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UNIVERSITÉ D'OTTAWA
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ABSTRACT

Two aminocyclitol antibiotics, hygromycin B and destomycin A, contain as a structural component optically active N-methyl(1,3/2,4,6)-4,6-diamino-1,2,3-trihydroxycyclohexanetriol (hyosamine). The (+)-enantiomer is present in the former antibiotic, and the (-)-enantiomer, in the latter. The present work describes enantiospecific syntheses of these two components, starting from a common chiral precursor, 1L-(1,3/2,4,6)-6-azido-1,2-Q-isopropylidene-4-nitro-1,2,3-cyclohexanetriol. The precursor was prepared in 9 steps from D-mannose according to a recently published procedure. Different sequential manipulations of its two unequal nitrogenous functions, converting these eventually into an amino and an N-methylamino group, led to the target compounds in enantiospecific fashion. Several approaches were explored, and one approach to (+)-hyosamine and two to (-)-hyosamine were successful.

The above mentioned azidonitro precursor was also used for a synthesis of a blocked derivative of 2-deoxystreptamine (1,3-diamino-4,5,6-trihydroxycyclohexane) stereospecifically glycosylated in the 6 - position. This was accomplished by conversion of the blocked azidonitrocyclitol into the corresponding diacetamido compound and coupling with acetobromoglucose.

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INTRODUCTION

A. Some Aspects of the Chemistry of Aminoglycoside Antibiotics

Antibiotics are chemical compounds elaborated by microorganisms and capable of inhibiting the growth of other microorganisms or tumors. Several thousand are known, and they encompass enormous structural varieties, but a large proportion including numerous clinically important drugs contain carbohydrate molecules either as appendages to other structures (e.g., the macrolides and anthracyclines) or as sole constituents. From the viewpoint of clinical efficacy, the most prolific producers of carbohydrate-related antibiotics are the actinomycetes (genus Actinomyces). Because of the characteristic prevalence of amino sugars and (or) aminocyclitols (i. e., aminopolyhydroxycyclohexanes) in antibiotics which are composed entirely of carbohydrate, such compounds are referred to as aminoglycoside or aminocyclitol antibiotics.^{1,2,3}

The first representative of this class of potent drugs was streptomycin, discovered⁴ in 1944 and soon to be followed in rapid succession by large numbers of similar antibiotics such as for example, the kanamycins⁵ and neomycins⁶ (Fig. 1).

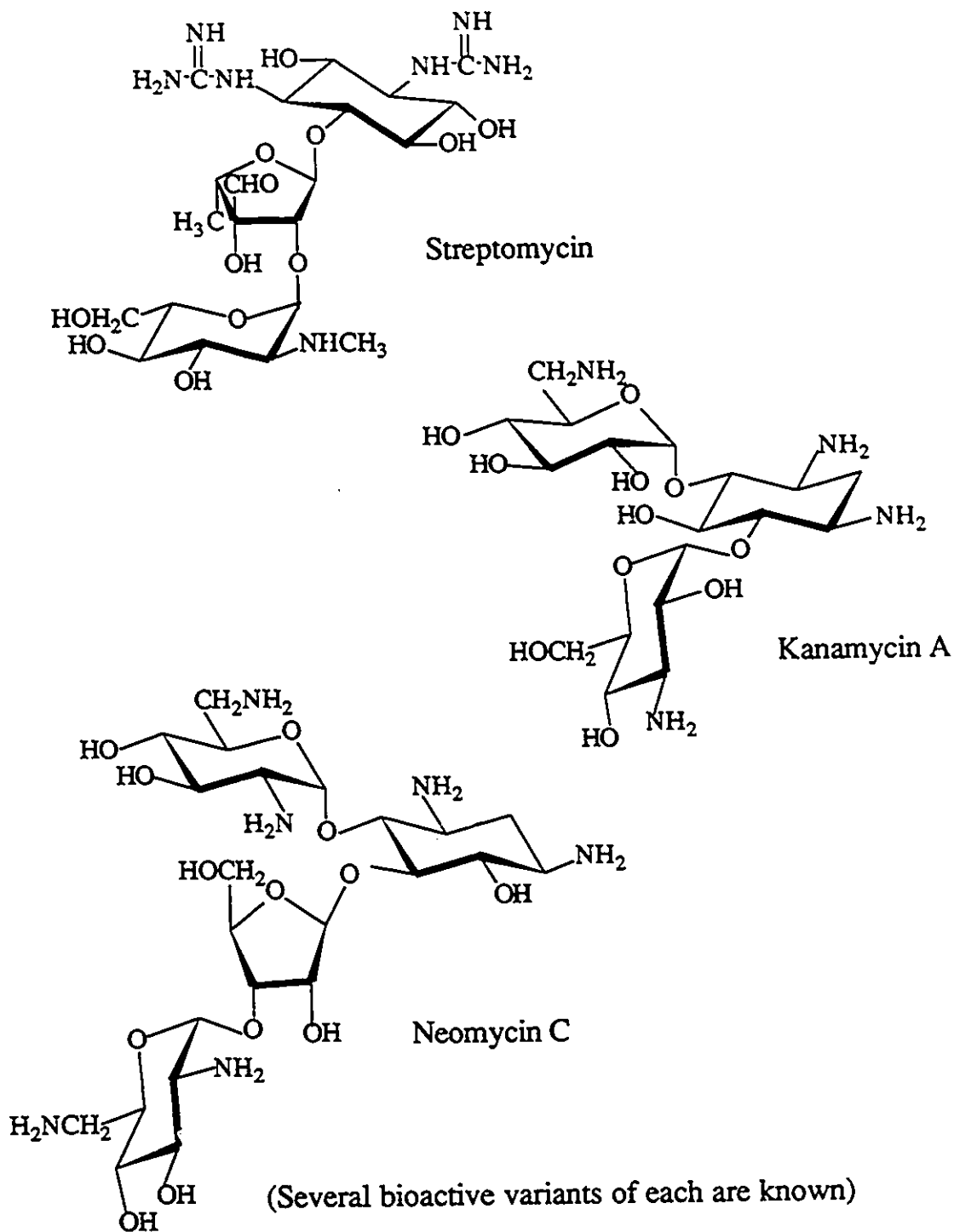


Fig. 1. Some aminocyclitol antibiotics

Streptomycin possesses a cyclitol constituent which, upon total hydrolysis of the antibiotic, emerges as 1,3-diamino-1,3-dideoxy-scyllo-inositol, systematically designated⁷ as (1,3,5/2,4,6)-4,6-diamino-1,2,3,5-cyclohexanetetrol but commonly referred to as streptamine (Fig. 2). By contrast, the neomycins, kanamycins, and many other aminocyclitols contain (1,3,5/4,6)-4,6-diamino-1,2,3-cyclohexanetriol, commonly called 2-deoxystreptamine (Fig. 2).

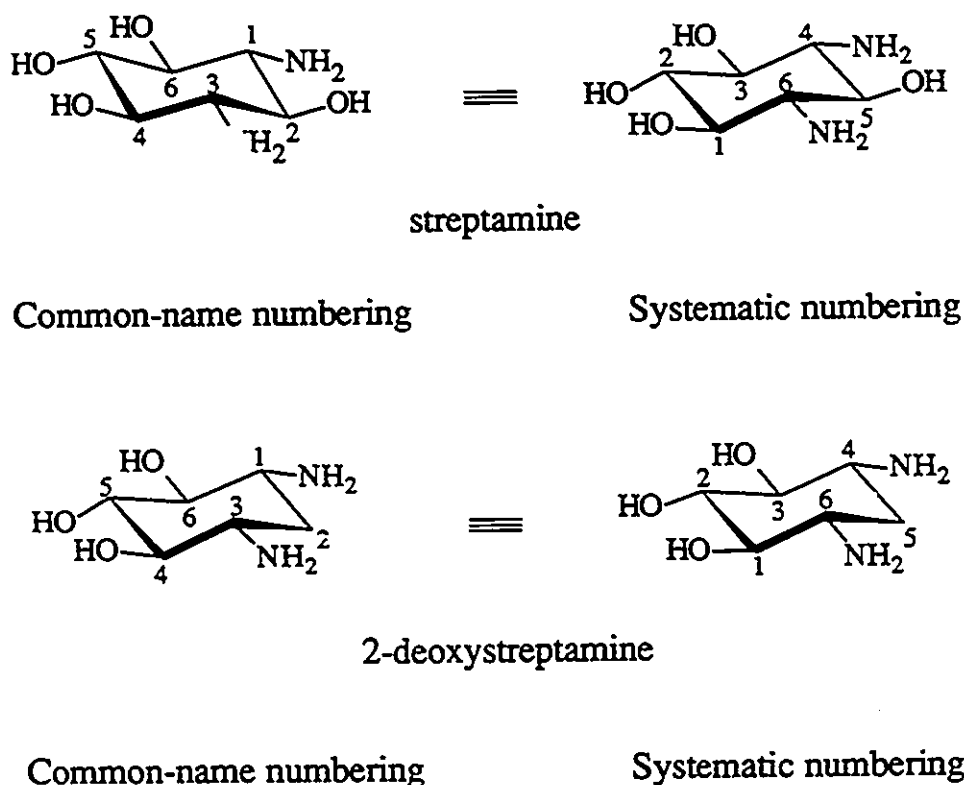


Fig. 2. Streptamine and 2-deoxystreptamine

As inspection of their formulas shows, streptomine and 2-deoxy-streptomine are meso compounds and must therefore be optically inactive. However, they contain prochiral centers, and this is an important point to be considered when these molecules are to be used as synthons in total synthesis of antibiotics or modified analogs. Thus, monosubstitution at the constitutionally equivalent but stereochemically non-equivalent position 1 and 3, or 4 and 6, by an achiral substituent leads to racemates (Fig. 3a, b); monosubstitution by a chiral substituent leads to a mixture of diastereomers (Fig. 3c).

For this reason, syntheses of aminocyclitol antibiotics starting from readily available (meso) streptomine or 2-deoxystreptomine must involve racemate resolution of intermediates at some stage, or separation of diastereomers, and these procedures are often laborious and not always highly efficient. Clearly, stereospecific methods of synthesis are desirable.

