sub> = 7.8 Hz, H-1'), 4.43 - 4.36 (m, 2H, H-6b, H-6b'), 4.07 (dd, 1H, J<sub>3,4</sub> = 8.6 Hz, H-3), 4.03 (dd, 1H, J<sub>4',5'</sub> < 1.0 Hz, H-4'), 3.99 (t, 1H, H-5), 3.92 (dd, 1H, J<sub>2',3'</sub> = 10.0 Hz, H-2'), 3.88 (m, 1H, H-5'), 3.70 (dd, 1H, J<sub>3',4'</sub> = 2.7 Hz, H-3'), 3.62 (dd, 1H, J<sub>4,5</sub> = 8.2 Hz, H-4); <sup>13</sup>C-NMR (125.7 MHz, Acetone-d<sub>6</sub>)  $\delta$  (ppm): 166.8, 166.3, 165.8 (3x C=O), 146.9 (C-para of SPhNO<sub>2</sub>), 144.5 (C-ipso of SPhNO<sub>2</sub>), 134.1 - 124.3 (24C, aromatic C), 105.4 (C-1'), 83.8 (C-1), 82.1 (C-

4), 77.4 (C-5), 75.2 (C-3), 74.3 (2C, C-3', C-5'), 72.9 (C-4'), 71.7 (C-2'), 69.8 (C-2), 65.3 (C-6), 64.5 (C-6').

Anal. Calcd for  $C_{56}H_{49}O_{17}N_1S_1$  (791.78): C, 59.16; H, 4.71; N, 1.77; S, 4.05. Found: C, 58.85; H, 4.60; N, 1.67; S, 4.17.

**Para-Nitrophenyl O-(6-O-tert-butyl-diphenylsilyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-6-O-tert-butyl-dimethylsilyl-1-thio- $\beta$ -D-glucopyranoside 116**

Compound 106 (2.0 g, 4.18 mmol) was dissolved in 50 mL dry pyridine. The solution was cooled to 0°C. Tert-butyl-diphenylsilyl chloride (3.4 mL) was added dropwise over a period of 15 min. The reaction mixture was stirred at room temperature for 6 hours. TLC (MeOH/CH<sub>2</sub>Cl<sub>2</sub>, 1:10 v/v) indicated that the starting material was completely converted to product 116 (R<sub>f</sub> = 0.43). The reaction mixture was concentrated directly under reduced pressure. Column chromatography (MeOH/CH<sub>2</sub>CH<sub>2</sub>, 1:15 in v/v) of the residue on silica gel (150 g) gave the titled product (3.75 g) in 90% yield: m.p. 105.7-158.0 °C; [ $\alpha$ ]<sub>D</sub> -68.8° (c = 1.0, CHCl<sub>3</sub>); IR (Thin film,  $\nu_{cm^{-1}}$ ): 3416, 3064, 2928, 2856, 1585, 1517, 1458, 1340, 1112, 1078, 747, 704; FAB-MS (glycerol) gave m/z (ion, relative intensity): 956.23 ([M+1]<sup>+</sup>, 2.2 %), 801.38 ([M+1-HSPhNO<sub>2</sub>]<sup>+</sup>, 6.8 %); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 8.01-7.28 (m, 24 H, aromatic H), 4.74 (d, 1H, J<sub>1,2</sub> = 9.6 Hz, H-1), 4.50 (d, 1H, H-4'), 4.36 (d, 1H, J<sub>1',2'</sub> = 7.5 Hz, H-1'), 3.97-3.45 (m, 11 H, H-2-H-6, H-2', H-3', H-5', H-6'), 1.04 (s, 9H, t-BuSi), 1.03 (s, 9H, tBuSi); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 146.9 (C-para of SPhNO<sub>2</sub>), 144.0 (C-ipso of SPhNO<sub>2</sub>), 136.4-124.5 (28C, aromatic C), 103.7 (C-1'), 86.2 (C-1), 79.8, 76.6, 75.8, 74.4, 73.0, 72.0, 69.0 (C-6), 62.9 (C-6'), 27.4 (6C, 2xtBu), 20.0 (Si-C $\equiv$ ), 19.7 (Si-C $\equiv$ ).

Anal. Calcd for  $C_{50}H_{61}O_{12}NSSi_2$  (956.23): C, 62.28; H, 6.44; N, 1.47. Found: C, 62.80; H, 6.46; N, 1.72.

**Para-Nitrophenyl O-(4,6-O-benzylidene- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1-thio- $\beta$ -D-glucopyranoside 117**

Compound **106** (0.5 g 1.04 mmol) was suspended in 50 mL of dry acetonitrile and the reaction was heated to dissolve most of **106**.  $\alpha,\alpha$ -Dimethoxytoluene (0.5 mL) and a catalytic amount of *p*-toluenesulfonic acid were added at room temperature and the reaction mixture was then stirred for 15 min at 50°C. TLC (chloroform-methanol 3:1) showed complete conversion of the starting material. Triethylamine was added until the pH became lightly basic. The solution was concentrated under reduced pressure. The residue was purified by silica gel chromatography (6% methanol/dichloromethane) to afford **117** (0.55 g) in 93 % yield: m.p. 195-196 °C;  $[\alpha]_D -81.0^\circ$  ( $c = 1.0$ , DMSO); IR (KBr,  $\nu_{\text{cm}^{-1}}$ ): 3367 (br, OH), 1578, 1509, 1458, 1375, 1338, 1162, 1038, 855, 737;  $^1\text{H-NMR}$  (DMSO- $d_6$  with  $\text{D}_2\text{O}$ )  $\delta$  (ppm): 8.18 (d, 1H,  $J_{\text{om}} = 9.1$  Hz, H-meta), 7.63 (d, 1H, H-ortho), 7.50-7.39 (m, 7 H, aromatic H), 5.59 (s,  $\text{CH}(\text{OMe})_2$ ), 5.04 (d, 1H,  $J_{12} = 9.9$  Hz, H-1), 4.44 (d, 1H,  $J_{1,2'} = 7.6$  Hz, H-1'), 4.45-3.29 (m, 12 H, H-2 - H-6, H-2' - H-6');  $^{13}\text{C-NMR}$  (DMSO- $d_6$ )  $\delta$  (ppm): 146.7 (C-para of  $\text{SPhNO}_2$ ), 146.4 (C-ipso of  $\text{SPhNO}_2$ ), 129.5 (C-meta of  $\text{SPhNO}_2$ ), 124.5 (ortho of  $\text{SPhNO}_2$ ), 110.1 (O-C-O), 104.3 (C-1'), 86.2 (C-1), 81.8, 80.6, 80.6, 80.0, 75.0, 74.5, 74.1, 74.0, 62.3 (C-6'), 62.2 (C-6), 63.8 (C-6), 28.4, 26.5 (2x $\text{CH}_3$ );  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 146.3-123.9 (12C, aromatic C), 102.9 (O-C-O), 99.9 (C-1'), 84.587, 8.6, 78.3, 75.9, 75.5, 72.0, 71.5, 69.9, 68.4, 66.3, 59.9.

Anal. Calcd for  $\text{C}_{25}\text{H}_{29}\text{NO}_{12}\text{S}\cdot\text{CH}_3\text{OH}$  (599.61): C, 52.07; H, 5.55; N, 2.34; S, 5.34. Found: C, 52.02; H, 5.55; N, 2.34; S, 5.34.

**Para-Nitrophenyl O-(4,6-O-benzylidene- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-6-O-tert-butyltrimethylsilyl-1-thio- $\beta$ -D-glucopyranoside **118****

To a solution of compound **117** (139 mg, 0.203 mmol) in 10 mL of dry pyridine-methylene chloride (1:20) was added t-butyltrimethylsilyl chloride (165 mg, 2.10 mmol) at 0 °C. The reaction mixture was then allowed to reach room temperature and stirring was continued for 12 hours. TLC (8:1 methanol-chloroform) showed that the starting material **117** ( $R_f = 0.15$ ) was converted to product **118** ( $R_f = 0.46$ ). Work up was done as standard procedure and the product was purified by silica gel column chromatography (2:1 ethyl acetate-hexane). Compound **118** (114.8 mg) in 83 % yield: m.p. 103-105 °C;  $[\alpha]_D -62.0^\circ$

( $c = 1.0$ ,  $\text{CHCl}_3$ ); IR (KBr,  $\nu_{\text{cm}^{-1}}$ ): 3385, 2909, 1595, 1514, 1457, 1374, 1340, 1253, 1163, 1060, 902, 838, 777, 741; FAB-MS (glycerol) gave  $m/z$  (ion, relative intensity): 682.2 ( $[\text{M}+1]^+$ , 7.3%), 781.3 ( $[\text{M}+1\text{-HSPHNO}_2]^+$ , 11.3%);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.10 (d, 1H,  $J_{\text{o,m}} = 9.0$ , H-meta), 7.61 (d, 1H, H-ortho), 7.48-7.35 (m, 7H, aromatic H), 5.52 (s,  $\text{CH}(\text{OMe})_2$ ), 4.72 (d, 1H,  $J_{1,2} = 9.8$  Hz, H-1), 4.40 (d, 1H,  $J_{1',2'} = 7.7$  Hz, H-1'), 4.27 (dd, 1H,  $J_{5,6a} = 1.2$ ,  $J_{6a,6b} = 12.7$  Hz, H-6a), 4.19 (dd, 1H,  $J_{3',4'} = 2.7$  Hz, H-4'), 4.07 (dd, 1H,  $J_{5,6a} = 1.7$  Hz, H-6b), 3.96-3.44 (m, 12H, H-2', H-4', H-5', H-6', H-2 - H-5);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 146.5 (C-para of  $\text{SPhNO}_2$ ), 143.1 (C-ipso of  $\text{SPhNO}_2$ ), 137.2 - 123.8 (10C, aromatic), 103.3 (O-C-O), 101.3 (C-1'), 85.8 (C-1), 79.4, 79.1, 76.2, 75.0, 72.6, 72.0, 71.1, 68.9 (C-6'), 66.9, 62.1 (C-6), 25.8 (3C,  $\text{Si}^t\text{Bu}$ ), 18.3 ( $\text{SiC}\equiv$ ), -5.2, -5.3 ( $\text{SiCH}_3$ ).

Anal. Calcd for  $\text{C}_{31}\text{H}_{43}\text{NO}_{12}\text{Si}$  (681.83): C, 54.61; H, 6.36; N, 2.05; S, 4.70. Found: C, 54.06; H, 6.57; N, 2.02; S, 4.52.

**Para-Nitrophenyl O-( $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy-1-thio- $\beta$ -D-glucopyranoside 115**

To a methanolic solution (10 mL) containing peracetylated N-acetyllactosamine 10 (860 mg, 1.12 mmol) was added a solution of NaOMe until the pH of the reaction mixture became ca. 9. The de-O-acetylation was completed within 30 min. The solution was neutralized by adding  $\text{H}^+$  resin (IR 120) and filtered. The filtrate was concentrated under reduced pressure to a yellowish solid which was pure enough for the next step as indicated by  $^1\text{H-NMR}$ . An analytical sample could be recrystallised from ethanol giving fine needles. The Zemplén deacetylation reaction was quantitative: m.p. 211.2-214.3  $^\circ\text{C}$ ;  $[\alpha]_{\text{D}} -1.73^\circ$  ( $c = 1.0$ , DMSO); FAB-MS (glycerol) gave  $m/z$  (ion, relative intensity): 521.2 ( $[\text{M}+1]^+$ , 6.4%), 366.15 ( $[\text{M}+1\text{-HSPHNO}_2]^+$ , 3.4%),  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )  $\delta$  (ppm): 8.21 (d, 2H,  $J_{\text{o,m}} = 9.1$  Hz, H-meta), 7.62 (d, 2H, H-ortho), 5.18 (d, 1H,  $J_{1,2} = 10.0$  Hz, H-1), 4.51 (d, 1H,  $J_{1',2'} = 7.2$  Hz, H-1'), 4.08-3.52 (m, 12 H, H2-H6, H2'-H6');  $^{13}\text{C-NMR}$  ( $\text{D}_2\text{O}$ )  $\delta$  (ppm): 175.2 (C=O, NHAc), 146.7 (C-para of  $\text{SPhNO}_2$ ), 144.3 (C-ipso of  $\text{SPhNO}_2$ ),

129.5 (C-meta of SPNO<sub>2</sub>), 124.9 (C-ortho of SPhNO<sub>2</sub>), 103.7 (C-1'), 85.4 (C-1), 79.8 (C-5), 78.8 (C-3), 76.2 (C-4), 74.4 (C-3'), 73.3 (C-5'), 71.8 (C-2'), 69.4 (C-4'), 61.9 (C-6), 61.0 (C-6'), 54.6 (C-2).

**Para-Nitrophenyl O-(6-O-t-butyldiphenylsilyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-6-O-t-butyldiphenylsilyl-2-deoxy-1-thio-β-D-glucopyranoside 119**

Dry pyridine (2x5 mL) was evaporated from **115** (340 mg, 0.66 mmol) and the residue was dissolved in 5 mL of dry pyridine again. The solution was cooled to 0°C. Tert butyldiphenylsilyl chloride (269.6 mg, 0.91 mmol, 1.5 eq per OH) was added. The reaction mixture was warmed to room temperature and stirred for 8 hours. The reaction mixture was concentrated under reduced pressure. Column chromatography using 4% methanol-dichloromethane as eluent gave the titled compound **119** (586 mg) in 89% yield: m.p. 119-120 °C; [α]<sub>D</sub> -53.2° (c = 1.0, CHCl<sub>3</sub>); FAB-MS (glycerol) gave m/z (ion, relative intensity): 1995.28 ([2M+1]<sup>+</sup>, 1.3%), 997.38 ([M+1]<sup>+</sup>, 7.5%), 842.38 ([M+1-HSPhNO<sub>2</sub>]<sup>+</sup>, 17.0%); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 7.93 (d, 2H, J<sub>o,m</sub> = 8.7 Hz, H-ortho), 7.44 (d, 2H, H-meta), 7.70-7.25 (m, 20H, 4xPh), 5.01 (d, 1H, J<sub>1,2</sub> = 10.0 Hz, H-1), 4.43 (d, 1H, J<sub>1',2'</sub> = 7.7 Hz, H-1'), 4.00 -3.40 (m, 12 H, H2-H6, H2'-H6'), 1.92 (s, 3H, NHAc), 1.01 (s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>), 0.99 (s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>); <sup>13</sup>C-NMR (125.7 MHz, CDCl<sub>3</sub>) δ (ppm): 171.2 (C=O, NHAc), 146.0 (C-para of SPhNO<sub>2</sub>), 144.5 (C-ipso of SPhNO<sub>2</sub>), 135.7 - 123.8 (m, 28C, Aromatic), 103.2 (C-1'), 84.5 (C-1), 79.2 (C-4), 78.8 (C-5), 75.2 (C-5'), 73.7 (C-3'), 73.1 (C-3), 71.4 (C-2'), 68.3 (C-4'), 62.6 (C-6), 62.2 (C-6'), 55.3 (C-2).

**Para-Nitrophenyl O-(4,6-O-benzylidene-β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside 120**

To a suspension of lactosamine derivative **115** (260 mg, 0.5 mmol) in acetonitrile (3 mL) was added benzaldehyde dimethylacetal (225 μL, 1.5 mmol), and p-toluenesulfonic acid monohydrate (catalytic amount). The mixture was stirred for 25 min at room temperature. Triethylamine (150 μL) was added, and the reaction mixture was

concentrated under reduced pressure. The residue was then purified by column chromatography on silica gel (5% ethanol in dichloromethane) to give **120** (270 mg) in 89% yield: m.p. 196-197 °C;  $[\alpha]_D -14.3^\circ$  (c = 1.0, DMSO); FAB-MS (glycerol) gave m/z (ion, relative intensity): 1217.91 ( $[2M+1]^+$ , 1.3%), 609.50 ( $[M+1]^+$ , 22.8 %), 454.2( $[M+1-HSPhNO_2]^+$ , 8.9%);  $^1H$ -NMR (500 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 8.12 (d, 2H,  $J_{o,m} = 9.0$  Hz, H-ortho of SPhNO<sub>2</sub>), 7.96 (d, 1H,  $J_{m,p} = 9.2$  Hz, H-para of Ph), 7.59 (d, 2H, H-meta of SPhNO<sub>2</sub>), 7.45-7.43 (m, 2H, H-meta of Ph), 7.36-7.34 (m, 2H, H-ortho of SPh), 5.56 (s, 1H, O-PhCHO), 5.05(d, 1H,  $J_{1,2} = 10.4$  Hz, H-1), 4.43 (d, 1H,  $J_{1',2'} = 7.7$  Hz, H-1'), 4.09 (dd, 1H,  $J_{3',4'} = 3.4$ ,  $J_{4',5'} < 1.0$  Hz, H-4'), 4.08 (dd, 1H,  $J_{5',6a'} < 1.0$ ,  $H_{6a',6b'} = 11.4$  Hz, H-6a'), 4.03 (dd, 1H,  $J_{5',6b'} < 1.0$  Hz, H-6a'), 3.82-3.31 (m, 9H, H-2 - H-6, H2', H-3', H5'), 1.81(s, 3H, NHAc);  $^{13}C$ -NMR (125.7 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 169.4 (C=O, NHAc), 145.8 (C-para of SPhNO<sub>2</sub>), 145.0 (C-ipso of SPhNO<sub>2</sub>), 138.5 (C-ipso of SPh), 128.7 (C-para of SPh), 128.0 (C-ortho of SPh), 127.6 (C-meta of SPhNO<sub>2</sub>), 126.2 (C-meta of SPh), 123.8 (C-ortho of SPhNO<sub>2</sub>), 103.1 (C-1'), 99.8 (O-C-O), 83.9 (C-1), 79.1 (2C, C-5, C-3), 75.7 (C-4), 73.2 (C-3'), 71.7 (C-5'), 70.0 (C-2'), 68.4 (C-6), 66.4 (C-4'), 60.0 (C-6'), 53.6 (C-2).

Anal. Calcd for C<sub>27</sub>H<sub>32</sub>O<sub>12</sub>N<sub>2</sub>S<sub>1</sub>H<sub>2</sub>O (626.18): C, 51.74, H, 5.47; N, 4.47; S, 5.11. Found: C, 50.22; H, 5.46; N, 4.27; S, 4.89.

**Para-Nitrophenyl O-(4,6-O-benzylidene- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2-acetamido-6-O-t-butylidiphenylsilyl-2-deoxy-1-thio- $\beta$ -D-glucopyranoside **121****

Lactosamine derivative **120** (200 mg, 0.32 mmol) was dissolved in dry pyridine (2 mL). The solution was cooled to 0°C. Tert-butylidiphenylsilyl chloride (78.5  $\mu$ L, 0.30 mmol, 1.8 eq per OH ) was added. The reaction mixture was stirred at room temperature for 12 hours. The reaction mixture was concentrated under reduced pressure. Column chromatography using 4% methanol-methylene chloride as eluent gave the titled compound (252 mg) in 90% yield: m.p. 149-151 °C;  $[\alpha]_D -57.0^\circ$  (c = 1.2, CHCl<sub>3</sub>); FAB-MS (glycerol) gave m/z (ion, relative intensity): 1694.5 ( $[2M+1]^+$ , 0.7%), 847.3 ( $[M+1]^+$ , 9.0%), 692.3 ( $[M+1-HSPhNO_2]^+$ , 18.4%);  $^1H$ -NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 7.94 (d,

2H,  $J_{\text{om}} = 9.0$  Hz, H-ortho of SPhNO<sub>2</sub>), 7.43 (d, 2H, H-meta of SPhNO<sub>2</sub>), 7.68-7.11 (m, 15H, 3xPh), 5.42 (s, 1H, PhCH), 4.80 (d, 1H,  $J_{1,2} = 8.9$  Hz, H-1), 4.47 (d, 1H,  $J_{1,2'} = 8.0$  Hz, H-1'), 4.17-3.34 (m, 12H, H-2-H-6, H-2'-H-6'), 1.97 (s, 3H, NHAc), 1.02 (s, 9H, <sup>t</sup>BuSi); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 171.1 (C=O, NHAc), 146.1 (C-para of SPhNO<sub>2</sub>), 144.5 (C-ipso of SPhNO<sub>2</sub>), 137.3-123.8 (22C, Aromatic), 103.1 (O-C-O), 101.3 (C-1'), 84.7 (C-1), 79.3, 75.2, 73.5, 72.7, 71.0, 68.8, 67.0 (C-6), 62.6 (C-6'), 54.9 (C-2).

**Para-Nitrophenyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosylate)-(2 $\rightarrow$ 3)-O-(6-O-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,6-di-O-benzoyl-1-thio- $\beta$ -D-glucopyranoside 122**

To a solution of lactosyl acceptor **113** (100 mg, 0.126 mmol) and methyl (phenyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero- $\alpha$ -D-galacto-nonulopyranosid) onate **12** (110 mg, 0.189 mmol) in dry propionitrile (5 mL) was added molecular sieves-4Å (200 mg) and the mixture was stirred for 4 hours at room temperature. The mixture was then cooled to -60°C and N-iodosuccinimide (56 mg, 0.252 mmol) and trifluoromethanesulfonic acid (10.4  $\mu$ L, 0.126 mmol) were added. The reaction mixture stirred at -60°C under nitrogen atmosphere. The progress of the reaction was closely monitored by TLC at 5 min a interval. The reaction was essentially finished within 50 min with less than 10% of the acceptor unreacted as judged from the TLC. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (15 mL) and filtered through a layer of celite. The filtrate was washed with 10 % Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (15 mL), saturated NaHCO<sub>3</sub> (2x15 mL) and brine (2x15 mL). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography using ethyl ether/ethyl acetate (1:1) as eluent to afford trisaccharide **122** (75.2 mg) in 47%: m.p. 129.2-131.3 °C;  $[\alpha]_{\text{D}} -12.8^{\circ}$  (c = 0.8, CHCl<sub>3</sub>); IR: (Thin film,  $\nu_{\text{cm}^{-1}}$ ): 3448 (br), 3067, 2956, 2925, 1733, 1589, 1581, 1518, 1451, 1370, 1341, 1272, 1228, 1070, 852, 713; (+)FAB-MS (glycerol) gave m/z (ion, relative intensity): 1265 ([M]<sup>+</sup>, 2.7%), 1205.3 ([M-AcOH]<sup>+</sup>, 4.4%), 1111.3 ([M-HSPhNO<sub>2</sub>]<sup>+</sup>, 10.6 %), 740.3 ([M-525.1]<sup>+</sup>, 16.2%); <sup>1</sup>H-NMR

(500 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 8.06-7.28 (m, 19H, aromatic), 5.37 (ddd, 1H,  $J_{8''9a''} = 2.6$ ,  $J_{8''9b''} = 6.7$  Hz, H-8''), 5.29 (dd, 1H,  $J_{2,3} = 9.0$  Hz, H-2), 5.27 (dd, 1H,  $J_{7''8''} = 8.2$  Hz, H-7''), 5.10 (dd, 1H,  $J_{5,6a} = 1.9$ ,  $J_{6a,6b} = 12.1$  Hz, H-6a), 5.24 (d, 1H,  $J = 9.8$  Hz, NH), 5.00 (d, 1H,  $J_{1,2} = 10.1$  Hz, H-1), 4.96 (ddd, 1H,  $J_{3a''4''} = 11.9$ ,  $J_{3e''4''} = 4.6$ ,  $J_{4''5''} = 10.4$  Hz, H-4''), 4.74 (dd, 1H,  $J_{6a',6b'} = 12.1$  Hz, H-6a'), 4.59 (d, 1H,  $J_{1',2'} = 7.9$  Hz, H-1'), 4.49 (dd, 1H,  $J_{5,6b} = 6.9$  Hz, H-6b), 4.35 (dd, 1H,  $J_{5',6b'} = 8.9$  Hz, H-6b'), 4.30 (dd, 1H,  $J_{9a'',9b''} = 12.5$  Hz, H-9a''), 4.16 (dd,  $J_{3',4'} = 3.2$  Hz, H-3'), 4.12 (dd, 1H,  $J_{6'',7''} = 1.7$  Hz, H-6''), 4.02 (dd, 1H,  $J_{3,4} = 8.7$  Hz, H-3), 4.00-3.95 (m, 4H, H-5, H-3'', H-5'', H-9b''), 3.90 (dd, 1H,  $J_{5',6a'} = 3.3$  Hz, H-5'), 3.80 (d, 1H,  $J_{2',3'} = 9.7$  Hz, H-2'), 3.74 (dd, 1H,  $J_{4,5} = 9.7$  Hz, H-4), 3.70 (d, 1H, H-4'), 2.69 (dd, 1H,  $J_{3e''4''} = 4.6$ ,  $J_{3e''3a''} = 12.9$  Hz, H-3e), 2.13, 2.06, 2.02, 1.95, 1.88 (5s, 5x Ac); <sup>13</sup>C-NMR (125.8 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 170.8, 170.5, 170.3, 170.2, 170.1 (OCOCH<sub>3</sub>, NCOCH<sub>3</sub>) 168.07 (C-1'',  $J_{C-1''H-3a''} = 4.2$  Hz), 166.6, 165.9, 165.3 (PhCO), 146.5 (C-para), 142.7 (C-ipso), 133.5-123.6 (Aromatic C), 104.4 (C-1'), 97.5 (C-2''), 84.1 (C-1), 82.0 (C-4), 76.9 (C-5), 76.5 (C-3'), 74.8 (C-3), 73.1, 73.0 (C-5', C-6''), 71.4 (C-2), 68.8 (C-8''), 68.7 (C-2'), 68.1, 68.1 (C-4', C-4''), 67.0 (C-7''), 63.6, 63.5 (C-6, C-6'), 62.4 (C-9''), 53.3 (OCH<sub>3</sub>), 49.7 (C-5''), 37.8 (C-3''), 23.1 (NCOCH<sub>3</sub>), 21.1, 20.8, 20.7, 20.6 (OCOCH<sub>3</sub>).

Anal. Calcd for C<sub>59</sub>H<sub>64</sub>O<sub>27</sub>N<sub>2</sub>S (1265.21): C, 56.01; H, 5.10; N, 2.21. Found: C, 55.78; H, 5.33; N, 2.19.

### Ethyl 2,3,4-tri-O-benzyl-1-thio- $\beta$ -L-fucopyranoside **93**

The synthesis of **93** was performed following a slight modification of previously described one pot two-step procedure. L-fucopyranose (1.0 g, 61 mmol) was treated with acetic anhydride-pyridine (30 mL, 2:1) at 100°C for 1 hour. The solution was concentrated and coevaporated with toluene (2×10 mL). The product was dissolved in dichloromethane (25 mL), ground molecular sieves (2.5 g, 4 Å), ethanethiol (1 mL), and stannum (IV) chloride (0.9 mL) were added at room temperature. The mixture was stirred for 1 hour, filtered through a layer of Celite, washed with ice-cold 1 M sulfuric acid, aqueous sodium bicarbonate, and water. The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>) and

concentrated. The residue was dissolved in methanol (25 mL) containing catalytic amount of sodium methoxide and was stirred until TLC revealed one major compound having Rf 0.14 (chloroform-acetone, 2:1). The solution was added diluted acetic acid in methanol until pH = 7. The solution was then concentrated under reduced pressure and the residue was dissolved in N,N-dimethylformamide (15 mL) containing benzyl bromide (3.2 mL). Sodium hydride as an oil suspension (50 %) (880 mg) was added at 0°C under nitrogen. After 1 hour, methanol (2 mL) was added, and the mixture was partitioned between toluene and water. The organic layer was washed with 5% HCl, water and concentrated. The residue was purified by column chromatography (ethyl acetate-hexane, 6:1) to afford **93** (1.79 g) in 61 % yield: m.p. 53.2-54.8 °C; [ $\alpha$ ]<sub>D</sub> +13.5° (c = 1.0, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 7.40-7.23 (m, 15H, 3xBn), 4.38 (d, 1H, J<sub>1,2</sub> = 9.6 Hz, H-1), 3.82 (dd, 1H, J<sub>2,3</sub> = 9.4 Hz, H-2), 3.61 (dd, 1H, J<sub>4,5</sub> < 1.0 Hz, H-4), 3.56 (dd, 1H, J<sub>3,4</sub> = 3.4 Hz, H-3), 3.47 (dq, 1H, J<sub>5,6</sub> = 6.4 Hz, H-5), 2.73 (m, SCH<sub>2</sub>), 1.29 (t, J = 10.3 Hz, SCH<sub>2</sub>CH<sub>3</sub>), 1.20 (d, H-6); <sup>13</sup>C-NMR (125.8 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 138.7-127.5 (18C, 3xBn), 85.0 (C-1), 84.5 (C-3), 78.4 (C-2), 76.6 (C-4), 75.7 (CH<sub>2</sub>), 74.6 (2C, CH<sub>2</sub>, C-5), 72.9 (CH<sub>2</sub>), 24.6 (SCH<sub>2</sub>), 17.2 (C-6), 15.0 (SCH<sub>2</sub>CH<sub>3</sub>).

**Para-Nitrophenyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosylate)-(2 $\rightarrow$ 3)-O-6-O-benzoyl- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-[(2,3,4-tri-O-benzyl- $\alpha$ -L-fucopyranosyl)-(1 $\rightarrow$ 3)] -2,6-di-O-benzoyl-1-thio- $\beta$ -D-glucopyranoside **123****

To a solution of GM<sub>3</sub> trisaccharide **122** (30 mg, 22.7  $\mu$ mol) and ethyl 2,3,4-tri-O-benzyl-1-thio- $\beta$ -L-fucopyranoside **93** (13.5 mg, 28.5  $\mu$ mol) in dry dichloromethane (1.5 mL) was added 4 Å molecular sieves (40 mg). The mixture was stirred under nitrogen atmosphere for 2 hours at room temperature and then cooled to -70°C. To the cooled mixture was added N-iodosuccinimide (9.6 mg, 42.6  $\mu$ mol) and triflic acid (0.42  $\mu$ L, 4.5  $\mu$ mol) and the solution was stirred for 30 min at -70 °C. The reaction mixture was filtered through a Celite pad. The filtrate was diluted with dichloromethane (5 mL) and washed with water (5 mL), 10 % Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (6 mL), saturated NaHCO<sub>3</sub> (8 mL) and water (5 mL).

The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered and evaporated to a syrup that was chromatographed on silica gel using 1:6 acetone-dichloromethane as eluent to afford **93** (24 mg) in 63% yield:  $R_f = 0.4$  (1:3 acetone-dichloromethane);  $[\alpha]_D^{20}$  12.2° ( $c = 0.58$ ,  $\text{CHCl}_3$ ); (+)FAB-MS (glycerol) gave  $m/z$  (ion, relative intensity): 1681 ( $[\text{M}]^+$ , 0.3%), 1425 ( $[\text{M}-\text{HSPHNO}_2]^+$ , 4.3%);  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 8.07-7.26 (m, 34H, aromatic), 5.58 (d, 1H,  $J_{1,2} = 4.1$  Hz, H-1, Fuc), 5.46 (ddd, 1H,  $J_{8,9a} = 2.6$ ,  $J_{8,9b} = 6.9$  Hz, H-8, NeuAc), 5.32 (dd, 1H,  $J_{2,3} = 9.1$  Hz, H-2, Glu), 5.27 (dd, 1H,  $J_{7,8} = 8.9$  Hz, H-7, NeuAc), 5.18 (d, 1H,  $J = 9.9$  Hz, NH), 5.16 (dd, 1H,  $J_{5,6a} = 1.9$ ,  $J_{6a,6b} = 12.1$  Hz, H-6a, Glu), 5.02 (d, 1H,  $J_{1,2} = 10.1$  Hz, H-1, Glu), 4.91 (d, 1H,  $J = 11.6$  Hz,  $\text{CH}_2$ ), 4.87 (ddd, 1H,  $J_{4,6} = 9.8$  Hz, H-4, NeuAc), 4.84-4.68 (m, 4H,  $\text{CH}_2 \times 2$ ), 4.67 (d, 1H,  $J_{1,2} = 8.0$  Hz, H-1, Gal), 4.59 (d, 1H,  $J = 11.6$  Hz,  $\text{CH}_2$ ), 4.51 (dd, 1H,  $J_{5,6b} = 4.2$  Hz, H-6b, Gal), 4.48 (dd, 1H,  $J_{5,6b} = 7.0$  Hz, H-6b, Glu), 4.28-4.25 (m, 2H, H-6a of Gal, H-9b of NeuAc), 4.23 (dd, 1H,  $J_{3,4} = 2.4$  Hz, H-3, Gal), 4.17 (dd, 1H,  $J_{6,7} = 2.6$  Hz, H-6, NeuAc), 4.12-4.01 (m, 5H, H-2 of Gal, H-3, H-5 of Glu, H-2 of Fuc, H-9 of NeuAc), 3.95 (m, 1H, H-5, Gal), 3.93 (dd, 1H,  $J_{5,6} = 10.2$  Hz, H-5), 3.87 (dd, 1H,  $J_{2,3} = 10.4$ ,  $J_{3,4} = 2.8$  Hz, H-3, Fuc), 3.84 (d, 1H,  $J_{3,4} = 2.0$  Hz, H-4, Gal), 3.80 (s, 3H,  $\text{OCH}_3$ ), 3.79 (dq, 1H, H-5, Fuc), 3.76 (dd, 1H,  $J_{4,5} = 9.0$  Hz, H-4, Glu), 3.65 (d, 1H, H-4, Fuc), 2.54 (dd, 1H,  $J_{3e,4} = 4.4$ ,  $J_{3e,3a} = 12.7$  Hz, H-3e, NeuAc), 2.17, 2.08, 2.01, 1.98, 1.88 (5s, 15H,  $\text{OAc}$ ,  $\text{NHAc}$ ), 1.03 (d, 1H,  $J_{5,6} = 6.5$  Hz, H-6, Fuc);  $^{13}\text{C-NMR}$  (125.7 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 170.4, 170.1, 169.8 (2C), 169.8 (2C), 167.5, 165.4, 164.9 (9x $\text{C}=\text{O}$ ), 151.9-123.3 (42C, Aromatic), 104.7 (C-1, Gal), 96.9 (2C, C-2 of NeuAc, C-1 of Fuc), 83.7 (C-1, Glu), 81.8 (C-4, Glu), 78.4 (C-3, Fuc), 77.6 (C-3, Gal), 77.5 (C-3, Glu), 76.0 (C-4, Fuc), 75.4 (C-5, Glu), 74.4 ( $\text{CH}_2$ ), 74.3 (C-2, Gal), 72.9 (2C, C-6 of NeuAc, C-5 of Gal), 72.5, ( $\text{CH}_2$ ), 72.4 ( $\text{CH}_2$ ), 71.2 (C-2, Glu), 70.0 (C-4, Gal), 68.1 (C-2, Fuc), 67.7\* (C-8, NeuAc), 67.6\* (C-4, NeuAc), 66.8 (C-5, Fuc), 66.4 (C-7, NeuAc), 53.6 (C-6, Glu), 63.3 (C-6, Gal), 62.1 (C-9, NeuAc), 53.0 ( $\text{OCH}_3$ ), 49.1 (C-5, NeuAc), 37.5 (C-3, NeuAc), 22.8, 20.9, 20.4 (2C), 20.3 (5x $\text{CH}_3$ ,  $\text{NHAc}$ ,  $\text{OAc}$ ), 16.4 (C-6, Fuc).

\* Assignment may be interchangeable.