As a contribution to methodology of stereospecific aminoglycoside synthesis, a new approach to 2-deoxystreptomine was recently developed in this laboratory,⁸ which starts from the inexpensive sugar D-mannose and leads to the target molecule via chiral intermediates throughout, thus

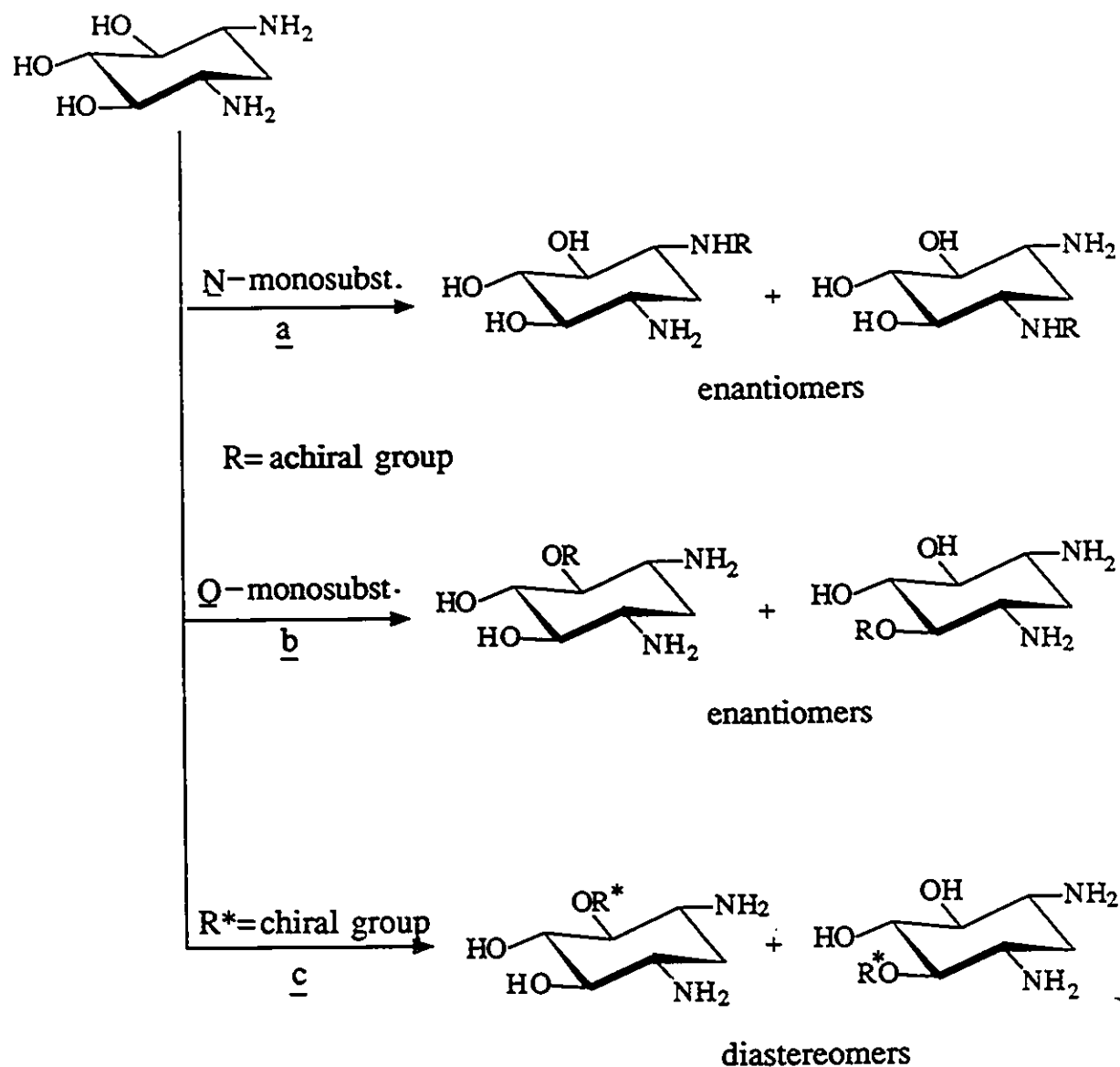


Fig. 3. Formation of racemic or diastereomeric product mixtures from 2-deoxystreptamine

B. The Aim of this Research: Enantiospecific Syntheses of (+)-and (-)-Hyosamine, and Stereospecific Glycosylation of 2-Deoxystreptamine

The syntheses contemplated relate in the first (and main) part of this research to the antibiotics hygromycin B⁹ and destomycin A¹⁰ (Fig. 5). These two compounds, elaborated from Streptomyces hygroscopicus and Streptomyces rimofaciens, respectively, differ structurally solely in the stereochemical location of an N-methyl group on the 2-deoxystreptamine moiety. (The central moiety is D-talose—a sugar extremely rare in nature—linked in orthoester fashion to a 6-amino-6-deoxyheptonic acid lactone.) The cyclitols produced on hydrolysis are (+)-hyosamine and (-)-hyosamine, to be designated systematically⁷ as 1L-(1,3/2,4,6)-4-amino-6-methylamino-1,2,3-cyclohexanetriol and 1D-(1,3/2,4,6)-4-amino-6-methylamino-1,2,3-cyclohexanetriol, respectively (Fig. 5).

It is seen that the key compound **1** (Fig. 4) bears three oxygen and two nitrogen functions in the same sequence and relative configurational relationship as the hyosamines. It was reasoned that the two nitrogenous functions, being of different oxidation states, should be able to undergo

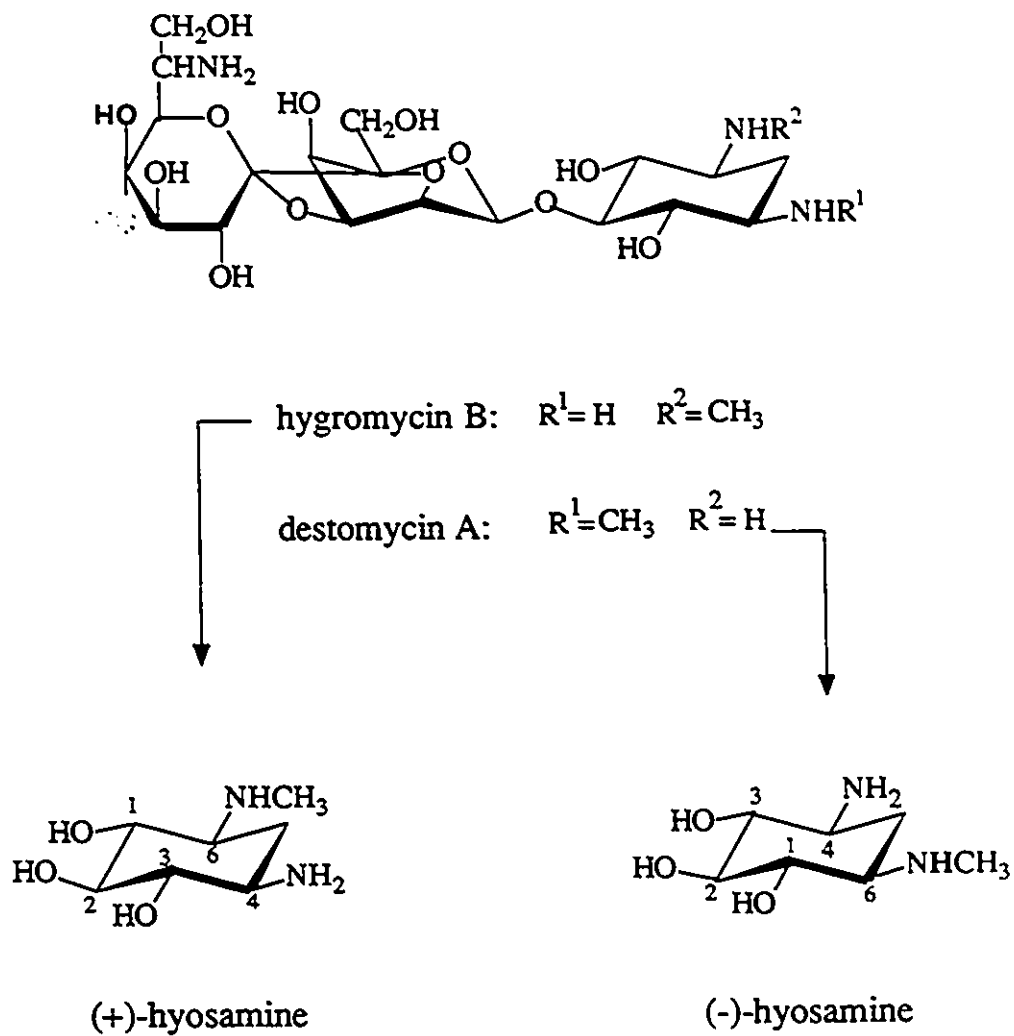


Fig. 5. The enantiomeric hyosamines

sequential reduction to the amino stage so that appropriate manipulations should make possible an individual N-methylation of either, leading enantiospecifically to the (+)-and (-)-hyosamines.

Unsymmetrical Q-substitution of 2-deoxystreptamine is also of interest. As is seen in Fig. 1, in neomycin this cyclitol moiety bears a glycosyl residue on only one of its prochiral carbinol positions; in kanamycin, both prochiral carbinols of 2-deoxystreptamine are glycosylated, but unequally so. It was considered that **1** should be a useful starting point to achieve such unsymmetrical glycosylation. One might contemplate glycosylation of OH-3 followed by removal of the 1,2-Q-isopropylidene protecting group; or alternatively, temporary blocking of OH-3, removal of the 1,2-blocking group, and glycosylation of OH-1. (The latter glycosylation might be satisfactorily regioselective if a sufficiently bulky protecting group chosen for OH-3 hinders reaction at OH-2.) Reduction of the nitrogenous functions to amino groups could either precede or follow these operations. This is schematically illustrated in Fig. 6. Some exploratory studies towards such diastereospecific glycosylation of 2-deoxystreptamine are to be included in this thesis research.