**Para-Nitrophenyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosylonate)-(2 $\rightarrow$ 3)-O-(6-O-tert-butylidiphenylsilyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-6-O-tert-butylidiphenylsilyl-2-acetamido-2-deoxyl-1-thio- $\beta$ -D-glucopyranoside 124**

To a solution of **93** (60 mg, 0.06 mmol) and methyl (phenyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero- $\alpha$ -D-galactononulopyranosid) onate **12** (60 mg, 0.103 mmol) in dry propionitrile (2 mL) was added 4 Å molecular sieves (120 mg). The mixture was stirred for 4 hours under nitrogen. The mixture was then cooled to -60 °C and N-iodosuccinimide (45 mg, 0.12 mmol) and trifluoromethanesulfonic acid (15.6  $\mu$ L, 0.06 mmol) were added. The reaction mixture stirring at -60 °C under nitrogen. The progress of the reaction was closely monitored by TLC at 5 min intervals. The reaction was essentially finished within 50 min with less than 15 % of the acceptor unreacted as judged from the TLC. The reaction mixture was then diluted with CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and filtered through a layer of celite. The filtrate was washed with 10 % Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (10 mL), saturated NaHCO<sub>3</sub> (2x10 mL), and brine (2x10 mL). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The foamy residue was purified by silica gel chromatography using 8% t-butanol in dichloromethane as eluent to afford trisaccharide **125** (43 mg) in 46% yield:  $[\alpha]_D$  -20.7° (c = 0.70, CHCl<sub>3</sub>); (+)FAB-MS (glycerol) gave m/z (ion, relative intensity): 1315.5 ([M+1-HSPhNO<sub>2</sub>]<sup>+</sup>, 1.6 %); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 7.84 (d, 2H, J<sub>o,m</sub> = 9.0 Hz, H-ortho), 7.61 (d, 2H, H-meta), 7.67-7.19 (m, 20H, aromatic), 5.57 (d, 1H, J<sub>2,NH</sub> = 8.3 Hz, NH), 5.33 (ddd, 1H, J<sub>8'',9a''</sub> = 2.5, J<sub>8'',9b''</sub> = 6.0 Hz, H-8''), 5.27 (dd, 1H, J<sub>7'',8''</sub> = 8.7 Hz, H-7''), 5.20 (d, 1H, J<sub>5'',NH</sub> = 9.8 Hz, NH), 5.02 (d, 1H, J<sub>1,2</sub> = 9.8 Hz, H-1), 4.92 (ddd, 1H, J<sub>3a'',4''</sub> = 11.9, J<sub>3e'',4''</sub> = 4.6, J<sub>4'',5''</sub> = 10.1 Hz, H-4''), 4.51 (d, 1H, J<sub>1',2'</sub> = 7.7 Hz, H-1'), 4.22 (dd, 1H, J<sub>9a'',9b''</sub> = 12.4 Hz, H-9a''), 4.16 (dd, 1H, J<sub>5,6a</sub> < 1.0, J<sub>6a,6b</sub> = 10.4 Hz, H-6a), 4.09 (dd, J<sub>3',4'</sub> = 3.6 Hz, H-3'), 4.07 (dd, 1H, J<sub>6'',7''</sub> = 1.8 Hz, H-6''), 3.96 (dd, J<sub>5'',6''</sub> = 10.1 Hz, H-5''), 3.94 (d, 1H, J<sub>5,6b</sub> = 6.0 Hz, H-6b), 3.92 (d, 1H, J<sub>8'',9b''</sub> = 6.0 Hz, H-9b''), 3.89 - 3.78 (m, , 4H, H-2, H-5, H-6a', H-6b'), 3.75 (d, 1H, J<sub>4',5'</sub> < 1.0 Hz, H-4'), 3.74 (s, 3H, OMe), 2.72-3.58 (m, 4H, H-3, H-4, H-2', H-5'), 2.69 (dd, 1H, J<sub>3e'',4''</sub> = 4.5, J<sub>3e'',3a''</sub> = 12.0 Hz, H-3e), 2.08, 2.02, 2.01, 1.94,

1.91 (5s, 5x Ac), 1.01 (s, 18H, 2x<sup>t</sup>BuSi); <sup>13</sup>C-NMR (125.7 MHz, CDCl<sub>3</sub>) δ (ppm): 170.9, 170.6, 170.4, 170.3, 170.1, (C=O), 168.3 (C-1), 145.9 (C-para), 145.1 (C-ipso), 133.7-123.8 (28C, Aromatic C), 102.9 (C-1'), 97.5 (C-2"), 85.1 (C-1), 80.5 (C-4), 79.6 (C-5), 76.5 (C-3'), 74.3 (C-3), 73.5 (C-5'), 72.7 (C-6"), 69.5 (C-2'), 68.6 (C-4"), 68.2 (C-8"), 67.0 (C-7"), 68.4 (C-6), 62.4 (C-9", C-6'), 54.6 (C-2), 53.2 (C-2', OCH<sub>3</sub>), 49.6 (C-5"), 37.7 (C-3"), 26.9 (CH<sub>3</sub> of <sup>t</sup>BuSi), 26.8 (CH<sub>3</sub> of <sup>t</sup>BuSi), 23.5, 23.2 (NHAc), 21.2, 20.8, 20.7 (2C) (4xOAc), 19.4 (C-Si), 19.2 (C-Si).

Anal. Calcd for C<sub>74</sub>H<sub>91</sub>N<sub>3</sub>O<sub>24</sub>SSi<sub>2</sub>·CH<sub>3</sub>OH (1502.79) C, 58.34; H, 6.37; N, 2.80.

Found: C, 58.44; H, 6.68; N, 2.51.

## Chapter 5 Glycopolymers

### 5.1 Introduction

Synthetic glycoconjugates are a family of carbohydrate derivatives which include glycoproteins, glycolipids, and glycopolymers.<sup>218</sup> Glycopolymers are gaining increasing interest owing to the many advantageous physical and immunochemical properties with which they can be designed.<sup>64,219,220</sup> Glycopolymers can possess uniform and stable structures having a wide range of molecular weights, carbohydrate densities and functionalities. Their purification and characterizations are easier. They can be produced inexpensively and in large scale. They can also be used advantageously as multivalent inhibitors or for cell targeting since they are non or poorly immunogenic.<sup>64</sup> In addition, they can be used directly in quantitative immunoprecipitation and in enzyme-linked immunosorbent assays (ELISA).<sup>221,222</sup> Moreover the well-recognized "cluster effect" of carbohydrate ligands for their specific receptors can be fully appreciated by virtue of the intrinsic multivalent structures of the polymers.<sup>223,224</sup>

Two decades have past since Horejsi and Kocourek<sup>225</sup> first described an entirely new approach to make useful artificial carbohydrate antigens. In this method, simple allyl glycosides were copolymerized with acrylamides with a radical initiator in an aqueous environment to yield multivalent antigenic polymers bearing pendant carbohydrate

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<sup>218</sup> Lee Y. C.; Lee, R. T. (Eds.), *Neoglycoconjugates: Preparation and Applications*, Academic Press, San Diego, CA, 1994, 261-285.

<sup>219</sup> Kosma, P.; Waldstätten, P.; Daoud, L.; Schulz, G.; Unger, F. M. *Carbohydr. Res.* 1989, 194, 145.

<sup>220</sup> Nishimura, S.-I.; Matsuoka, K.; Furuike, T.; Nishi, N.; Tokura, S.; Nagani, K.; Murayama, S.; Kurita, K. *Macromolecules* 1994, 27, 157.

<sup>221</sup> Roy, R.; Tropper, F. D. *Glycoconjugate J.* 1988, 5, 203.

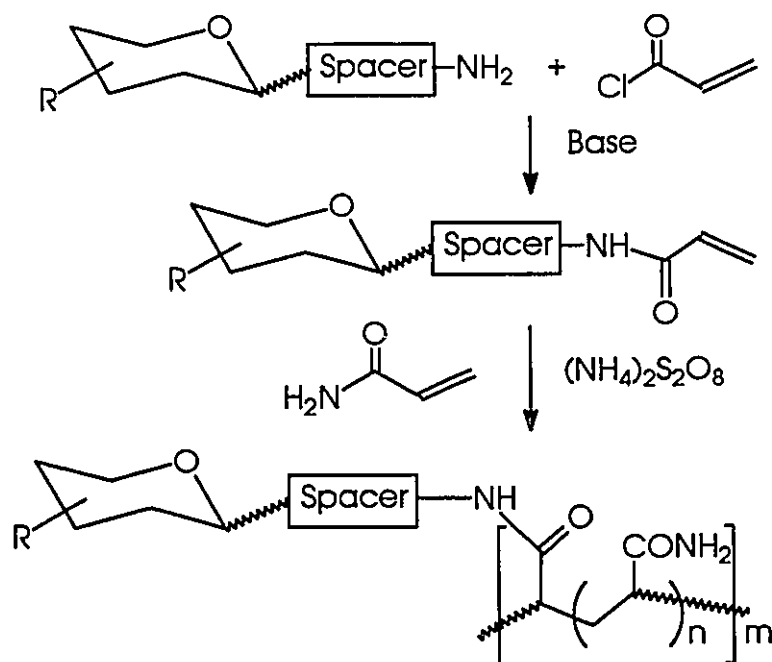
<sup>222</sup> Roy, R.; Tropper, F. D. *J. Chem. Soc., Chem. Commun.* 1988, 1058.

<sup>223</sup> Lee, Y. C.; Townsend, R. R.; Hardy, M. R.; Lönnngren, J.; Arnap, J.; Haraldsson, M.; Lönn, H. *J. Bio. Chem.* 1983, 258, 155.

<sup>224</sup> Lee Y. C.; Lee, R. T.; Rice, K.; Ichikawa, Y.; Wong, T.-C. *Pure Appl. Chem.* 1991, 63, 499.

<sup>225</sup> Horejsí, V.; Kocourek, J. *Biochem. Biophys. Acta* 1973, 297, 346.

residues. Allyl<sup>226</sup> glycosides have also been used in co-polymerizations with acrylamide. The reactivity of alkenyl glycoside monomers in radical copolymerizations is lower than that of acrylamide. Therefore, excess of carbohydrate monomer is needed if a high density of saccharide is desired in the final glycopolymers. In contrast, glycosides bearing acryloyl residues have reactivities similar to that of acrylamide, thereby making it possible to control saccharide density in the co-polymer by controlling the monomer ratios in the polymerization mixture.<sup>227</sup> Furthermore, the incorporation of acrylamidoalkyl glycosides into glycopolymers is practically quantitative.



**Scheme 5.1 Common Strategy for the Synthesis of Polyacrylamide Based Glycopolymers**

<sup>226</sup> Bovin, N. V.; Khorlin, A.; Ya. In "Proc. XII Mendeleev Congress Pure Appl. Chem. Part 2", 1981, 120-121, Nauka Publisher, Moscow.

<sup>227</sup> Bovin, N. V.; Khorlin, A.; Ya. *Bioorgan. Khimia* 1985, 11, 420.

Glycosides having acrylamide groups in the aglycon are accessible via acryloylations of glycosides bearing amine groups in the aglycon. Copolymerization of acrylamido containing glycosides with acrylamide can proceed in the presence of ammonium persulfate. A general synthetic strategy for the preparation of polyacrylamide-based conjugates is illustrated in Scheme 5.1.

In the following section, lactose- and GM<sub>3</sub>-containing glycopolymer syntheses using pre-formed N-acryloylated carbohydrates as monomer precursors are described. The carbohydrate monomer and co-monomer (acrylamide) contain common terminal functionality in order to maximize the homogeneous polymerization environment between the monomers and thus minimize discrete block polymer formation.

## 5.2 Para-Nitrophenyl Thiolactoside as Precursors to Glycoconjugates

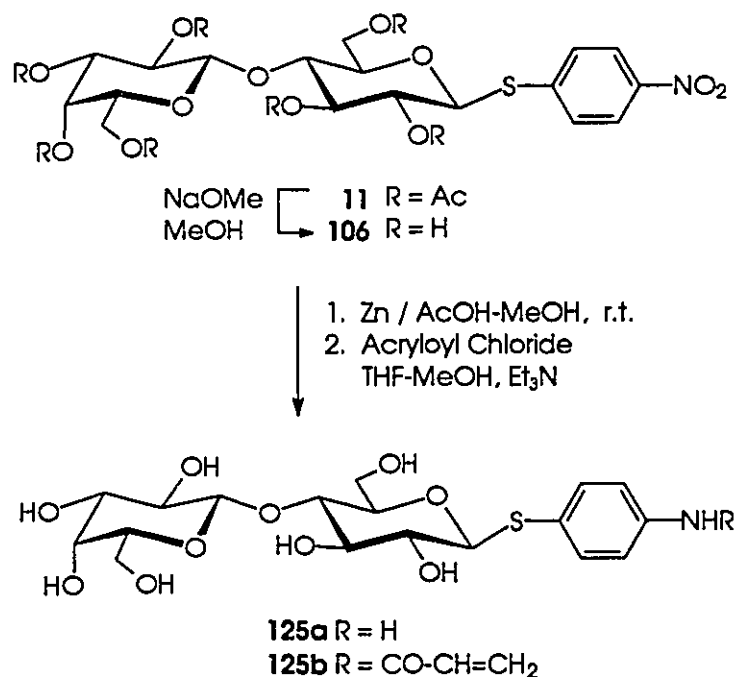
Lactose was chosen as a good ligand model because it is an important hepatocyte receptor,<sup>228</sup> and because suitably protected lactosyl copolymer precursor could be readily available in large scale. Peracetylated para-nitrophenyl thiolactoside **11** was prepared as described in the Section 2.1 under phase transfer catalyzed (PTC) conditions. Zemplén de-O-acetylation of peracetylated lactoside **11** afforded **106** quantitatively. The aromatic nitro group of **106** can be reduced using tin (II) chloride as discussed previously in Section 3.3. In a more efficient way, we found later that the nitro group could be also reduced into amine under even milder conditions. As model compound, **106** was dissolved in a mixture solvent of acetic acid and methanol (1:5) at room temperature and the resulting solution was added a large excess of Zinc dust (10 equiv.). The reduction was finished within 30 min. Once complete, filtration and evaporation of the solution under reduced pressure provided *p*-aminophenyl thioglycoside **125a**. Amine **125a** was directly acryloylated without purification.

N-Acryloylation of **125a** was performed in methanol solution with acryloyl chloride in the presence of triethylamine as base. The terminal amine group was selectively

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<sup>228</sup> Lee, R. T.; Lee, Y. C. *Glycoconjugate J.* 1987, 4, 317.

acylated by dropwise addition of a stock solution of acryloyl chloride in tetrahydrofuran to afford monomer precursor **125b** in 91% yield for the two steps.



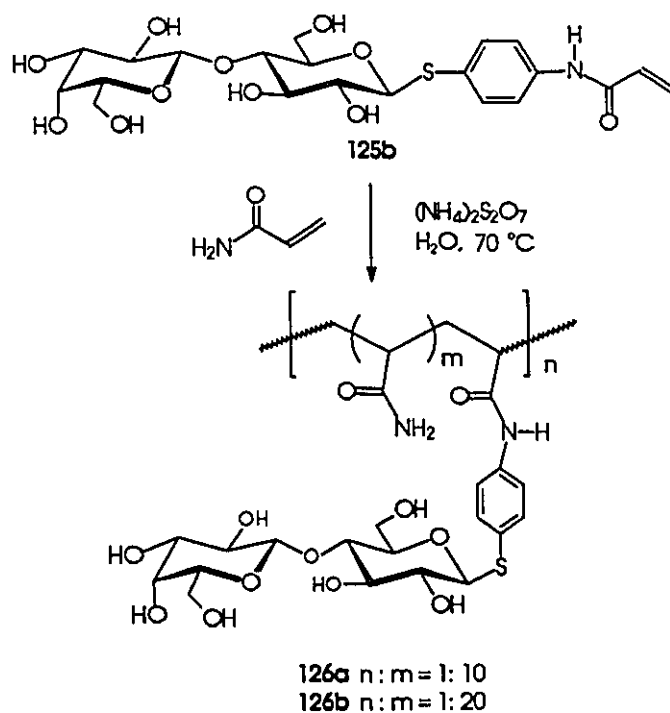
**Scheme 5.2 Preparation of Para-N-Acrylamidophenyl 1-thio-β-D-Lactopyranoside 125b**

With suitable glycosyl precursor **125b** in hand, para-N-acrylamidophenyl 1-thio β-D-lactoside-co-acrylamide (Lactoside-S-Ø-CPA) **126a** and **126b** were prepared by copolymerization of **125b** with acrylamide (1:10 and 1:20 molar ratios respectively) using ammonium persulfate [(NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub>] as radical initiator<sup>229</sup> with heat (90°C, 25 min.). The copolymerizations proceeded smoothly and gave **126a** and **126b** in 57% and 59% yields respectively. The glycopolymers produced were purified and easily isolated by dialysis and freeze drying to give white, spongy solids containing typically between 9 and 15% of retained water. The monomer ratios incorporated within the glycopolymers were found to

<sup>229</sup> Riggs, J. P.; Rodrigues, F. J. *J. Polym. Sci.* 1967, 5, 3167.

be almost identical to the load ratios as judged by  $^1\text{H-NMR}$  spectra. In aryl glycoside glycopolymers, the aromatic proton resonance in the  $^1\text{H-NMR}$  spectra ( $\text{D}_2\text{O}$ ) provided a good reporting group observed downfield (7.3-7.8 ppm, br) from the sugar resonances ( $\delta$  3.4-4.0 ppm, ring H) and the polymer backbone signal at  $\delta$  2.47-2.12 (br, methine) and at 1.97-1.32 (methylene). The N-Acetyl groups were found as relatively sharp singlets at 2.0 ppm, while much of the remaining  $^1\text{H-NMR}$  spectrum was composed of broadened signals.

It is worthy to mention that Lactoside-S- $\emptyset$ -CPA **126** described here, differs from the Lactoside-O- $\emptyset$ -CPA prepared in this group before.<sup>65b</sup> The incorporation of an S-glycoside instead of O-glycoside should render the glycopolymer more stable toward the enzymatic hydrolysis by glucohydrolases.



**Scheme 5.3** Synthesis of Lactosyl Glycopolymer

### 5.3 GM<sub>3</sub> as Precursor to Glycoconjugates

As shown in scheme 5.4, para-nitrophenyl thiotrisaccharide GM<sub>3</sub> **122** was efficiently reduced using zinc dust as catalyst in a mixture of acetic acid and dichloromethane (4:25) to give para-aminophenyl thiotrisaccharide **127**. Reductions were usually finished within 30 min at room temperature and conversion was quantitative as judged by TLC. The amino derivative **127** was directly subjected to N-acryloylation without purification. N-Acryloylation of aminothiophenyl GM<sub>3</sub> **128** was performed with acryloyl chloride in dichloromethane using triethylamine as base at 0°C. The reaction was finished within 60 min and the key GM<sub>3</sub> monomer **128** was obtained in high yield (85%). N-Acryloylated **128** had the same R<sub>f</sub> value as the starting amino compound **127**, but completion of the reaction could be monitored based on the color change of the spots on TLC. The structures of the GM<sub>3</sub> derivatives were confirmed by <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra. Examples of <sup>1</sup>H-NMR and <sup>1</sup>H-<sup>1</sup>H COSY, <sup>1</sup>H-<sup>13</sup>C HMQC NMR spectra of N-acryloylated GM<sub>3</sub> **128** are shown in figures 5.1-5.3.

The preparation of unprotected GM<sub>3</sub> monomer **131** by Zemplén de-O-acetylation (NaOMe, MeOH) and saponification (NaOH, H<sub>2</sub>O), was then needed. However, when **128** was first treated under Zemplén de-O-acetylation conditions, extensive addition of methoxyl anions to the N-acrylamido function (detected by <sup>1</sup>H and <sup>13</sup>C NMR analyses) resulted in **129**. The molar ratio of the desired N-acryloylated GM<sub>3</sub> monomer **131** to conjugate addition product **129** was found to be 32:68 by analysis of the <sup>1</sup>H-NMR signals. The acryloyl signals at δ 6.5-5.95 ppm were smaller than expected and a new triplet signal appearing at δ 2.78 ppm was reported and attributed to the NCH<sub>2</sub>CH<sub>2</sub>OMe group. A similar phenomena was previously observed during the course of preparation of sialic acid containing glycopolymer.<sup>65c</sup> Selected <sup>13</sup>C-NMR data for GM<sub>3</sub> derivatives **122**, **127**, **128**, **131** are given in table 5.1.

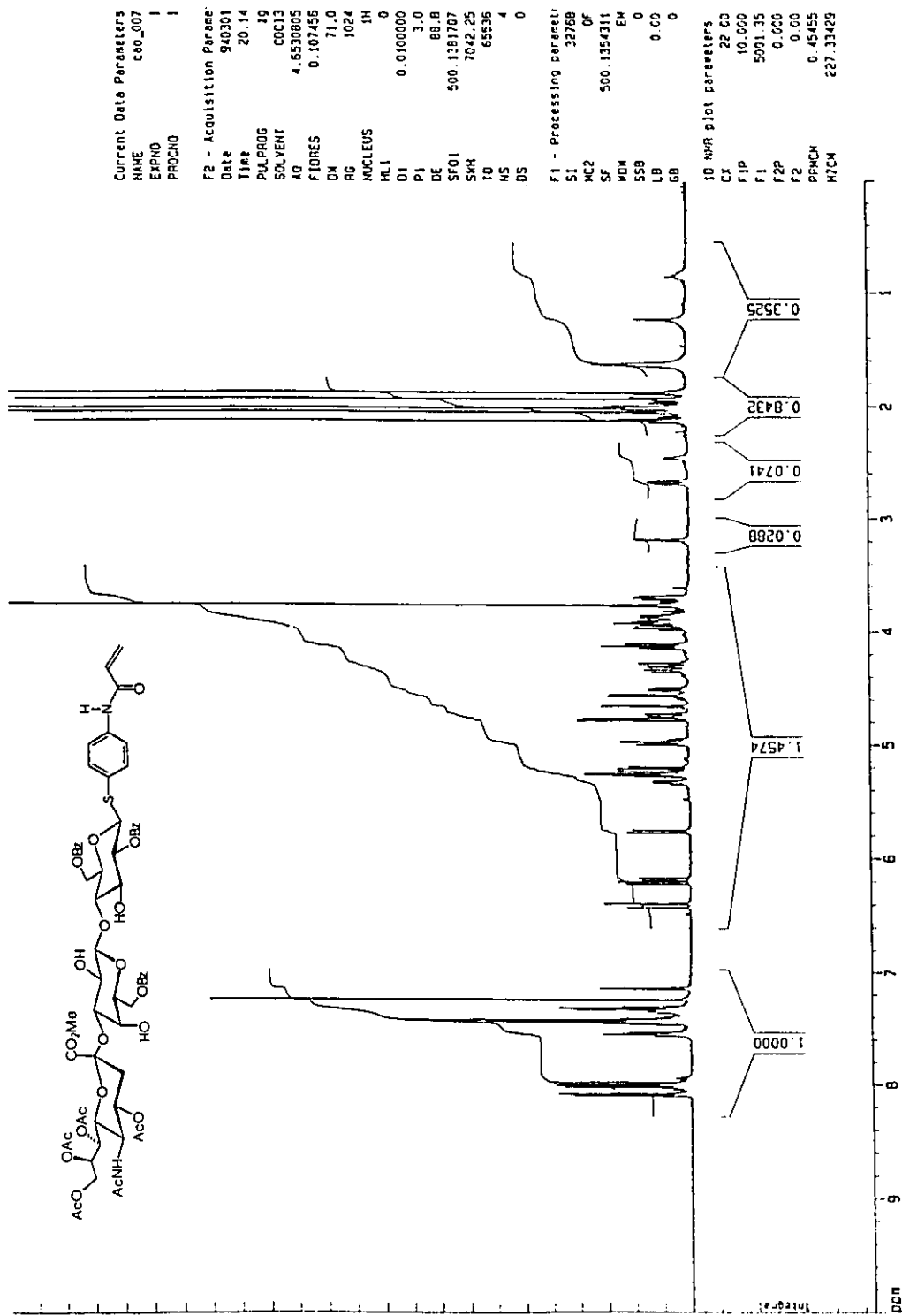


Figure 5.1 <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) Spectrum of GM<sub>3</sub> Trisaccharide 128

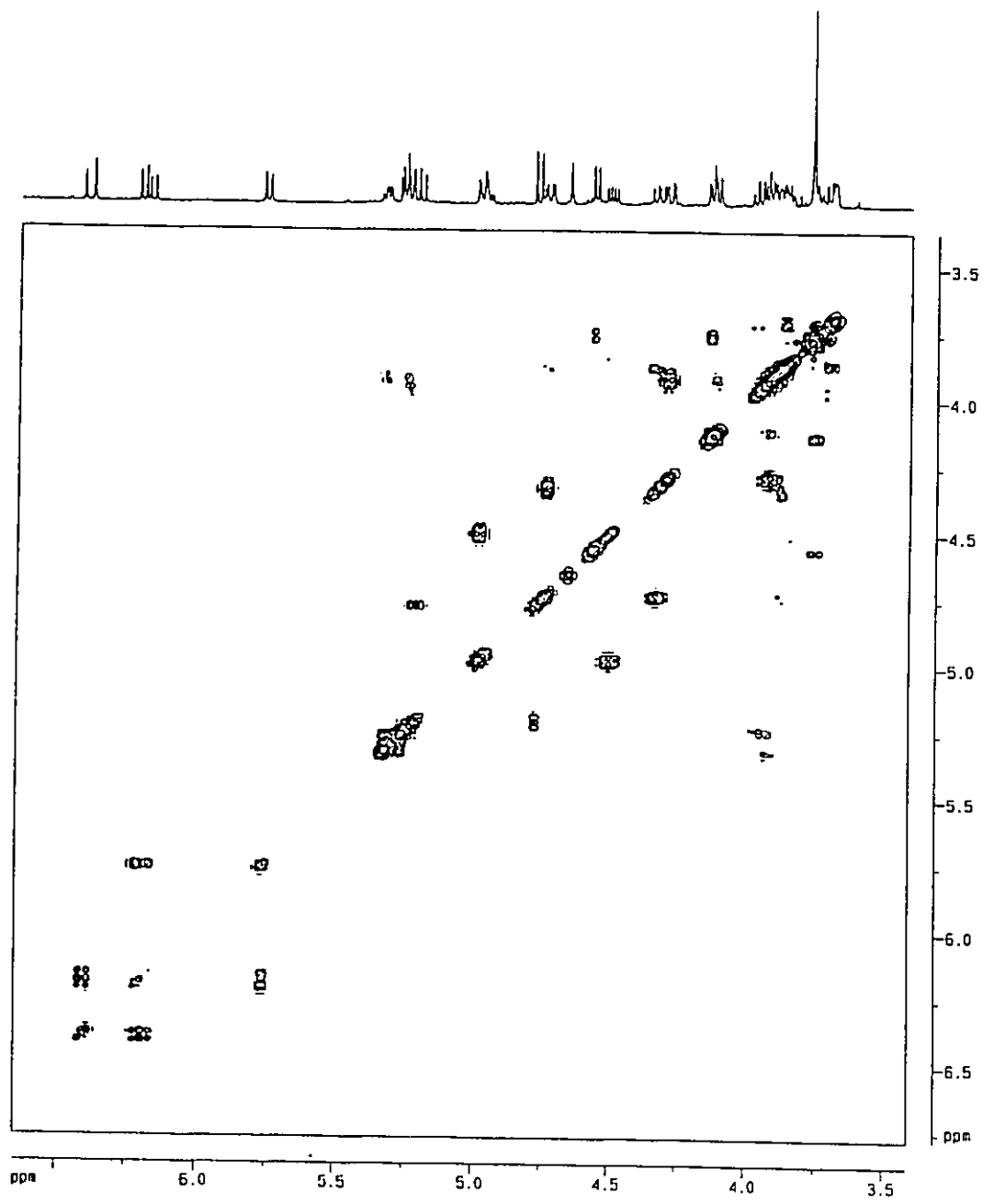


Figure 5.2  $^1\text{H}$ - $^1\text{H}$ -COSY Spectrum (500 MHz,  $\text{CDCl}_3$ ) of  $\text{GM}_3$  Trisaccharide 128

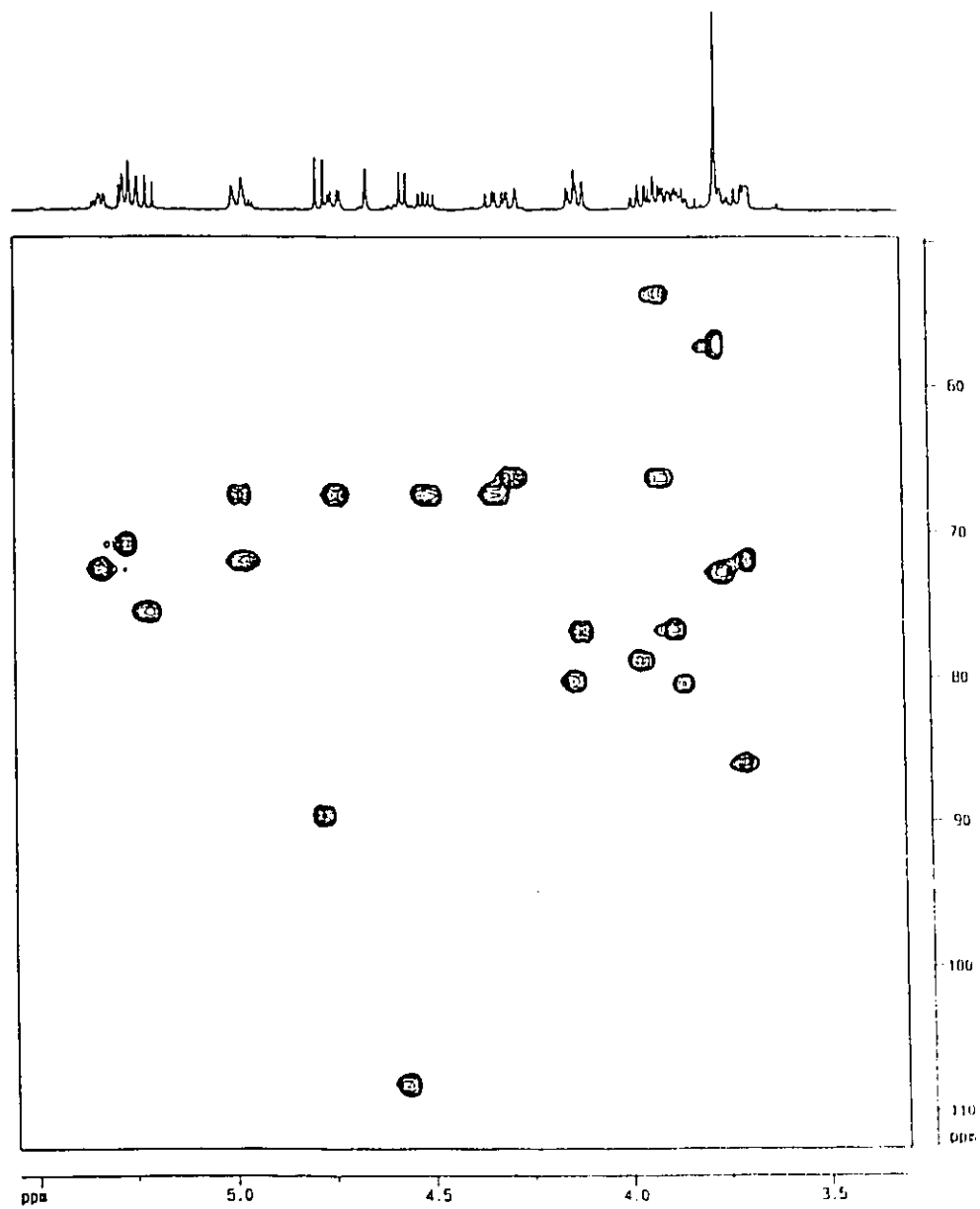


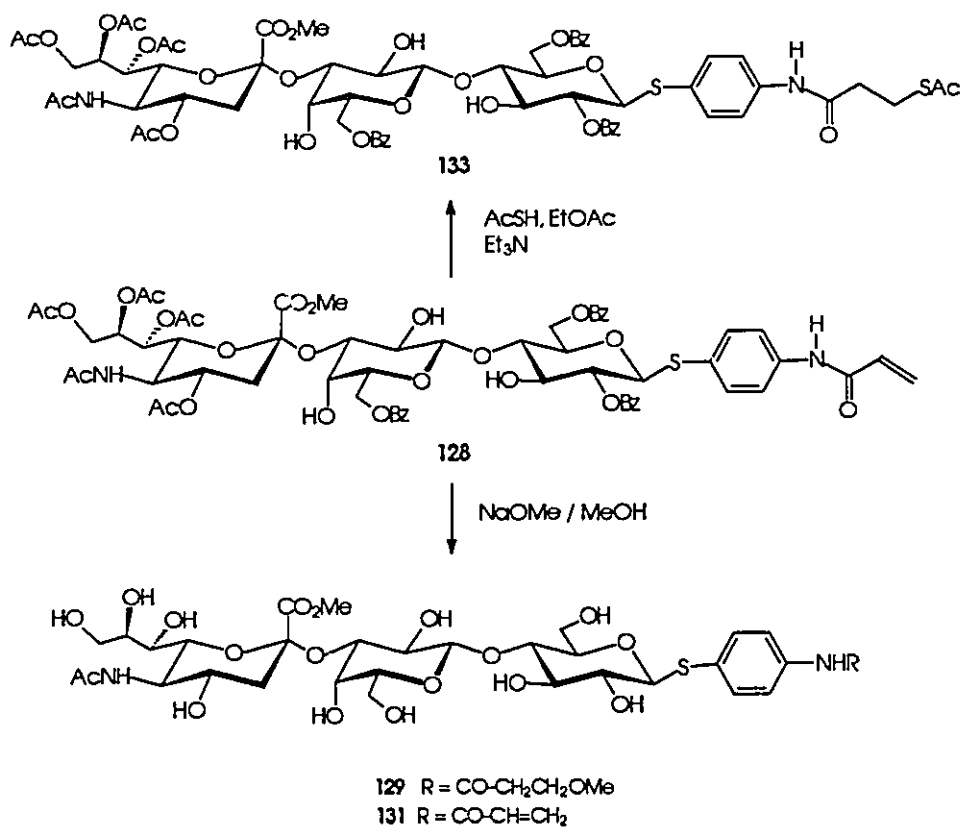
Figure 5.3  $^1\text{H}$ - $^{13}\text{C}$ -HMQC Spectrum (125.7 MHz,  $\text{CDCl}_3$ ) of  $\text{GM}_3$  Trisaccharide 128



**Table 5.1 Selected  $^{13}\text{C}$ -NMR Data for GM<sub>3</sub> Derivatives**

Atom	122	127	128	133
<b>NeuAc</b>				
C-1	168.1	168.0	168.1	168.1
C-2	97.5	97.5	97.5	97.5
C-3	37.8	37.8	37.7	37.7
C-4	68.2	68.2	68.2	68.2
C-5	49.7	49.8	49.8	49.8
C-6	73.1	73.1	73.0	73.0
C-7	67.0	67.0	67.0	68.1
C-8	68.8	68.9	68.8	71.7
C-9	62.4	62.5	62.4	62.4
OMe	53.3	53.3	53.3	53.3
NAc	23.1	23.2	23.2	23.2
<b>Gal</b>				
C-1	104.4	104.4	104.4	104.4
C-2	68.7	68.8	68.1	69.0
C-3	76.5	76.5	76.4	76.6
C-4	68.1	68.1	68.1	68.8
C-5	73.0	73.0	73.0	73.0
C-6	63.5	63.6	63.6	63.6
<b>Glc</b>				
C-1	84.1	85.1	85.8	85.9
C-2	71.4	71.7	71.4	72.9
C-3	74.8	75.0	75.0	76.4
C-4	82.0	82.2	82.1	82.2
C-5	76.9	76.5	76.6	76.6
C-6	63.5	63.7	63.6	67.0

Chemical shifts  $\delta$  are given in ppm relative to internal TMS and were recorded in 125.8 MHz for  $\text{CDCl}_3$  solution (ref. 77.0 ppm). Assignment are based the  $^{13}\text{C}$ -NMR spectra of the precursors, adept and  $^1\text{H}$ - $^{13}\text{C}$  HMQC experiments.