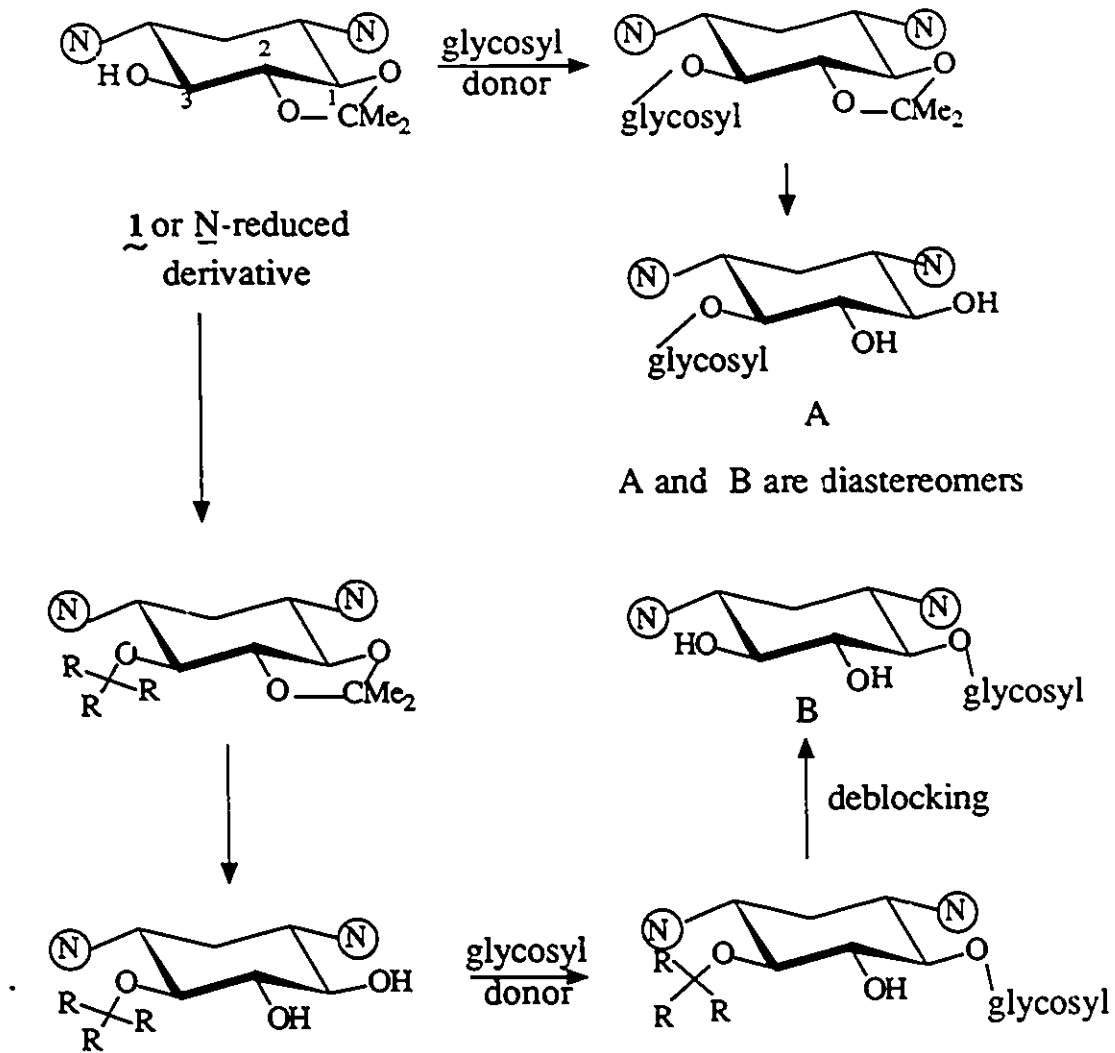


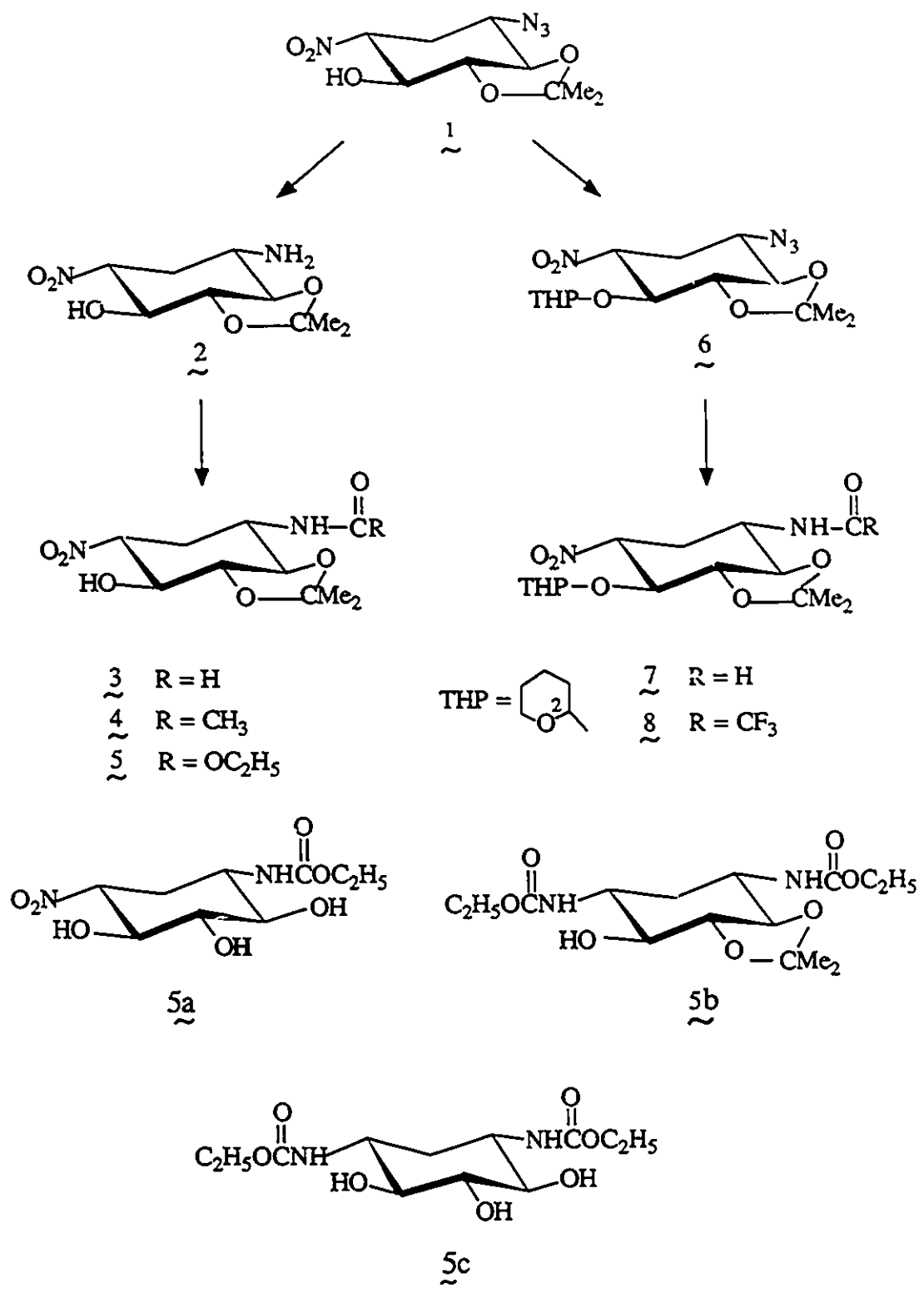
Fig. 6. Strategies for obtaining unsymmetrically glycosylated 2-deoxystreptamines

RESULTS AND DISCUSSION

A. Preparation of Key Intermediates

To begin with, the crystalline azidonitro compound **1** was prepared according to the published procedure⁸ and then derivatized to furnish a number of intermediates (**3-8**) from which various approaches to the optically active hyosamines were to depart (Scheme 1).

Catalytic transfer hydrogenation¹¹ of **1** over palladium on charcoal in the presence of 1,4-cyclohexadiene as the hydrogen donor selectively reduced the azido group, to afford the aminonitro cyclitol **2**. This reaction had to be planned and executed with some circumspection in order to avoid or minimize possible complications. Compound **1** is base-sensitive; it can undergo base-catalyzed epimerization at C-3 adjacent to the nitro group, through a retro nitroaldol reaction⁸, and although **1** is thermodynamically preferred over its 3-epimer, even a minor amount of epimerization would be undesirable. (Epimerization at C-4 is theoretically possible also but in practice poses no risk because of the well-established, great preference of nitro groups on six-membered sugar and cyclitol rings for adopting, or remaining in, an equatorial orientation¹²). On the other hand, **1** is also far more acid-sensitive than ordinary acetals because of the strained nature of the trans-fused oxolane ring which is cleaved rapidly at ordinary



Scheme 1

temperatures by moderately strong acid. Fortunately, **1** was found to tolerate the presence of acetic acid in low concentration (and even of *p*-toluenesulfonic acid in catalytic amounts) for short periods of time, and acetic acid was therefore added to the transfer hydrogenation medium in a quantity sufficient to neutralize the engendered amine **2** and thus prevent the medium from becoming basic. Nevertheless, it was considered inadvisable to try and isolate **2** for characterization because of its expected instability. Therefore, the product was *N*-formylated *in situ* by addition of formyl acetate immediately after completion of the hydrogenation. The formamide **3** was obtained, crystalline, in 90% yield.

Secondly, the transfer hydrogenation of **1** was performed in the presence of acetic acid and acetic anhydride, which led to the crystalline acetamide **4** in 97% yield.

Thirdly, crude **2** obtained as in the preparation of **3** was treated in aqueous solution with ethyl chloroformate in the presence of either pyridine or sodium bicarbonate, to produce the carbamate **5**. Although high yields of crude **5** were obtained in several experiments, the product tended to contain more-polar by-products and had to be purified by column chromatography, after which pure crystalline **5** was isolated in yields of 40-57%. One of the by-products, isolated in one instance in

10.4% yield, proved to be the corresponding triol **5a** resulting from hydrolytic loss of the isopropylidene group. This loss was revealed by comparison of the mass spectrum and the ^1H - and ^{13}C -n.m.r. spectra with the spectra of **5**. A second crystalline by-product isolated chromatographically (13.7% yield) in the same experiment had a polarity (t.l.c) intermediate between **5** and **5a**, and spectroscopy gave a somewhat unexpected result: The product had retained the acetonide group but the nitro group was absent, as evidenced by the lack of a proton signal in the range of δ 4.7-4.5 where **3-8** exhibit characteristic multiplets for the nitromethine proton H-4. Instead, a second ethoxy-carbonylamino group was present (formula **5b**). This was clear from a 6:6:4 intensity ratio of the ^1H -n.m.r. signals attributable to ethyl CH_3 , isopropylidene CH_3 , and ethoxy CH_2 , and from the presence of two separate, low-field, exchangeable NH doublets. The ^{13}C -n.m.r. spectrum contained two separate carbonyl resonances near δ 161 (see data in the Experimental). Compound **5b** has been described in the literature¹³ before, but only as the racemate. Its deacetalation with 50% acetic acid at room temperature gave known¹³ 1,3-di-N-ethoxycarbonyl-2-deoxystreptamine (**5c**).

The results presented thus far allow the following conclusions to be drawn. Evidently, **1** can very well be converted into nitro-amides. The reactions were clean and high-yielding especially for **3** and **4**, requiring no

chromatographic purification of the products when proper care was exercised. However, the results also indicate that a danger of deisopropylideneation exists, although it is not clear whether the triol by-product **5a** owed its origin to the use of an impure sample of **1** that may have become partly deblocked prior to use, or to the conditions prevailing during the reactions or subsequent processing. Although t.l.c. of the crude **5** did suggest the presence of **5a**, it may well be that its proportion increased by exposure to the silica gel during the chromatography designed to obtain pure **5**.

A more intriguing and rather unexpected observation was the formation of a significant amount of **5b** in at least one experiment. Although nitroarenes are readily reduced to aminoarenes by transfer hydrogenation under a variety of conditions, the same does not normally seem to be the case for nitroalkanes. A comprehensive review¹¹ of the method makes no mention of nitroalkane reduction, which is why we chose it for selective azide hydrogenation in the first place, with obvious success. But evidently, NO_2 can undergo slow reduction to NH_2 . No attempts were made to pinpoint the cause; this might be difficult because, for such a heterogeneous reaction, precise reproducibility of conditions is hard to achieve. The quality of the batch of catalyst used or the efficacy of agitation (by ultrasound) may have played a role.

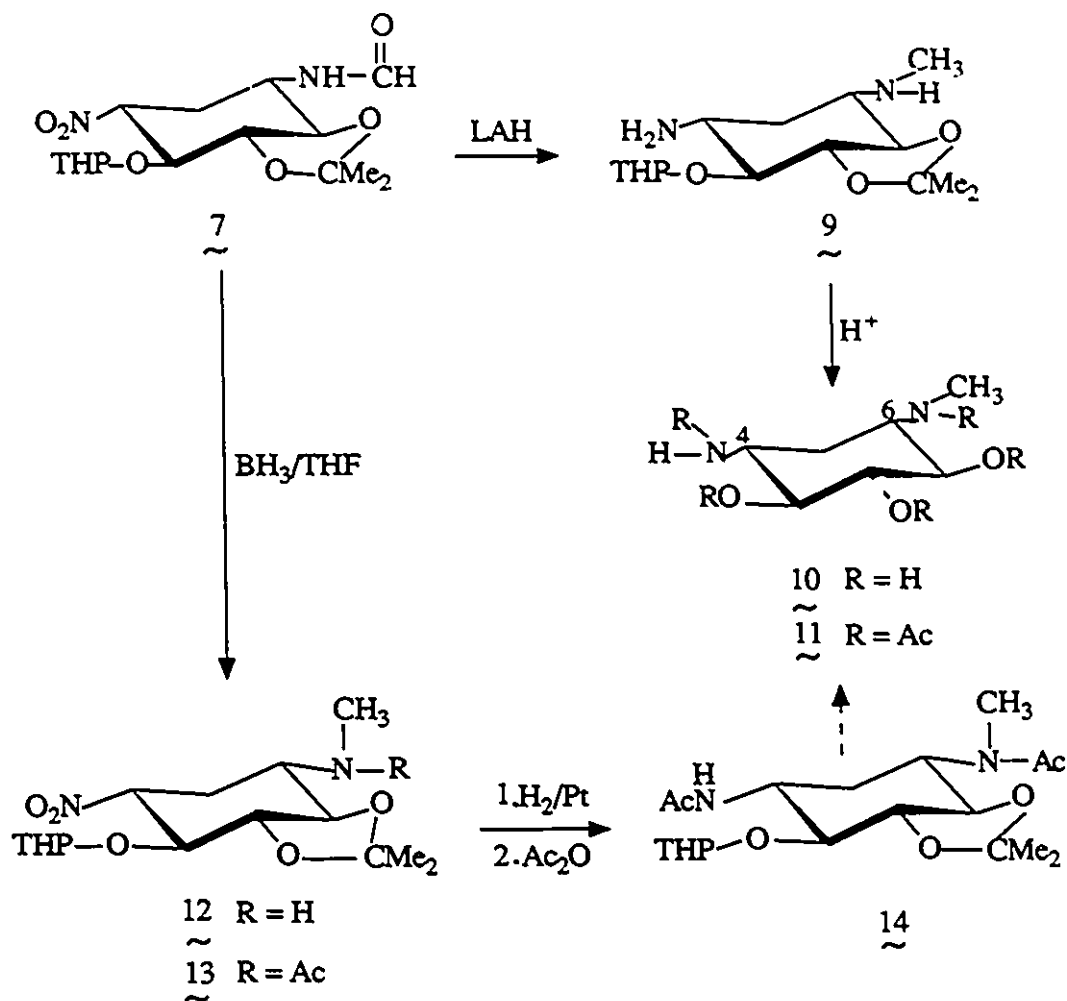
A further intermediate required for the planned synthesis was an O-3 protected derivative of **3**. The tetrahydropyranyl ether **7** was chosen, and this was prepared in 93% yield by reaction of **3** with 2,3-dihydropyran catalyzed by *p*-toluenesulfonic acid. Alternatively, the azidonitro alcohol **1** was first tetrahydropyranylated by the same procedure to give almost quantitatively the protected azidonitro derivative **6**, which was subsequently hydrogenated and *N*-formylated (as for the preparation of **3**), to furnish crystalline **7** in 86% yield. Finally, the trifluoroacetamide **8** was required. It was obtained from **6** by transfer hydrogenation followed by *N*-acylation of the resulting amine with trifluoroacetic anhydride. Purification by column chromatography and recrystallization of the product was needed, and **8** could be elaborated in 41% yield only.

The tetrahydropyranyl group is a good protecting group for many purposes because of its facile introduction, high stability under basic, oxidizing, or reducing conditions, and its easy removal by acid. It does have one disadvantage, however. It is the fact that tetrahydropyran-2-yl ethers always tend to arise, in reactions of alcohols with dihydropyran, as mixtures of epimers due to asymmetry at the acetalic position 2. As a result, interpretation of n.m.r. spectra may be complicated. Being diastereomeric, the components usually arise in different proportions and sometimes one or the other may be isolable pure by chromatography or crystallization. Compounds **6**, **7**, and **8** (and compounds derived from them in later section

of this work) all were such mixtures of epimers as indicated by separate ^1H -n.m.r. signals for the acetalic H-2 in the tetrahydropyanyl ring (narrow triplets in the range δ 5.1-4.7). For example, **6** could be partially separated by column chromatography into a crystalline, single epimer and a crystalline mixture of both epimers. Both samples gave correct microanalytical data and essentially identical mass spectra, but the second sample exhibited ^1H -n.m.r. signals for two epimers (see Experimental). Similarly **7** consisted of two epimers which emerged unseparated from column chromatography in one experiment; part of the material (48%) then crystallized from ethyl acetate-hexane with a strong preponderance of one epimer while the mother liquor yielded a syrupy mixture (45%) containing both epimers in comparable proportions as deduced from the ^1H -n.m.r. spectrum (see Experimental). Generally, it is unnecessary to attempt epimer separation; the mixture can be used for further chemical transformations.

B. Synthesis of (+)-Hyosamine

Although racemic hyosamine has been synthesized^{14,15}, no enantio-specific synthesis of (+)-hyosamine (**10**) appears to have been recorded in the literature. Such a synthesis has now been accomplished as shown in Scheme 2.



Scheme 2

Compound 7 was treated with lithium aluminum hydride in 1,4-dioxane at the reflux temperature. Concurrent reduction of the nitro group to an amino group and of the formamido group to a methylamino group took place*. The resulting, O-protected cyclitol 9 was not characterized (other than by giving an immobile, ninhydrin-positive spot

in t.l.c.), but was hydrolyzed by trifluoroacetic acid to furnish the target compound **10** as an amorphous powder. It gave a single spot (R_F 0.25) in t.l.c. with acetonitrile-aq. ammonia as the irrigant and was so compared with an authentic sample of the corresponding 4,6-diamine (2-deoxystreptamine), which had R_F 0.35. The product gave a molecular ion peak $M^+ + 1$ at m/z 177 in the chemical-ionization mass spectrum, as calculated for $C_7H_{16}N_2O_3$ (mol. wt. 176). For definitive characterization the material was peracetylated with acetic anhydride and pyridine to give the crystalline penta-N,Q-acetyl derivative **11** in 23% yield based on **7**, melting at 156-157°C as reported¹⁶ for the enantiomer, and showing $[\alpha]_D -4.4^\circ$ (in methanol), close to the reported¹⁷ value of -7.5° . It must be noted here that natural **10**-dihydrochloride is dextrorotatory ($[\alpha]_D +10.7^\circ$)^{9b}, but its pentaacetyl derivative is levorotatory.¹⁷ Conversely, natural (-)-hyosamine from destomycin A is levorotatory and its pentaacetyl derivative is

*This reduction was originally tried with the unprotected alcohol **3** but it failed because of solubility problems. Upon addition of the hydride the starting material precipitated from the solution (ether or dioxane), presumably because of formation of insoluble alkoxide, and no reduction was observed. It was for this reason that the fully blocked derivative **7** was synthesized and used.

dextrorotatory.¹⁷ The mass spectrum of 11 displayed an $M^+ + 1$ peak at m/z 387 in agreement with the composition $C_{17}H_{26}N_2O_8$ (mol. wt. 386). The 1H -n.m.r. spectrum (see Experimental) agreed with data recorded¹⁴ for the pentaacetyl derivative of synthetic hyosamine (racemate), exhibiting the same curious phenomenon: The compound showed two N-methyl signals (a broadened and a sharp singlet at δ 2.81 and 2.72, respectively) in a ratio of $\sim 3:2$, integrating together to three protons, and instead of the expected five 3-proton singlets for the acetyl groups around δ 2.0, there were numerous singlets (13-14) of varying intensities but correctly integrating to five times the combined N-methyl signal intensity. Furthermore, there were two NH doublets at low field (δ 5.70 and 5.63), integrating together to one proton. This spectral pattern has been attributed¹⁴ without further comment "probably to the space requirement of the methylacetamido group". It could be that free rotation of that group about the N-C⁶ bond is hindered by the neighboring acetoxy substituent sufficiently for individual rotamers to be recognizable on the n.m.r. time scale. For an alternative explanation, one could assume a sufficiently hindered rotation about the amidic NH-CO bond due to resonance, so that individual rotamers become recognizable by the spectrometer (as is the case for N,N-dimethylformamide). Possibly both factors are operative, and since the molecule contains two acetamido groups a rather complicated spectrum should not be surprising. Related cases will be discussed in section C.4 (see compounds 25 and 26).

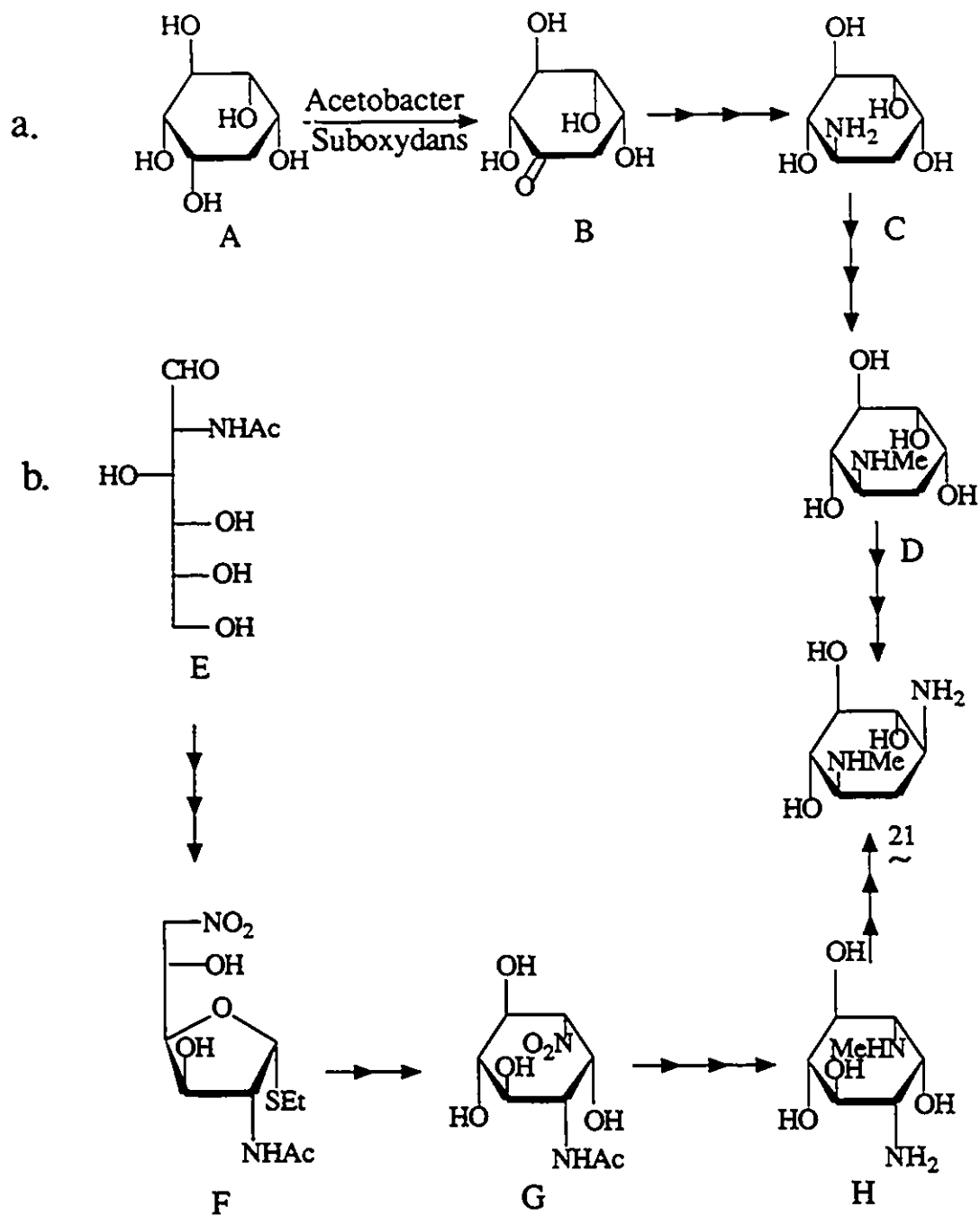
Another approach to **10** was pursued in an exploratory way (Scheme 2). The same starting compound, **7**, was treated with borane-tetrahydrofuran complex, which effected reduction of the formamido group to a methylamino group but left the nitro group intact. The crude product (**12**) that was obtained was crystalline but gave a poor ^1H -n.m.r. spectrum, probably because it was contaminated by some boron complex. Nevertheless, the spectrum clearly showed that an N-methyl group was engendered* and the formamido group of **7** had disappeared. Moreover, the mass spectrum showed a strong $M^+ + 1$ peak (m/z 331) in accord with formula **12**. Upon N-acetylation of **12**, crystalline and spectroscopically well-characterized **13** was obtained. Catalytic hydrogenation of **13** over platinum, followed by N-acetylation of the product, afforded the blocked 4-acetamido-6-(N-methyl) acetamido cyclitol **14** as a glassy material showing the expected molecular ion peak $M^+ + 1$ at m/z 385 and displaying, like **11**, two N-methyl singlets and multiple acetyl singlets in the ^1H -n.m.r. spectrum. It should no doubt be possible remove the Q-protecting groups in **14** by mild acid hydrolysis,

*Actually, there were two doublets for NH-CH₃ at δ 2.69 and 2.53, in a 2:1 intensity ratio. The probable reason for this will be discussed in connection with compound **26** (see section C.4).

and the N-acetyl groups by more vigorous acid hydrolysis or by saponification with barium hydroxide, and so arrive at **10** by this alternative route. However, these experiments were not done.