### Scheme 5.4 Preparation of GM<sub>3</sub> Derivatives

However, addition of thioacetic acid to **128** was also desired. The addition product **133** is a valuable precursor for the preparation of dendritic GM<sub>3</sub>.<sup>230,231</sup> Thioacetate derivative **133** was efficiently synthesized in 85% yield by adding thioacetic acid<sup>232</sup> to a solution of **128** in ethyl acetate in the presence of triethyl amine in 85% yield. Disappearance of signals attributed to the acryloyl group in the <sup>1</sup>H-NMR spectrum at  $\delta$

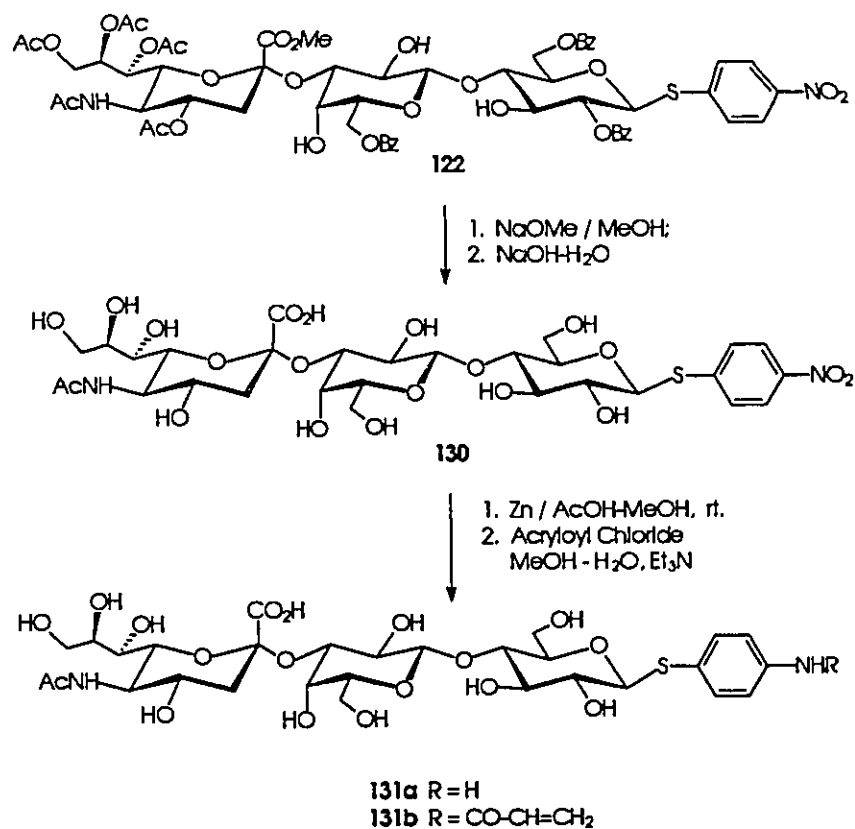
<sup>230</sup> Roy, R.; Zanini, D.; Meunier, S.; Romanowska, A. *J. Chem. Soc., Chem. Commun.* **1993**, 1869.

<sup>231</sup> Thioacetate derivative **133** could be de-S-acetylated with using hydrazinium acetate. The resulting thiol can react with various dendritic electrophiles to afford dendritic GM<sub>3</sub> compounds. The synthetic studies along this line are under current investigation in this laboratory.

<sup>232</sup> Park, W. K. C.; Meunier, S. J.; Zanini, D.; Roy, R. *Carbohydr. Lett.* **1994**, *1*, 179.

6.40-5.75 ppm and appearance of new signals at  $\delta$  3.19 ppm ( $\text{SCH}_2$ ),  $\delta$  2.64 ppm ( $\text{CH}_2\text{CO}$ ), and at  $\delta$  2.33 ppm (s, SAc) clearly indicated the formation of derivative **133**.

An alternative strategy was proposed as illustrated in Scheme 5.5. Zemplén de-O-acetylation (NaOMe, MeOH) and saponification (NaOH, H<sub>2</sub>O) of **122** were executed before the reduction and N-acryloylation in order to eliminate the problem associated with the conjugate addition of methoxyl anions. In fact, this synthetic route was very efficient. Zemplén deacetylation and saponification of **122** afforded deprotected GM<sub>3</sub> trisaccharide **130** in 95% yield. Subsequent reduction and N-acryloylation of **130** under conditions similar to the preparation of the lactose-containing monomer **125b**, afforded desired GM<sub>3</sub> monomer **131b** in 83% yield. It should be noted that the amine intermediate had the same R<sub>f</sub> as that of the nitro compound **130** in several solvent systems. A large excess of Zinc dust and longer reaction time (60 min) were used to eliminate any possibility of under reduction.



**Scheme 5.5** Synthesis of Unprotected GM<sub>3</sub> Monomer **131**

The structure of deprotected GM<sub>3</sub> monomers **130** and **131** were fully characterized by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy. The assignments were based on 2D NMR spectra (<sup>1</sup>H-<sup>1</sup>H COSY and <sup>1</sup>H-<sup>13</sup>C HMQC). Selected <sup>13</sup>C-NMR data of **130** and **131** are given in table 5.3. As an example, the <sup>1</sup>H- and <sup>13</sup>C-NMR spectra (D<sub>2</sub>O) are depicted in Figures 5.4 and 5.5.

**Table 5.2 Selected <sup>13</sup>C NMR Data for GM<sub>3</sub> Derivatives 130 and 131<sup>a</sup>**

Atom	Compound 130			Compound 131		
	NeuAc	Gal	Glc	NeuAc	Gal	Glc
C-1	173.4	102.2	85.1	173.4	102.1	86.8
C-2	99.4	68.9	71.0	99.4	68.9	71.0
C-3	39.2	75.3	74.8	39.2	75.0	74.7
C-4	67.9	67.1	78.4	67.9	67.0	78.3
C-5	51.3	77.4	75.1	51.2	77.4	75.3
C-6	72.5	60.6	59.7	72.4	60.6	59.7
C-7	67.7			67.7		
C-8	71.3			71.3		
C-9	62.2			62.1		
C=O	174.6			174.6		
aglycons	145.6 (C-ipso), 142.8 (C-para), 128.7 (C-meta), 123.7 (C-ortho),			166.4 (COCH=CH <sub>2</sub> ), 136.5 (C-ipso), 132.5 (C-meta), 129.8 (C- para), 128.2 (-CH=), 127.5 (=CH <sub>2</sub> ), 121.6 (C-ortho),		

<sup>a</sup> Chemical shifts values are in ppm from solution in D<sub>2</sub>O (500 MHz) with acetone as internal reference (δ<sub>CH<sub>3</sub></sub> = 31.1 ppm). Assignments are based on 2D <sup>1</sup>H-<sup>1</sup>H COSY and <sup>1</sup>H-<sup>13</sup>C HMQC

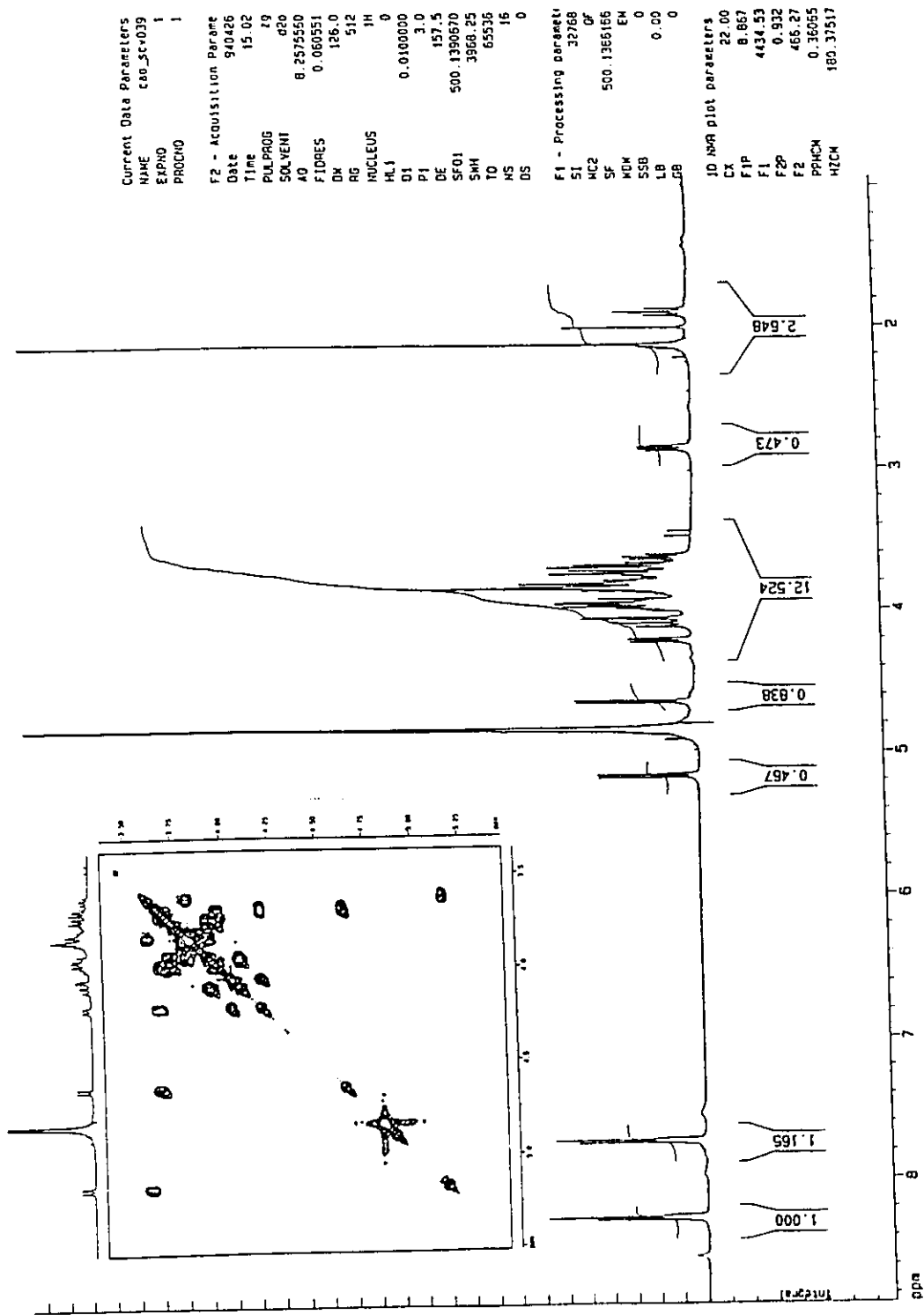


Figure 5.4 <sup>1</sup>H-NMR and <sup>1</sup>H-<sup>1</sup>H-COSY Spectra (500 MHz, D<sub>2</sub>O) of Trisaccharide 130

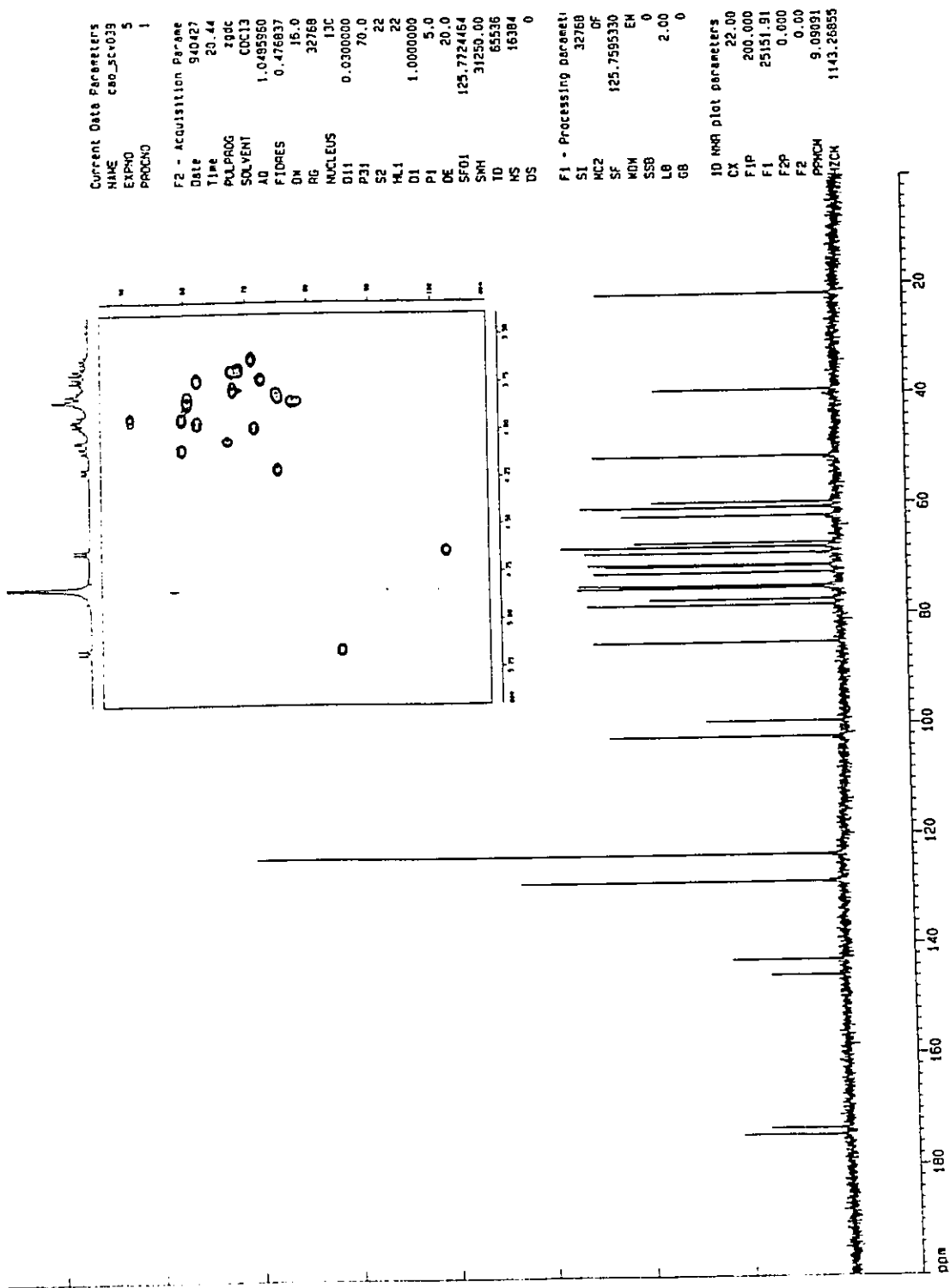
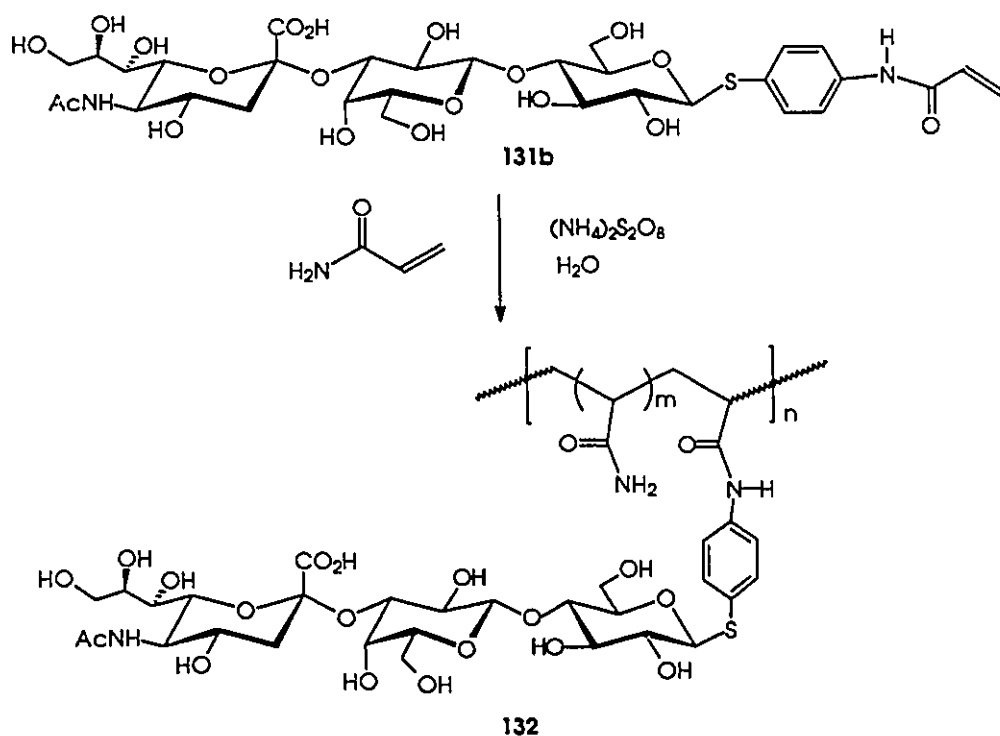


Figure 5.5 <sup>13</sup>C-NMR and <sup>1</sup>H-<sup>13</sup>C-HMQC Spectra (125.7 MHz, D<sub>2</sub>O) of GM<sub>3</sub> Trisaccharide 130

Similar to the preparation of lactosyl glycopolymer **126**, the monomeric N-acrylamidophenyl thioglycoside **131b** was copolymerized with acrylamide using ammonium persulfate as initiator in deoxygenated water (obtained by multiple cool-thaw cycles and bubbling nitrogen). The copolymerization generally proceeded at 90°C and finished within one hour. Good yields (50-70%) of GM<sub>3</sub> copolymers with different GM<sub>3</sub> monomer incorporation were obtained. As described earlier, the GM<sub>3</sub> monomer ratios incorporated within the glycopolymers were found to be almost identical to the load ratio. This is especially true when lower amounts of acrylamide monomers were used (1/5,1/10). The GM<sub>3</sub> glycopolymers produced here were purified and easily isolated by dialysis and freeze drying to give white, spongy solids.



**Scheme 5.6 Copolymerization of GM<sub>3</sub> Monomer and Acrylamide**

Alternatively, GM<sub>3</sub> glycopolymer could be prepared directly from the protected monomer 128 with acrylamide in deoxygenated DMSO using AIBN as initiator.<sup>233</sup> The obtained copolymer, after successive Zemplén de-O-acetylation (NaOMe, MeOH) and saponification (NaOH, H<sub>2</sub>O) gave the same glycopolymer 132 as that prepared from the deprotected monomer 131 using ammonium persulfate as initiator.

The homogeneity of the glycopolymer structures was established by <sup>13</sup>C-NMR spectroscopy. The spectrum of glycopolymer 132 showed a single set of relatively sharp resonances for the GM<sub>3</sub> residues. The aromatic ortho carbon as well as the anomeric carbon attached to the phenyl ring were sharp but slightly broadened when compared to other carbohydrate resonances. The backbone methine and methylene resonances appeared in their usual position as broad peaks near 43 and 35 ppm, respectively. The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of GM<sub>3</sub> polymer are given in Figure 5.6 and 5.7.

The most important feature of glycopolymer is probably the carbohydrate density. Multivalency is an important factor in adhesion phenomena. Various methods have been utilized<sup>62</sup> to quantitate the carbohydrate content of glycopolymers. Carbohydrate incorporation has been determined by colorimetric chemical assays,<sup>234</sup> by elemental analysis and from <sup>1</sup>H-NMR spectrum integrations.

<sup>1</sup>H-NMR spectroscopy has proven to be a very simple, quick and effective tool for accurately determining the carbohydrate content in acrylate based glycopolymers. In the <sup>1</sup>H-NMR spectra of glycopolymers, resonances belonging to the sugar residue are found in the same chemical shift range as in the glycosides. Their position between 3.4 and 5 ppm are well removed from the residues arising from the polymer backbone found between 1 and 2.5 ppm. By comparing the relative integration ratios of these areas, an unambiguous value for the average carbohydrate density can be determined once the backbone resonance contribution from the glycoside comonomer has been taken into account. A typical <sup>1</sup>H-NMR spectra of a poly(acrylamide-co-GM<sub>3</sub>) glycopolymer is shown in Figure 5.6. We found that one proton has an average integration value of ~1

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<sup>233</sup> Chytrý, V.; Driguez, H. *Makromol. Chem., Rapid Commun.* 1992, 13, 499.

<sup>234</sup> Dubois, M.; Gilles, K.A.; Hamilton, J. K.; Robers, P. A.; Smith, F. *Anal. Chem.* 1956, 28, 350.

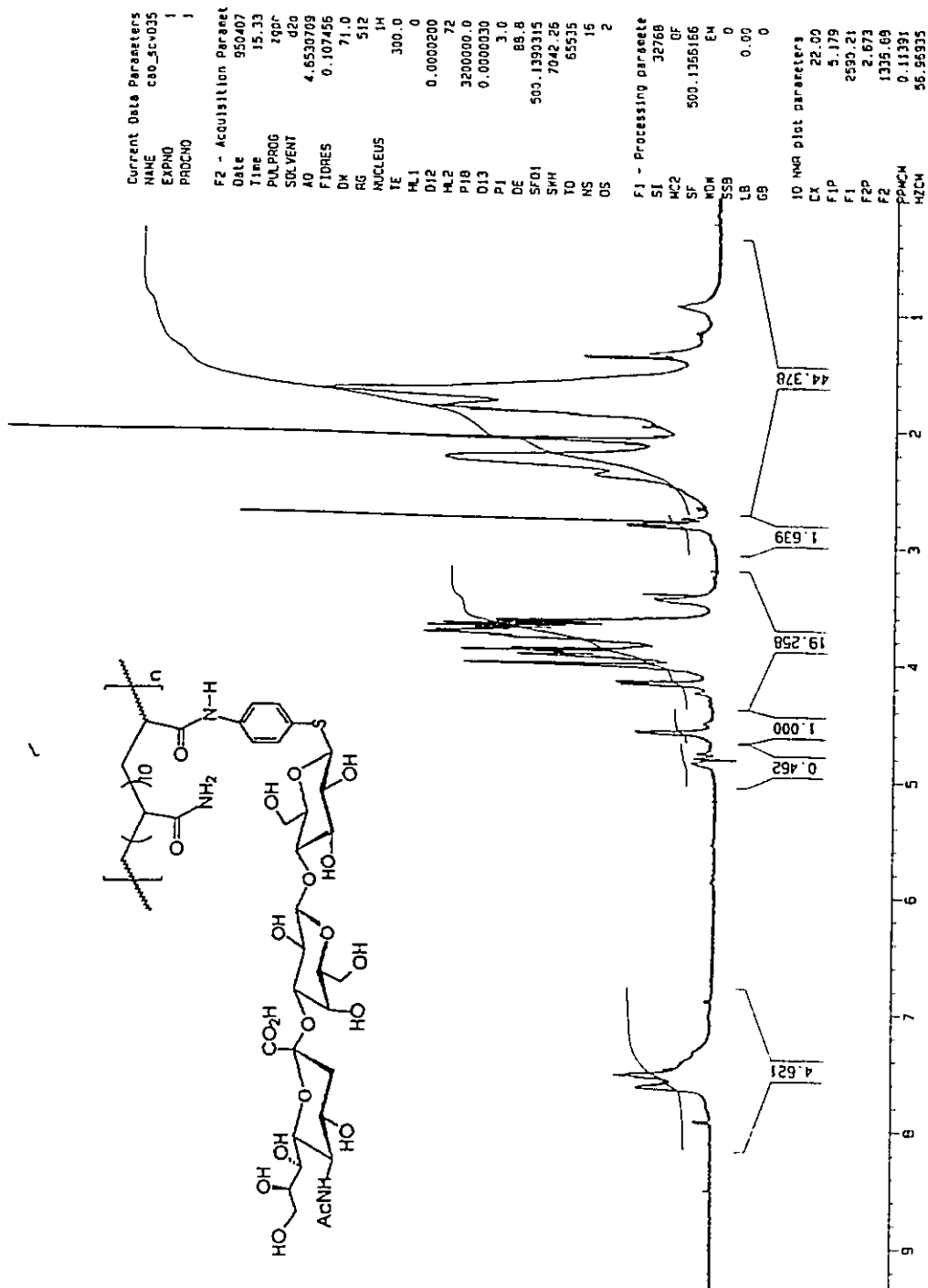


Figure 5.6 <sup>1</sup>H-NMR (500 MHz, D<sub>2</sub>O) Spectrum of GM<sub>1</sub>-S-O-CPA

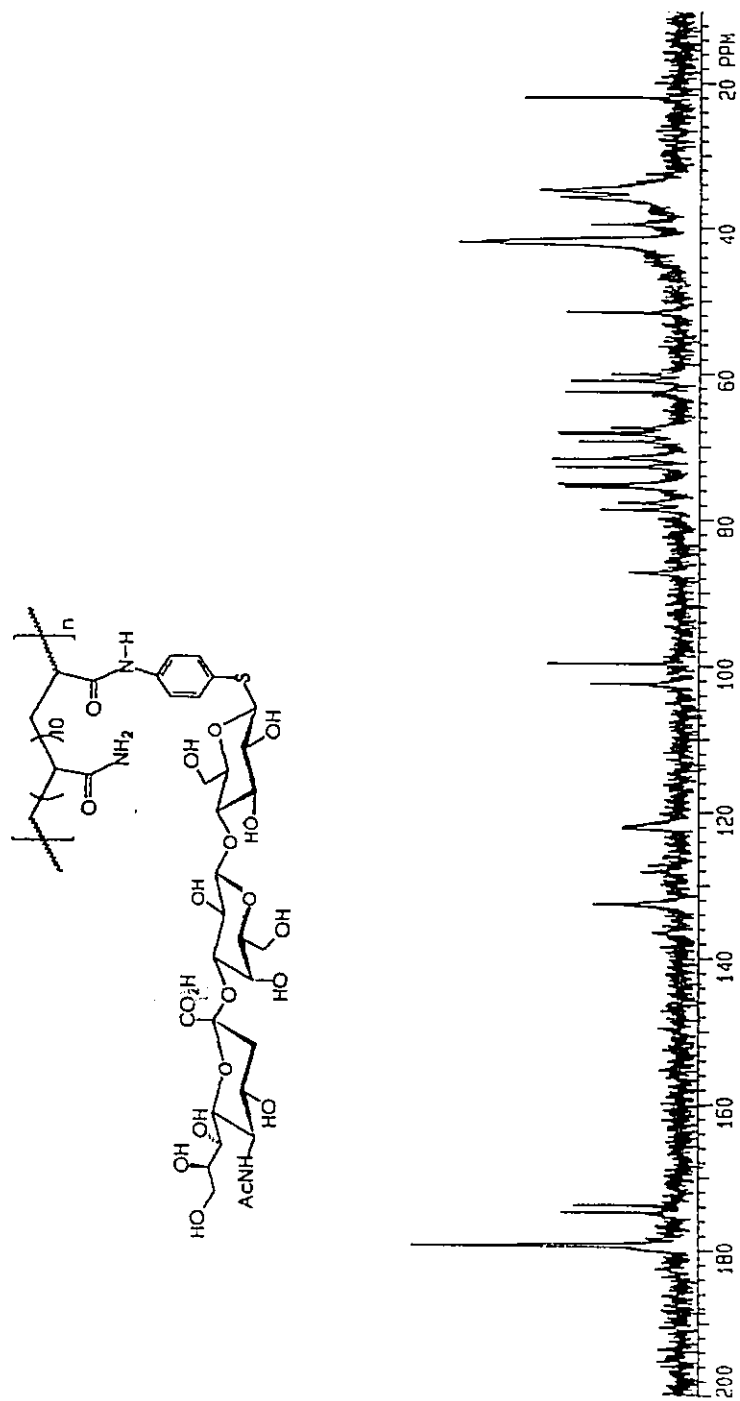


Figure 5.7 <sup>13</sup>C-NMR (500 MHz, D<sub>2</sub>O) Spectrum of GM<sub>3</sub>-S-O-CPA

unit. The total methylene and methine signals from all monomer units in the polymer backbone integrate for 37 units. The incorporation ratio of GM<sub>3</sub> in this copolymer is roughly 1:11, as deduced by subtracting the 4 proton contributions of the sialic acid moiety (NHAc and H-3a).

#### 5.4 Evaluation of Antigenicity

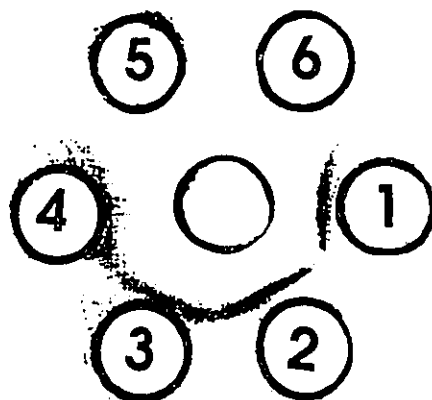
Double radial diffusion assays through agarose gel were performed with plant lectins to test the antigenicity of GM<sub>3</sub>-containing glycopolymers (GM<sub>3</sub>-S-Ø-CPA) 132 and lactose-containing glycopolymers (Lactose-S-Ø-CPA) 126. In a first set of experiments, GM<sub>3</sub>-S-Ø-CPA with sugar contents of 1:5.2, 1:11; 1:27, 1:48 and Lactose-S-Ø-CPA with sugar contents of 1:11, 1:21 were allowed to diffuse against wheat germ agglutinin (WGA). This carbohydrate-specific lectin<sup>235</sup> is known to bind with Neu5Ac as well as GlcNAc. A photograph of the gel is given in Figure 5.8 and the results of double immunodiffusion of the polymers are reported in Table 5.3. As expected, all the GM<sub>3</sub>-S-Ø-CPA 132, except the one with a very low sugar ratio (1:48), were proved to be antigenic towards WGA, while the Lactose-S-Ø-CPA 126 (wells 5 and 6) showed no antigenicity toward this plant lectin. It is not surprising that the glycopolymers with very low sugar contents showed no antigenicity considering the requirement of multivalent interaction for binding between antigens and lectins. In the second set of experiments, Peanut Lectin (*Arachis Hypogea*) was used against all the glycopolymers used in the first set of experiments. Opposite results were obtained, the lactose-S-Ø-CPA 126 showed precipitation bands and while GM<sub>3</sub>-S-Ø-CPA 132 showed no bands.

Diffusion studies were also used to provide an indication of the relative molecular weight. Generally, the higher the molecular weight, the slower the diffusion rate in the gel.

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<sup>235</sup> Goldstein, I. J.; Poretz, R. D. in *The Lectins. Properties, Functions, and Applications in Biology and Medicine*. Liener, I. E.; Sharon, N.; Goldstein, I. J. (eds.) Academic Press, Orlando Florida, 1986.

It is apparent from figure 5.8 that the diffusion rates of all GM<sub>3</sub> containing glycopolymers were much smaller than that of WGA lectin as precipitation bands are closer to the polymer wells than to the lectin well. It is known that WGA has a molecular weight of 43 kDa. According to the observed diffusion ratio, the estimated molecular weight of all the GM<sub>3</sub> containing glycopolymers prepared here were larger than 50 kDa. It is known that molecular weights of glycopolymers increase as the concentration of the initiator decreases.<sup>236</sup> In our copolymerization process, small amounts of ammonium persulfate were used, so it is not surprising that the glycopolymers obtained had relatively high molecular weights.



**Figure 5.8** Agar gel diffusion assay of WGA (central well) against:  
 GM<sub>3</sub>-S-Ø-CPA 132 (1:5) well 1; GM<sub>3</sub>-S-Ø-CPA (1:11), well 2;  
 GM<sub>3</sub>-S-Ø-CPA (1:27), well 3; GM<sub>3</sub>-S-Ø-CPA (1:48), well 4;  
 Lactose-S-Ø-CPA 126 (1:11), well 5; Lactose-S-Ø-CPA (1:21), well 6.

<sup>236</sup> Kallin, E.; Lönn, H.; Norberg, T.; Elofsson, M. *J. Carbohydr. Chem.* 1989, 8, 597.

**Table 5.3** Double radial diffusion assay for lectins and GM<sub>3</sub>-S-Ø-CPA 132 and Lactose-S-Ø-CPA 126

Antigen		Lectins	
Hapten	Sugar Content*	Wheat Germ Agglutinin	<i>Arachis Hypogea</i>
GM <sub>3</sub>	1:5	+	-
GM <sub>3</sub>	1:11	+	-
GM <sub>3</sub>	1:27	+	-
GM <sub>3</sub>	1:48	-	-
Lactose	1:11	-	+
Lactose	1:21	-	+

\* Sugar contents in copolymers were determined by <sup>1</sup>H-NMR (500 Hz) spectroscopy

## 5.5 Conclusion

In summary, lactose and GM<sub>3</sub> containing glycopolymers with different sugar incorporations were successfully prepared. The glycopolymers prepared have approximate molecular weight  $\geq 50$  kDa and are water soluble. Preliminary double immunodiffusion experiments with the polymers revealed binding specificity between the sugar and plant lectins. Polymers with high sugar contents (1:5 and 1:11) showed good antigenicities, while glycopolymers with very low sugar contents (1:48) showed no detectable interaction between the glycopolymer and the lectins. Further quantitative evaluation of the antigenicities for these new glycopolymers and the influence of the sugar density on binding affinity will be pursued in due course.

## 5.6 Experimental Methods

### Para-N-acrylamidophenyl O-( $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1-thio- $\beta$ -D-glucopyranoside **125b**

To a solution of para-nitrophenyl O-( $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1-thio- $\beta$ -D-glucopyranoside **106** (500 mg, 1.04 mmol) in methanol and acetic acid (5 mL, 5:1 v/v) was added zinc dust (340 mg, 5.20 mmol) at 0 °C and the mixture was stirred at room temperature for 30 min. TLC (MeOH:CH<sub>2</sub>Cl<sub>2</sub>, 4:5, v/v) showed conversion of **106** (R<sub>f</sub> = 0.53) to amine intermediate **125a** (R<sub>f</sub> = 0.37). The reaction was filtered through a layer of Celite with methanol used for rinsing. Evaporation of the solvent under reduced pressure gave a yellowish solid. The crude product was dried over KOH overnight to remove the trace of remaining acid. The residue was then dissolved in methanol (5 mL). The pH of the solution was adjusted to *ca* 8. To this cooled solution (ice bath) was added 350  $\mu$ l of acryloyl chloride in 2 mL tetrahydrofuran. The reaction mixture was then stirred at room temperature for 2.5 hours. TLC (MeOH:CH<sub>2</sub>Cl<sub>2</sub>, 4:5, v/v) showed a clean and complete conversion of the amine (R<sub>f</sub> = 0.37) to acrylamide **125** (R<sub>f</sub> = 0.39). The reaction mixture was neutralized with H<sup>+</sup> resin and concentrated under reduced pressure to drying mass. Purification by flash chromatography with a gradient of MeOH/CH<sub>2</sub>Cl<sub>2</sub> (from 1:5 to 1:1 in v/v) gave **125** as yellowish solid (475 mg) in 91% yield:  $[\alpha]_D -34.8^\circ$  (c = 1.3, H<sub>2</sub>O); MS (FAB) gave m/e (ion, relative intensity): 504.2 ([M+1]<sup>+</sup>, 4.0 %); <sup>1</sup>H-NMR (D<sub>2</sub>O)  $\delta$  (ppm): 7.63-7.51 (m, 4H, Ph ), 6.47 (dd, 1H, J<sub>gem</sub> = 1.1, J<sub>trans</sub> = 17.0 Hz, =CH<sub>2trans</sub>), 6.39 (dd, 1H, C(O)CH= ), 5.93 (dd, 1H, J<sub>cis</sub> = 10.1 Hz, =CH<sub>2cis</sub>), 4.81 (d, 1H, J<sub>1,2</sub> = 10.1 Hz, H-1), 4.50 (d, 1h, J<sub>1,2'</sub> = 7.8 Hz, H-1'), 4.03- 3.59 (m, 12 H, H-2-6, H-2'-6'); <sup>13</sup>C-NMR (D<sub>2</sub>O)  $\delta$  (ppm): 166.3 (C=O), 136.5 (C-ipso), 132.5 (C-meta), 129.8(-CH=), 128.1 (CH<sub>2</sub>=), 127.38 (C-para), 121.51 (C-ortho), 102.39 (C-1'), 86.17 (C-1), 78.23 (C-5'), 77.52 (C-5), 75.31 (C-3), 74.86 (C-3'), 72.05 (C-4), 70.92 (C-2), 70.46 (C-2'), 68.08 (C-4), 60.53 (C-6), 59.68 (C-6').