C. Synthesis of (-)-Hyosamine

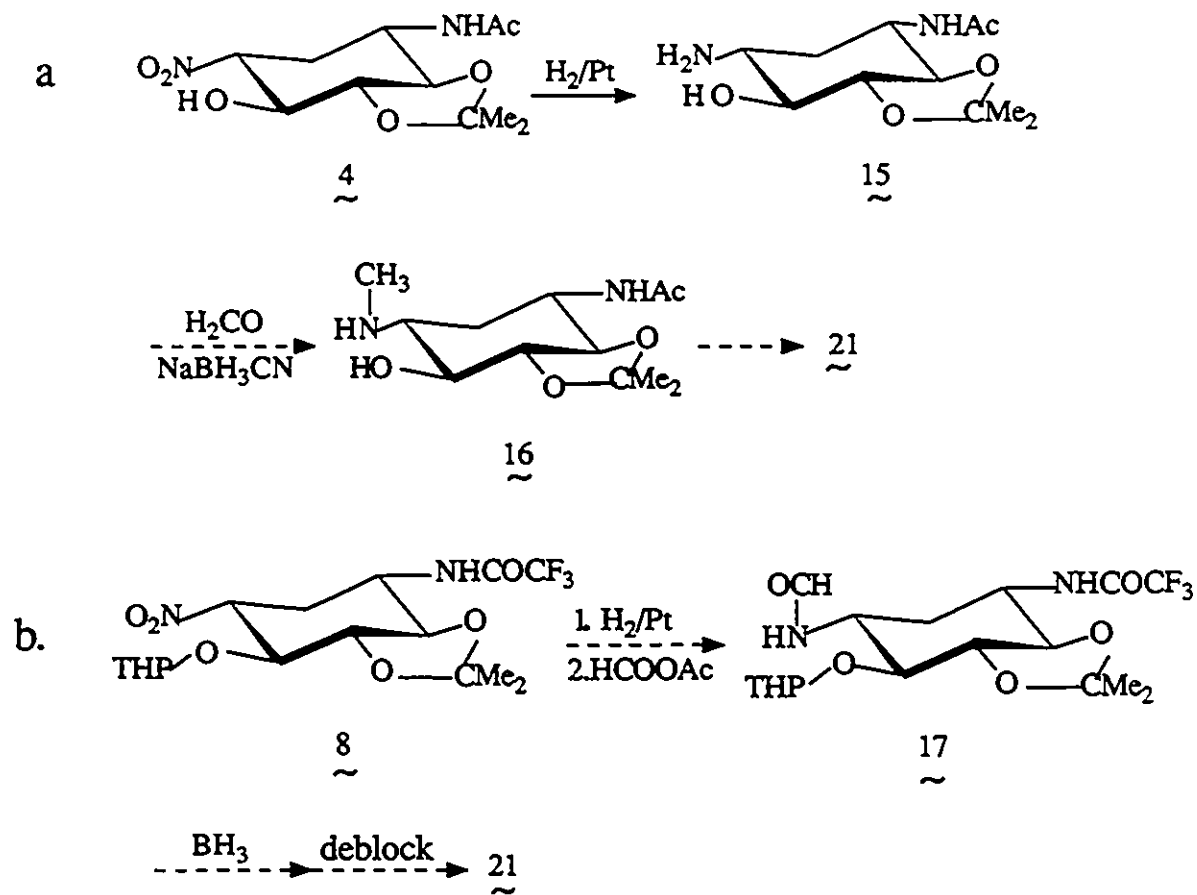
C.1. Previous syntheses. Levorotatory hyosamine (**21**) has been synthesized by two Japanese groups of investigators. Nakajima and his coworkers¹⁷ started from achiral 1,2,4,5/3-cyclohexanepentol (A) and used enantiospecific biochemical oxidation by Acetobacter suboxydans as the key step of generating chirality. They obtained the chiral ketone B which was converted, via sodium amalgam reduction of its oxime, into the chiral monoamine C. Numerous further steps, involving N-methylation to D and introduction of a second amino group, eventually led to **21** in low overall yield (Fig. 7a). Incidentally, the same sequence had been executed previously by these authors¹⁴ in their synthesis of racemic hyosamine referred to earlier, with the difference that a regioselective chemical oxidation (catalytic, with platinum and oxygen according to Heyns¹⁸) had been used in the step A to B (Fig. 7a).



The second synthesis¹⁶ of **21** (Fig. 7b) started from natural N-acetyl-D-glucosamine (**E**), which was first functionalized to the nitro thioglycoside **F**. The further course of the approach was based on the principle of nitroalkane cyclization¹⁹, similar to the chemistry portrayed in Fig. 4, to produce the chiral acetamidonitrocyclohexanetetrol **G** as the key intermediate. The latter was converted into **21** in several steps involving reduction and N-methylation to **H**, bromine displacement of the axial OH-5 in **H** and finally, reductive debromination of the product with Raney nickel. The overall yield was very poor, with the yield of **21** from **H** being 6%. Again, a racemic synthesis referred to earlier¹⁵ followed the same course, starting with racemic **H** elaborated from an achiral cyclitol derivative.

C.2. Unsuccessful exploratory approaches. It was thought that the acetamidonitro compound **4** could be hydrogenated to the amine **15** which could then be N-monomethylated to **16** and deprotected to furnish the target compound **21** (Scheme 3, a).

Catalytic hydrogenation of **4** using Adams catalyst proceeded very well, with the calculated volume of hydrogen being consumed overnight at ordinary temperature and pressure. Thin-layer chromatography revealed absence of **4** and showed a strong, ninhydrin-positive spot for **15**. Processing gave syrupy **15** that exhibited an $M^+ + 1$ peak at m/z 245 in



Scheme 3

agreement with the composition $\text{C}_{11}\text{H}_{20}\text{N}_2\text{O}_4$, as the base peak of its mass spectrum. The substance was not purified or analytically characterized further, but its authenticity was corroborated by its successful use in crude form for a subsequent reaction (see section C.3).

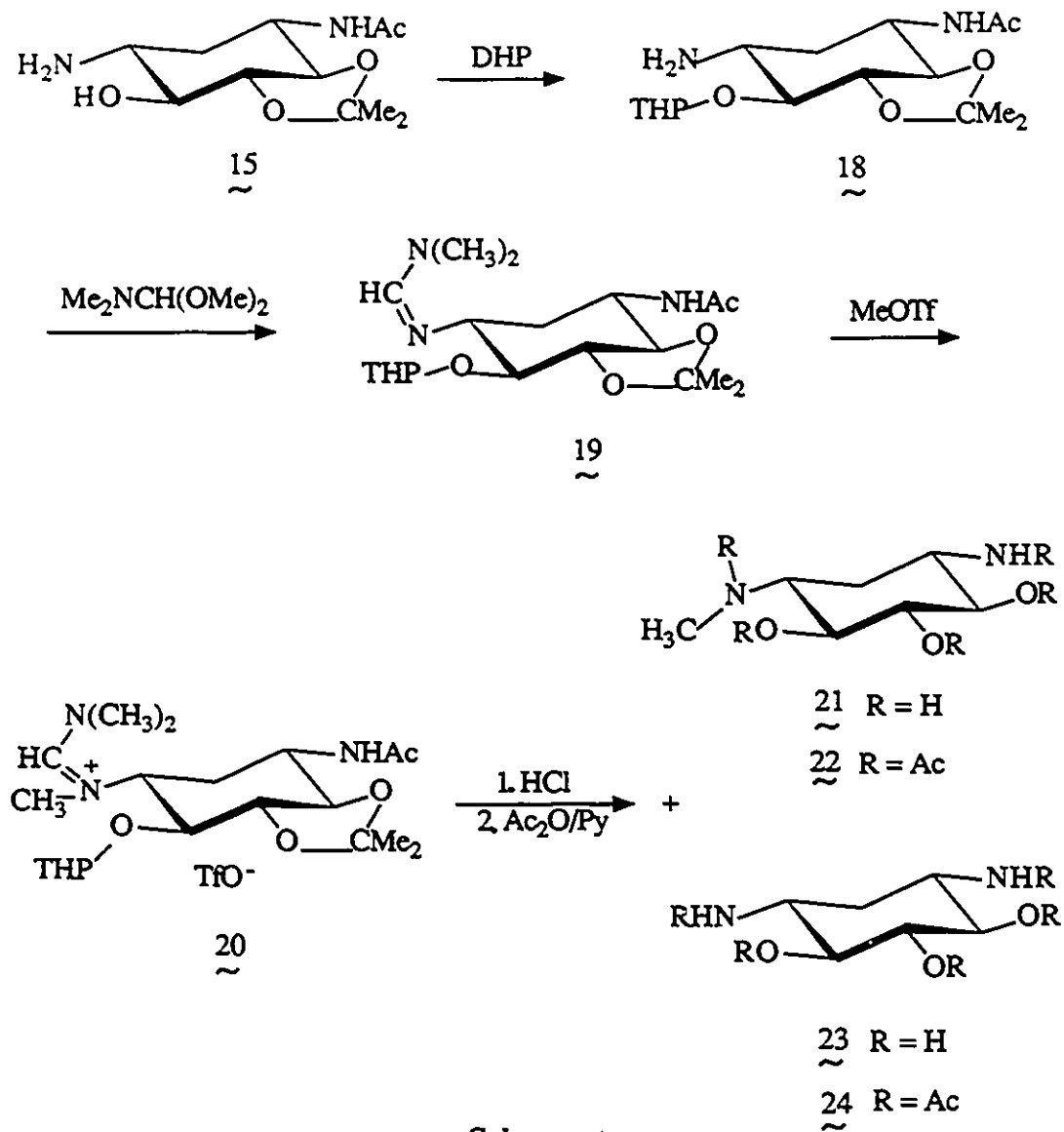
However, attempted N-monomethylation of **15** failed. Treatment of the compound with aqueous formaldehyde followed by sodium cyanoborohydride, in the hope of effecting reductive alkylation via intermediary imine²⁰, led to a complex pattern of products, according to t.l.c., and this approach was abandoned. Other procedures of N-methylation might perhaps have been tried. Thus, S. Sani *et al.*¹⁶ in their work just cited (section C.1, Fig. 7b) obtained compound H by hydrogenating the amino precursor in formalin solution with palladium catalyst, but they achieved only a 28% yield along with 18% of the corresponding N,N-dimethylamino derivative and 37% of unmethylated diamine, which did not seem particularly attractive. Nakajima *et al.*¹⁷ (see section C.1, Fig. 7a) accomplished N-methylation by making the N-benzyloxycarbonyl derivative of their amine (C) and cleaving the latter reductively with lithium aluminum hydride. This method, of course, was ruled out for **15** as it would have produced an N-methyl-N'-ethyl derivative.

Instead, another approach was tried (Scheme 3,b). It was considered that the formamido trifluoroacetamido cyclitol **17**, if available, might be amenable to selective reduction of the formamido group by means of borane under carefully chosen condition. In contrast to metal hydrides or borohydrides, which are nucleophilic reductants, borane is a strong Lewis acid and is expected to attack preferentially at centers of high electron density²¹. Ordinary amides including formamides are readily reduced to

alkylamines by that reagent²² (as was also demonstrated in this research by the reaction **7** to **12**, see Scheme 2), but the electron-deficient carbonyl of trichloroacetaldehyde, for example, is not attacked to any great extent²¹, nor are acid chlorides²¹, and it seemed possible that the same might hold for trifluoroacetamides. It is true that trifluoroethylamines have been prepared by reduction of trifluoroacetamides²³ using sodium borohydride-aluminum trichloride and sodium borohydride-boron trifluoride mixtures in which the active reductant is believed to be borane, and it might therefore have appeared questionable whether borane-tetrahydrofuran complex would be suitable for the intended purpose. However, Dr. J. Giszewicz of this Laboratory has performed a number of pilot experiments on two model compounds, 1-formamido- and 1-trifluoroacetamido-1-deoxy-D-xylitol tetraacetates (A and B), evaluating the reactions by t.l.c. and mass spectrometry. He found that both amides were in fact reduced by borane under identical conditions, but that A reacted about 10 times faster than B. Therefore it appeared worthwhile to study the method with the aim of optimizing reaction condition for maximum selectivity. After an acceptably selective reduction of **17** the trifluoroacetyl group would be readily removable by hydrolysis with cold aqueous base. It was with this strategy in mind that compound **8** had been synthesized (section A). Unfortunately it turned out that catalytic hydrogenation of **8**, expected to provide an amine that could be N-formylated to **17**, was quite unsatis-

factory. Reaction of **8** with platinum catalyst and hydrogen was extremely sluggish, could not be accelerated by liberal quantities of fresh catalyst, and after many days led to a 34% recovery of unchanged **8**. Following N-formylation of any amine presumed to be present in the reaction mixture, a mixture of at least three unidentified products was obtained among which **17** may or may not have been present. This experience contrasted starkly with the facile hydrogenations of the nitro compounds **4** and **13**, and the approach was not pursued any further.

C.3. First synthesis of (-)-hyosamine. The first successful approach to **21** was undertaken as depicted in Scheme 4. Crude **15**, prepared from **4** as already mentioned, was tetrahydropyranylated to yield 83% (based on **4**) of the O-3 protected derivative **18** after chromatographic purification. Like **15**, **18** was amorphous and characterized only by its mass spectrum which showed a molecular ion peak $M^+ + 1$ at m/z 329 as calculated for the formula. Crude **18** was subjected to N-methylation by the method of O' Donnell *et al.*²⁴. This is a one-pot procedure which involves generation of a formamidine and reaction of the latter with methyl triflate followed by acid hydrolysis. Thus, the amine **18** was treated with N,N-dimethylformamide dimethyl acetal to form the intermediary amidine **19**, not isolated but giving the expected $M^+ + 1$ peak at m/z 384 as the base peak of its mass spectrum. Methyl triflate was then added to **19** in dichloromethane



Scheme 4

solution and allowed to react overnight at room temperature, to generate the amidinium salt **20**. The solvent was removed and the product immediately boiled with 6 M hydrochloric acid, to effect hydrolysis of the amidinium function to a methylamino group²⁴. Of course, this treatment simultaneously split off all the protecting groups from the molecule, and

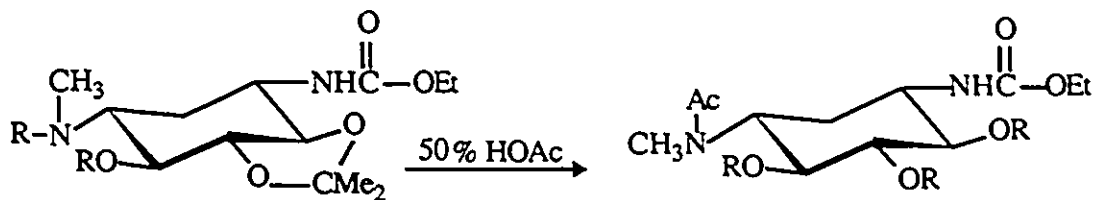
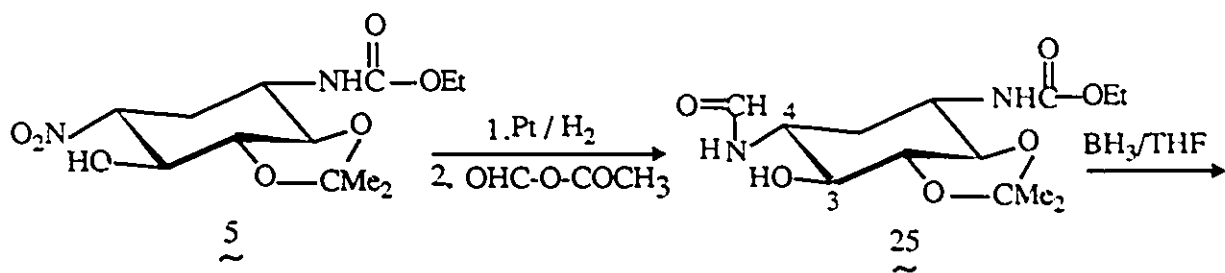
(-)-hyosamine (**21**) was expected to arise, as a dihydrochloride. The product of hydrolysis was not homogeneous, however. It showed two closely-spaced, ninhydrin-positive spots in t.l.c., one of which migrated like an authentic sample of 2-deoxystreptamine (**23**) spotted for comparison; the other spot was attributed to **21**. Preparative separation by chromatography appeared unpromising at this stage, and the material was therefore peracetylated by treatment with acetic anhydride and pyridine. Chromatographic separation of the mixture of peracetylated compounds succeeded, furnishing the crystalline (-)-hyosamine N,Q-pentaacetyl derivative **22** in 19.4% yield, and the known²⁵ 2-deoxy-streptamine N,Q-pentaacetyl derivative **24**, also crystalline, in 14% yield (yields based on **18**). Isolation of the latter indicated that N-methylation by this procedure²⁴ was incomplete. It seems that efficient N-monomethylation presents a recurrent problem in aminocyclitol chemistry; the experience of Suami¹⁶ mentioned in section C.2 is recalled at this point. However, the present procedure at least precludes unwanted N,N-dimethylation.

The target compound **22** had the same melting point (156-157°C) as its enantiomer **11**, and the same specific rotation but with opposite sign ($[\alpha]_D +4.6^\circ$ in methanol; reported¹⁷, $+5.4^\circ$ and¹⁶ $+7.5^\circ$). It showed m/z 387 as the base peak in the mass spectrum, corresponding to $M^+ + 1$ for formula **22**, and its ¹H-n.m.r. spectrum was indistinguishable from that of **11**.

C.4. Second synthesis of (-)-hyosamine. In view of the troubles encountered in N-methylation as discussed in preceding sections, an additional approach to (-)-hyosamine was sought, in the hope of overcoming the problem. In section C.2, some of the characteristics of the electrophilic reductant, borane, have been referred to. An additional feature of that reagent is that it selectively reduces amides to amines without affecting carbamate functions present in the same molecule^{22b,26,27}. For this reason the ethyl carbamate **5** was prepared (see section A), and the reaction sequence shown in Scheme 5 was examined.

Catalytic hydrogenation of **5** using Adams catalyst proceeded smoothly, and the amine produced was N-formylated in situ by addition of formyl acetate to give formamido carbamate **25** in 76% yield, fully characterized by analytical and spectroscopic data.

This formamide (**25**) showed a noteworthy phenomenon in its n.m.r. spectra, which the previously described formamides **3** and **7** did not display. It gave two signals for the formyl proton, in an intensity ratio of ~ 4:1 (integrating to 1 H), namely a somewhat broadened singlet at δ 8.13



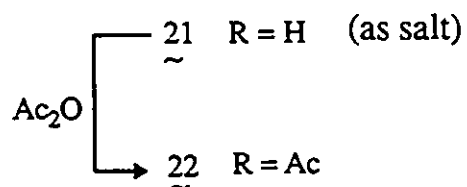
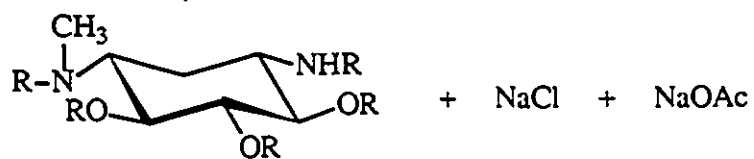
26 R = H

28 R = Ac

27 R = H

29 R = Ac

1. HCl
2. NaOH
3. AcOH



Scheme 5

and sharp doublet at δ 8.03 ($J = 11$ Hz); and two corresponding, exchangeable NH signals in the same ratio, namely a doublet at δ 7.3 ($J_{4,NH} \sim 7$ Hz) and a triplet at δ 6.7 Hz ($J_{4,NH} \approx J_{NH,CHO} \approx 11$ Hz), both broadened and with chemical shifts slightly variable in several spectra. In the δ 4.8-4.5 region were two exchangeable doublets assignable to OH-3 ($J_{3,OH} = 4.7$ Hz for both), again in a $\sim 4:1$ ratio, integrating to 1 H, and with somewhat variable shifts. (A broadened, exchangeable one-proton signal near δ 6.4 was attributable to the carbamate NH.) Upon deuterium exchange, the larger CHO signal (δ 8.13) became a sharp singlet, and the accompanying smaller doublet (δ 8.03) collapsed to a sharp singlet. The ^{13}C -n.m.r. spectrum likewise showed duplication of signals, for most carbons (see Experimental). It was concluded that hindrance of free rotation about the amidic CO-NH bond due to resonance, which is a known cause of magnetic nonequivalence of appended groups (e.g., in *N,N*-dimethylformamide), is apparently not significant enough in **3** and **7** to affect their spectra; but in **25**, two rotameric conformations populated in unequal proportion interconvert slowly enough on the n.m.r. time scale to be recorded as separate species. Dipole or hydrogen bonding interaction with the neighboring OH-3 may perhaps be responsible for this.

The formamido carbamate **25** was treated with borane in refluxing tetrahydrofuran for 5 h, by which the formamido group was reduced to a methylamino group. At the end of the reduction it is necessary to destroy

excess borane present and to hydrolyze the amine-borane complex formed, which is usually done^{22b,26,27} by acidification of the reaction mixture with hydrochloric acid. In a first experiment this procedure was used although it was recognized that simultaneous hydrolysis of the isopropylidene protecting group would take place. The latter circumstance did not matter in principle; in fact, removal of the acetonide in a subsequent step was required anyway. However, it turned out that the reduced and hydrolyzed product was difficult to process and purify, and no clean material could be obtained. Nevertheless, the crude product showed a strong $M^+ + 1$ peak (68%) in the mass spectrum at m/z 249 as required for a compound $C_{10}H_{20}N_2O_5$ (mol.wt. 248), and the 1H -n.m.r. spectrum showed substituent resonances for N-methyl (δ 2.5) and Q-ethyl (δ 3.95 and 1.1) but no longer the low-field N-formyl resonances of **25**, indicating that the desired reduction of the formamide group with retention of the carbamate group had occurred. The borane reduction of **25** was then repeated as before, but at the end the reaction mixture was processed by addition of water and acidification with a stream of carbon dioxide instead of hydrochloric acid. In this way, the acetonide group was largely retained, and pure 6-ethoxycarbonylamido-1,2-Q-isopropylidene-4-methylamino-1,2,3-cyclohexanetriol **26** could be isolated by chromatography, albeit only in a yield of 35%.