Anal. Calcd for C<sub>21</sub>H<sub>29</sub>O<sub>11</sub>N<sub>1</sub>S<sub>1</sub> · 1.5 H<sub>2</sub>O (530.55): C, 47.54, H, 6.02, N, 2.64, Found: .C, 47.39; H, 5.89; N, 2.55.

**Poly (Para-N-acrylamidophenyl O-(β-D-galactopyranosyl)-(1→4)-1-thio-β-D-glucopyranoside-co-acrylamide) 126a and 126b**

Compound **125** (10 mg, 19.8 μmol) and acrylamide (14 mg, 197.9 μmol) were dissolved in deoxygenated water (300 μL) obtained by multiple cool-thaw cycles and bubbling nitrogen. A small amount of (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (0.18 mg, 0.792 μmol) was added by injection of 36 μL of a stock solution, 5 mg/500 μL water). The reaction mixture was stirred at 90 °C for 15 min. TLC (MeOH/CH<sub>2</sub>Cl<sub>2</sub>, 1:1 in v/v) showed remaining monomers. Ammonium persulfate 36 μL of the stock solution was added again with stirring for an additional 12 min at 90°C. The reaction mixture was then diluted with water (1 mL) and dialyzed (2,000 MW cut off) exhaustively against multiple 1 L volumes of distilled water. Lyophilization gave 13.7 mg (57%) of Lactose-S-CPA **126a** as a spongy white solid. The lactose/acrylamide ratio within the polymer was 1:11 as determined by <sup>1</sup>H NMR spectroscopy.

In a parallel manner Lactose-S-CPA **126b** (lactose/acrylamide ratio within the polymer was 1:21) was prepared in 59 % yield: <sup>1</sup>H-NMR (500 MHz, D<sub>2</sub>O) δ (ppm): 7.62-7.43 (br, 4H, Ph), 4.81 (1H, H-1), 4.51 (1H, H-1'), 4.03- 3.41 (m, 12 H, H-2-6. H-2'-6'), 2.47-2.12 (br, methine of backbone), 1.97-1.32 (methylene of backbone); <sup>13</sup>C-NMR (125.8 MHz, D<sub>2</sub>O) δ (ppm): 179.1 (C=O, backbone), 174.5 (C=O), 136.2 (C-ipso), 132.4 (C-meta), 121.6 (br, C-para, C-ortho), 102.4 (C-1'), 86.9 (C-1), 78.2 (C-5'), 77.7 (C-5), 75.3 (C-3), 74.9 (C-3'), 72.1 (C-4), 71.0 (C-2), 70.5 (C-2'), 68.1 (C-4), 60.6 (C-6), 59.7 (C-6'), 41.7-41.4 (br, methine of backbone), 38.3-33.6 (methylene of backbone).

**Para-Aminophenyl O-(Methyl 5-acetamido-4,7,8,9-tera-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylate)-(2→3)-O-(6-O-benzoyl-β-D-galactopyranosyl)-(1→4)-O-2,6-di-O-benzoyl-1-thio-β-D-glucopyranoside 127**

To a solution of para-nitrophenyl O-(methyl 5-acetamido-4,7,8,9-tera-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylate)-(2→3)-O-(6-O-benzoyl-β-

D-galactopyranosyl)-(1→4)-O-2,6-di-O-benzoyl-1-thio-β-D-glucopyranoside **122** (50 mg, 39.5 μmol) in dichloromethane (2.5 mL) was added acetic acid (400 μL) and zinc dust (30 mg). The mixture was stirred for 30 min at room temperature. TLC (3% methanol in dichloromethane) indicated the reaction to be finished (product R<sub>f</sub> = 0.44 and ninhydrin positive). The excess zinc dust was filtered out and the reaction mixture was concentrated under reduced pressure. The crude product was purified by radial silica gel chromatography (Chromatotron 1 mm plat, 3% ethanol in dichloromethane) to afford **127** (45.4 mg) as amorphous solid in 97% yield: [α]<sub>D</sub> -7.9° (c = 0.70, CHCl<sub>3</sub>); IR: (Thin film, ν<sub>cm</sub>): 3443 (br), 3068, 2947, 1731, 1601, 1585, 1535, 1451, 1370, 1255, 1119, 1070, 800, 714; <sup>1</sup>H-NMR δ (CDCl<sub>3</sub>): 8.10 -7.28 (m, 19H, Aromatic), 5.35 (ddd, 1H, J<sub>8',9a''</sub> = 2.7, J<sub>8',9b''</sub> = 6.8 Hz, H-8''), 5.32 (dd, 1H, J<sub>2,3</sub> = 9.3 Hz, H-2), 5.29 (d, 1H, NH), 5.28 (dd, 1H, J<sub>7'',8''</sub> = 8.9 Hz, H-7''), 5.08 (dd, 1H, J<sub>5,6a</sub> ≤ 1.0, J<sub>6a,6b</sub> = 11.8 Hz, H-6a), 4.97 (ddd, 1H, J<sub>4'',5''</sub> = 10.1 Hz, H-4''), 4.98 (d, 1H, J<sub>1,2</sub> = 10.0 Hz, H-1), 4.75 (dd, 1H, J<sub>5',6a'</sub> = 3.2, J<sub>6a',6b'</sub> = 12.0 Hz, H-6a'), 4.58 (d, 1H, J<sub>1',2'</sub> = 7.9 Hz, H-1'), 4.48 (dd, 1H, J<sub>5,6b</sub> = 6.6 Hz, H-6b), 4.34 (dd, 1H, J<sub>5',6b'</sub> = 8.9 Hz, H-6b'), 4.32 (dd, 1H, J<sub>9a'',9b''</sub> = 12.3 Hz, H-9a''), 4.15 (dd, J<sub>3',4'</sub> = 2.6 Hz, H-3'), 4.12 (dd, 1H, J<sub>6'',7''</sub> ≤ 1.0 Hz, H-6''), 4.03 (dd, 1H, J<sub>3,4</sub> = 9.0 Hz, H-3), 3.98-3.88 (m, 4H, H-5, H-5', H-5'', H-9b''), 3.79 (d, 1H, J<sub>2',3'</sub> = 10.5 Hz, H-2'), 3.78 (s, 3H, OCH<sub>3</sub>), 3.75 (dd, 1H, J<sub>4,5</sub> = 8.7 Hz, H-4), 3.68 (d, 1H, H-4'), 2.68 (dd, 1H, J<sub>3e'',4''</sub> = 4.4, J<sub>3e'',3a''</sub> = 12.9 Hz, H-3e), 2.14, 2.06, 2.02, 1.93, 1.88 (5s, 5x Ac); <sup>13</sup>C-NMR (CDCl<sub>3</sub>), δ (ppm): 170.8, 170.5, 170.3, 170.2 (OCOCH<sub>3</sub>, NCOCH<sub>3</sub>) 168.0(C-1''), 166.6, 166.0, 165.3 (PhCO), 133.2-122.5 (24 C, aromatic C), 104.4 (C-1'), 97.5 (C-2''), 85.1 (C-1), 82.2 (C-4), 76.8 (C-5), 76.5 (C-3'), 75.0 (C-3), 73.1, (C-6''), 72.9 (C-5'), 71.7 (C-2), 68.9(C-8''), 68.8 (C-2'), 68.2 (C-4''), 68.1 (C-4'), 67.0 (C-7''), 63.7 (C-6), 63.6(C-6'), 62.5 (C-9''), 53.3 (OCH<sub>3</sub>), 49.8 (C-5''), 37.8 (C-3''), 23.2 (NCOCH<sub>3</sub>), 21.1, 20.8, 20.7, 20.61 (OCOCH<sub>3</sub>).

**Para-N-Acrylamidophenyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosylonate)-(2 $\rightarrow$ 3)-O-(6-O-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-O-2,6-di-O-benzoyl-1-thio- $\beta$ -D-glucopyranoside 128**

Compound 127 (49 mg, 0.0397 mmol) was dissolved in dichloromethane (3 mL). To this solution was added triethyl amine to adjust the pH *ca.* 8.0. The solution was then cooled to 0°C and acryloyl chloride (460  $\mu$ L) in tetrahydrofuran (1% v/v) was added in a 15 min. period. The solution was then allowed to reach room temperature and stirring continued for an additional 2 hours. The solution was directly concentrated under vacuum to dryness. The residue was purified by silica gel chromatography using 2% ethanol in dichloromethane as an eluent to afford the expected product 128 (43.6 mg) in 85% yield as amorphous solid:  $[\alpha]_D -5.1^\circ$  ( $c = 0.75$ ,  $\text{CHCl}_3$ ); IR: (Thin film,  $\nu_{\text{cm}}$ ): 3469-3461 (br), 3067, 2956, 2925, 1735, 1594, 1529, 1451, 1370, 1254, 1121, 1038, 835, 716; (+)FAB-MS (glycerol) gave  $m/e$  (ion, relative intensity): 1289.4 ( $[\text{M}]^+$ , 1.3%), 1110.3 ( $[\text{M-aglycon}]^+$ , 4.7%);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.09-7.13 (m, 19H, Aromatic), 6.40 (dd, 1H,  $J_{\text{gem}} = 1.1$ ,  $J_{\text{trans}} = 16.8$  Hz, C=CH<sub>2</sub>, trans), 6.20 (dd, 1H,  $J_{\text{cis}} = 10.3$  Hz, C(O)CH=), 5.75 (dd, 1H, C=CH<sub>2</sub>, cis), 5.33 (ddd, 1H,  $J_{8'',9a''} = 2.5$ ,  $J_{8'',9b''} = 6.6$  Hz, H-8''), 5.26 (dd, 1H,  $J_{7'',8''} = 8.2$  Hz, H-7''), 5.24 (d, 1H,  $J = 9.8$  Hz, NH), 5.21 (dd, 1H,  $J_{2,3} = 9.3$  Hz, H-2), 4.99 (dd, 1H,  $J_{5,6a} = 1.9$ ,  $J_{6a,6b} = 12.1$  Hz, H-6a), 4.97 (ddd, 1H,  $J_{3a'',4''} = 11.5$ ,  $J_{3e'',4''} = 4.6$ ,  $J_{4'',5''} = 10.0$  Hz, H-4''), 4.77 (d, 1H,  $J_{1,2} = 10.0$  Hz, H-1), 4.74 (dd, 1H,  $J_{5',6a'} = 3.5$ ,  $J_{6a',6b'} = 12.0$  Hz, H-6a'), 4.56 (d, 1H,  $J_{1',2'} = 7.9$  Hz, H-1'), 4.50 (dd, 1H,  $J_{5,6b} = 6.1$  Hz, H-6b), 4.33 (dd, 1H,  $J_{5',6b'} = 8.8$  Hz, H-6b'), 4.29 (dd, 1H,  $J_{9a'',9b''} = 12.5$  Hz, H-9a''), 4.13 (dd,  $J_{3',4'} = 3.4$  Hz, H-3'), 4.11 (dd, 1H,  $J_{6'',7''} = 1.6$  Hz, H-6''), 3.97 (dd, 1H,  $J_{3,4} = 9.0$  Hz, H-3), 3.94-3.82 (m, 5H, H-5, H-5', H-3'', H-5'', H-9b''), 3.76 (d, 1H,  $J_{2',3'} = 9.4$  Hz, H-2'), 3.69 (dd, 1H,  $J_{3,4} \leq 1.0$ ,  $J_{4,5} = 9.0$  Hz, H-4), 3.69 (dd, 1H, H-4'), 2.67 (dd, 1H,  $J_{3e'',4''} = 4.6$ ,  $J_{3e'',3a''} = 13.0$  Hz, H-3e), 2.12, 2.05, 2.01, 1.93, 1.88 (5s, 5x Ac);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 170.8, 170.5, 170.3, 170.2 (2C), (OCOCH<sub>3</sub>, NCOCH<sub>3</sub>) 168.1(C-1''), 166.6, 166.1, 165.3 (PhCO), 134.5-128.4 (26C, aromatic C, CH=CH<sub>2</sub>), 104.4 (C-1'), 97.5 (C-2''), 85.8 (C-1), 82.1 (C-4), 76.6 (C-5), 76.4 (C-3'), 75.0 (C-3), 73.0 (C-6''), 72.9 (C-5),

71.4 (C-2), 69.0 (C-2') 68.8(C-8''), 68.2, 68.1 (C-4', C-4''), 67.1 (C-7''), 63.6 (2C, C-6, C-6'), 62.4 (C-9''), 53.3 (OCH<sub>3</sub>), 49.8 (C-5''), 37.7 (C-3''), 23.2 (NCOCH<sub>3</sub>), 21.1, 20.80, 20.7, 20.6 (OCOCH<sub>3</sub>).

Anal Calcd for C<sub>62</sub>H<sub>68</sub>O<sub>26</sub>N<sub>2</sub>S (1289.28): C, 57.75; H, 5.32; N, 2.17. Found: C, 58.14; H: 5.92; N, 2.02.

**Para-Nitrophenyl (5-acetamido-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosylonic acid)-(2 $\rightarrow$ 3)- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1-thio- $\beta$ -D-glucopyranoside 130**

To a methanolic solution (3 mL) containing **122** (50 mg, 0.04 mmol) was added sodium methoxide (1.0 M) until the pH of the reaction mixture became *ca.* 8.5. The reaction mixture was stirred for 3 hours at room temperature. To this mixture was added 350  $\mu$ L of 1.0 M NaOH solution and stirring was continued for additional 8 hours. TLC (15 % water/acetonitrile) showed complete conversion of the starting material ( $R_f$  = 0.93) to product ( $R_f$  = 0.23). The mixture was then neutralized with H<sup>+</sup> resin (IR 120) and filtered. The filtrate and washings were combined and concentrated under reduced pressure. The resulting residue was then purified by radial silica gel chromatography (10 % water/acetonitrile) to afford **130** (29.7 mg) in 95% yield as an amorphous mass:  $[\alpha]_D^{25}$ : -60.1° (c = 0.88, water); IR: (Thin film,  $\nu_{cm^{-1}}$ ): 3395, 2929, 1704, 1610, 1515, 1393, 1342, 1222, 1064; (+)FAB-MS (glycerol) gave m/e (ion, relative intensity): 793.2 ([M+1]<sup>+</sup>, 1.6 %), 638.2 ([M-HSPHNO<sub>2</sub>]<sup>+</sup>, 0.6 %); <sup>1</sup>H-NMR (500 MHz, D<sub>2</sub>O)  $\delta$  (ppm): 8.28 (d, 2H,  $J_{o,m}$  = 7.1 Hz, H-mata), 7.70 (d, 2H, H-ortho), 5.16 (d, 1H,  $J_{1,2}$  = 9.9 Hz, H-1), 4.64 (d, 1H,  $J_{1',2'}$  = 7.9 Hz, H-1'), 4.20 (dd,  $J_{3',4'}$  = 3.1 Hz, H-3'), -3.74 (H-4''), 3.67 (d, 1H,  $J_{2,3}$  = 9.7 Hz, H-2'), 3.62 (dd, 1H,  $J_{2,3}$  = 8.5 Hz, H-2), 2.85 (dd, 1H,  $J_{3e',4e'}$  = 4.6,  $J_{3e',3a'}$  = 12.5 Hz, H-3e), 2.12 (s, NHAc), 1.89 (t,  $J_{3a',4a'}$  = 11.7 Hz, H-3a); <sup>13</sup>C-NMR (125.8 MHz, D<sub>2</sub>O),  $\delta$  (ppm): 174.6 (NCOCH<sub>3</sub>) 173.4 (C-1'') 145.6 (C-ipso), 142.8 (C-para), 128.7 (C-mata), 123.7 (C-ortho), 102.2 (C-1'), 99.4 (C-2''), 85.1 (C-1), 78.4 (C-4), 77.4 (C-5'), 75.3 (C-3'), 75.1 (C-5), 74.8 (C-3), 72.5 (C-6''), 71.3 (C-8''), 71.0 (C-2), 68.9 (C-2'), 67.9

(C-4''), 67.7 (C-7''), 67.1 (C-4'), 62.2 (C-9'), 60.6 (C-6'), 59.7 (C-6), 51.3 (C-5''), 39.2 (C-3''), 21.6 (NCOCH<sub>3</sub>).

Anal Calcd for C<sub>29</sub>H<sub>50</sub>N<sub>2</sub>O<sub>24</sub>SNa: C, 40.02; H, 5.71; Found: C, 39.83, H, 5.63.

**Para-N-Acrylamidophenyl (5-Acetamido-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosylonic acid)-(2 $\rightarrow$ 3)- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1-thio- $\beta$ -D-glucopyranoside 131**

To a solution of **130** (52 mg, 0.066 mmol) in 3 mL of methanol-acetic acid (5:3) was added Zinc dust (12 mg). The mixture stirred for 3 hours at room temperature. TLC (15% water/acetonitrile) showed reduction of the nitro group into amine (R<sub>f</sub> = 0.21). Triethylamine was added to neutralize the reaction mixture. The reaction mixture was filtered and concentrated under vacuum to dryness. The crude product was dissolved in methanol (3 mL). To the solution was added triethylamine and the pH of the reaction mixture was adjusted to *ca.* 8.0. The solution was then cooled to 0°C and 800  $\mu$ L of acryloyl chloride in dry THF (1% v/v) was added at 0°C over a five minute period. The solution was stirred at room temperature for 1 hour and an additional 8.6  $\mu$ L of acryloyl chloride was added. The progress of the reaction was monitored by TLC (15 % water/acetonitril). After 3 hours, TLC showed that complete conversion of the amine to acrylamide was achieved. The solution was concentrated under vacuum to dryness. The residual mass was purified by radial silica gel chromatography (1 mm) using a gradient of 5%-15% water in acetonitrile. Compound **131** (47 mg) was obtained as amorphous solid. The total yield for the two steps was 83%: [ $\alpha$ ]<sub>D</sub> -68.2° (c = 0.80, water); (+)FAB-MS (glycerol) gave m/e (ion, relative intensity): 817.3 ([M+1+Na]<sup>+</sup>, 2.0 %); <sup>1</sup>H-NMR (500 MHz, D<sub>2</sub>O)  $\delta$  (ppm): 7.68 (d, 2H, J<sub>o,m</sub> = 8.7 Hz, H-meta), 7.59 (d, 2H, H-ortho), 6.50 (dd, 1H, J<sub>cis</sub> = 10.1, J<sub>trans</sub> = 17.1 Hz, C(O)CH=), 6.41 (dd, 1H, J<sub>gem</sub> = 1.3 Hz, C=CH<sub>2</sub>, trans), 5.95 (dd, 1H, C=CH<sub>2</sub>, cis), 4.86 (d, 1H, J<sub>1,2</sub> = 9.8 Hz, H-1), 4.59 (d, 1H, J<sub>1,2</sub> = 7.9 Hz, H-1'), 4.17 (dd, J<sub>3,4</sub> = 3.1 Hz, H-3'), 4.02 (dd, J<sub>4,5</sub> < 1.0 Hz, H-4'), -3.79 (H-4''), 3.63 (d, 1H, J<sub>2,3</sub> = 10.0 Hz, H-2'), 3.45 (dd, 1H, J<sub>2,3</sub> = 9.6 Hz, H-2), 2.82 (dd, 1H,

$J_{3e',4''} = 4.6$ ,  $J_{3e',3a''} = 12.5$  Hz, H-3e), 2.10 (s, NHAc), 1.86 (t,  $J_{3a'',4''} = 11.7$  Hz, H-3a);  $^{13}\text{C}$ -NMR (125.8 MHz,  $\text{D}_2\text{O}$ )  $\delta$  (ppm): 174.6 (NCOCH<sub>3</sub>), 173.4 (C-1''), 166.4 (COCH=CH<sub>2</sub>), 136.5 (C-ipso), 132.5 (C-meta), 129.8 (C-para), 128.2 (-CH=), 127.5 (=CH<sub>2</sub>), 121.6 (C-ortho), 102.1 (C-1'), 99.4 (C-2''), 86.8 (C-1), 78.3 (C-4), 77.4 (C-5'), 75.3 (C-5), 75.0 (C-3'), 74.7 (C-3), 72.4 (C-6''), 71.3 (C-8''), 70.9 (C-2), 68.9 (C-2'), 67.9 (C-4''), 67.7 (C-7''), 67.0 (C-4'), 62.1 (C-9'), 60.6 (C-6'), 59.7 (C-6), 51.2 (C-5''), 39.2 (C-3''), 21.6 (NCOCH<sub>3</sub>).

**Copoly (Para-N-Acrylamidophenyl (5-acetamido-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosylonic acid)-(2 $\rightarrow$ 3)- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1-thio- $\beta$ -D-glucopyranoside) 132**

Method A:

GM<sub>3</sub> derivative **131** (8.4 mg, 10.6  $\mu\text{mol}$ ) and acrylamide (7.4 mg, 106  $\mu\text{mol}$ ) were dissolved in deoxygenated water (250  $\mu\text{L}$ ) obtained by multiple cool-thaw cycles and bubbling nitrogen. A small amount of  $(\text{NH}_4)_2\text{S}_2\text{O}_8$  (0.1 mg, 0.424  $\mu\text{mol}$ ) was added by injecting of 20  $\mu\text{l}$  of stock solution 10 mg/200  $\mu\text{l}$  in water. The reaction mixture was stirred 90 °C for 15 min. TLC (MeOH/ $\text{CH}_2\text{Cl}_2$ , 1:1 in v/v) showed no monomer left. The reaction mixture was diluted with water (1 mL) and dialyzed (2,000 MW cutoff) against multiple 1 l volumes of distilled water. Lyophilization gave 9 mg (57%) of GM<sub>3</sub> glycopolymer **132** as a sponge solid. The GM<sub>3</sub> monomer incorporation in the glycopolymer was 1:11 which was determined using  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ ) spectroscopy. In a parallel manner, GM<sub>3</sub> glycopolymers with ratios of monomer to acrylamide 1:5, 1:27, 1:48 were prepared with load ratio of 1:5, 1:20, 1:30 respectively in 50-70 % yields.

Method B:

GM<sub>3</sub> derivative **131** (14 mg, 10.86  $\mu\text{mol}$ ) and acrylamide (7.7 mg, 108.6  $\mu\text{mol}$ ) were dissolved in 250  $\mu\text{L}$  of DMSO in screw-capped test tube and the solution was frozen and degassed three times under reduced pressure by placing the tube in a dry ice-acetone bath. The vial was sealed with Teflon tape. The reaction mixture was then warmed 45 °C

and injected with 37  $\mu\text{L}$  of a 10 mg/mL solution of AIBN (2.7 mol %). The polymerization was left to proceed at 45  $^{\circ}\text{C}$  for 3 hours. The solution was then diluted with 1 mL of DMSO. Lyophilization gave 21 mg of spongy solid. Zemplén deacetylation and saponification was done by adding 150  $\mu\text{L}$  of 1M NaOMe/MeOH to the crude product in 2 mL methanol-DMSO (1:1) and the resulting mixture was stirred for 12 hours at room temperature. 1 M NaOH (300  $\mu\text{L}$ ) was then added to the mixture which was stirred for an additional 18 hours at room temperature, neutralized with Amberlite IR-120 ( $\text{H}^+$ ) resin and filtered. The resin was washed with methanol, and the combined filtrates and washings were concentrated under reduced pressure. To residue was then added 1 mL of water and the solution was dialyzed (2,000 MW cutoff) exhaustively against distilled water. The aqueous solution of the polymer was lyophilized to afford GM<sub>3</sub>-S-CPA 132 (9.8 mg) in 61% yield as a white spongy solid:  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ ) analysis gave a 1/10.3 molar ratio of GM<sub>3</sub>/acrylamide incorporation in glycopolymer.

$^1\text{H}$ -NMR  $\delta$  (500 MHz,  $\text{D}_2\text{O}$ ): 7.62 (br, H-meta), 7.55 (br, H-ortho), 4.83 (d, 1H,  $J_{1,2} = 9.2$  Hz, H-1), 4.60 (d, 1H,  $J_{1,2} = 7.1$  Hz, H-1'), 4.18 (1H, H-3'), 4.03 (1H, H-4'), 3.97-3.62 (m, 16H), 3.43 (1H, H-2), 2.83 (dd, 1H,  $J_{3e,4a} = 3.7$ ,  $J_{3e,3a} = 11.3$  Hz, H-3e), 2.40-2.12 (br, methine of backbone), 2.10 (s, NHAc), 2.06-1.71 (methylene of backbone);  $^{13}\text{C}$ -NMR (125.8 MHz,  $\text{D}_2\text{O}$ ),  $\delta$  (ppm): 179.1 (br, NHAc of backbone), 174.6 (NCOCH<sub>3</sub>) 173.5 (C-1"), 132.4 (br, C-ortho), 121.6 (br, C-meta), 102.2 (C-1'), 99.4 (C-2"), 86.9 (C-1), 78.3 (C-4), 77.5 (C-5'), 75.4 (C-5), 75.1 (C-3'), 74.4 (C-3), 72.5 (C-6"), 71.4 (C-8"), 71.0 (C-2), 69.0 (C-2'), 68.0 (C-4"), 67.7 (C-7"), 67.1 (C-4'), 62.2 (C-9'), 60.6 (C-6'), 59.7 (C-6), 51.3 (C-5"), (43.2-41.3) (br, methine of backbone), 39.2 (C-3"), 36.4-34.2 (br, methylenes of backbone), 21.67 (CH<sub>3</sub> of NHAc).

**N-(3-thioacetyl) propanamidophenyl O-(methyl 5-acetamido-4,7,8,9-tera-O-acetyl-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosylate)-(2 $\rightarrow$ 3)-O-(6-O-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,6-di-O-benzoyl-1-thio- $\beta$ -D-glucopyranoside**  
**133**

To an ethyl acetate solution (1.5 mL) containing **128** (50 mg, 38.8  $\mu$ mol) was added thioacetic acid (3.5  $\mu$ L) at room temperature. To the reaction mixture was added triethylamine from a stock solution (1M in ethyl acetate) to adjust the pH to *ca* 8.0. The reaction mixture was stirred under N<sub>2</sub> atmosphere for 5 hours at room temperature. TLC (5% ethanol in dichloromethane) showed a completion of reaction. The reaction mixture was concentrated to dryness under reduced pressure and the residue was subjected to silica gel chromatography (2% ethanol in dichloromethane) to give compound **133** (22.14 mg) in 84% yield: m.p. 129-131°C;  $[\alpha]_D = -4.4^\circ$  (c = 0.74, CHCl<sub>3</sub>); IR: (Thin film,  $\nu_{cm}$ ): 3471, 3459 br, 3065, 2956, 2926, 1743, 1728, 1693, 1527, 1494, 1450, 1370, 1274, 1227, 1121, 1070, 1039, 834, 713; (+)FAB-MS (glycerol) gave m/e (ion, relative intensity): 2731.6 (2xM<sup>+</sup>, 2.6%), 1365.4 (M<sup>+</sup>, 5.7%), 1110.5 (M<sup>+</sup>-aglycon, 7.4 %); <sup>1</sup>H-NMR  $\delta$  (CDCl<sub>3</sub>): 8.08-7.24 (m, 19H, Aromatic), 5.33 (ddd, 1H,  $J_{8'',9a''} = 2.5$ ,  $J_{8'',9b''} = 6.6$  Hz, H-8''), 5.26 (dd, 1H,  $J_{7'',8''} = 8.1$  Hz, H-7''), 5.20 (dd, 1H,  $J_{2,3} = 9.3$  Hz, H-2), 4.99-4.94 (m, 2H, H-6a, H-4''), 4.76 (d, 1H,  $J_{1,2} = 10.1$  Hz, H-1), 4.73 (dd, 1H,  $J_{5,6a'} = 3.7$  Hz,  $J_{6a',6b'} = 12.1$  Hz, H-6a'), 4.56 (d, 1H,  $J_{1',2'} = 7.9$  Hz, H-1'), 4.50 (dd, 1H,  $J_{5,6b} = 6.3$ ,  $J_{6a,6b} = 11.9$  Hz, H-6b), 4.34 (dd, 1H,  $J_{5',6b'} = 7.1$  Hz, H-6b'), 4.29 (dd, 1H,  $J_{9a'',9b''} = 12.5$  Hz, H-9a''), 4.14 (dd,  $J_{3',4'} = 3.3$  Hz, H-3'), 4.12 (dd, 1H,  $J_{6'',7''} = 1.7$  Hz, H-6''), 3.97 (dd, 1H,  $J_{3,4} = 8.9$  Hz, H-3), 3.93-3.84 (m, 4H, H-5, H-5', H-5'', H-9b''), 3.77 (s, 3H, OCH<sub>3</sub>), 3.77 (d, 1H,  $J_{2',3'} = 10.4$  Hz, H-2'), 3.69-3.6 (m, 2H, H-4, H-4'), 3.19 (t, 2H, J = 6.9 Hz, SCH<sub>2</sub>), 2.67 (dd, 1H,  $J_{3e'',4''} = 4.7$ ,  $J_{3e'',3a''} = 13.0$  Hz, H-3e), 2.64 (t, 2H, J = 6.9 Hz, CH<sub>2</sub>CO), 2.33 (s, 3H, SAc), 2.13, 2.06, 2.02, 1.93, 1.88 (5s, 15 H, NHAc, OAc); <sup>13</sup>C-NMR (CDCl<sub>3</sub>),  $\delta$  (ppm): 170.8, 170.5, 170.2, 170.2 (5C, OCOCH<sub>3</sub>, NCOCH<sub>3</sub>), 168.6 (SAc), 168.1 (C-1''), 166.6, 166.1, 165.3 (PhCO), 134.4-119.8 (24C, aromatic C), 104.4 (C-1'), 97.5 (C-2''), 85.9 (C-1), 82.2 (C-4), 76.6 (C-5, C-3'), 76.4 (C-3), 75.0 (C-6''), 73.0 (C-5'), 72.9 (C-2),

71.7 (C-8''), 69.0 (C-2'), 68.8 (C-4') , 68.2 (C-4''), 68.1 (C-7''), 67.0 (C-6), 63.6 (C-6'), 62.4 (C-9''), 53.3 (OCH<sub>3</sub>), 49.8 (C-5''), 37.7 (C-3''), 37.6 (SCH<sub>2</sub>), 30.6 (SAc), 24.8 (CH<sub>2</sub>CONH), 23.2 (NCOCH<sub>3</sub>), 21.1, 20.8, 20.7, 20.6 (OAc).

Anal Calcd for (C<sub>64</sub>H<sub>72</sub>O<sub>27</sub>N<sub>2</sub>S<sub>2</sub>): C, 56.30, H, 5.32, N, 2.05. Found: C, 55.55; H, 5.39; N, 2.07.

#### **Agar gel double radial diffusion assays<sup>237</sup>**

Double radial diffusion assays were performed on 1% (w/v) agarose (BDH) containing 2% polyethylene glycol (PEG, molecular weight 8000, Sigma) in 0.1 M phosphate-buffered saline (PBS) pH = 7.2. Both glycopolymers and lectins were used at concentrations of 1 mg/mL in PBS. Aliquots (20 µL) of the respective polymer antigens and lectins were used to fill the wells perforated in the agarose gel slabs (≈ 1 mm thickness). Precipitin bands were allowed to form overnight at 4°C.

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## Chapter 6 Synthesis of Lewis X and Sulfated Lewis X Oligosaccharide as Selectin Ligands

### 6.1 Introduction

Sialyl Lewis X (SLe<sup>x</sup>), a terminal tetrasaccharide ( $\alpha$ -Neu5Ac-(2 $\rightarrow$ 3)- $\beta$ -D-Gal-(1 $\rightarrow$ 4)-[ $\alpha$ -L-Fuc-(1 $\rightarrow$ 3)]- $\beta$ -GlcNAc) of cell-surface glycoproteins and glycolipids, is a ligand for the endothelial leukocyte adhesion molecule-1 (ELAM-1), which mediates the early stage of adhesion of leukocytes to activated endothelial cells.<sup>238</sup> Given the connection of ELAM-1 to leucocytes and its role in their recruitment to inflammation sites, SLe<sup>x</sup> is emerging as an important biological tool and potential agent for the treatment of inflammation and related disorders.<sup>239</sup> Furthermore, the carbohydrate fragment of SLe<sup>x</sup> is found to be a tumor-associated oligosaccharide<sup>240</sup> and, thus, it may serve as a marker for tumor cell targeting.

Recently, Feizi and co-workers<sup>37</sup> reported the isolation of an equimolar mixture of sulfated Le<sup>x</sup> and Le<sup>a</sup> trisaccharides derived from an ovarian cystadenoma glycoprotein that binds more strongly to L-Selectin than to Le<sup>x</sup> and Le<sup>a</sup>. The sulfo-Le<sup>x</sup> not only is a ligand of selectins but also shows substantially greater binding activity for selectins than SLe<sup>x</sup> epitope.<sup>37</sup> Owing to the biological importance of these ligands in cell-adhesion processes, coupled with difficulties associated with their isolation from natural sources, their efficient chemical syntheses are in demand.

In oligosaccharide synthesis, two aspects have to be mastered. The first is stereoselectivity or, preferably, stereospecific glycosylation. The second one concerns protecting group manipulation in order to make sure that only specific hydroxyl groups

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<sup>239</sup> Mulligan, M. S.; Paulson, J. C.; Defrees, S.; Z.-L, Zheng; Lowe, J. B.; Ward, P A. *Nature* 1993, 364, 149.

<sup>240</sup> Fukushima, K.; Hirota, H.; Terasaki, P. I.; Waranabe, A.; Togashi, H.; Chia, D.; Sayama, N.; Fukushi, Y.; Nudelman, S.; Hakomori, S. *Cancer Res.* 1984, 44, 5279.

are glycosylated. Normally this means that all hydroxy groups not to be glycosylated have to be suitably protected. This in turn tends to make synthetic paths lengthy and time-consuming. One of the major breakthroughs in new strategies for oligosaccharide synthesis has been the use of “lightly protected” acceptors, i.e. acceptors where there are several OH groups unprotected, especially near the position at which one wishes the glycosidic bond to be formed. This “open” glycosylation, in which two or several hydroxyl groups are free and in which one hydroxyl group is preferentially glycosylated in the presence of the other(s) may offer shorter routes to oligosaccharide synthesis.

Previous synthetic strategies for sialyl Le<sup>x</sup><sup>241</sup> and sufo-Le<sup>x</sup><sup>242</sup> epitopes are first syntheses of the Le<sup>x</sup> trisaccharide building block. 4-O-Unprotected N-acetylglucosamine acceptor was first allowed to react with galactosyl donors<sup>243</sup> and then, after 3-O-deprotection, a fucosyl residue was attached<sup>244</sup> to give the Le<sup>x</sup> trisaccharide building block. Our synthetic strategy of sialyl Le<sup>x</sup> and sulfo-Le<sup>x</sup> is depicted in scheme 6.1. Retrosynthetic analysis of sialyl Le<sup>x</sup> and sufo-Le<sup>x</sup> led us to design an efficient glycosylation route to access Le<sup>x</sup> family. A selectively 6-O-TBDPS protected N-acetylglucosamine derivative **136** was used as key acceptor. Regioselective introduction of the galactosyl moiety allowed us to easily access the key intermediate N-acetyllactosamine building block. Subsequent fucosylation should give the Le<sup>x</sup> trisaccharide. Thus, the Le<sup>x</sup> trisaccharides can now be easily constructed with a short route from synthons derived from D-galactose, N-acetyl glucosamine and L-fucose respectively.

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<sup>241</sup> (a) Kameyama, A.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1991**, 209, C1-C4.

(b) Kamegawa, A.; Ishida, H.; Kiso, M.; Hasegawa, A.; *J. Carbohydr. Chem.* **1991**, 10, 549. (c)

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<sup>242</sup> (a) Nicolaou, K. C.; Bockovich, N. J.; Carcanague, D. R. *J. Am. Chem. Soc.*, **1993**, 115, 8843.

(b) Lubineau, J.; Gallic, J. L.; Lemoine, R. *J. Chem. Soc., Chem. Commun.* **1993**, 1419. (c)

Hasegawa, A.; Ito, K.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1995**, 14, 353.

<sup>243</sup> (a) Sato, S.; Ito, Y.; Ogawa, T.; *Tetrahedron Lett.* **1988**, 29, 5267; (b) Sato, S. Ito, Y.;

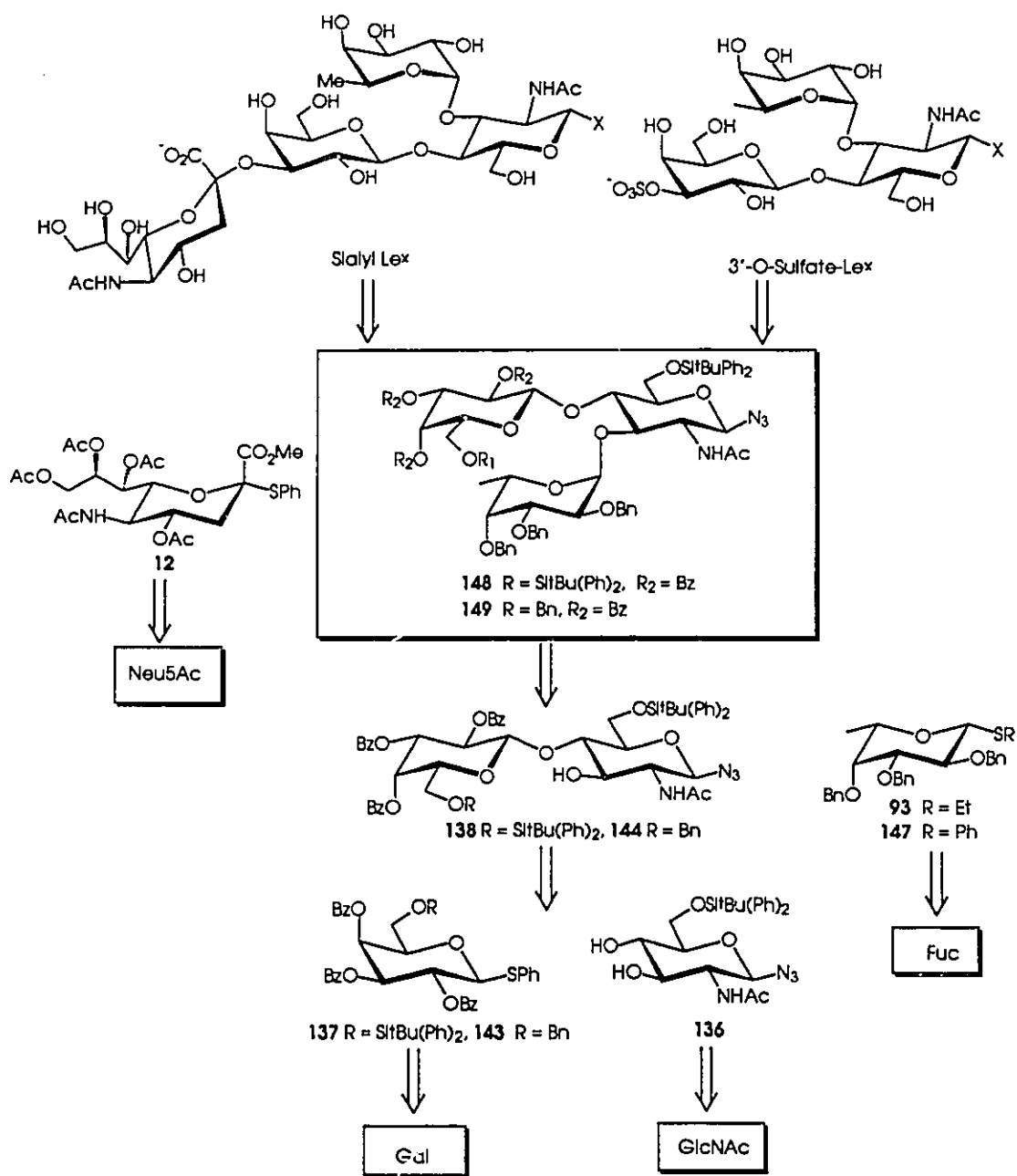
Nukads, T.; Nakahara, Y.; Ogawa, T.; *Carbohydr. Res.* **1987**, 167, 197.

<sup>244</sup> (a) Nicolaou, K. C.; Casulfield, T. J.; Katacka, H.; Styliadides, N. A. *J. Am. Chem. Soc.*

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*Chem. Soc.* **1992**, 114, 3126.

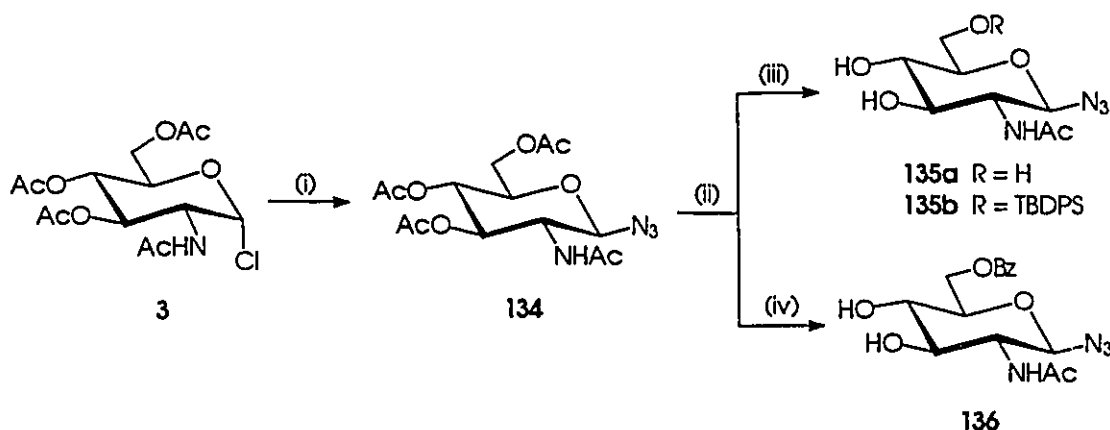


**Scheme 6.1** Structure and Retrosynthetic Disconnection of Sialyl Le<sup>x</sup> and 3'-O-Sulfate Le<sup>x</sup>

## 6.2 Result and discussion

### 6.2.1 Preparation of “Lightly” Protected Glycosyl Acceptors

For the synthesis of Le<sup>x</sup> trisaccharide, the first step was the synthesis of N-acetyllactosamine building block. Compound **135b** was selected as the key acceptor in the first glycosylation step. This compound was obtained from acetochloro N-acetylglucosamine **3**<sup>245</sup> in three steps as showed in scheme 6.2.



#### Scheme 6.2 Preparation of N-Acetyl Glucosamine Acceptors

(i) PTC: EtOAc/sat. NaHCO<sub>3</sub>, TBAHS, r.t., 2 h; 94%; (ii) NaOMe/MeOH, quantitative; (iii) TBDPSCI (1.5 equiv.), pyridine, r.t., 3 h, 92%; (iv) 1.5 equiv. BzCl, Pyridine: CH<sub>2</sub>Cl<sub>2</sub>, 1:2; -50 °C, 80.5%

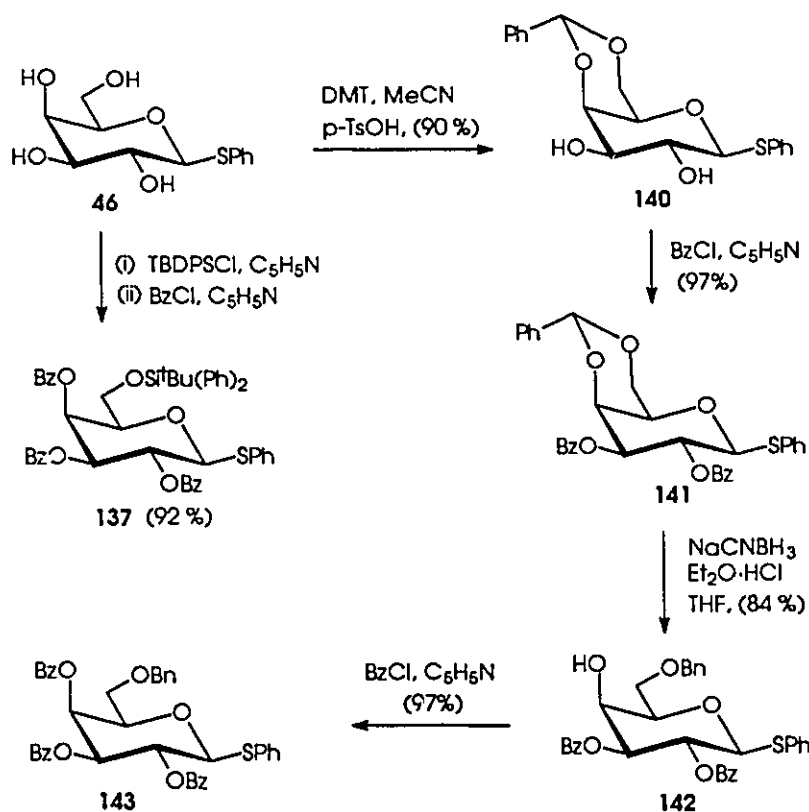
Acetochloro N-acetylglucosamine **3** was treated with sodium azide under PTC conditions<sup>58</sup> to give glycosyl azide **134** which was purified by crystallization from ethanol to give **134** in 94% yield. The azide **134** has a  $\beta$  configuration as confirmed by <sup>1</sup>H NMR spectroscopy (H-1 at  $\delta$  4.74 ppm,  $J_{1,2} = 9.3$  Hz). Subsequent Zemplén deacetylation and selective silylation of OH-6 with TBDPSCI in pyridine gave azide acceptor **135b** in 88% overall yield. This facile chemistry allowed us to prepare the key acceptor **135b** in large scale without any difficulty. Another glycosyl acceptor **136** could be obtained in similar

<sup>245</sup> Chapter one of this thesis.

fashion except that a selective 6-O-benzoylation was executed under kinetically controlled conditions (-55°C) and afforded 6-O-benzoylated acceptor **136** in 81% yield. The location of the 6-O-benzoyl group in **136** was verified by <sup>1</sup>H NMR spectroscopy. The primary H-6 protons appeared at low field δ 4.56-4.36 ppm, indicating that the benzoylation had occurred at OH-6.

## 6.2.2 Preparation of Galactosyl Donors

As discussed in Chapter 3.3, galactosyl donor **137** (Scheme 6.3) was easily available by a simple one pot, two step reaction from phenyl thiogalactoside **46**. Thus successive treatment of triol **46** with tert-butyldiphenylsilyl chloride (1.2 equiv.) and benzoyl chloride (3.6 equiv.) in dry pyridine gave galactosyl donor **137** in 92% overall yield. In contrast, starting from the same starting material **46**, four steps were needed to obtain galactosyl donor **143**. Triol **46** was first treated with benzaldehyde dimethyl acetal in dry acetonitrile under acid catalysis (*p*-TsOH) to give acetal **140** in 90% yield. Subsequent benzoylation with benzoyl chloride in dry pyridine gave compound **141** in 97% yield. The treatment of benzylidene acetal **141** with NaCNBH<sub>3</sub> and HCl·Et<sub>2</sub>O (obtained by bubbling hydrogen chloride gas into a diethyl ether solution for 25 min) gave galactosyl derivative **142** in 84 % yield. Finally, treatment of **142** with benzoyl chloride in pyridine again finished the preparation of galactosyl donor **143**.



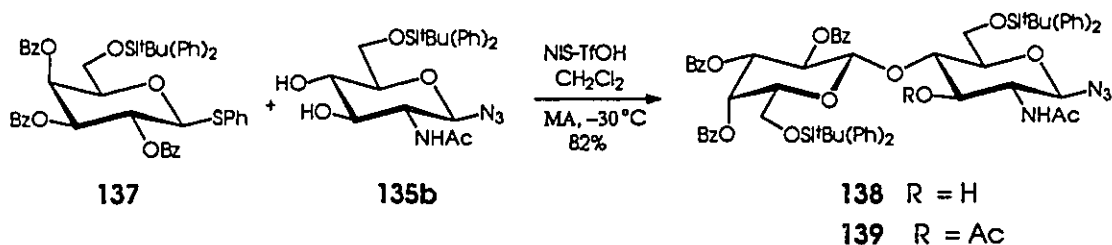
### Scheme 6.3 Synthesis of Galactopyranosyl Donors

The reductive benzylidene ring opening of **141** is worthy of additional comments. It was found that the benzylidene ring opening was very slow and incomplete if the reaction system was under basic or neutral conditions. Enough hydrogen chloride should be added to keep the pH of the solution at approximately 5. The ring opening reaction was very fast (usually finished in 10 min) and highly regioselective and the benzoyl groups are compatible under these acidic conditions. Quite surprisingly, the  $R_f$  of the ring opened product **142** with a 4-OH free was higher than that of the fully protected precursor **141** using 2% methanol in dichloromethane as eluent. The regioselectivity of the ring opening of 4,6-benzylidene acetal was confirmed by  $^1\text{H-NMR}$  spectroscopy, in a  $^1\text{H-}^1\text{H}$  COSY spectrum of **142**, a coupling of H-4 with 4-OH was observed indicating that the free OH was at C-4 rather than at C-6. Furthermore, after benzoylation, the strong downfield shift

for the H-4 signal at  $\delta$  5.94 ppm ( $\Delta\delta = +1.54$  ppm) in **143** confirmed the regiochemistry of the benzylidene ring opening reaction.

### 6.2.3 Synthesis of N-Acetylactosamine, a Key Building Block for the Synthesis of Le<sup>x</sup> Family

With suitable glycosyl donors and acceptors in hand, condensation of diol acceptor **135b** with donor **137** (Scheme 6.4) was performed in dichloromethane in the presence of NIS/TfOH at -30 °C. The reaction afforded the desired  $\beta$ -(1 $\rightarrow$ 4)-linked disaccharide **138** in 82% yield. Very impressively, no  $\beta$ -(1 $\rightarrow$ 3)-linked regioisomer was detected during this coupling reaction. Disaccharide **138** is an important acceptor for the synthesis of Lewis x derivatives.



**Scheme 6.4** Regioselective Synthesis of N-Acetylactosamine Derivative **138**

The  $\beta$ -configuration of the disaccharide **138** was deduced from the  $^1\text{H-NMR}$  spectrum which showed H-1' as a doublet at  $\delta$  5.06 ppm ( $J_{1',2'} = 8.0$  Hz). The regiochemistry of the newly introduced glycosidic linkage of **138** was proved by converting **138** to its corresponding acetylated (Py-Ac<sub>2</sub>O) derivative **139** which showed H-3 at  $\delta$  4.98 ppm (dd,  $J_{3,4} = 9.2$  Hz,  $\Delta\delta = +1.04$  ppm) as a deshielded signal in its  $^1\text{H-NMR}$  spectrum. As examples, the  $^1\text{H-NMR}$ ,  $^1\text{H-}^1\text{H}$  COSY,  $^{13}\text{C-NMR}$ , HMQC spectra of **138** are given in Figures 6.1 and 6.2.

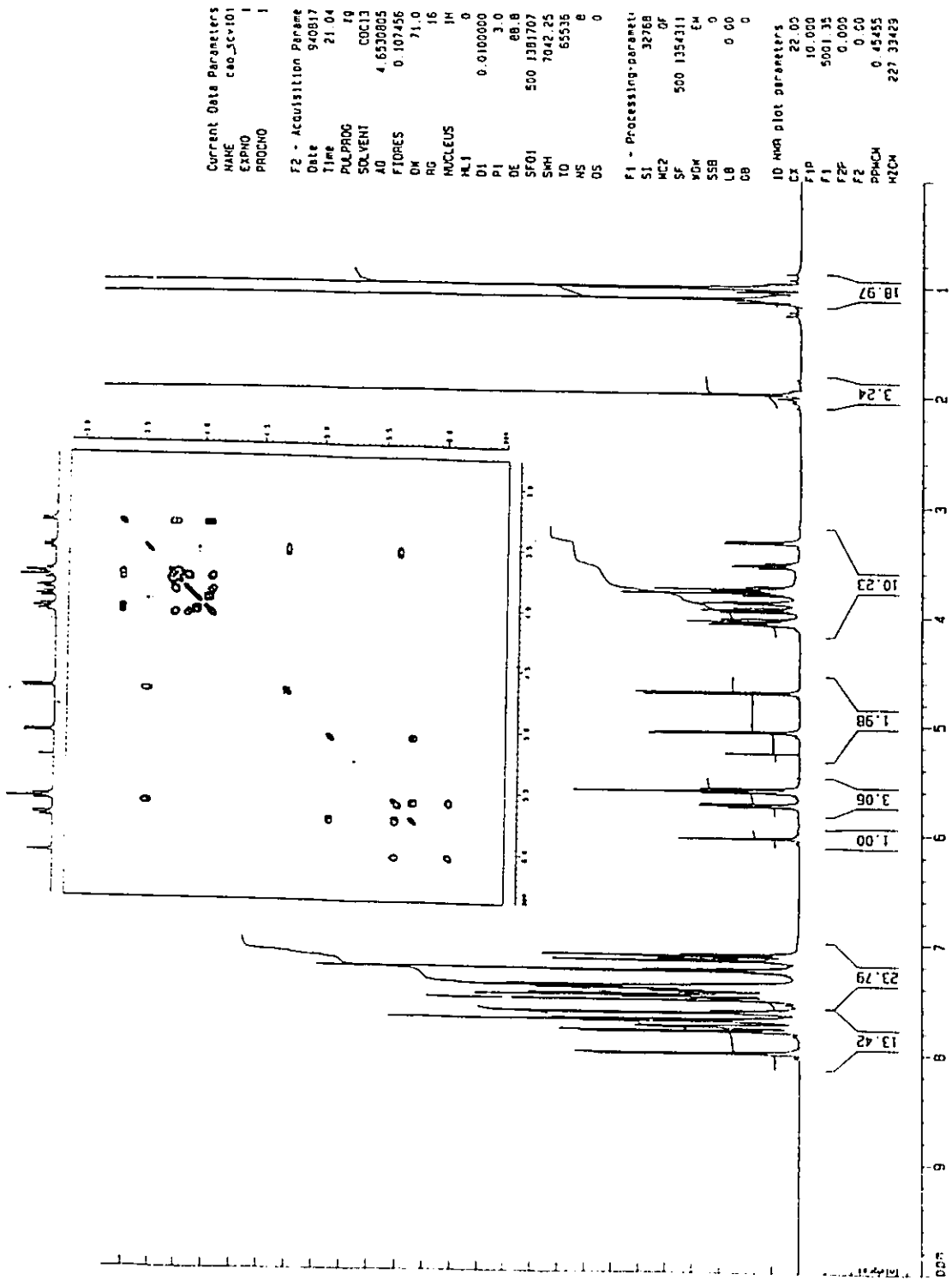


Figure 6.1 <sup>1</sup>H-NMR and <sup>1</sup>H-<sup>1</sup>H COSY Spectra (500 MHz, CDCl<sub>3</sub>) of 138

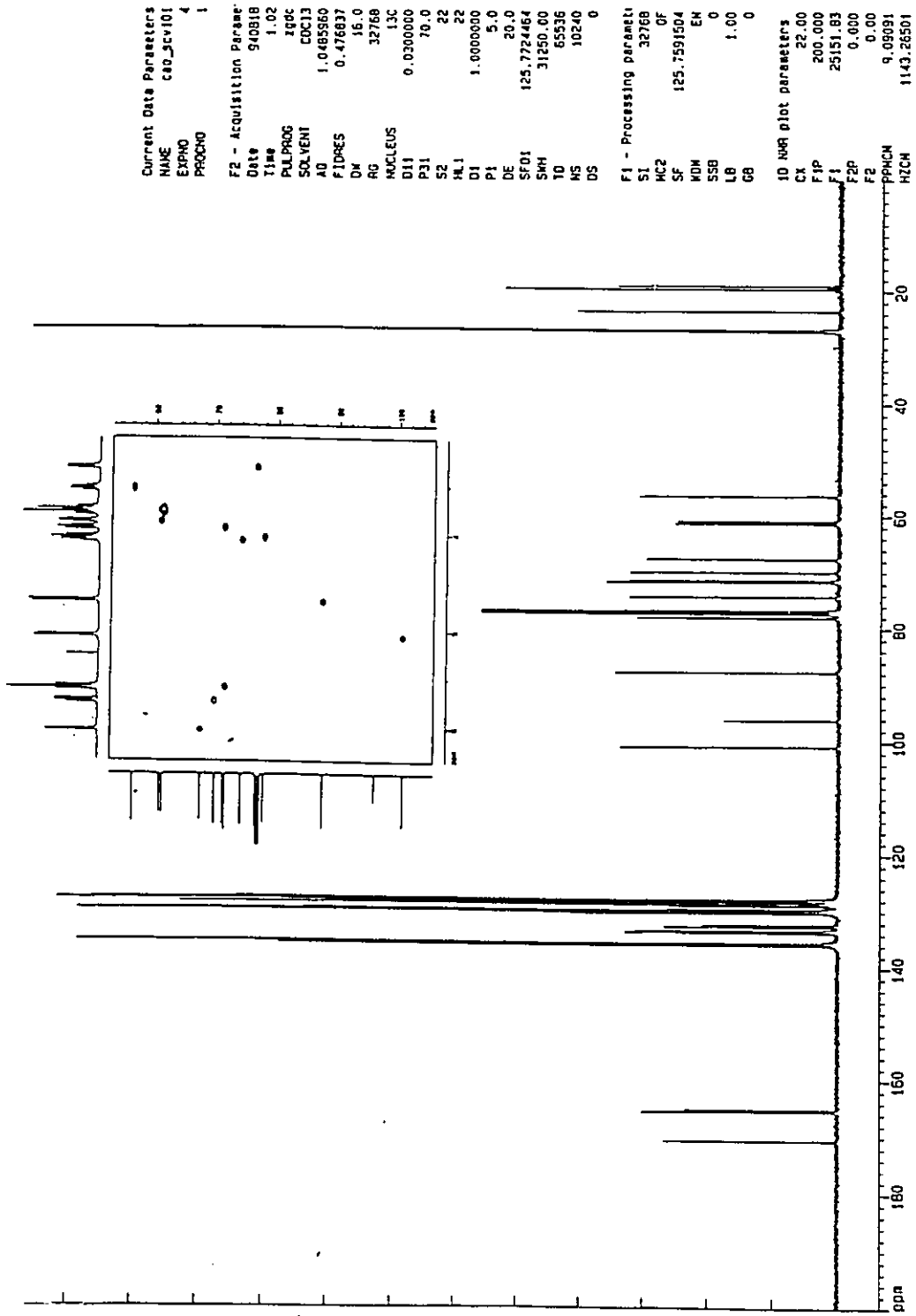
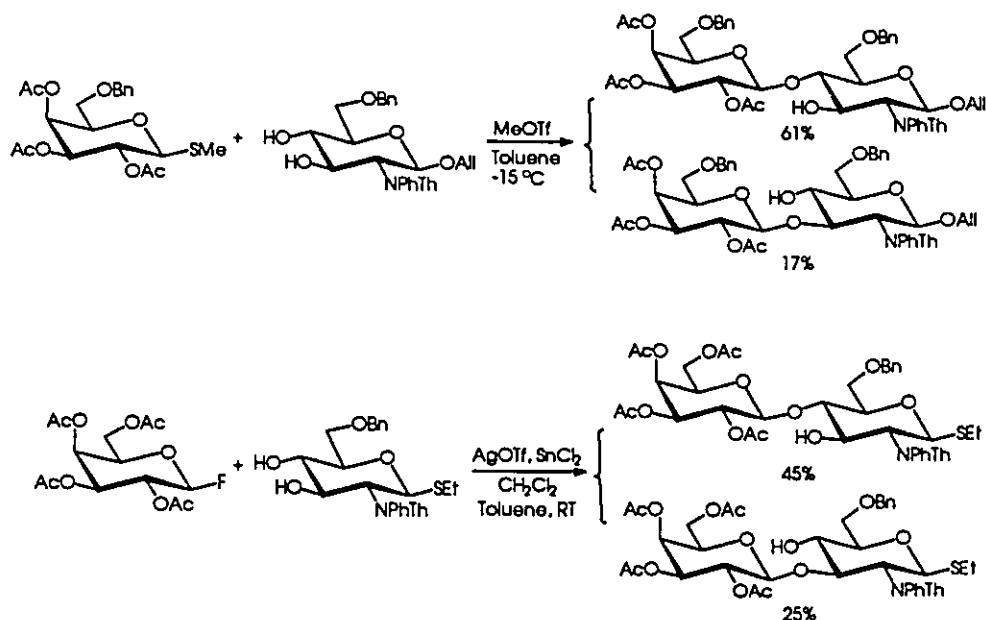


Figure 6.2  $^{13}\text{C}$ -NMR and  $^1\text{H}$ - $^{13}\text{C}$ -HMQC Spectra (125.7 MHz,  $\text{CDCl}_3$ ) of 138

The regioselectivity of this glycosylation reaction was remarkably high as compared with results reported by Ogawa's group<sup>246</sup> and very recently by Sinaÿ's group.<sup>247</sup> In these cases both the  $\beta(1\rightarrow4)$  and  $\beta(1\rightarrow3)$  linked disaccharides were obtained in 61 and 17% yield by Ogawa's group, while Sinaÿ *et.al.*<sup>247</sup> obtained  $\beta(1\rightarrow4)$  and  $\beta(1\rightarrow3)$  linked disaccharide in a 2:1 ratio (Scheme 6.5).



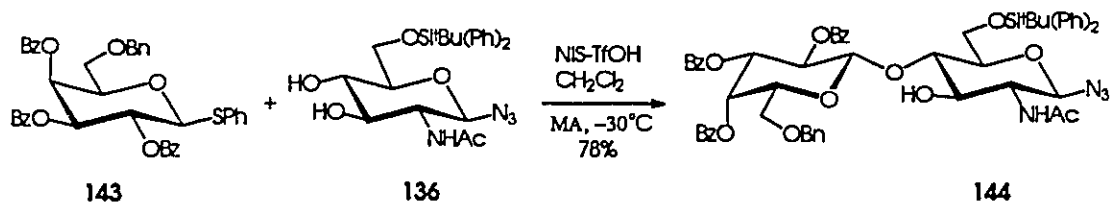
**Scheme 6.5** Observed Regioselectivity in the Glycosylation of GlcNAc Derivatives. Above (Ogawa *et al.*), below (Sinaÿ *et al.*)

We first reasoned that the steric effect of the *tert*-butyldiphenylsilyl protecting groups played a key role in the outcome of the regioselective glycosylation. The very bulky protecting 6-O-TBDPS groups on both the glycosyl donor 137 and acceptor 136

<sup>246</sup> Numomura, S.; Iida, M.; Numata, M.; Sugimoto, M.; Ogawa, T. *Carbohydr. Res.* 1994, 263, C1

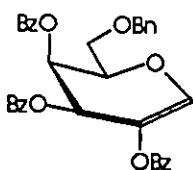
<sup>247</sup> Zhang, Y.-M.; Brodzky, A.; Sinaÿ, P.; Saint-Marcoux, G.; Perly, B. *Tetrahedron Asymmetry* 1995, 6, 1195.

may have contributed to the remarkable regioselectivity. To test this hypothesis, a similar glycosylation reaction between the glycosyl donor **142** with a less bulky 6-O-benzyl protecting group and acceptor **136** was conducted (Scheme 6.6).



**Scheme 6.6** Regioselective Synthesis of N-Acetyllactosamine **144**

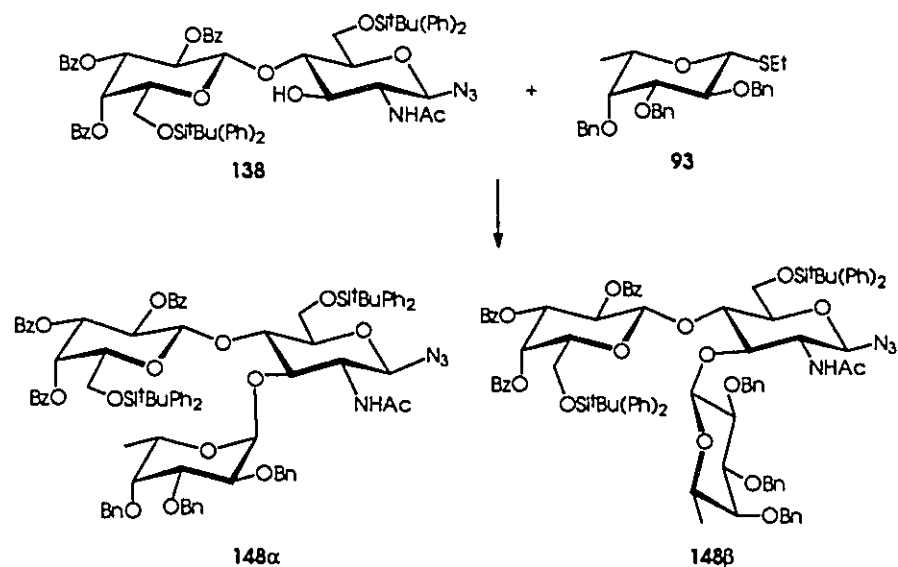
To our surprise, again, a remarkable regioselective outcome was obtained. The  $\beta(1\rightarrow4)$  linked product **144** was obtained in 78% yield and no  $\beta(1\rightarrow3)$  coupling product was detected during the reaction. The only by-product isolated from the reaction mixture was the elimination product **143a**. Following these observations it seemed that the bulky 6-O-TBDPS protecting group in acceptor **136** played the crucial role for the high regioselectivity.



**143a**

#### 6.2.4 Synthesis of Le<sup>x</sup> trisaccharide

Having the key glycosyl acceptor **138**, the crucial coupling with ethyl 2,3,4-tri-O-benzyl-1-thio- $\beta$ -L-fucopyranoside **93** was attempted (Scheme 6.7). The glycosylation conditions and results are given in Table 6.1.



**Scheme 6.7 Preparation of Le<sup>x</sup> Trisaccharide 148**

**Table 6.1 Reaction Conditions and Results of the Synthesis of Le<sup>x</sup>**

Entry	D:A <sup>b</sup> (equiv.)	Solvent (v/v)	Promoter (equiv.) <sup>c</sup>	Reaction Time Temperature	Product yield <sup>a</sup> (%)	
					148α	148β
1	2:1	CH <sub>3</sub> CN/CH <sub>2</sub> Cl <sub>2</sub> (5:1)	NIS/TfOH (1.8/0.2)	45 min -45 °C	26	37
2	1.5:1	C <sub>6</sub> H <sub>6</sub> /CH <sub>2</sub> Cl <sub>2</sub> (5:1)	DMTST (2.5)	120 min 0 °C	84	-

a: Determined by individual isomer separation.

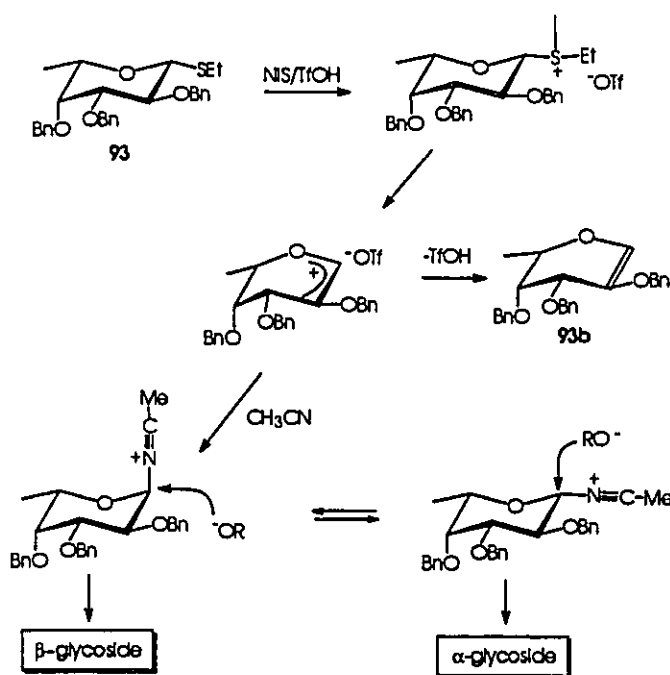
b. D: donor 93, A: Acceptor 138.

c: The equivalents of promoters were calculated on the basis of the donor used.

Given the poor solubility of NIS in dichloromethane, a solvent mixture of acetonitrile-dichloromethane (5:1, v/v) was used in our first attempt. The coupling reaction was conducted with NIS/TfOH at -45°C. After routine work up, the reaction afforded two compounds in a ratio of ~2:3. The chromatographically faster-moving component proved to be the desired α-glycoside 148α (R<sub>f</sub> = 0.35) and the slow-moving

component was the  $\beta$ -isomer **148 $\beta$**  ( $R_f = 0.32$ ). This  $\alpha,\beta$  anomer mixture was successfully separated by radial silica gel chromatography. Unfortunately, the desired trisaccharide **148 $\alpha$**  was obtained in only 26% yield and the  $\beta$  anomer in 37% yield. It should be mentioned that extensive elimination of the very reactive fucosyl donor **93** into **93b** was observed during the glycosylation process. Because of this side reaction, the L-fucosyl donor was consumed without coupling, as two equivalents of donor **93** were added, about 15% of unreacted glycosyl acceptor could be recovered. Further addition of the glycosyl donor during the glycosylation reaction did not have dramatic effect on the results.

These results could be explained by a solvent participating mechanism<sup>248</sup> as illustrated in Scheme 6.8 below.



**Scheme 6.8** Possible Mechanism for the Solvent Mediated Glycosylation Process

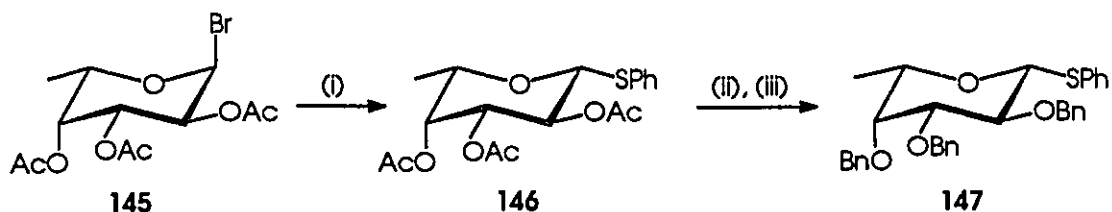
<sup>248</sup> (a) Gorden, D. M.; Danishefsky, S. J. *J. Org. Chem.* **1991**, *56*, 3713. (b) Marra, A.; Esnault, J.; Veyières, A.; Sinaÿ, P. *J. Am. Chem. Soc.* **1992**, *114*, 6354.