Again noteworthy n.m.r.-spectral phenomena were observed. Compound **26** showed two well-separated doublets ($J = 6$ Hz) at δ 2.60 and 2.44, in an intensity ratio of 1:1.2 and integrating to 3 protons for the N-methyl group (^1H spectrum); in the ^{13}C spectrum, most carbons gave closely spaced double peaks with similar intensity ratios. It is to be recalled here that the methylamino-nitro compound **12** also showed two similar N-methyl proton doublets, and that the N-acetyl-methylamino compounds **11** (or enantiomer **22**) and **14** displayed two N-methyl proton singlets. (Strangely, **13** did not.) Different conformers of some sort, interconverting sufficiently slowly on the n.m.r. time scale, must exist in all these cases. Whereas restricted rotation in amide or carbamate groups may be part of the explanation as discussed earlier, this cannot be invoked for compound **12** where obviously some other structural feature must be responsible, — perhaps a lack of molecular flexibility owing to the trans-fused dioxolane ring. The same rigidity is present in **26**. To assess its possible influence on spectral behavior, it was decided to remove the acetonide ring. When **26** was treated with 50% aqueous acetic acid at room temperature (compare the deacetonation of **5b** to **5c**), the ring was hydrolyzed but the product emerged as the N-acetyl derivative **27**, rather than a methylammonium acetate salt*. This was evident from the ^{13}C -n. m.r. spectrum, which lacked a signal near δ 180 attributable to acetate ion but displayed instead a carbonyl signal at δ 173.1 for N-acetyl, and from the ^1H -n.m.r. spectrum, which contained a sharp, 3-proton N-acetyl signal at δ 1.87.

Now, the interesting feature of both spectra was that they gave no evidence for conformational (or other) inhomogeneity of 27; there was no signal duplication, and in particular, the N-methyl proton signal was a clear singlet at δ 2.29. It may therefore be concluded that in the inflexible bicyclic system of 26 (and of 12 and 14, by implication), free rotation of the methylamino substituent is significantly impeded. It remains unclear, though, why a similar effect was not observed in 13.

As was mentioned in a preceding paragraph, compound 26 was obtained in pure form after column chromatography in a yield of only 35%. However, some additional, impure 26 was contained in inhomogeneous chromatographic fractions; although this syrupy material appeared to consist largely of 26, some of it was suspected of having lost its isopropylidene group probably during processing or perhaps even during the chromatography. In an attempt at purification by way of a crystalline derivative, the impure material was acetylated with acetic anhydride and pyridine. Unfortunately, no crystalline product emerged,

*It is not uncommon to observe slow acetylation even of sugar hydroxyls by hot 70-80% acetic acid. N-Acetylation should occur more readily and, in the present case, may have happened during evaporation of the reaction mixture at moderately elevated temperature.

but spectroscopic analysis of the acetylated mixture supported the notion that it was mainly the N,Q-diacetyl derivative (**28**) of **26**, accompanied by a minor proportion of the tri-Q-acetyl derivative (**29**) of **27** (see Scheme 5). This conclusion was based on the mass spectrum showing strong $M^+ + 1$ and fragmentation peaks attributable to **28** and similar but weak peaks attributable to **29**, and on the pattern and intensities of substituent resonances seen in the ^1H - and ^{13}C -n.m.r. spectra. The spectral data are recorded in the Experimental Section.

Complete deprotection of **26** or **27** was expected to furnish the target (-)-hyosmine. As the supply of either compound was exhausted at this stage of the work, the deprotection was performed on the mixture of acetylated derivatives **28** and **29**. Although the acetonide group of **28** was known to be easily acid-hydrolyzable and the N- and Q-acetyl groups present in both compounds could be expected to be cleavable by hot, dilute mineral acid, hydrolysis of the carbamate group was anticipated to require rather forcing conditions. The material was therefore treated with half-concentrated hydrochloric acid (6 M) at 100°C for 2 h. Examination of the hydrolyzate by ^{13}C -n.m.r. indicated that the isopropylidene and all the acetyl groups were removed as expected, but that the carbamate function had resisted hydrolysis: The spectrum showed signals at δ 158.1 (C=O), 61.7 (Q-CH₂),

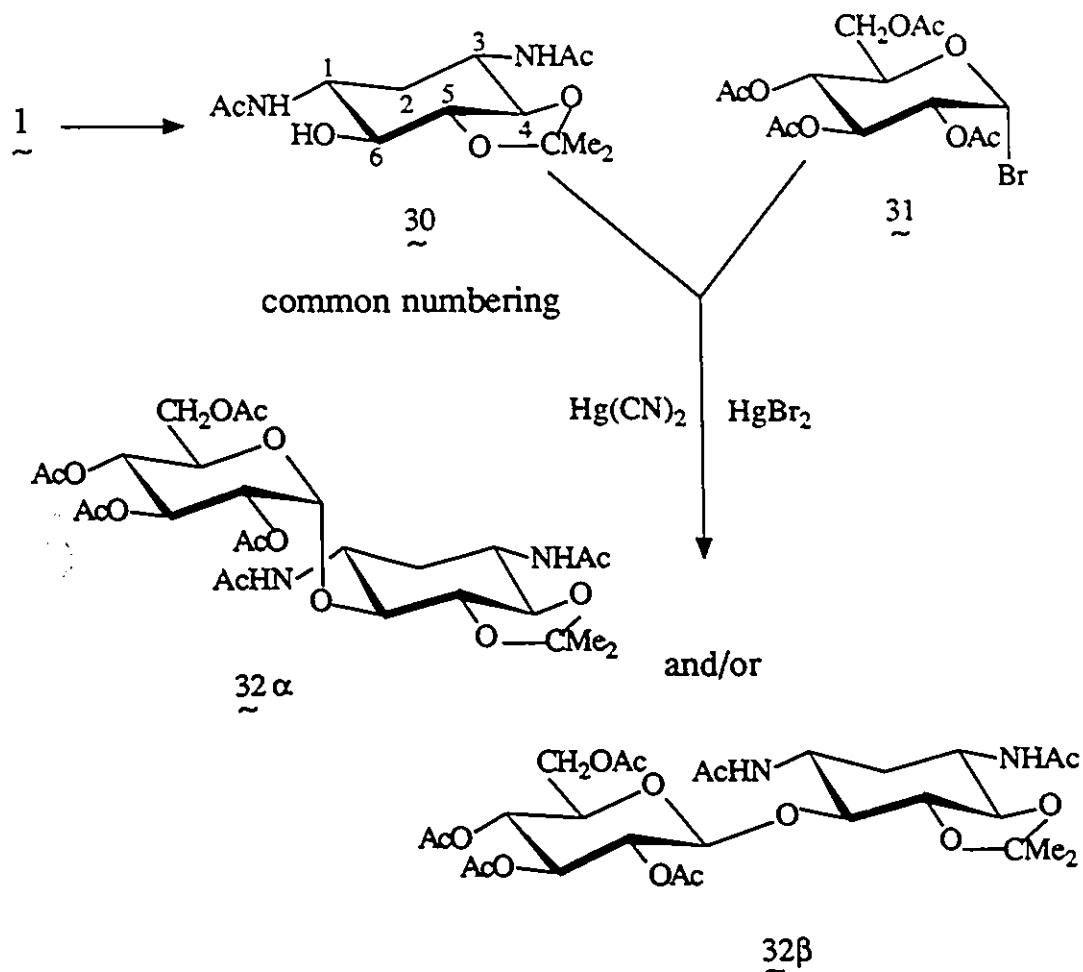
and 13.6 (CH₂-CH₃) for ethyl carbamate and 29.6 for N-methyl as the only substituent resonances in addition to the signals for the 6 ring carbon atoms.

In retrospect this result seemed understandable and might perhaps have been predicted. Acid hydrolysis of carbonamides generally is faster than that of carbamates; therefore, loss of the N-acetyl group from the other nitrogen occurs first and the molecule then exists as a methylammonium ion in which the positive charge electrostatically inhibits further proton attack and thus, hydrolysis of the carbamate. The situation is reminiscent of the well-known fact that, for the same reason, glycosides of free amino sugars are extremely difficult to hydrolyze by acid.

However, subsequent alkaline hydrolysis of the partially deprotected product with 2 M sodium hydroxide at 100°C for 3 h removed the ethoxycarbonyl group, and after neutralization with acetic acid a mixture of salts containing the desired (-)-hyosamine (**21**) was obtained. The latter was extracted in 60% yield following peracetylation, and characterized as the crystalline pentaacetyl derivative **22** whose physical and spectral data agreed with those of **22** from the first synthesis and with literature data.^{16,17}

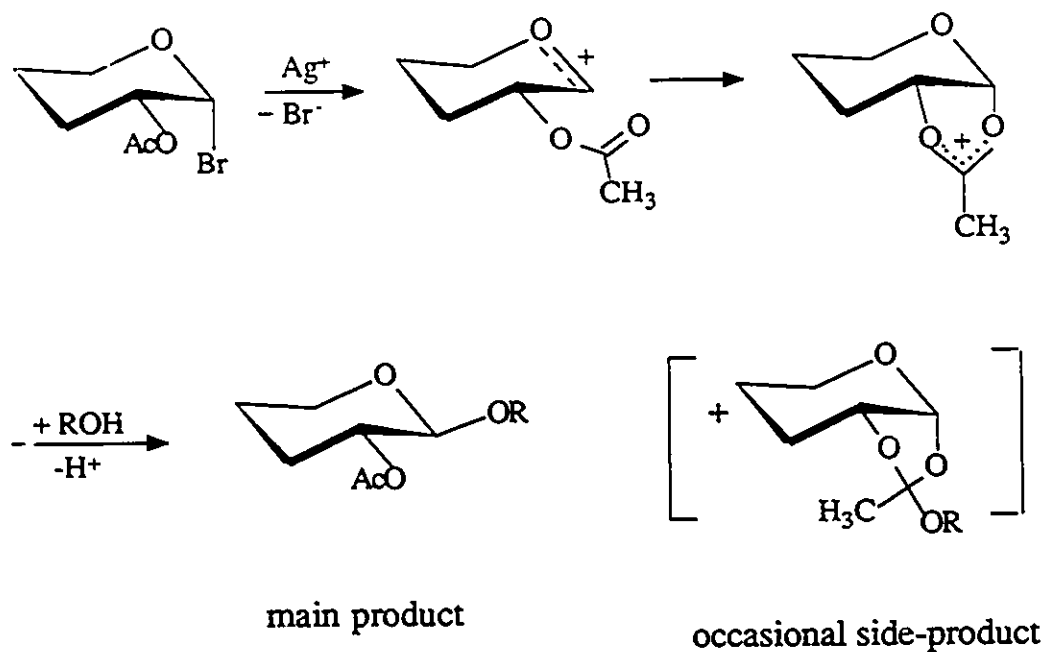
D. Unsymmetrical Glycosylation of 2-Deoxystreptamine

It was outlined in section B that compound **1** should be a useful starting point for achieving diastereospecific, unsymmetrical glycosylation of 2-deoxystreptamine as portrayed in Fig. 6. In all previous syntheses² of 2-deoxystreptamine-based aminocyclitol antibiotics such as the kanamycins and neomycins (Fig. 1), partially blocked racemic derivatives of the cyclitol served as starting materials and consequently, glycosylation led to mixtures of diastereomers as schematically depicted in Fig. 3. Diastereoselectivity was often poor, laborious separation procedures were necessary, and low overall yields resulted in several cases²⁸⁻³⁰. Therefore, the possibility of avoiding these difficulties by performing glycosylation of a chiral deoxystreptamine synthon was to be investigated on a first, simple example in the course of this thesis work. It was decided to use as a substrate the optically active 1L-(1,3/2,4,6)-4,6-diacetamido-1,2-Q-isopropylidene-1,2,3-cyclohexanetriol **30** (or 1,3-di-N-acetyl-4,5-Q-isopropylidene-2-deoxystreptamine by the common nomenclature, see Fig. 2), which is readily available⁸ by platinum-catalyzed hydrogenation of **1** followed by N-acetylation, and to employ as a glycosyl donor the most conveniently accessible one, namely tetra-Q-acetyl- α -D-glucopyranosyl bromide (**31**, "acetobromoglucose"). Coupling of the two components would lead regiospecifically to a 6-Q-glucosyl-2-deoxystreptamine, the



Scheme 6

4,5-positions being blocked (Scheme 6). Whether an α -glucoside (32 α) or a β -glucoside (32 β) would predominantly arise would to some extent depend on the conditions of the coupling reaction and was not considered relevant in these exploratory studies which focused on regiochemistry. Nevertheless, expectations with regard to α , β selectivity will be briefly discussed.