In the mechanism shown in Scheme 6.8, formation of an  $\alpha$ -nitrilium species, followed by an  $S_N2$  displacement at the anomeric center provides the  $\beta$  anomer, while a  $\beta$ -nitrilium species gives the  $\alpha$  anomer.

The configurations of the newly introduced anomeric centers in **148 $\alpha$**  and **148 $\beta$**  were unambiguously assigned from these  $^1\text{H}$  NMR spectra. The anomeric proton H-1'' appeared as a doublet at  $\delta$  4.71 ppm ( $J_{1'',2''} = 7.6$  Hz) for **148 $\beta$**  and at  $\delta$  5.25 ppm ( $J_{1'',2''} = 3.5$  Hz) for **148 $\alpha$** . For comparison, the  $^1\text{H}$  NMR and COSY spectra of **148 $\alpha$**  and **148 $\beta$**  are illustrated in Figures 6.3 and 6.4.

A dramatic improvement was achieved when the glycosylation reaction between **93** and **138** was executed in a non-participating solvent system (benzene-dichloromethane; 5:1, v/v) using the milder DMTST as catalyst at 0 °C (Entry 2 in table 6.1). The desired  $\alpha$  glycoside **148 $\alpha$**  was obtained in good yield (84%) and no  $\beta$ -anomer **148 $\beta$**  was observed under the glycosylation conditions.

In another similar glycosylation reaction, peracetylated phenyl 1-thio- $\beta$ -L-fucopyranoside was used as donor which could be readily prepared as described in Scheme 6.9. Acetobromofucose was treated with thiophenol under PTC conditions as we described in Section 2.2 to afford phenyl 2,3,4-tri-O-acetyl-1-thio- $\beta$ -L-fucopyranoside **146** in quantitative yield. The  $\beta$ -configuration of **146** was assigned from the  $^1\text{H}$ -NMR spectrum which showed H-1 as a doublet at  $\delta$  4.68 ppm ( $J_{1,2} = 10.0$  Hz). Simple Zemplén deacetylation and routine benzylation afforded **147** in 90% yield.



**Scheme 6.9** Synthesis Fucosyl Donor

(i) PTC: PhSH, 1M Na<sub>2</sub>CO<sub>3</sub>, EtOAc, TBAHS, r.t., 1 h, 93%; (ii) NaOMe, MeOH, quantitative; (iii) BnBr, DMF, NaH, 90%.

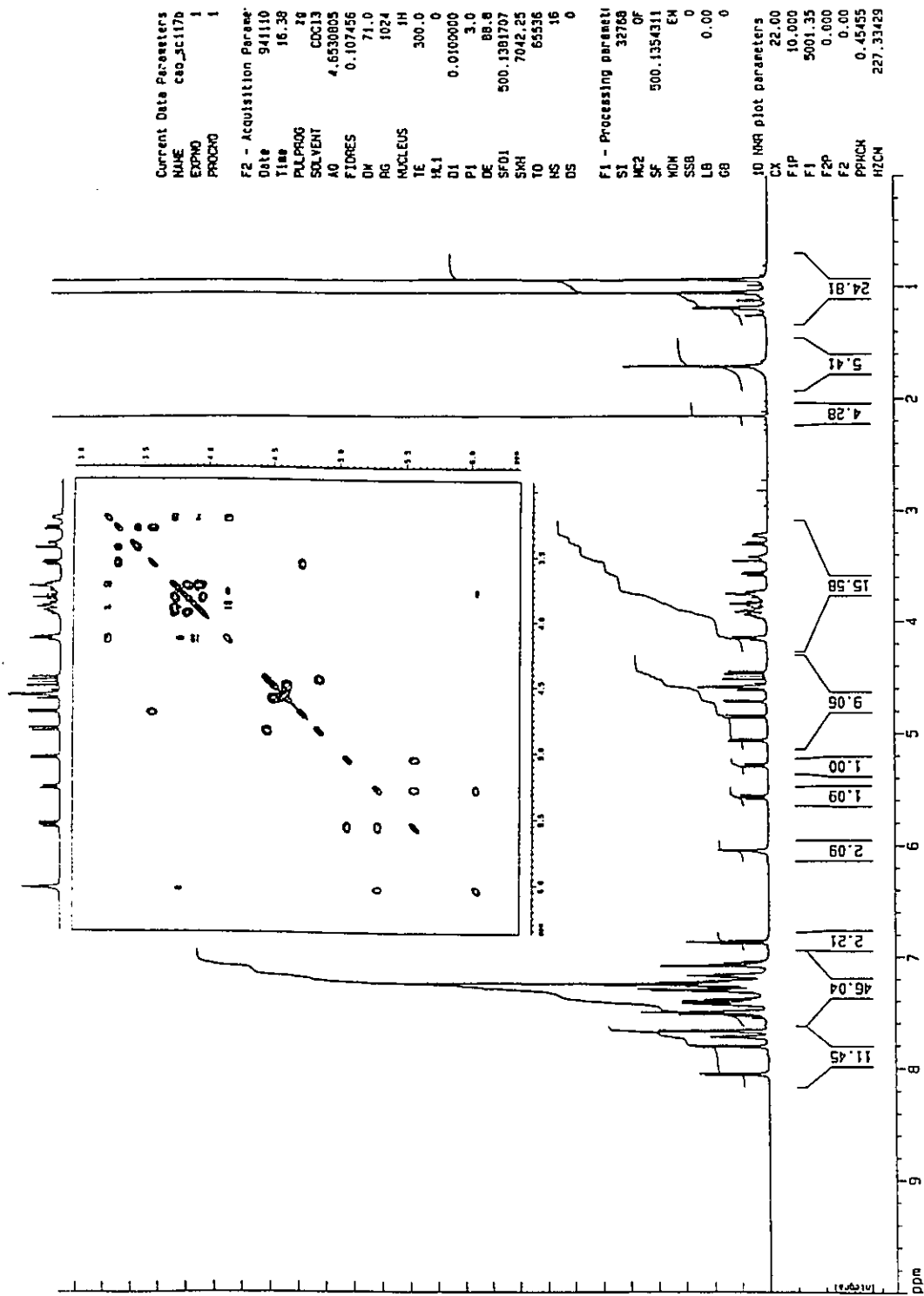


Figure 6.3  $^1\text{H-NMR}$  and  $^1\text{H-}^1\text{H COSY}$  Spectra (500 MHz,  $\text{CDCl}_3$ ) of 148 $\alpha$

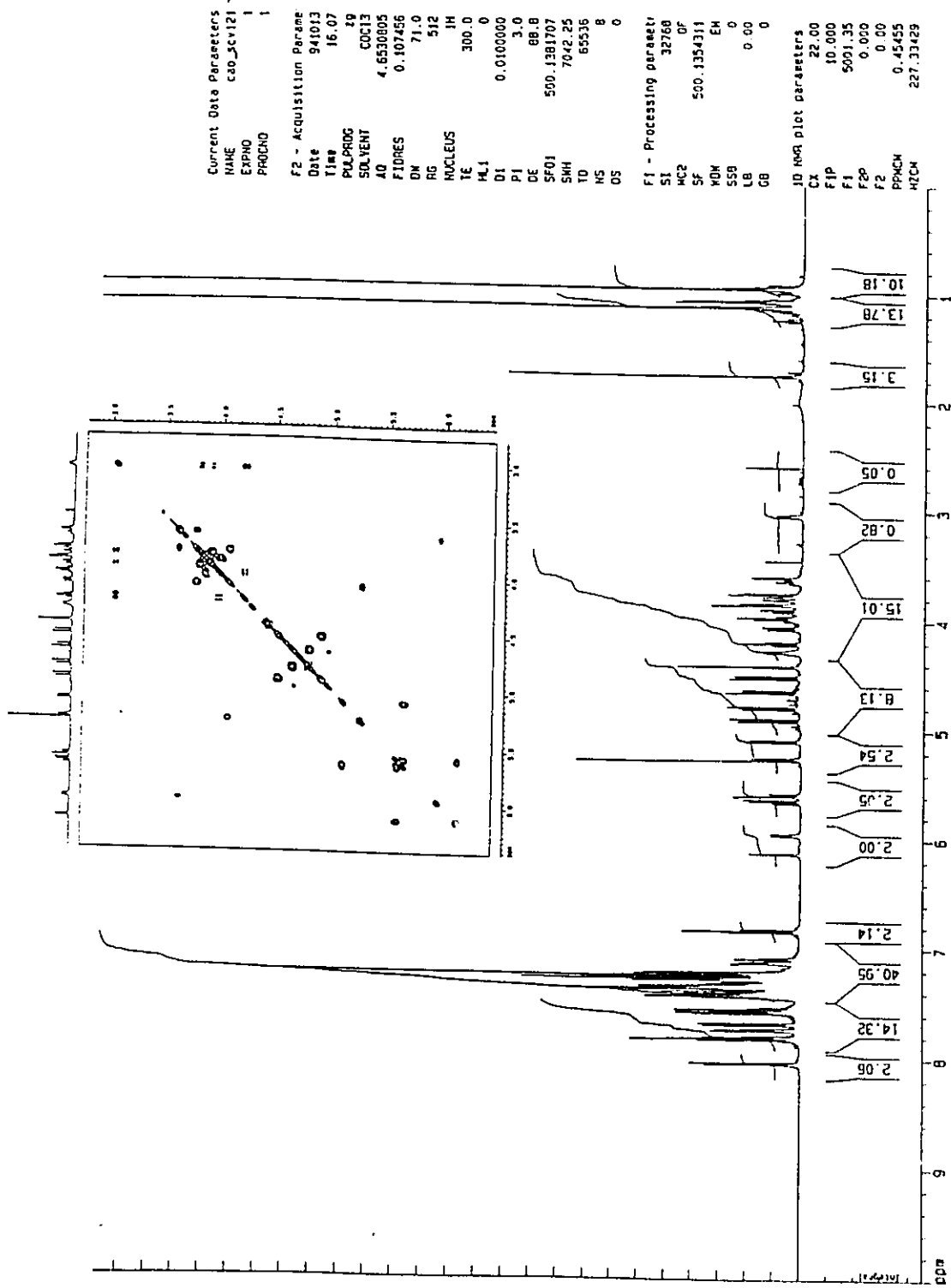
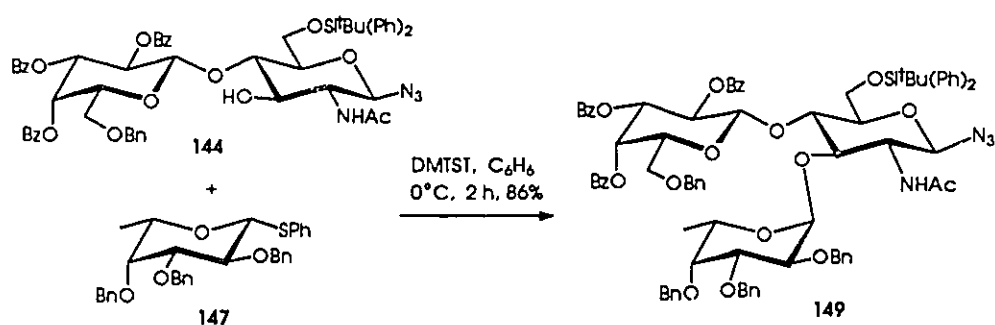


Figure 6.4  $^1\text{H}$ -NMR and  $^1\text{H}$ - $^1\text{H}$ -COSY Spectra (500 MHz,  $\text{CDCl}_3$ ) of 148 $\beta$

Condensation of phenyl 2,3,4-tri-O-benzyl-1-thio- $\beta$ -L-fucopyranoside **147** with N-acetyllactosamine acceptor **144** (Scheme 6.10) under condition similar to that in table 6.1 entry 2 afforded trisaccharide **149** in good yield (86%). Again, no  $\beta$ -trisaccharide anomer was detected during the reaction. The successful introduction of the L-fucosyl unit was confirmed from the  $^1\text{H}$  NMR data of **149** which showed the H-1'' signal at  $\delta$  5.30 ppm ( $J_{1,2} = 2.3$  Hz).

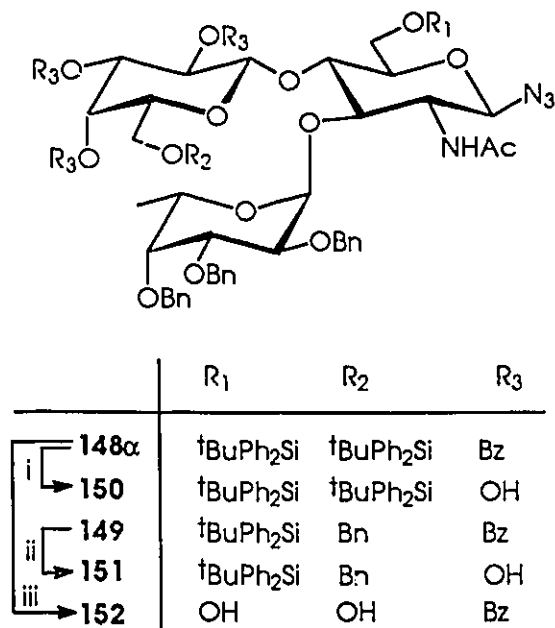


Scheme 6.10 Synthesis of  $\text{Le}^x$  Trisaccharide **149**

### 6.2.5 Synthesis of Sulfo- $\text{Le}^x$ and Sialyl $\text{Le}^x$

$\text{Le}^x$  trisaccharide **148** and **149** were evaluated as potential acceptors for the preparation of sulfo- $\text{Le}^x$  and sialyl- $\text{Le}^x$ . The benzoyl protecting groups of  $\text{Le}^x$  trisaccharides **148** and **149** were removed routinely ( $\text{NaOMe-MeOH-CH}_2\text{Cl}_2$ ) to give trisaccharides **150** and **151** in almost quantitative yield. However the removal of 6'- and 6-O-TBDPS protecting groups from trisaccharide **150** was not as easy as expected using the well established fluoride-anion treatment. Reacting **150** with an excess of tetrabutylammonium fluoride under reflux for 5 hours gave a monodesilylated product (as judged by NMR spectroscopy). In contrast, if **148** was treated under the same condition as

described above, silyl group removal was relatively easier and afforded the expected trisaccharide **152** in 81% yield.



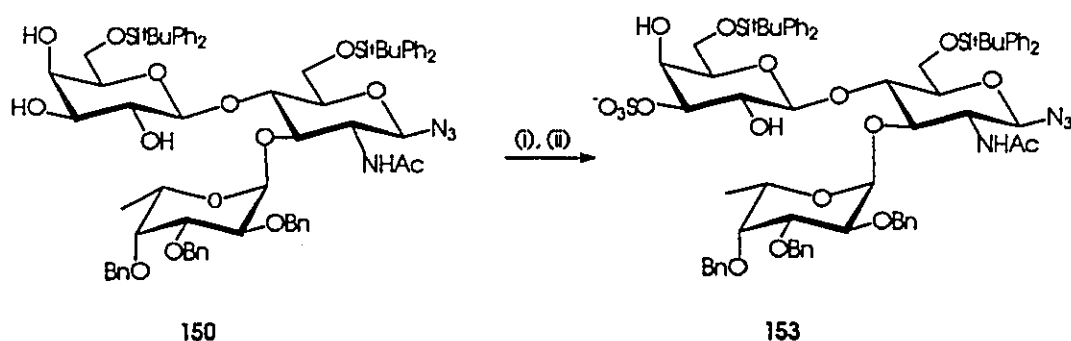
### Scheme 6.11 Preparation of Le<sup>x</sup> Derivatives

(i) NaOMe, MeOH, r.t. quantitative; (ii) same as (i); (iii) TBAF, THF, reflux, 5 h, 86%.

One of the original goals of this research was to prepare sulfated Le<sup>x</sup>. Sulfations of sugars are usually performed with sulfuric acid, chlorosulfuric acid or sulfuryl chloride which generally gives complex mixtures. On the other hand, the use of sulfur trioxide complexes with pyridine or tertiary amines in solvents such as pyridine or N,N-dimethylformamide gave generally good yields of sulfation, provided that only the hydroxyl groups to be sulfated are unprotected in the starting material. The regioselectivity is usually poor, except that of primary alcohol.<sup>249</sup> The regioselectivity in sugar chemistry is often solved using the well-known stannylene methodology discussed in Section 4.2.2. More recently, a chemical synthesis of sulfated Le<sup>x</sup> using stannylene

<sup>249</sup> (a) Yurcaym J. R. *Adv. Carbohydr. Chem. Biochem.* 1965, 20, 183. (b) Haines, A. H. *Adv. Carbohydr. Chem. Biochem.* 1976, 33, 11.

mediated method were reported by Lubineau *et al.*<sup>250</sup> Our synthesis of sulfated Le<sup>x</sup> is illustrated in Scheme 6.12.



#### Scheme 6.12 Preparation of 3'-O-Sufated Lewis<sup>x</sup>

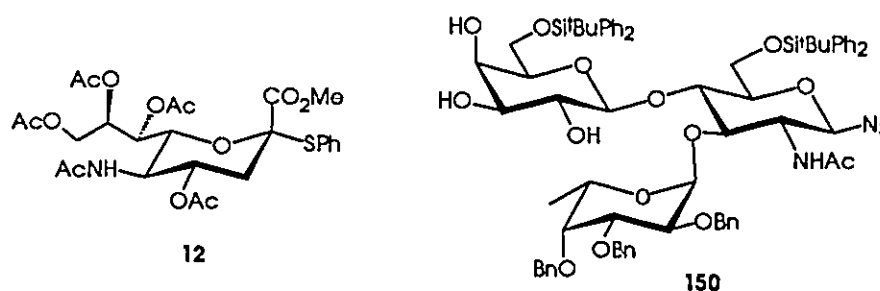
(i) Bu<sub>2</sub>SnO, toluene, reflux, 14 h; (ii) SO<sub>3</sub>·NMe<sub>3</sub>, THF-pyridine (1:1), 24 h, r.t., 91%

Le<sup>x</sup> trisaccharide **150** having three secondary unprotected hydroxyl groups at C-2, C-3 and C-4 was first treated with dibutyltin oxide (1.3 equiv.) in toluene by refluxing under nitrogen for 14 hours. Evaporation of toluene and addition of SO<sub>3</sub>·Me<sub>3</sub>N (2 equiv.) in a 1:1 tetrahydrofuran-pyridine mixture at room temperature for 24 hours gave 3'-O-sulfate **153** in 91% yield. The successful introduction of the sulfo-group was confirmed by FAB-MS which gave a molecular ion (*m/z* 1380.54, [M]<sup>+</sup>, 0.4%). Sulfo-Le<sup>x</sup> **153** has been fully characterized by NMR using <sup>1</sup>H-<sup>1</sup>H COSY and <sup>1</sup>H-<sup>13</sup>C HMQC experiments. In particular, the C-3' signal of sulfated Le<sup>x</sup> **153** was found at δ 80.7 ppm, 7.1 ppm downfield compared with that of the signal of C-3' in Le<sup>x</sup> trisaccharide **150** observed at δ 73.6 ppm, showing unambiguously the presence of the sulfate on O-3 of the galactose moiety.

Our attention was turned to the preparation of the next target: the sialyl Le<sup>x</sup> tetrasaccharide. The first attempts at the condensation of sialyl donor **12** with Le<sup>x</sup> trisaccharide **150** in propionitrile-dichloromethane (5:1, v/v) using NIS/TfOH as promoter

<sup>250</sup> (a) Lubineau, A.; Lemoine, R. *Tetrahedron lett.* 1994, 47, 8795. (b) Singh, K.; Fernández-Mayoralas, A.; Martín-Lomas, M. *J. Chem. Soc., Chem. Commun.* 1994, 775.

(-60 °C) gave no trace of condensation product. Instead, a sizable cleavage of the  $\alpha$ -L-fucopyranosyl residue from the Le<sup>x</sup> acceptor moiety was observed. A similar phenomena was also recently observed by Matta's group.<sup>251</sup> Using DMTST as a promoter was equally frustrating. Despite a large number of attempts, no desired condensation between sialyl donor **12** and Le<sup>x</sup> trisaccharide **150** occurred. Instead, sialic acid glycal and hemiacetal were consistently obtained from the reaction mixture. Steric mismatch was assumed to be the cause. The bulky 6,6'-bis(O-TBDPS) ethers were probably responsible for the steric hindrance. In another synthesis of sialyl Le<sup>x</sup> reported by Danishefsky et.al,<sup>38f</sup> a similar synthetic strategy was applied. Although they also prepared 6,6'-O-disilylated Le<sup>x</sup> trisaccharide, they did not use this derivative as a glycosyl acceptor with sialyl acid. Instead, they used a 6-O-TBDPS-6'-O-Bn Le<sup>x</sup> as glycosyl acceptor. The authors have not commented this choice. To eliminate the possibility of steric hindrance, replacement of the 6'-O-TBDPS group with 6'-O-Bn was taken as an alternative. To this end, Le<sup>x</sup> trisaccharide **149** was synthesized as described in section 6.3.5.



**Scheme 6.13** Coupling Sialyl Donor **12** with Le<sup>x</sup> Trisaccharide **150**

Glycosyl donors are also important for successful coupling reactions. It was reported<sup>252</sup> that the use of perbenzoylated phenyl thiosialyl donor gave better glycosylation results than those from peracetylated sialyl donors. To test this point, we

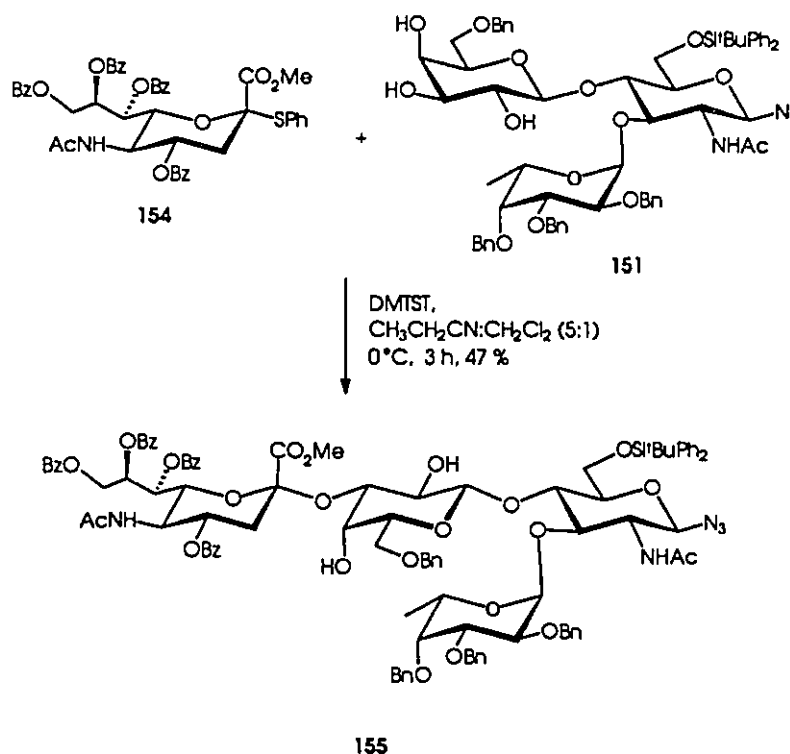
<sup>251</sup> Jain, R. K.; Vig, R.; Rampal, R.; Chandrasekaran, E. V.; Matta, K. *J. Am. Chem. Soc.* **1994**, *116*, 12123.

<sup>252</sup> Ehara, T.; Kameyama, A.; Yamada, Y.; Ishida, H.; Kiso, M.; Hasegawa, A. Abstracts of the *XVIIth International Carbohydrate Symposium*, Ottawa, Canada, B1.88, **1994**.

prepared perbenzoylated sialyl donor **154** prepared quantitatively by a simple Zemplén deacetylation and benzylation of **12**.

A crucial coupling of the sialyl donor **154** and Le<sup>x</sup> trisaccharide acceptor **151** was performed in a mixture of propionitrile and dichloromethane (5:1) containing 4Å molecular sieves using DMTST as promoter. Promising results were obtained, the predicted sialyl Le<sup>x</sup> tetrasaccharide **155** was obtained in 47% purified yield (Scheme 6.14).

Successful introduction of a sialic acid moiety into the Le<sup>x</sup> trisaccharide was confirmed by NMR and mass spectroscopic evidences. These include the signals of sialyl acid in the <sup>1</sup>H-NMR spectra at  $\delta$  2.76 ppm (dd,  $J_{3e,4} = 4.9$ ,  $J_{3e,3a} = 13.1$  Hz) for H-3e, 2.27 ppm (dd,  $J_{3a,4} = 11.4$  Hz) for H-3a, 3.21 ppm (s) for OMe and the <sup>13</sup>C-NMR singals at 168.6 ppm for C=O (C-1) and 62.4 ppm for C-3. Positive ion FABMS corresponding to the tetrasaccharide fragment at  $m/z$  1728.4 ( $[M-N_3-Bz]^+$ , 16.2%) was observed.



**Scheme 6.14** Synthesis of Sialyl Le<sup>x</sup> **155**

### 6.3 Conclusion

A very efficient synthetic route towards Le<sup>x</sup> trisaccharide family was developed. This work demonstrates an efficient synthesis of an N-acetyllactosamine donor, as a key building block for further transformation to Le<sup>x</sup> trisaccharide. This procedure takes advantage of a 6-O-TBDPS protected N-acetylglucosamine derivative, allowing the introduction of a galactosyl moiety with high regioselectivity. In addition, the fucosyl moiety could be introduced to the N-acetyllactosamine building block in a stereocontrolled manner even without anchimeric group participation. Facile chemistry allowed us to prepare Le<sup>x</sup> trisaccharide in large scale without difficulty.

Regioselective introduction of the sulfate group and sialic acid residues onto Le<sup>x</sup> afforded important sulfo-Le<sup>x</sup> and sialyl Le<sup>x</sup> oligosaccharides as selectin ligands. Further development of these important oligosaccharides to multivalent glycoconjugates is currently being pursued in this laboratory.

### 6.4 Experimental Methods

#### 2-Acetamido-3,4,6 tri-O-acetyl-2-deoxy-β-D-glucopyranosyl azide **134**

To a solution of acetochloro N-acetylglucosamine **3** (1.0 g, 2.73 mmol), TBAHS (1.39 g, 4.10 mmol) and sodium azide (0.89 g, 13.65 mmol) in methylene chloride (10 mL) was added saturated aqueous NaHCO<sub>3</sub> (10 mL). The two phase reaction mixture was vigorously stirred at room temperature for 2 hours. TLC (benzene/acetone, 1:1 v/v) indicated complete transformation of the halide **3** (R<sub>f</sub> = 0.65) to product **134** (R<sub>f</sub> = 0.60). EtOAc (60 mL) was added, the organic phase was separated and successively washed with sat. NaHCO<sub>3</sub>, water (2x) and sat. NaCl. The combined extracts were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and evaporated under reduced pressure to give essentially pure **134** (0.94 g) in 94 % yield as judged by <sup>1</sup>H NMR spectroscopy. Compound **134** was crystallized from ethanol: m.p. 159.2-161.3 °C; [α]<sub>D</sub>

-45.7° (c = 0.9, CHCl<sub>3</sub>); CI-MS (ether) gave m/z (ion, relative intensity): 373.4 ([M+1]<sup>+</sup>, 45.3 %), 329.9 ([M+1-N<sub>3</sub>]<sup>+</sup>, 100%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 5.77 (1H, NH), 5.23 (dd, 1H, J<sub>3,4</sub> = 9.8 Hz, H-3), 5.07 (dd, 1H, J<sub>4,5</sub> = 9.6 Hz, H-4), 4.74 (d, 1H, J<sub>1,2</sub> = 9.3 Hz, H-1), 4.24 (dd, 1H, J<sub>6a,6b</sub> = 12.5 Hz, H-6a), 4.13 (dd, 1H, J<sub>5,6b</sub> = 2.4 Hz, H-6b), 3.92 (dd, 1H, J<sub>2,3</sub> = 10.1 Hz, H-2), 3.72 (ddd, 1H, J<sub>5,6b</sub> = 4.7 Hz, H-5), 2.07, 2.02, 2.01, 1.96 (OAc, NHAc). <sup>13</sup>C-NMR δ (CHCl<sub>3</sub>): 171.1, 170.7 (2C), 170.4 (2C) 169.2 (6 C=O), 88.3 (C-1), 73.8 (C-5), 72.0 (C-3), 68.1 (C-4), 61.8 (C-6), 53.9 (C-2), 23.0 (NHAc), 20.6, 20.4, 20.3 (OAc).

### 2-Acetamido-6-O-tert-butylidiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide 135b

To a cold solution of 2-acetamido-2-deoxy-β-D-glucopyranosyl azido 135a (1.7g, 6.9 mmol) in dry pyridine (20 mL), tert-butylchlorodiphenylsilane (2.1 mL, 8.2 mmol) was added in one portion. The mixture was stirred for 3 hours at room temperature and poured onto ice water, extracted with dichloromethane, and washed with saturated sodium hydrogen carbonate. The organic extract was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated. The crude product was subjected to column chromatography (methanol-methylene chloride, 1:10) to give pure 135b as a foamy solid (3.08 g) in 92 % yield: [α]<sub>D</sub> -53.6° (c = 1.0, CHCl<sub>3</sub>); IR (thin film, ν<sub>cm-1</sub>): 3329, 3073, 2938, 2858, 2116 (N<sub>3</sub>), 1652, 1553, 1428, 1375, 1313, 1092, 942, 823, 704; (+)FAB-MS (glycerol) gave m/z (ion, relative intensity): 485.0 ([M]<sup>+</sup>, 6.2 %), 440.5 ([M-N<sub>3</sub>]<sup>+</sup>, 100 %); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 7.34-7.67 (m, 10H, aromatic); 6.14 (d, 1H, J<sub>2,NH</sub> = 7.1 Hz, NH), 4.61 (d, 1H, J<sub>1,2</sub> = 9.1 Hz, H-1), 3.88 (m, 2H, H-6, H-6'), 3.66 (dd, 1H, J<sub>3,4</sub> = 9.4 Hz, H-3), 3.60 (dd, 1H, J<sub>4,5</sub> = 9.1 Hz, H-4), 3.53 (dd, 1H, J<sub>2,3</sub> = 9.1 Hz, H-2), 3.44 (ddd, 1H, J<sub>5,6a</sub> = 4.3, J<sub>5,6b</sub> = 4.5 Hz, H-5), 2.02 (s, 3H, NHAc), 1.02 (s, 9H, SiCMe<sub>3</sub>); <sup>13</sup>C-NMR (125.7 MHz, CDCl<sub>3</sub>) δ (ppm): 125.7 172.2 (C=O, NHAc), 135.6-127.8 (12C, aromatic), 88.0 (C-1), 77.5 (C-5), 74.8 (C-3), 71.7 (C-5), 63.7 (C-6), 56.5 (C-2), 26.8 (3C, SiCMe<sub>3</sub>), 23.5 (NHAc), 19.3 (SiCMe<sub>3</sub>).

Anal. calcd for C<sub>24</sub>H<sub>32</sub>O<sub>5</sub>N<sub>4</sub>Si (484.63): C, 59.48; H, 6.66; N, 11.57. found: C, 59.51; H, 6.55; N, 11.91.

### 2-Acetamido-6-O-benzoyl-2-deoxy- $\beta$ -D-glucopyranosyl azide 136

To a suspension of **134** (1.0 g, 2.68 mmol) in 50 mL of methanol was added 350  $\mu$ L of 1 M NaOMe (pH = 9-10). The mixture was stirred at room temperature for 35 min, TLC (1/4, Ethanol/CH<sub>2</sub>Cl<sub>2</sub>) showed that Zemplén deacetylation was finished. The solution was neutralized with H<sup>+</sup> resin (Dowex 50W-X8) filtered, concentrated under reduced pressure and coevaporated with pyridine two times. The residue was dissolved in dry pyridine/CH<sub>2</sub>Cl<sub>2</sub>, 1:2 v/v. The solution was cooled to -55 °C and benzoyl chloride (0.45 mL) in 10 mL of dichloromethane was added drop by drop in a period of 30 min. The reaction mixture was stirred under nitrogen at -55 °C for additional 15 min. TLC (EtOAc/CH<sub>2</sub>Cl<sub>2</sub>, 1:4) indicated that the starting material (R<sub>f</sub> = 0.17) was completely consumed. To this mixture was added methanol (1.5 mL) and the solution was stirred for an additional 30 min. The crude product obtained after evaporation was chromatographed on silica gel (EtOAc/CH<sub>2</sub>Cl<sub>2</sub>, 1:6). The titled compound (755 mg) was obtained in 80% yield as a white solid: m.p. 226-228 °C; [ $\alpha$ ]<sub>D</sub> -30.3° (c = 1.0, CH<sub>3</sub>OH); (+)FAB-MS (glycerol) gave m/z (ion, relative intensity): 351.15 ([M+1], 30.1 %), 308.13 ([M-N<sub>3</sub>]<sup>+</sup>, 20.4 %); <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>)  $\delta$  (ppm): 5.49 (1H, OH), 5.21 (1H, OH), 4.56 (dd, 1H, J<sub>5,6a</sub> = 2.8, J<sub>6a, 6b</sub> = 12.0 Hz, H-6a), 4.49 (d, 1H, J<sub>1,2</sub> = 10.3 Hz, H-1), 4.36 (dd, 1H, J<sub>5,6b</sub> = 5.7 Hz, H-6b), 3.69-3.43 (m, 4H, H-2, H-3, H-4, H-5) 1.83 (s, 3 H, NHAc); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>)  $\delta$  (ppm): 170.7 (2C), 170.0 (C=O, NHAc) 165.7 (C=O, Bz), 132.3-128.7 (6C, Aromatic), 88.6 (C-1), 76.1 (C-5), 73.8 (C-3), 70.0 (C-4), 63.9 (C-6), 55.0 (C-2), 23.1 (NHAc).

Anal calcd for (C<sub>15</sub>H<sub>18</sub>N<sub>4</sub>O<sub>6</sub>) (350.32): C, 51.43; H, 5.18, N, 95.99. found: C, 51.40; H, 5.14; N, 15.67.

### Phenyl 2,3,4-tri-O-benzoyl-6-O-tert-butylidiphenylsilyl-1-thio- $\beta$ -D-galactopyranoside 137

To a cooled (ice bath) solution of phenyl 1-thio- $\beta$ -D-galactopyranoside **64** (3.3 g, 12.1 mmol) in dry pyridine (15 mL) was added TBDPSCI (3.5 mL, 13.3 mmol). The reaction mixture was then allowed to reach room temperature. The stirring was continued for 4 hours, after which time TLC showed complete conversion of **64** to silylated

intermediate **136**. The mixture was cooled to 0°C, and benzoyl chloride (5.0 mL, 44 mmol) was added. The mixture was stirred at room temperature for an additional 5 hours and then poured onto ice. The solution was then extracted with chloroform. The extracts were collected and washed with saturated sodium bicarbonate and brine. The solution was dried over sodium sulfate and evaporated to dryness under reduced pressure. The product was purified by silica gel chromatography using ethyl acetate/hexane (1:4, v/v) as eluent to give **137** (9.2 g) in 92% yield:  $[\alpha]_D +110^\circ$  (c = 1.0, CHCl<sub>3</sub>); M.S. (C.I. ether) (m/z): 688.9 ([M+1]<sup>+</sup>, 3.6%), 579.1 ([M+1-SPh]<sup>+</sup>, 100 %); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 7.98-7.14 (m, 30H, aromatic), 6.06 (dd, 1H, J<sub>4,5</sub> < 1.0 Hz, H-4), 5.66 (dd, 1H, J<sub>1,2</sub> = 9.8 Hz, H-2), 5.60 (dd, 1H, J<sub>3,4</sub> = 3.1 Hz, H-3), 4.98 (d, 1H, J<sub>1,2</sub> = 9.8 Hz, H-1), 4.14 (m, 1H, H5), 3.90 (dd, 1H, J<sub>5,6a</sub> = 6.1, J<sub>6a,6b</sub> = 10.3 Hz, H-6a), 3.79 (dd, 1H, J<sub>5,6b</sub> = 7.6 Hz, H-6b), 1.02 (s, 9H, SiMe<sub>3</sub>); <sup>13</sup>C-NMR (125.7 MHz, CDCl<sub>3</sub>) δ (ppm): 165.6, 165.2, 165.1 (C=O, 3xBz), 85.7 (C-1), 77.8 (C-5), 73.4 (C-3), 68.1 (C-4), 68.1 (C-2), 61.6 (C-6), 26.7 (3c, SiCMe<sub>3</sub>), 19.1(SiCMe<sub>3</sub>).

#### Phenyl 4,6-O-benzylidene-1-thio-β-D-galactopyranoside **140**

Phenyl 1-thio-β-D-galactopyranoside (201 mg, 0.74 mmol) in acetonitrile (2 mL) was heated at 60 °C until the starting material was completely dissolved. The solution was then cooled to room temperature, benzaldehyde dimethyl acetal (0.33 mL, 2.2 mmol) and *p*-toluenesulfonic acid monohydrate (catalytic amount) were then added. The mixture was stirred for 4 hours at room temperature, neutralized with triethylamine and concentrated under reduced pressure. The residue was then purified by column chromatography (MeOH/CH<sub>2</sub>Cl<sub>2</sub>, 1:10) on silica gel to give **140** (234 mg) in 90% yield:  $[\alpha]_D -24.0^\circ$  (c = 1.0, CHCl<sub>3</sub>); M.S.(C.I. ether) (m/z): 361.0 ([M+1]<sup>+</sup>, 54.5 %), 250.9 ([M+1-SPh]<sup>+</sup>, 100 %); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 7.70-7.10 (m, 10H, 2xPh), 5.41 (s, 1H, PhCH), 4.39 (d, 1H, J<sub>1,2</sub> = 9.2 Hz, H-1), 4.29 (dd, 1H, J<sub>5,6a</sub> = 1.4, J<sub>6a,6b</sub> = 12.4 Hz, H-6), 4.11 (d, 1H, J<sub>3,4</sub> < 1.0 Hz, H-4), 3.94 (dd, 1H, J<sub>5,6b</sub> = 1.7 Hz, H-6b), 3.58 (m, 2H, H-2, H-3), 3.46 (1H, H-5).

Anal. Calcd for C<sub>19</sub>H<sub>20</sub>O<sub>5</sub>S (360.42): C, 63.32; H, 5.59. found: C, 64.51; H, 5.31.

### Phenyl 2,3-di-O-benzoyl-4,6-O-benzylidene-1-thio-β-D-galactopyranoside 141

Phenyl 4,6-benzylidene-1-thio-β-D-galactopyranoside **140** (1.00 g, 2.8 mmol) was dissolved in 15 mL of dry pyridine. The solution was cooled to 0 °C and benzoyl chloride (1.62 mL, 7 mmol) was added slowly. The mixture was then stirred for overnight. TLC showed a complete conversion starting material to product. 1 mL of methanol was added stirred for additional 15 min. The mixture was concentrated under reduced pressure. Column chromatography (ethyl acetate/Hexane, 1:1) of the residue on silica gel gave **141** (1.54 g) in 97% yield:  $[\alpha]_D +54.9^\circ$  (c = 1.0, CHCl<sub>3</sub>); M.S.(C.I. ether) (m/z): 368.7 ([M]<sup>+</sup>, 1.7 %), 459.2 ([M+1-SPh]<sup>+</sup>, 100 %); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 7.95-7.10 (m, 20H, 4Ph), 5.66 (t, 1H, J<sub>2,3</sub> = 10.0 Hz, H-2), 5.41 (s, 1H, PhCH), 5.22 (dd, 1H, J<sub>3,4</sub> = 3.2 Hz, H-3), 4.84 (d, 1H, J<sub>1,2</sub> = 9.7 Hz, H-1), 4.50 (d, 1H, J<sub>4,5</sub> < 1.0 Hz, H-4), 3.87 (dd, 2H, H-6a, H-6b), 3.67 (1H, H-5).

Anal. Calcd for C<sub>33</sub>H<sub>28</sub>O<sub>7</sub>S: C, 69.70; H, 4.96. found: C, 69.82; H, 4.90.

### Phenyl 2,3-di-O-benzoyl-6-O-benzyl-1-thio-β-D-galactopyranoside 142

To a solution of **141** (2.0 g, 3.52 mmol) in dry THF (20 mL) was added powdered molecular sieves 4 Å (1.2 g). The mixture was stirred for 2 hours at room temperature and sodium cyanoborohydride (3.0 g, 47.7 mmol) was gradually added. After the reagent had dissolved, saturated solution of hydrogen chloride in ether (obtained by bubbling HCl gas in ether for 25 min) was added dropwise at room temperature until the evolution of gas ceased. TLC (EtOAc/Hexane 2:3) indicated that starting material **141** (R<sub>f</sub> = 0.46) was cleanly converted to product **142** (R<sub>f</sub> = 0.54) after 10 min. The mixture was diluted with dichloromethane, filtered, washed with saturated sodium hydrogen carbonate and water, dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated. Column chromatography on silica gel using ethyl acetate-hexane (1:3) as a eluent gave **142** (1.7 g) in 84% yield:  $[\alpha]_D +77.0^\circ$  (c = 1.0 CHCl<sub>3</sub>); (+)FAB-MS (glycerol) gave m/z (ion, relative intensity): 571.3 ([M+1]<sup>+</sup>, 4.3 %), 440.5 ([M-SPh]<sup>+</sup>, 82.5 %); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ (ppm): 7.99-7.20 (m, 20H, aromatic), 5.75 (dd, 1H, J<sub>2,3</sub> = 9.8 Hz, H-2), 5.29 (dd, 1H, J<sub>3,4</sub> = 3.2 Hz, H-3), 4.91 (d, 1H,

$J_{1,2} = 9.9$  Hz, H-1), 4.56 (AB pattern, 2H, CH<sub>2</sub> Ph), 4.41 (dd, 1H,  $J_{3,4} = 3.2$ ,  $J_{4,OH} = 1.4$  Hz,  $J_{4,5} < 1.0$  Hz, H-4), 3.91-3.83 (m, 3H, H5, H-6a, H-6b), 2.67 (d, 1H,  $J_{4,OH} = 1.4$  Hz, OH); <sup>13</sup>C-NMR (125.7 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 165.3, 165.8 (C=O, 2xBz), 86.7 (C-1), 77.3 (C-5), 75.5 (C-3), 73.9 (CH<sub>2</sub>), 69.6 (C-6), 68.4 (C-4), 68.0 (C-2).

Anal. Calcd for C<sub>33</sub>H<sub>30</sub>O<sub>7</sub>S: C, 69.45; H, 5.30. found: C, 69.32; H, 5.23.

#### Phenyl 2,3,4-tri-O-benzoyl-6-O-benzyl-1-thio- $\beta$ -D-galactopyranoside 143

Compound **143** was treated as described for the synthesis of **142**. Column chromatography (1%, methanol-dichloromethane) of the residue on silica gel gave **142** (651mg) in 97% yield as an amorphous mass:  $[\alpha]_D^{25} +113.5^\circ$  (c, 1.0, CHCl<sub>3</sub>); (+)FAB-MS (glycerol) gave m/z (ion, relative intensity): 675.2 ([M+1]<sup>+</sup>, 0.5%), 565.2 ([M+1]<sup>+</sup>, 5.2%); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm) 7.19-7.96 (m, 25H, aromatic), 5.94 (dd, 1H,  $J_{4,5} < 1.0$  Hz, H-4), 5.68 (dd, 1H,  $J_{2,3} = 9.9$  Hz, H-2), 5.53 (dd, 1H,  $J_{3,4} = 3.0$  Hz, H-3), 4.98 (d, 1H,  $J_{1,2} = 9.9$  Hz, H-1), 4.52 (AB pattern, 1H,  $J = 11.8$  Hz, H-A of CH<sub>2</sub>Ph), 4.45 (AB pattern, 1H, H-B of CH<sub>2</sub>Ph), 4.19 (m, 1H, H-5), 3.73 (dd, 1H,  $J_{5,6a} = 6.3$ ,  $J_{6a,6b} = 9.8$  Hz, H-6a), 3.62 (dd, 1H,  $J_{5,6b} = 6.3$  Hz, H-6b); <sup>13</sup>C-NMR (125.7 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm) 165.5, 165.3, 165.2 (C=O, 3xBz), 85.7 (C-1), 76.7 (C-5), 73.7 (CH<sub>2</sub>), 73.2 (C-3), 68.6 (C-4), 68.2 (C-6), 68.0.(C-2).

Anal. Calcd. For C<sub>40</sub>H<sub>34</sub>O<sub>8</sub>S (674.20): C, 71.20; H, 5.08. found: C, 71.28; H, 4.96.

#### O-(2,3,4-tri-O-benzoyl-6-O-tert-butyldiphenylsilyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2-acetamido-6-O-tert-butyldiphenylsilyl-2-deoxy- $\beta$ -D-glucopyranosyl azide 138

Compound **135b** (100 mg, 0.21 mmol, 1.0 eq) and **137** (203.6 mg, 0.25 mmol, 1.2 eq) was dissolved in dry dichloromethane (3 mL) under nitrogen and powdered molecular sieves (4 Å) was added. The mixture was stirred at room temperature for 30 min and then cooled to -30 °C. N-Iodosuccinimide (92.7 mg, 0.41 mmol) and trifluoromethanesulfonic acid (11.7  $\mu$ L, 0.6 eq) were added. The reaction was essentially complete (as judged by TLC) after 35 min. The mixture was then diluted with dichloromethane (10 mL) and filtered through celite. The filtrate was washed with 10% aqueous sodium thiosulfate (10

mL), saturated aqueous sodium bicarbonate (2x10 mL), and brine (10 mL). The solution was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated to a foam that was chromatographed on silica gel (2% methanol in dichloromethane) to afford disaccharide 138 (202.6 mg) in 82% yield: m.p. 108-110 °C;  $[\alpha]_D^{25} +45.2^\circ$  (c = 1.0,  $\text{CHCl}_3$ ); IR (Thin film,  $\nu_{\text{cm}^{-1}}$ ): 3367, 3067, 2937, 2858, 2114, 1732, 1282, 1106, 814, 734, 706; FAB-MS (glycerol) gave m/z (ion, relative intensity): 1154.5 ( $[\text{M}-\text{HN}_3]^+$ , 3.2 %);  $^1\text{H-NMR}$  (500 Hz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 7.99-7.10 (m, 35H, Aromatic H), 6.03 (d, 1H,  $J_{4',5'} < 1.0$  Hz, H-4'), 5.73 (dd, 1H,  $J_{2',3'} = 10.5$  Hz, H-2'), 5.61 (d, 1H,  $J_{3,\text{NH}} = 8.3$ , NH), 5.59 (dd, 1H,  $J_{3',4'} = 3.3$  Hz, H-3'), 5.06 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'), 4.70 (d, 1H,  $J_{1,2} = 9.2$  Hz, H-1), 4.07 (m, 1H, H-5'), 4.04 (dd, 1H,  $J_{4,5} = 9.5$  Hz, H-4), 3.94 (dd, 1H,  $J_{3,4} = 8.4$  Hz, H-3), 3.88 (dd, 1H,  $J_{5',6a'} = 6.3$ ,  $J_{6a',6b'} = 10.1$  Hz, H-6a'), 3.82-3.76 (m, 2H, H-6b', H-6a), 3.74 (dd, 1H,  $J_{5,6b} = 2.3$ ,  $J_{6a,6b} = 11.6$  Hz, H-6b), 3.54 (ddd, 1H,  $J_{2,3} = 9.4$  Hz, H-2), 3.32 (1H, H-5), 1.98 (s, 3H, NHAc), 1.11 (s, 9H, tBu), 1.01 (s, 9H, tBu);  $^{13}\text{C-NMR}$  (125.8,  $\text{CDCl}_3$ )  $\delta$  (ppm) : 170.6, 165.4, 165.2, 165.3 (4x C=O), 135.9-127.7 (42C, Aromatic), 100.8 (C-1'), 87.7 (C-1), 77.9 (C-4), 76.7 (C-5), 74.3 (C-5'), 71.6 (C-3'), 71.3 (C-3), 69.9 (C-2'), 67.6 (C-4'), 61.3 (C-6), 60.9 (C-6'), 56.4 (C-2), 27.0 (tBu), 26.7 (tBu), 23.5 (NHAc), 19.5 (Si-C), 19.0 (Si-C).

Anal. Calcd for  $\text{C}_{67}\text{H}_{72}\text{N}_4\text{O}_{13}\text{Si}_2$  (1197.50): C, 67.20; H, 6.06; N, 4.68. Found: C, 66.46; H, 6.01; N, 4.71.

**O-(2,3,4-tri-O-benzoyl-6-O-tert-butylidiphenylsilyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2-acetamido-3-O-acetyl-6-O-tert-butylidiphenylsilyl-2-deoxy- $\beta$ -D-glucopyranosyl azide 139**

Compound 138 (200 mg, 0.167 mmol) was dissolved in pyridine (3 mL) and acetic anhydride (200  $\mu$ L) was added at 0 °C. The reaction mixture was stirred at room temperature for 30 min. TLC (5% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) showed that the acetylation was completed. Direct evaporation of the solvent under reduced pressure and coevaporation with toluene afforded crude product 139 which was subjected to chromatography purification (2% MeOH in CH<sub>2</sub>Cl<sub>2</sub>). Compound 139 (207 mg) was obtained quantitatively: m.p.  $[\alpha]_D +26.2^\circ$  (c = CHCl<sub>3</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 7.98-6.88 (m, 35H, Aromatic H), 6.07 (d, 1H, J<sub>3,NH</sub> = 8.1, NH), 6.06 (d, 1H, J<sub>4',5'</sub> < 1.0 Hz, H-4'), 5.57 (dd, 1H, J<sub>2',3'</sub> = 10.4 Hz, H-2'), 5.46 (dd, 1H, J<sub>3',4'</sub> = 3.4 Hz, H-3'), 5.03 (d, 1H, J<sub>1',2'</sub> = 8.0 Hz, H-1'), 4.98 (dd, 1H, J<sub>3,4</sub> = 9.2 Hz, H-3), 4.46 (d, 1H, J<sub>1,2</sub> = 9.2 Hz, H-1), 4.25 (dd, 1H, J<sub>4,5</sub> = 9.5 Hz, H-4), 4.06 (dd, 1H, J<sub>2,3</sub> = 10.1 Hz, H-2), 3.96 (dd, 1H, J<sub>5',6a'</sub> = 5.5, J<sub>5',6b'</sub> = 8.8 Hz, H-5'), 3.78 (dd, 1H, J<sub>6a',6b'</sub> = 9.7 Hz, H-6a'), 3.70 (dd, 1H, J<sub>5,6a</sub> = 1.4, J<sub>6a,6b</sub> = 12.0 Hz, H-6a), 3.64 - 3.60 (m, 2H, H-6b', H-6a), 3.06 (1H, H-5), 1.96 (s, 3H, NHAc), 1.76 (s, 3H, OAc), 1.10 (s, 9H, tBu), 0.99 (s, 9H, tBu); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 171.4, 170.2, 165.37, 165.1, 164.7 (5x C=O), 135.9-127.6 (42C, Aromatic), 99.7 (C-1'), 88.7 (C-1), 76.8 (C-5), 73.3 (C-5'), 73.0 (C-3), 72.6 (C-4), 71.9 (C-3'), 70.2 (C-2'), 67.6 (C-4'), 60.8 (C-6'), 60.7 (C-6), 53.1 (C-2), 26.9 (tBu), 26.7 (tBu), 23.3 (NHAc), 20.73 (OAc), 19.4 (Si-C), 19.0 (Si-C).

**O-(2,3,4-tri-O-benzoyl-6-O-benzyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2-acetamido-6-O-tert-butylidiphenylsilyl-2-deoxy- $\beta$ -D-glucopyranosyl azide 144**

To a solution of 143 (181 mg, 0.268 mmol) and 2-acetamido-6-O-(tert-butylidiphenylsilyl)-2-deoxy- $\beta$ -D-glucopyranosyl azide 136 (100 mg, 0.206 mmol) in 4 mL of acetonitrile and dichloromethane (1:1, v/v) was added powdered molecular sieves 4 Å

(200 mg). The mixture was stirred for 2 hours at room temperature, then cooled to -45 °C. NIS (94.5 mg, 0.42 mmol) and TfOH (8.5  $\mu$ L, 0.161 mmol) were added and the reaction mixture was stirred for 45 min at -45 °C. TLC (ethyl acetate-hexane, 1:1) showed complete reaction. The reaction mixture was diluted with 5 mL of dichloromethane, filtered and then washed with dichloromethane. The combined filtrate and washings were successively washed with 10% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, sat. NaHCO<sub>3</sub>, and brine. The dried (Na<sub>2</sub>SO<sub>4</sub>) solution was concentrated under reduced pressure. The syrupy residue was purified by silica gel chromatography (ethyl acetate/hexane, 1:2) to give **144** (168 mg) in 78% yield as an amorphous mass:  $[\alpha]_D^{25} +56.5^\circ$  (c = 1.0, CHCl<sub>3</sub>); (+)FAB-MS (glycerol) gave m/z (ion, relative intensity): 1049 ([M+1]<sup>+</sup>, 4.8 %); IR (Thin film,  $\nu_{\text{cm}^{-1}}$ ): 3390 (NH), 2936, 2865 (CH<sub>3</sub>), 2114 (N<sub>3</sub>), 1728, 1666 and 1267 (C=O), 1090 (C-O), 3066 and 713 (Ph); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 8.01-7.12 (m, 30H, aromatic), 5.91 (dd, 1H,  $J_{4',5'} < 1.0$  Hz, H-4'), 5.73 (dd, 1H,  $J_{2',3'} = 10.5$  Hz, H-2'), 5.57 (d, 1H,  $J_{\text{NH}_2} = 8.1$  Hz, NH), 5.51 (dd, 1H,  $J_{3',4'} = 3.4$  Hz, H-3'), 5.02 (d, 1H,  $J_{1,2} = 8.1$  Hz, H-1'), 4.74 (d, 1H,  $J_{1,2} = 9.3$  Hz, H-1), 4.52 (AB pattern, 1H, J = 11.8, H-A of CH<sub>2</sub>Ph), 4.40 (AB pattern, 1H, H-B of CH<sub>2</sub>Ph), 4.15 (dd, 1H,  $J_{5',6a'} = 6.3$ ,  $J_{5',6b'} = 6.5$  Hz, H-5'), 4.00-4.03 (m, 2H, H-5, H-4), 3.79 (dd, 1H,  $J_{6,6a} = 1.4$ ,  $J_{6a,6b} = 11.8$  Hz, H-6a), 3.71 (dd, 1H,  $J_{5,6b} = 2.7$  Hz, H-6b), 3.69 (dd, 1H, H-6a'), 3.61 (dd, 1H,  $J_{5',6b'} = 6.5$  Hz, H-6b'), 3.52 (dd, 1H,  $J_{2,3+\text{NH}_2} = 18.8$  Hz, H-2), 3.36 (m, 1H, H5), 2.01 (s, 3H, AcHN), 1.06 (s, 9H, Me<sub>3</sub>Si); <sup>13</sup>C-NMR (125.8 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 170.6 (C=O, AcO), 165.4, 165.4 and 165.1 (C=O, 3xBz), 127.8-137.2 (24C, aromatic), 100.9 (C-1'), 87.6 (C-1), 78.6 (C-3), 76.6 (C-5), 73.7 (CH<sub>2</sub>), 73.0 (C-5'), 71.6 (C-3'), 71.4 (C-4), 68.8 (C-2'), 68.1 (C-4'), 67.3 (C-6'), 61.5 (C-6), 56.5 (C-2), 26.9 [SiC(CH<sub>3</sub>)<sub>3</sub>], 23.6 (NHAc), 19.5 [SiC(CH<sub>3</sub>)<sub>3</sub>].

Anal. Calcd. For C<sub>58</sub>H<sub>60</sub>O<sub>13</sub>N<sub>4</sub>Si(1048.38): C, 66.39; H, 5.77; N, 5.34. found: C, 66.11; H, 5.68; N, 5.32.

#### Phenyl 2,3,4-tri-O-acetyl-1-thio- $\beta$ -L-Fucopyranoside **146**

To a solution of acetobromofucopyranose **145** (974 mg, 2.76 mmol), TBAHS (0.96 g, 2.76 mmol, 1 equiv.) and thiophenol (718 mg, 4.64 mmol) in ethyl acetate (10 mL) was added

saturated aqueous 1M Na<sub>2</sub>CO<sub>3</sub> (10 mL). The two phase reaction mixture was vigorously stirred at room temperature for 2 hours. EtOAc (10 mL) was added, and the organic phase was separated and successively washed with sat. NaHCO<sub>3</sub>, water (2x), and sat. NaCl. The combined extracts were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and evaporated under reduced pressure. The residue was purified by silica gel chromatography (EtOAc/Hexane 1:3) to provide pure **6** in 91 % yield:  $[\alpha]_D^{20} -9.6^\circ$  (c = 1.0, CHCl<sub>3</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 7.49-7.27 (m, 5H, Ph), 5.24 (dd, 1H, J<sub>4,5</sub> = 1.0 Hz, H-4), 5.20 (dd, 1H, J<sub>2,3</sub> = 10.0 Hz, H-2), 5.03 (dd, 1H, J<sub>3,4</sub> = 3.3 Hz, H-3), 4.68 (d, 1H, J<sub>1,2</sub> = 10.0 Hz, H-1), 3.80 (dq, 1H, J<sub>5,6</sub> = 6.4 Hz, H-5), 2.12, 2.06, 1.95 (s, Me, 3xOAc), 1.21 (d, 3H, H-6); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 170.6, 170.1, 169.5 (3xOAc), 132.9 (C-*ipso*), 132.4 (2C, C-*ortho*), 128.9 (2C, C-*meta*), 127.9 (C-*para*), 86.5 (C-1), 73.2 (C-5), 72.4 (C-3), 70.3 (C-4), 67.4 (C-2), 20.8, 20.6, 20.6 (s, 3xMe), 16.5 (C-6).

#### Phenyl 2,3,4-tri-O-benzyl-1-thio- $\beta$ -L-fucopyranoside **147**

To a methanolic solution (10 mL) of phenyl 2,3,4-tri-O-acetyl-1-thio- $\beta$ -L-fucopyranoside **144** (1.2 g, 3.11 mmol) was added 1M of sodium methoxide solution in methanol until pH of the reaction mixture became 9. The de-O-acetylation was finished after 25 min. H<sup>+</sup> Resin (IR 120) was added and the solution was stirred for an additional 5 min. The reaction mixture was filtered and concentrated under reduced pressure. The residue was dissolved in N,N-dimethylformamide (15 mL) containing benzyl bromide (1.2 mL). Sodium hydride (450 mg, 18.75 mmol) was added at 0 °C and the resulting mixture was then stirred under nitrogen for 3 hours. Methanol (2 mL) was added to the reaction mixture to destroy excess sodium hydride. The mixture was extracted using toluene and iced water. The organic layer was washed with 5% HCl (10 mL), water and concentrated. The residue was purified by column chromatography (ethyl acetate-hexane, 6:1) to afford **145** (1.37 g) in 84 % yield: IR (thin film,  $\nu_{\text{cm}^{-1}}$ ): 3061, 3030, 2983, 2882, 1584, 1496, 1453, 1357, 1087, 872, 740, 697; M.S. (C.I. ether, m/z): 527.0 ([M+1]<sup>+</sup>, 2.1%), 416.9 ([M+1-PhSH]<sup>+</sup>, 13.8%); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm), 7.60-7.20 (m, 15H, 3xBn), 5.03-4.67 (6H, 3 AB pattern, 3xCH<sub>2</sub>), 4.61 (d, 1H, J<sub>1,2</sub> = 9.7 Hz, H-1), 3.94 (dd, 1H, J<sub>2,3</sub> = 9.2 Hz, H-2), 3.64 (dd, 1H, J<sub>4,5</sub> < 1.0 Hz, H-4), 3.60 (dd, 1H, J<sub>3,4</sub> = 2.8 Hz, H-

3), 3.53 (dq, 1H,  $J_{5,6} = 6.4$  Hz, H-5), 1.27 (d, H-6);  $^{13}\text{C}$  NMR (125.7 MHz,  $\text{CDCl}_3$ ),  $\delta$  (ppm): 138.8-127.0 (18C, 3xBn), 87.60 (C-1), 84.6 (C-3), 77.2 (C-2), 76.7 (C-4), 75.6 ( $\text{CH}_2$ ), 74.7 ( $\text{CH}_2$ ), 74.6 (C-5), 72.9 ( $\text{CH}_2$ ), 17.3 (C-6).

### Preparation of $\text{Le}^x$ trisaccharide

#### Method A:

To a solution of **138** (100 mg, 0.0835 mmol) and ethyl 2,3,4-tri-O-benzyl-1-thio- $\alpha$ -L-fucopyranose **93** (103 mg, 0.209 mmol) in 3 mL of  $\text{CH}_3\text{CN}/\text{CH}_2\text{Cl}_2$  (5:1) was added powdered molecular sieves 4 Å (MS-4 Å; 150 mg). The mixture was stirred for 3 hours at room temperature. The reaction mixture was cooled to  $-45$  °C, NIS (83.1 mg, 0.369 mmol) and TfOH (3.7  $\mu\text{L}$ , 0.042 mmol) were added. The solution was stirred at  $-45$  °C for 45 minutes. TLC showed complete conversion of the donor and gave two coupling products ( $R_f = 0.21$  and  $R_f = 0.25$ , 2% methanol in dichloromethane). The reaction mixture was filtered through celite, and washed with dichloromethane. The combined filtrate and washings were washed with 10% sodium thiosulfate, saturated sodium bicarbonate and brine. It was then dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated to a syrup. After dissolution in the minimum volume of  $\text{CH}_2\text{Cl}_2$ , the mixture was successfully purified by radial silica gel chromatography using 1% MeOH- $\text{CH}_2\text{Cl}_2$  as eluent to give **148 $\beta$**  anomer (44.1 mg) in 37% yield and **148 $\alpha$**  anomer (31.6 mg) in 26% yield.

#### Method B:

To a solution of **138** (100 mg, 0.0835 mmol) and ethyl 2,3,4-tri-O-benzyl-1-thio- $\alpha$ -L-fucopyranoside **93** (60 mg, 0.125 mmol) in 5 mL of 5:1 benzene/dichloromethane was added powdered molecular sieves 4 Å (MS-4 Å; 100 mg). The mixture was stirred for 2 hours at room temperature. DMTST (70 mg, 0.324 mmol) and 4Å MS (50 mg) was added to the stirred mixture at 0 °C. After 1 hour, TLC (2% MeOH in  $\text{CH}_2\text{Cl}_2$ ) showed complete conversion of the donor. Then triethylamine (100  $\mu\text{L}$ ) and methanol (200  $\mu\text{L}$ ) were added to the reaction mixture which was stirred for an additional 25 min. Routine

work up procedure afforded **148 $\alpha$**  (86.5 mg) in 78% yield as an amorphous mass which could be crystallized from isopropanol to give **148** as needles. Traces of acceptor (17.6 mg) were recovered.

**O-(2,3,4-Tri-O-benzoyl-6-O-tert-butyldiphenylsilyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-O-[(2,3,4-tri-O-benzyl  $\alpha$ -L-fucopyranosyl)-(1 $\rightarrow$ 3)]-2-acetamido-6-O-tert-butyldiphenylsilyl-2-deoxy- $\beta$ -D-glucopyranosyl azide **148 $\alpha$****

Compound **148 $\alpha$**  had:  $[\alpha]_D = -15.1^\circ$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ); IR (Thin film,  $\nu_{\text{cm}^{-1}}$ ): 3068, 3032, 2935, 2859, 2114, 1733, 1675, 1601, 1532, 1495, 1453, 1428, 1281, 1262, 1102, 1068, 912, 824, 809, 736, 705; FAB-MS (glycerol) gave  $m/z$  (ion, relative intensity): 1571.7 ( $[\text{M}+1-\text{N}_3]^+$ , 4.8%);  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.04-6.82 (m, 50H, Aromatic H), 6.13 (dd, 1H,  $J_{4',5'} = 0.9$  Hz, H-4'), 5.95 (d, 1H,  $J_{2,\text{NH}} = 7.9$  Hz, NH), 5.65 (dd, 1H,  $J_{2,3'} = 10.5$  Hz, H-2'), 5.59 (dd, 1H,  $J_{3',4'} = 2.4$  Hz, H-3'), 5.25 (d, 1H,  $J_{1'',2''} = 3.5$  Hz, H-1''), 5.09 (d, 1H,  $J_{1,2'} = 7.8$  Hz, H-1'), 4.91-4.50 (4H,  $J = 11.2$  Hz, 2 AB pattern,  $2 \times \text{CH}_2$ ), 4.42 (s, 2H,  $\text{CH}_2$ ), 4.28 (d, 2H,  $J_{1,2} = 9.0$  Hz,  $J_{5'',6''} = 6.7$  Hz, H-1, H-5), 4.21 (dd, 1H,  $J_{4,5} = 8.0$  Hz, H-4), 4.08 (dd, 1H,  $J_{2'',3''} = 10.2$  Hz, H-2''), 4.02-3.95 (m, 2H, H-5', H-3), 3.90 (dd, 1H,  $J_{5,6a} = 2.4$ ,  $J_{6a,6b} = 11.7$  Hz, H-6a), 3.86-3.84 (m, 2H, H-6a', H-6b'), 3.85-3.75 (m, 2H, H-6b, H-3''), 3.64 (dd, 1H,  $J_{2,3} = 9.0$  Hz, H-2), 3.61 (d, 1H,  $J_{4'',5''} < 1.0$  Hz, H-4), 3.04 (m, 1H, H-5), 1.76 (s, 3H, NHAc), 1.12 (s, 9H,  $\text{SiC}(\text{CH}_3)_3$ ), 1.07 (d, 3H,  $J_{5'',6''} = 6.6$  Hz, H-6''), 0.95 (s, 9H,  $\text{SiC}(\text{CH}_3)_3$ );  $^{13}\text{C-NMR}$  ( $\text{CHCl}_3$ )  $\delta$  (ppm): 170.3, 165.5, 165.3, 165.2 ( $4 \times \text{C}=\text{O}$ , BzO, AcNH), 138.9 - 127.2 (60C, Aromatic), 99.59 (C-1'), 97.37 (C-1''), 88.13 (C-1), 79.8 (C-3), 78.3 (C-4''), 77.5 (2C, C-5, C-2''), 75.3 ( $\text{CH}_2$ ), 75.1 (C-5'), 74.2 ( $\text{CH}_2$ ), 73.7 (C-4), 73.5 (C-3'), 72.2 ( $\text{CH}_2$ ), 71.6 (C-3'), 70.3 (C-2'), 67.5 (C-4), 67.0 (C-5''), 61.5 (C-6), 60.3 (C-6'), 55.2 (C-2), 26.9 (tBuSi), 26.6 (tBuSi), 23.2 (NHAc), 19.4 (Si-C), 18.9 (Si-C), 16.9 (C-6'').

Anal. Calcd for  $\text{C}_{94}\text{H}_{100}\text{N}_4\text{O}_{17}\text{Si}_2$  (1614.02): C, 69.95; H, 6.24; N, 3.47, Found: C, 69.84; H, 6.27; N, 3.71.

**O-(2,3,4-Tri-O-benzoyl-6-O-tert-butyl-diphenylsilyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-O-[(2,3,4-tri-O-benzyl  $\beta$ -L-fucopyranosyl)-(1 $\rightarrow$ 3)]-2-acetamido-6-O-tert-butyl-diphenylsilyl-2-deoxy- $\beta$ -D-glucopyranosyl azide 148 $\beta$**

Compound 148 $\beta$  had:  $[\alpha]_D = +17.0^\circ$  (c = 1.0, CHCl<sub>3</sub>); IR (Thin film,  $\nu_{\text{cm}^{-1}}$ ): 3067, 3031, 2935, 2858, 2114, 1733, 1683, 1601, 1527, 1494, 1452, 1428, 1281, 1262, 1103, 1069, 912, 815, 738, 705; FAB-MS (glycerol) gave m/z (ion, relative intensity): 1571.7 ([M+1-N<sub>3</sub>]<sup>+</sup>, 9.0%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 8.06-6.85 (m, 50H, Aromatic H), 6.04 (dd, 1H,  $J_{4',5'} < 1.0$  Hz, H-4'), 5.57 (dd, 1H,  $J_{2',3'} = 10.3$  Hz, H-2'), 5.29 (dd, 1H,  $J_{3',4'} = 3.4$  Hz, H-3'), 5.07 (d, 1H,  $J_{1',2'} = 8.0$  Hz, H-1'), 4.71 (d, 1H,  $J_{1'',2''} = 7.6$  Hz, H-1''), 4.88-4.14 (6H, 3xAB pattern, 3xCH<sub>2</sub>), 4.15 (dd, 1H,  $J_{4,5} = 7.9$  Hz, H-4), 3.96-3.72 (m, 7 H, H-2, H-3, H-5', H-6a, H-6b, H-6a', H-6b'), 3.57 (dd, 1H,  $J_{2'',3''} = 9.8$  Hz, H-2''), 3.45 (d, 1H,  $J_{4'',5''} < 1.0$  Hz, H-4), 3.42 (dd, 1H,  $J_{5'',6''} = 6.2$  Hz, H-5''), 3.30 (dd, 1H,  $J_{3'',4''} = 2.7$  Hz, H-3''), 3.22 (ddd, 1H,  $J_{5,6a} = 3.1$  Hz,  $J_{5,6b} = 7.3$  Hz,  $J_{6a,6b} = 11.2$  Hz, H-5), 1.71 (s, 3H, NHAc), 1.18 (d, 3H,  $J_{5'',6''} = 6.2$  Hz, H-6''), 1.05 (s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>), 0.93 (s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 171.9, 165.3, 165.2, 165.2 (4xC=O, BzO, AcNH), 138.6 - 127.0 (60C, Aromatic), 102.0 (C-1''), 99.4 (C-1'), 89.1 (C-1), 82.8 (C-3''), 78.2 (C-5), 78.0 (C-2''), 76.6 (C-4''), 75.4 (C-5'), 74.8 (CH<sub>2</sub>), 74.2 (CH<sub>2</sub>), 74.1 (C-2'), 75.4 (C-4), 73.2 (CH<sub>2</sub>), 71.6 (C-3'), 72.0 (C-3'), 71.0 (C-2'), 70.6 (C-5''), 67.4 (C-4), 62.1 (C-6), 60.6 (C-6'), 53.6 (C-2), 26.8 (tBuSi), 26.7 (tBuSi), 22.9 (NHAc), 19.3 (Si-C), 19.0 (Si-C), 16.8 (C-6'').

**O-(2,3,4-Tri-O-benzoyl-6-O-benzyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-O-[(2,3,4-tri-O-benzyl  $\alpha$ -L-fucopyranosyl)-(1 $\rightarrow$ 3)]-2-acetamido-6-O-tert-butyl-diphenylsilyl-2-deoxy- $\beta$ -D-glucopyranosyl azide 149**

To a solution of 144 (80 mg, 0.076 mmol) and phenyl 2,3,4-tri-O-benzyl-1-thio- $\beta$ -L-fucopyranoside 147 (80.3 mg, 0.152 mmol) in benzene (3 mL) was added powdered molecular sieves 4 Å (200 mg). The mixture was stirred for 2 hour at room temperature,

and cooled to 0 °C. To the cooled solution, dimethyl(methylthio)sulfonium triflate (98 mg, 0.38 mmol) was added and the reaction mixture was stirred under nitrogen atmosphere for 2 hours at 0 °C. The course of the reaction was monitored by TLC. After reaction completion, triethylamine (100  $\mu$ L) and methanol (200  $\mu$ L) were added and the mixture was stirred for an additional 25 min. The precipitates were filtered, and washed with dichloromethane. The filtrate and washings were combined, dried ( $\text{Na}_2\text{SO}_4$ ), and concentrated to give a syrup which was chromatographed on silica gel using 1% methanol-dichloromethane to give **148** (96.7 mg) in 87% yield as an amorphous mass:  $[\alpha]_D = -27.0^\circ$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ); (+)FAB-MS (glycerol) gave  $m/z$  (ion, relative intensity): 1422.58 ( $[\text{M}-\text{N}_3]^+$ , 1.8 %); IR (Thin film,  $\nu_{\text{cm}^{-1}}$ ): 3323, 3064, 2906, 2114, 1733, 1675, 1452, 1267, 1101, 1068, 823.4, 738, 705;  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 8.02-7.11 (m, 45H, 9 Ph), 5.93 (dd, 2H,  $J_{4,5'} = 1.4$  Hz, H-4', NH), 5.69 (dd, 1H,  $J_{2',3'} = 10.5$  Hz, H-2'), 5.51 (dd, 1H,  $J_{3',4'} = 3.4$  Hz, H-3'), 5.30 (d, 1H,  $J_{1'',2''} = 2.3$  Hz, H-1''), 5.11 (d, 1H,  $J_{1,2} = 8.2$  Hz, H-1'), 4.89-4.35 (4xAB pattern, 8H, 4xPhCH<sub>2</sub>), 4.41 (1H, H-5''), 4.36 (d, 1H, H-1), 4.25 (dd, 1H,  $J_{4,5} = 8.2$  Hz, H-4), 4.12 (dd, 1H,  $J_{2'',3''} = 10.2$  Hz, H-2''), 4.06-3.99 (M, 2H, H-3, H-5), 3.93-3.81 (m, 3H, H-3', H-6a, H-6b), 3.70-3.62 (m, 4H, H-2, H-4'', H-6a', H-6b'), 3.08 (d, 1H, H-5), 1.79 (s, 3H, NHAc), 1.23 (d, 3H,  $J_{5'',6''} = 6.2$  Hz, H-6''), 1.11 (s, 9H,  $\text{SiCMe}_3$ );  $^{13}\text{C NMR}$  (125.7 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 170.3 (C=O, OAc), 165.7, 165.3, 165.1 (4xC=O,  $\nu_z$ ), 135.9-127.7 (54C, aromatic), 99.7 (C-1'), 97.4 (C-1''), 88.1 (C-1), 79.9 (C-3''), 78.4 (4''), 77.5 (C-5), 77.3 (C-2''), 75.2 (CH<sub>2</sub>), 74.8 (C-3), 74.1 (CH<sub>2</sub>), 73.6 (C-4), 73.6 (CH<sub>2</sub>), 72.6 (C-5'), 72.4 (CH<sub>2</sub>), 71.6 (C-3'), 70.2 (C-2'), 68.3 (C-4'), 67.2 (C-6'), 67.1 (C-5''), 61.4 (C-6), 55.5 (C-2), 26.9 ( $\text{SiCMe}_3$ ), 23.3 (NHAc), 19.5 [ $\text{SiC}(\text{CH}_3)_3$ ], 17.0 (C-6'').

Anal. Calcd For  $\text{C}_{85}\text{H}_{88}\text{O}_{17}\text{N}_4\text{Si}$  (1465.74): C, 69.65; H, 6.05; N, 3.82. found: C, 69.78; H, 5.94; N, 3.83.

**O-(6-O-tert-butylidiphenylsilyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-O-tert-butylidiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide 150**

To a solution of **148α** (100 mg, 0.0619 mmol) in 5 mL (MeOH:CH<sub>2</sub>Cl<sub>2</sub>, 9:1) was added 200 μL of sodium methoxide (1.0 M) and the mixture was stirred at room temperature for 4 hours. TLC (7% methanol in dichloromethane) showed clear conversion of **148α** to debenzoylated product **149**. The reaction mixture was neutralized with Amberlite IR-120 (H<sup>+</sup>) resin and filtered. The resin was washed with methylene chloride. The combined filtrate and washings were concentrated under reduced pressure to a syrup that was chromatographed on radial silica gel plate with 5% methanol in dichloromethane to give **150** (80.2 mg) in 97 % yield:  $[\alpha]_D^{25} -51.5^\circ$  (c = 1.13, CHCl<sub>3</sub>); IR (Thin film,  $\nu_{\text{cm}^{-1}}$ ): 3404, 3055, 2933, 2858, 2114, 1661, 1590, 1532, 1496, 1463, 1089, 912, 820, 737, 703; FAB-MS (glycerol) gave m/z (ion, relative intensity): 1258.5 ([M+1-HN<sub>3</sub>]<sup>+</sup>, 1.8%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 7.72-7.03 (m, 35H, Aromatic H), 5.87(d, 1H,  $J_{2,\text{NH}}$  = 6.4 Hz, NH), 5.05 (d, 1H,  $J_{1,2}$  = 9.0 Hz, H-1), 4.94 (d, 1H,  $J_{1'',2''}$  = 3.6 Hz, H-1''), 4.87-4.55 (2H,  $J$  = 11.5, AB pattern, CH<sub>2</sub>), 4.69 (d, 1H,  $J_{1',2'}$  = 7.9 Hz, H-1'), 4.82-4.47 (2H,  $J$  = 11.4 Hz, AB pattern, CH<sub>2</sub>), 4.45 (d, 1H,  $J_{4'',5''}$  < 1.0 Hz, H-5''), 4.28-4.23 (2H,  $J$  = 11.0 Hz, AB pattern, CH<sub>2</sub>), 4.18 (dd, 1H,  $J_{3,4}$  = 9.4 Hz, H-3), 4.13-4.11 (m, 3H, H-4, H-4', H-6a), 4.00 (dd, 1H,  $J_{2'',3''}$  = 10.2 Hz, H-2''), 3.92-3.87 (m, 2H, H-6a', H-6b), 3.82 (dd, 1H,  $J_{5',6b'}$  = 5.0,  $J_{6a',6b'}$  = 9.2 Hz, H-6b'), 3.76 (dd,  $J_{3'',4''}$  = 2.6 Hz, H-3''), 3.54-3.41 (m, 5H, H-2', H-3', H-5', H-4'', H-5), 3.16 (dd, 1H,  $J_{2,3}$  = 9.4 Hz, H-2), 1.55 (s, 3H, NHAc), 1.06 (s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>), 1.05 (d, 3H,  $J_{5'',6''}$  = 6.6 Hz, H-6''), 0.99 (s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>); <sup>13</sup>C-NMR (CHCl<sub>3</sub>)  $\delta$  (ppm): 170.9 (C=O), 138.8-127.4 (42C, Aromatic), 100.8 (C-1'), 98.5 (C-1''), 87.0 (C-1), 79.9 (C-3''), 77.5 (C-4''), 77.4 (C-5), 76.8 (C-2''), 75.7 (C-3), 75.1 (CH<sub>2</sub>), 74.8 (CH<sub>2</sub>), 74.1 (C-5'), 73.6 (C-3'), 73.2 (C-4), 72.4 (C-2'), 71.9 (CH<sub>2</sub>), 67.1 (C-4'), 66.9 (C-5''), 61.8 (C-6), 60.8 (C-6'), 58.4 (C-2), 26.8 (tBuSi), 26.7 (tBuSi), 23.1 (NHAc), 19.5 (Si-C), 19.1 (Si-C), 16.8 (C-6'').

Anal. Calcd for  $C_{73}H_{88}N_4O_{14}Si_2 \cdot H_2O$  (1319.71): C, 66.44; H, 6.87; N, 4.25, Found: C,66.056; H, 6.65; N, 4.24.

**O-(2,3,4-Tri-O-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-O-[(2,3,4-tri-O-benzyl  $\alpha$ -L-fucopyranosyl)-(1 $\rightarrow$ 3)]-2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl azide 152**

Compound 148 $\alpha$  (80.7 mg, 0.05 mmol) was dissolved in a mixture of 1 M tetrabutylammonium fluoride in tetrahydrofuran (3 mL) and tetrahydrofuran (2 mL). To this solution was added acetic acid to adjust pH of reaction solution to 7. The resulting mixture was refluxed for 5 hours. TLC (5% methanol in dichloromethane) showed complete conversion of 147 to desilylated product 152. Evaporation under reduced pressure and column chromatography (2%, MeOH/CH<sub>2</sub>Cl<sub>2</sub>) of the residue on silica gel gave 152 (43.9 mg) in 76 % yield as amorphous solid:  $[\alpha]_D -9.1^\circ$  (c = 1.0, CHCl<sub>3</sub>); FAB-MS (glycerol) gave m/z (ion, relative intensity): 1137.5 9 ([M]<sup>+</sup>, 0.8%), 1094.4 ([M-HN<sub>3</sub>]<sup>+</sup>, 3.6%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 8.07-7.16 (m, 30H, Aromatic H), 5.95 (d, 1H, J<sub>2,NH</sub> = 7.2 Hz, NH), 5.82 (dd, 1H, J<sub>2,3</sub> = 10.3 Hz, H-2'), 5.80 (dd, 1H, J<sub>4',5'</sub> < 1.0 Hz, H-4'), 5.77 (dd, 1H, J<sub>3',4'</sub> = 3.4 Hz, H-3'), 5.24 (d, 1H, J<sub>1',2</sub> = 7.8 Hz, H-1'), 5.21 (d, 1H, J<sub>1,2</sub> = 8.9 Hz, H-1), 5.12 (d, 1H, J<sub>1'',2''</sub> = 4.8 Hz, H-1''), 4.79 -4.07 (6H, 3xAB pattern, 3xCH<sub>2</sub>), 4.66 (dd, 1H, J<sub>5',6'</sub> = 6.2 Hz, H-5''), 4.42 (dd, 1H, J<sub>3,4</sub> = 9.5 Hz, H-3), 4.25 (dd, 1H, J<sub>5',6a'</sub> = 4.9, J<sub>5',6b'</sub> = 9.4 Hz, H-5'), 4.08-4.02 (m, 2H, H-4, H-2''), 3.95 (dd, 1H, J<sub>3'',4''</sub> = 2.6 Hz, H-3''), 3.83 -3.78 (m, 4H, H-6a, H-6b, H-6a', H-6b'), 3.46 (d, 1H, J<sub>4'',5''</sub> < 1.0 Hz, H-4''), 3.28 (d, 1H, J<sub>2,3</sub> = 9.2 Hz, H-2), 3.14 (m, H-5), 1.75 (s, 3H, NHAc), 1.41 (d, 3H, J<sub>5'',6''</sub> = 6.2 Hz, H-6''); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 170.8, 166.5, 165.3, 165.2 (4xC=O, BzO, AcNH), 138.8 - 127.1 (36C, Aromatic), 101.1 (C-1'), 96.9 (C-1''), 87.7 (C-1), 79.6 (C-3''), 78.8 (C-4''), 77.6 (C-5), 76.3 (C-2''), 75.6 (C-5'), 75.1 (CH<sub>2</sub>), 74.1 (CH<sub>2</sub>), 73.8 (C-4), 72.6 (C-3), 72.2 (CH<sub>2</sub>), 71.9 (C-3'), 70.2 (C-4'), 96.9 (C-2'), 67.0 (C-5''), 61.8 (C-6'), 59.4 (C-6), 58.4 (C-2), 23.2 (NHAc), 16.8 (C-6'').

Anal. Calcd for  $C_{62}H_{64}N_4O_{17}H_2O$  (1155.22): C, 64.46; H,5.76; N, 4.85. Found: C,64.54; H, 5.76; N, 4.78.

**O-(6-O-Benzyl-β-D-galactopyranosyl)-(1→4)-O-[(2,3,4-tri-O-benzyl α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-O-tert-butylidiphenylsilyl-2-deoxy-β-D-glucopyranosyl azide 151**

To a solution of **149** (420 mg, 0.287 mmol) in methanol (5 mL) was added sodium methoxide (0.5 mL). The mixture was stirred for 6 h at room temperature. The solution was then neutralized with Amberlite IR-120 (H<sup>+</sup>) resin and filtered. The filtrate was concentrated to a syrup that was chromatographed on a column of silica gel with ethyl acetate-hexane (2:1, v/v) as eluent to give **151** (314 mg) in 95% yield as an amorphous mass:  $[\alpha]_D -61.7^\circ$  (c, 1.0, CHCl<sub>3</sub>); (+)FAB-MS (glycerol) gave m/z (ion, relative intensity): 1153.5 ([M+1]<sup>+</sup>, 0.1%), 1111.8 ([M+1-N<sub>3</sub>]<sup>+</sup>, 5.9%); IR (Thin film,  $\nu_{\text{cm}^{-1}}$ ): 3397, 2905, 2115, 1659, 1454, 1368, 1083.6, 738, 701; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 7.69-7.19 (m, 30H, aromatic), 5.92 (d, 1H,  $J_{\text{NH},2} = 6.8$  Hz, NH), 5.06 (d, 1H,  $J_{1'',2''} = 3.5$  Hz, H-1''), 4.98 (d, 1H,  $J_{1,2} = 9.1$  Hz, H-1'), 4.93-4.54 (6H, 3xAB pattern), 4.68 (d, 1H,  $J_{1',2'} = 7.6$  Hz, H-1'), 4.47-4.43 (2H, AB pattern), 4.44 (H-5''), 4.18 (dd, 1H,  $J_{4,5} = 8.2$  Hz, H-4), 4.16 (dd, 1H,  $J_{5,6a} = 2.9$ ,  $J_{6a,6b} = 12.2$  Hz, H-6a), 4.09 (dd, 1H,  $J_{2'',3''} = 10.3$  Hz, H-2''), 3.97 (dd, 1H,  $J_{3'',4''} = 2.7$  Hz, H-3''), 3.95 (1H, H-4'), 3.94 (dd, 1H, H-6b), 3.71 (dd, 1H,  $J_{5',6a'} = 7.2$ ,  $J_{6a',6b'} = 9.5$  Hz, H-6a'), 3.70 (1H, H-4''), 3.63 (dd, 1H,  $J_{5',6b'} = 5.2$  Hz, H-6b), 3.53- 3.44 (m, 3H, H-2', H-5', H-5), 3.42 (dd, 1H,  $J_{2',3'} = 9.5$ ,  $J_{3',4'} = 3.2$  Hz, H-3'), 3.5 (1H, H-2), 19.90 (s, 3H, NHAc), 1.12 (d, 3H,  $J_{5'',6''} = 6.2$  Hz, H-6''), 1.04 (s, 9H, SiCMe<sub>3</sub>); <sup>13</sup>C-NMR (125.7 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 170.8 (C=O, NHAc), 138.6 - 127.2 (30H, aromatic), 100.7 (H-1'), 98.4 (C-1''), 87.3 (C-1), 80.0 (C-3''), 77.7 (C-4''), 77.5 (C-5), 77.1 (C-2''), 76.1 (C-4), 75.1 (CH<sub>2</sub>), 74.7 (CH<sub>2</sub>), 73.6 (C-3'), 73.5 (2C, CH<sub>2</sub>, C-3), 72.8 (C-5'), 72.3 (C-2'), 72.3 (CH<sub>2</sub>), 68.5 (C-6'), 68.2 (C-4'), 67.1 (C-5''), 61.9 (C-6), 57.9 (C-2), 26.8 (SiCMe<sub>3</sub>), 23.1 (NHAc), 19.4 [SiC(CH<sub>3</sub>)<sub>3</sub>], 16.9 (C-6'').

Anal. Calcd For C<sub>64</sub>H<sub>76</sub>O<sub>14</sub>N<sub>4</sub>Si: C, 66.64; H, 6.65; N, 4.86. found: C, 66.40; H, 6.65; N, 4.71.

**O-(6-O-tert-butyl-diphenylsilyl-3-O-Sulfo- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-O-[(2,3,4-tri-O-benzyl  $\alpha$ -L-fucopyranosyl)-(1 $\rightarrow$ 3)]-2-acetamido-6-O-tert-butyl-diphenylsilyl-2-deoxy- $\beta$ -D-glucopyranosyl azide 153 (Le<sup>x</sup>-3'-O-Sulfate)**

To a solution of Le<sup>x</sup> derivative 150 (100 mg, 0.0769 mmol) in toluene (3 mL) was added 23.5 mg (0.0923 mmol) of dibutyltin oxide. The mixture was refluxed under nitrogen for 14 hours. The solution was concentrated under reduced pressure. The dry dibutylstannylene complex was treated with SO<sub>3</sub>·NMe<sub>3</sub> (21.7 mg, 0.154 mmol) in 1:1 tetrahydrofuran-pyridine mixture (3 mL) at room temperature for 24 hours. The reaction mixture was then diluted with methanol (3 mL), filtered and concentrated under reduced pressure. The residue was purified on a radial silica gel plate (1 mm) using 6% to 15% methanol in dichloromethane as gradient eluent to give 153 (97.2 mg) in 92 % yield: R<sub>f</sub> = 0.45 (MeOH/CH<sub>2</sub>Cl<sub>2</sub>, 1:8); m.p. 119-121 °C; [ $\alpha$ ]<sub>D</sub> = -30.2° (c = 1.5, CHCl<sub>3</sub>); IR (Thin film,  $\nu_{\text{cm}^{-1}}$ ): 3381, 3021, 2934, 2857, 2113, 1661, 1543, 1472, 1428, 1233, 1157, 1006, 1068, 991, 809, 751, 703; FAB-MS (glycerol) gave m/z (ion, relative intensity): 1380.2 ([M]<sup>+</sup>, 0.4 %); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 7.71-6.97 (m, 35H, Aromatic H), 5.06 (d, 1H, J<sub>1'',2''</sub> = 1.7 Hz, H-1''), 4.85 (d, 1H, J<sub>1,2</sub> = 7.7 Hz, H-1'), 4.83 (d, 1H, J<sub>1,2</sub> = 8.6 Hz, H-1), 4.78, 4.42 (2H, J = 11.1, AB pattern, CH<sub>2</sub>), 4.73, 4.61 (2H, J = 11.5 Hz, AB pattern, CH<sub>2</sub>), 4.54 (d, 1H, J<sub>4',5'</sub> < 1.0 Hz, H-5'), 4.47 (d, 1H, J<sub>4',5'</sub> < 1.0 Hz, H-4'), 4.35 (dd, 1H, J<sub>3',4'</sub> = 3.0 Hz, H-3'), 4.34-4.16 (m, 4H, H-4, H-6a, CH<sub>2</sub>), 4.11 (dd, 1H, J<sub>3,4</sub> = 9.1 Hz, H-3), 3.94-3.89 (m, 2H, H-2'', H-6a'), 3.97 (dd, 1H, J<sub>5',6'</sub> = 4.6 Hz, H-6b'), 3.97-3.75 (m, 1H, H-6b, H-2'), 3.73 (dd, 1H, J<sub>2'',3''</sub> = 9.8, J<sub>3'',4''</sub> = 2.7 Hz, H-2''), 3.56 (m, 1H, H-5'), 3.51 (1H, H-4''), 3.47 (1H, H-5), 2.90 (1H, H-2), 1.62 (s, 3H, NHAc), 1.04 (s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>), 1.03 (d, 3H, J<sub>5'',6''</sub> = 6.5 Hz, H-6''), 0.94 (s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>); <sup>13</sup>C-NMR (CHCl<sub>3</sub>)  $\delta$  (ppm): 171.0 (C=O), 138.9-127.3 (42C, Aromatic), 100.3 (C-1'), 98.0 (C-1''), 87.9 (C-1), 80.7 (C-3'), 79.5 (C-3''), 77.8 (C-4''), 77.5 (C-5), 76.8 (C-2''), 76.0 (C-3), 75.2 (CH<sub>2</sub>), 73.8 (CH<sub>2</sub>), 73.7 (C-5'), 73.3 (C-4), 72.1 (CH<sub>2</sub>), 70.5 (C-2'), 66.9 (C-5''), 66.4 (C-4'), 61.6 (C-6), 60.8 (C-6'), 50.8 (C-2), 26.8 (tBuSi), 26.7 (tBuSi), 23.2 (NHAc), 19.5 (Si-C), 19.1 (Si-C), 16.7 (C-6'').

**Methyl (phenyl-5-acetamido-4,7,8,9-tetra-O-benzoyl-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-thio-nonulopyranosid)onate 154**

To a solution of methyl (phenyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-thio-nonulopyranosid)onate **12** (0.5 g , 0.86 mmol ) in 10 mL of methanol was added 1 M NaOMe until pH = 8.5. The reaction mixture was stirred for 25 min. The solution was evaporated under reduced pressure and coevaporated with dry pyridine (5 mL). The resulting dried compound was dissolved in dry pyridine (5 mL) and benzoyl chloride (0.55 mL, 4.8 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 3 hours. The excess chloride was then quenched with ice water and the solution was extracted with methylene chloride (10 mLx2). The extracts were washed with sodium bicarbonate, water and brine. The Organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography using EtOAc/hexane (2:5) as eluent to give **154** (0.66 g) in 92 % yield:  $[\alpha]_D$ : -18.2° ( c = 1.0, CHCl<sub>3</sub>); (+)FAB-MS (glycerol) gave m/z (ion, relative intensity): 832.3 ([M+1]<sup>+</sup>, 4.8%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 8.09-7.27 (m, 25H, aromatic), 6.16 (d, 1H, J<sub>5,NH</sub> = 7.8 Hz, NH), 5.62 (ddd, 1H, J<sub>8,9a</sub> = 2.3, J<sub>8,9b</sub> = 4.8 Hz, H-8), 5.16 (ddd, 1H, J<sub>3a,4</sub> = 11.5, J<sub>4,5</sub> = 10.5 Hz, H-4), 5.02 (dd, 1H, J<sub>9a,9b</sub> = 12.3 Hz, H-9a), 4.73 (dd, 1H, J<sub>8,9b</sub> = 4.8 Hz, H-9b), 4.15 (dd, 1H, J<sub>5,6</sub> = 10.3 Hz, H-5), 4.00 (dd, 1H, J<sub>7,8</sub> = 8.5 Hz, H-7), 3.49 (dd, 1H, J<sub>6,7</sub> = 1.3 Hz, H-6), 2.99 (dd, 1H, J<sub>3c,4</sub> = 4.7 Hz, H-3e), 2.83 (s, 3H, OCH<sub>3</sub>), 2.18 (dd, 1H, J<sub>3a,4</sub> = 11.5, J<sub>3a,3c</sub> = 12.5 Hz, H-3a), 1.90 (NHAc); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 172.8, 168.5, 167.7, 166.1, 165.0 (C=O), 136.5-128.3 (30C, aromatic), 87.9 (C-2), 76.7 (C-6), 71.2 (C-8), 69.7 (C-4), 67.7 (C-7), 63.6 (C-9), 52.2 (OMe), 52.1 (C-5), 37.9 (C-3), 23.0 (Me, NHAc).

**O-(Methyl 5-acetamido-4,7,8,9-tetra-O-benzoyl-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosylonate)-(2 $\rightarrow$ 3)-O-(6-O-benzyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-O-[(2,3,4-tri-O-benzyl  $\alpha$ -L-fucopyranosyl)-(1 $\rightarrow$ 3)]-2-acetamido-6-O-tert-butyl-diphenylsilyl-2-deoxy- $\beta$ -D-glucopyranosyl azide (SLe<sup>x</sup>) 155**

To a solution of 151 (58 mg, 0.05 mmol) and methyl (phenyl 5-acetamido-4,7,8,9-tetra-O-benzoyl-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-thio-nonulopyranosid)onate 154 (104 mg, 0.125 mmol) in 3 mL dry propionitrile-dichloromethane (5:1) was added powdered molecular sieves 4 Å (200 mg). The mixture was stirred for 2 hours at room temperature, and cooled to -30°C. To the cooled solution, dimethyl(methylthio)sulfonium triflate (26 mg, 0.25 mmol) was added and the reaction mixture was stirred under nitrogen atmosphere for 2 hours at 0 °C. The progress of the reaction was monitored by TLC (EtOAc/Hexane 3:1). After reaction completion, triethylamine (50  $\mu$ L) and methanol (100  $\mu$ L) were added to the mixture. The mixture was then stirred for an additional 15 min and then filtered. The combined filtrates and washings were washed with water, dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated. Chromatography on silica gel using ethyl acetate/hexane (1:1) as eluent gave 155 (44.2 mg) in 47% yield as an amorphous mass:  $[\alpha]_D = -0.64^\circ$  (c = 1.0, CHCl<sub>3</sub>); (+)FAB-MS (glycerol) gave m/z (ion, relative intensity): 1728.38 ([M-N<sub>3</sub>-Bz]<sup>+</sup>, 16.2 %); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 8.17-7.18 (m, 50H, 10xPh), 6.25 (1H, d, J<sub>NH,5''</sub> = 8.9 Hz, NH of Neu5Ac), 6.12 (1H, d, J<sub>NH,2</sub> = 7.2 Hz, NH of Glu), 5.69 (ddd, 1H, J<sub>8,9a</sub> = 2.5, J<sub>8,9b</sub> = 5.4, J<sub>7,8</sub> = 9.1 Hz, H-8 of Neu5Ac), 5.17 (d, J<sub>1,2</sub> = 3.5 Hz, H-1 of Fuc), 5.01-4.41 (3xAB pattern, 6H, 3xPhCH<sub>2</sub>), 4.85 (d, J<sub>1,2</sub> = 7.7 Hz, H-1 of Gal), 4.82 (d, J<sub>1,2</sub> = 9.8 Hz, H-1 of GlcNAc); 4.68-4.57 (AB pattern, 2H, PhCH<sub>2</sub>), 4.43 (dq, 1H, J<sub>4,5</sub> = 6.0 Hz, H-5 of Fuc), 3.21 (s, 3H, OMe, Neu5Ac), 2.68 (dd, J<sub>3e,4</sub> = 4.9, J<sub>3e,3a</sub> = 13.1 Hz, H-3e of Neu5Ac), 2.26 (t, 1H, J<sub>3a,4</sub> = 11.4 Hz, H-3a), 1.94 (s, 3H, NHAc of Neu5Ac), 1.67 (s, 3H, NHAc of GlcNAc), 1.15 (d, 3H, H-6 of Fuc), 1.08 (s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>); <sup>13</sup>C NMR (125.7 MHz, CDCl<sub>3</sub>):  $\delta$  172.9, 170.6 (C=O, NHAc), 168.6, 167.5, 166.1, 165.8, 165.0 (C=O, Bz), 138.9-125.5 (60C, Aromatic), 101.1 (C-1, Gal), 98.4 (C-1, Fuc), 98.1 (C-1, Glc), 87.9 (C-3, Fuc), 79.9 (C-4, Fuc), 87.9, 79.9, 77.8, 77.7, 76.1, 75.1, 74.6, 74.4, 73.3 (2C), 73.0, 72.6, 72.2, 70.5, 70.0, 69.3, 68.4 (C-6, Gal), 68.0, 67.3,

64.2 (C-9, Neu5Ac), 62.4 (C-6, Glc), 52.7 (OMe), 51.9 (C-5, Fuc), 37.0, 27.0 [3C, SiC(CH<sub>3</sub>)<sub>3</sub>], 23.2 (Me, NHAc), 23.0 (Me, NHAc), 19.5 (Si-C).

## Conclusions

The research presented in this thesis has demonstrated the feasibility and generality of the “active-latent” glycosylation strategy in oligosaccharide synthesis including biologically important sialosides and glycoconjugates.

To demonstrate the generality of this new glycosylation strategy, an extensive amount of glycosylation reactions using various thioglycosyl donors and acceptors were conducted. The potential usefulness of the “latent” nature of the nitro group was nicely illustrated by the chemoselective glycosylation of partially benzoylated para-nitrophenyl thioglycosides with “active” thioglycosyl donors using NIS/TfOH as promoter. The results discussed in this thesis clearly indicate that NIS/TfOH is a valuable promoter for the chemoselective glycosylation of “latent” acceptors with “active” thioglycosyl donors. Furthermore, our studies also revealed that the amount of triflic acid used was crucial for the selective activation of an “active” thioglycoside over a relatively “latent” thioglycoside.

The combination of “active-latent” and “armed-disarmed” glycosidation methodologies, *i.e.* changing the electron donating and withdrawing abilities of the substituents on the thiophenyl ring and the protecting groups at the C-2 position, could expand the relative reactivities of glycosyl donors or acceptors and thus widen the scope of this glycosylation strategy. This new strategy is clearly advantageous for the syntheses of complex oligosaccharides.

The “active-latent” glycosylation strategy was successfully extended to the synthesis of sialosides. Because the formation of the sialic acid glycosyl linkage with other sugar moieties is hindered electronically as well as sterically by the presence of the geminal carboxylate group, formation of this type of bond is a **true test** of the utility of a given method of glycosylation. After careful optimization of reaction conditions, the NIS/TfOH promoted glycosylation of an “active”  $\alpha$ -thiosialoside of Neu5Ac over a “latent” *p*-nitrophenyl thioglycosyl acceptor was achieved. GM<sub>3</sub> trisaccharide and GM<sub>3</sub> related sialosides have been synthesized in good yields.

An advantage of using “active-latent” glycosylation strategy to prepare a GM<sub>3</sub> sialoside over other methodologies is that the GM<sub>3</sub> trisaccharide prepared can be easily transformed back into an “active” form ready for further coupling to other sugar moieties. In addition, the nitro group on the thiophenyl ring of GM<sub>3</sub> trisaccharides could be quantitatively reduced to amino group which allows the introduction of an acryloyl group or other optional spacers to develop GM<sub>3</sub> glycopolymers and or glycoconjugates.

GM<sub>3</sub> and lactose-containing glycopolymers, with different incorporation ratios, were prepared by copolymerization of GM<sub>3</sub> and lactosyl monomers with acrylamide. Qualitative double immunodiffusion assays were conducted for evaluation of the interaction between the glycopolymers and the corresponding lectins and showed selective bindings.

Finally, a very efficient synthetic route to the sialyl Le<sup>x</sup> was developed. Remarkable regioselectivity in the glycosylation reaction between a galactosyl donors and a N-acetylglucosamine acceptor allowed us to synthesize N-acetyl-lactosamine in a very convergent way. Stereocontrolled introduction of a fucosyl moiety to the N-acetyl-lactosamine acceptor provided to the important Le<sup>x</sup> trisaccharide. After removal of the protecting groups of the galactosyl moiety of Le<sup>x</sup>, a sulfate group or sialic acid moiety was regioselectively introduced to complete the synthesis of 3-O-sulfo-Le<sup>x</sup> and sialyl Le<sup>x</sup> respectively.

In order for complex oligosaccharide conjugates to become useful in therapeutic industry, much more work remains to be done in their practical syntheses. In addition, the preparation of dendritic Le<sup>x</sup>, 3-O-sulfo Le<sup>x</sup> and sialyl Le<sup>x</sup> generates much scientific and industrial interests. The research along this line is being actively pursued in this laboratory.

## Claims to Original Research

### Novel Synthetic methodology:

Development of “active-latent” glycosylation strategy for the synthesis of complex oligosaccharides and glycoconjugates.

Development of phase transfer catalyzed procedure for the efficient synthesis of different type of glycosyl derivatives: aryl and alkyl thiolates, xanthates, cyanates and N-hydroxysuccinimides.

Development of improved procedure for the synthesis of important partially protected glycosyl acceptor para-nitrophenyl O-(2,6-di-O-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1-thio- $\beta$ -D-glucopyranoside 113.

Novel synthesis of GM<sub>3</sub> [Neu5Ac $\alpha$ (2 $\rightarrow$ 3)Gal $\beta$ (1 $\rightarrow$ 4)GlcNAc] trisaccharide using “active-latent” glycosylation strategy.

Development of efficient synthesis of Lewis<sup>x</sup> and 3'-sulfate Lewis<sup>x</sup> and sialyl Lewis<sup>x</sup>. High regioselectivities were obtained during the preparation of N-acetyllactosamine derivatives.

### Important oligosaccharides and glycoconjugates:

Para-Nitrophenyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosylonate)-(2 $\rightarrow$ 6)-2,3,4-tri-O-benzoyl-1-thio- $\alpha$ -D-galactopyranoside 102

Para-Nitrophenyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosylonate)-(2 $\rightarrow$ 3)-O-(6-O-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,6-di-O-benzoyl-1-thio- $\beta$ -D-glucopyranoside 122

Para-Nitrophenyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosylonate)-(2 $\rightarrow$ 3)-6-O-benzoyl- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-[(2,3,4-tri-O-benzyl- $\alpha$ -L-fucopyranosyl)-(1 $\rightarrow$ 3)]-2,6-di-O-benzoyl-1-thio- $\beta$ -D-glucopyranoside 123

N-(3-thioacetyl) propanamidophenyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosylate)-(2 $\rightarrow$ 3)-O-(6-O-benzoyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,6-di-O-benzoyl-1-thio- $\beta$ -D-glucopyranoside **133**

Copoly (Para-N-Acrylamidophenyl (5-acetamido-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosylonic acid)-(2 $\rightarrow$ 3)- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-1-thio- $\beta$ -D-glucopyranoside) **132**

O-(6-O-Benzyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-O-[(2,3,4-tri-O-benzyl- $\alpha$ -L-fucopyranosyl)-(1 $\rightarrow$ 3)]-2-acetamido-6-O-tert-butyl-diphenylsilyl-2-deoxy- $\beta$ -D-glucopyranosyl azide **151**

O-(6-O-tert-butyl-diphenylsilyl-3-O-sulfo- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-O-[(2,3,4-tri-O-benzyl- $\alpha$ -L-fucopyranosyl)-(1 $\rightarrow$ 3)]-2-acetamido-6-O-tert-butyl-diphenylsilyl-2-deoxy- $\beta$ -D-glucopyranosyl azide **153** (Le<sup>x</sup>-3'-O-Sulfate)

O-(Methyl 5-acetamido-4,7,8,9-tetra-O-benzoyl-3,5-dideoxy-D-glycero- $\alpha$ -D-galacto-2-nonulopyranosylate)-(2 $\rightarrow$ 3)-O-(6-O-benzyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 4)-O-[(2,3,4-tri-O-benzyl- $\alpha$ -L-fucopyranosyl)-(1 $\rightarrow$ 3)]-2-acetamido-6-O-tert-butyl-diphenylsilyl-2-deoxy- $\beta$ -D-glucopyranosyl azide (SLe<sup>x</sup>) **155**.

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Cao, S.; William, K. C. Park.; F. Hernandez-Mateo, S. Aravind, Roy, R. "Synthesis of Glycopolymers Containing in Sialyl  $\alpha$ -(2 $\rightarrow$ 3)-Lactosides" *XVIIth International Carbohydrate Symposium*, Ottawa, Canada, July 17-22, 1994.

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