Scheme 7

In the classical Koenigs-Knorr condensation³¹, with silver oxide or silver salts (usually carbonate) being used as condensing agent, 1,2-cis glycosyl halides having a participating group at C-2 (such as **31**) are known to give β -glycosides in a highly selective way, although 1,2-orthoesters may be side products (Scheme 7). Yields with primary alcohols as glycosyl acceptors are generally good, but yields with secondary alcohols, especially cyclic ones such as **30**, are sometimes modest.³¹ The Helferich modification³² of the Koenigs-Knorr reaction, which uses a mixture of

mercuric cyanide and mercuric bromide as the condensing agent, has been claimed to afford improved yields but it tends to give α , β glycoside mixtures. Numerous examples have been cited³¹ where either one or the other anomer predominated, and no reasonable mechanism has yet been proposed to correlate the seemingly contradictory results. (When a 1,2-cis glycosyl halide having a nonparticipating group at C-2 is used as the donor, such as for example the tetra-Q-benzyl analog of **31**, the α -glycoside is usually the preponderant but seldom the exclusive product, both under Koenigs-Knorr and Helferich condition³¹; in at least one study³³ it was found that the glycosidic configuration arising depended on the stereochemistry of the glycosyl acceptor!). In view of this general lack of predictability we were curious to find out what results the Helferich procedure would yield in the particular case of coupling between **30** and **31**.

Thus, the acceptor **30** and a 1-molar excess of the donor **31** were allowed to interact, overnight at room temperature, in 1,2-dichloroethane solution in the presence of mercuric cyanide and bromide. All of the **30** was consumed, according to thin-layer chromatography, and processing of the reaction mixture by column chromatography removed the excess of unreacted **31** and furnished in 50% yield a crystalline mixture of a major and a minor disaccharidic product, distinguishable in t.l.c. The mixture gave a mass spectrum (m/z 617 [43%] for $M^+ + 1$) and an elemental analysis in accord with structure **32**, and the ¹H-n.m.r. signals representing the major

component clearly proved its constitution by indicating the presence of 6 acetyl and 2 isopropylidene methyl groups as well as the required pattern of ring protons. The anomeric proton signal was a clear doublet at δ 4.68 with a coupling constant $J_{1,2} = 8$ Hz, indicative of a 1,2-diaxial proton arrangement and hence, the β -glycosidic configuration. The H-2 signal of the glucose residue was a doublet of doublets (δ 4.93) with $J_{1,2} = 8.0$ and $J_{2,3} = 9.7$ Hz, and the H-3 and H-4 signals were triplets (δ 5.22 and 5.04) with spacings of 9.5-9.7 Hz. Thus, the major product was 32β . Had the α -configuration applied, a $J_{1,2}$ coupling of 3-4 Hz for a leg, 2ax proton arrangement would have occurred in the H-1 and H-2 signals.

That the minor condensation product was the anomer 32α can only be surmised on the basis of precedent in such reactions; the n.m.r. signals present in the mixture spectrum were mostly obscured by the strong signals of 32β and could not be analyzed unambiguously. It would have been gratifying had 32α been the main product, for it is the α -glycosidic linkage that normally connects hexopyranosyl rings to 2-deoxystreptamine in antibiotics (compare Fig. 1). In fact, 6-O- α -D-glucopyranosyl-2-deoxystreptamine, i.e., the compound derivable from 32α by complete deprotection, is a constituent of two kanamycin-related antibiotics (recorded as NK-1001 and NK-1012-1)² which are produced by mutants of the actinomycete Streptomyces kanamyceticus that produces kanamycin. The present work has achieved the regiospecific attachment of a glycosyl residue

to one of the enantiotopic carbinol positions (OH-6) in 2-deoxystreptamine; it remains for future research to examine possible modifications of the condensation reaction here reported so as to promote formation of the α -linkage, and also, to extend the strategy with a view to glycosylation specifically of the other enantiotopic position (OH-4), perhaps along the lines sketched in Fig. 6.

EXPERIMENTAL

General Methods

Thin layer chromatography (t.l.c) was performed on precoated silica gel plates (Merck 60 F₂₅₄). Spots were made visible by spraying the plates with 5% H₂SO₄ in ethanol or, for amines, with 2% ninhydrin in ethanol, and heating them on a hot-plate. Column chromatography was performed with silica gel Merck 9385 (particle size 20-45 μ m) or equivalent material. The following solvent combinations (v/v) were used for chromatography, unless otherwise specified: methanol-chloroform 1:1 (A), 1:5 (B), 1:9 (C), 1:19 (D) and 1:49 (E); ethyl acetate-hexane 3:1 (F), 3:2 (G), 1:1 (H), 1:3 (I), and 1:9 (J); ether-hexane 1:1 (K) and 1:4 (L); methanol-ethyl acetate 1:6 (M); acetonitrile-conc. aq. ammonia-water 3:2:1 (N) and 4:1:2 (O); and methanol-dichloromethane 1:9 (P) and (1:49) (Q).

Melting points were determined in capillaries with a Gallenkamp electrothermal apparatus and are uncorrected. Optical rotations were measured at room temperature in a Perkin Elmer 241 polarimeter. Infrared data (ν_{\max}) refer to spectra obtained from Nujol mulls for solid substances, or from thin films for syrups, unless otherwise indicated; only bands of particular structural significance are listed. Mass-spectral data

(m/z) were obtained by the chemical ionization mode using ether as the ionizing gas.

Nuclear magnetic resonance (n.m.r.) data given without special notation refer to spectra taken from chloroform-d solution at 300 MHz (for ^1H) or 75.43 MHz (for ^{13}C) with a Varian XL-300 instrument. Data denoted by 200 MHz (for ^1H) or 50.3 MHz (for ^{13}C) were obtained with a Varian Gemini 200 instrument. Solvents other than CDCl_3 are stated. In presenting the data, atoms or groups of atoms responsible for the signal in question are indicated in regular type, whereas atoms or groups of atoms that serve as locants for the resonating species are underlined (when necessary to avoid ambiguity). Thus, for example, $\text{CO}\underline{\text{C}}\text{H}_3$ and $\underline{\text{C}}\text{OCH}_3$ in ^{13}C data refer to the carbonyl and methyl resonances, respectively, of an acetyl group; $\underline{\text{C}}\text{Me}_2$ and $\underline{\text{C}}\text{Me}$ may be used to indicate the quaternary carbon and methyl carbon resonances, respectively, in an isopropylidene group.

In accord with current practice in many research journals, inorganic compounds are given by molecular formula, and some frequently-used organic solvents or reagents are given by widely accepted abbreviations (MeOH for methanol, EtOH for ethanol, EtOAc for ethyl acetate, AcOH for acetic acid, THF for tetrahydrofuran, DMF for N,N-dimethyl-formamide, DMSO for dimethyl sulfoxide).

A. Preparation of Key Intermediates 3 - 8

The starting compound required for the synthesis of 3 - 8, namely 1L-(1,3/2,4,6)-6-azido-1,2-Q-isopropylidene-4-nitro-1,2,3-cyclohexanetriol (**1**) was prepared from D-mannose exactly as described⁸, without procedural changes. Schematically portrayed in Fig. 4, the synthesis involves in detail the following steps: (a) base-catalyzed addition of nitromethane (Henry-reaction) to D-mannose, followed by peracetylation of the 2-epimeric mixture of 1-deoxy-1-nitro-D-glycero-D-galacto- and D-glycero-D-talohexitols produced³⁴; (b) reductive dehydroacetoxylation of the nitroheptitol hexaacetates by sodium borohydride in acetonitrile solvent, leading to 1,2-dideoxy-1-nitro-D-manno-heptitol pentaacetate; (c) Q-deacetylation of the latter with sodium methoxide in methanol; (d) acetonation of the product with 2,2-dimethoxypropane in the presence of CuSO₄ and H₂SO₄ to furnish 1,2-dideoxy-4,5:6,7-di-Q-isopropylidene-1-nitro-D-manno-heptitol; (e) trifluoromethylsulfonylation of the latter at OH-3; (f) azide displacement in the triflate, to afford 3-azido-1,2,3-trideoxy-4,5:6,7-di-Q-isopropylidene-1-nitro-D-gluco-heptitol; (g) selective hydrolysis of the 6,7-acetonide in the latter by 90% trifluoroacetic acid in toluene at -30°C; (h) periodate cleavage between C-6 and C-7 of the resulting diol and immediate, base-catalyzed cyclization of the nitro-aldehyde so produced, to yield **1**. (In this cyclization, the 3-epimer of **1** arises under kinetic control as a minor product, most of which can be converted into the more-stable, crystalline **1** by methoxide-catalyzed equilibration.) All reactions were performed on multigram scales, and yields for each step (a) to (g) were in the 80-90% range as reported. The

yield of pure **1** in steps (h) was 67%. The product had m.p. 170°C (lit.⁸ m.p.171°C) and was free from 3-epimer as a ascertained by its ¹H-n.m.r. spectrum. The spectral data for **1** and the epimer have been reported⁸. The originals were available for comparison; they are exceedingly well resolved and clearly distinct from each other, so that mutual contamination is readily discernible. The ¹³C-n.m.r. data (50.32 MHz, CDCl₃), not previously recorded, were: δ113.3 (O₂CMe₂), 86.4 (C-4), 79.0, 78.4 (C-1,2), 71.5 (C-3), 57.0 (C-6), 32.9 (C-5), 26.4 (CMe₂).

A.1. 1L-(1,3/2,4,6)-6-Formamido-1,2-O-isopropylidene-4-nitro-1,2,3-cyclohexanetriol (3)

To a solution of azidonitro compound⁸ **1** (516 mg, 2 mmol) in MeOH (20 mL) containing AcOH (0.18 mL) was added 10% Pd-C (400 mg, suspended in a small amount of MeOH), followed by 1,4-cyclohexadiene (1.6 mL). An N₂ atmosphere was provided, and the flask was immersed in an ultrasonic bath at 45°C. After sonication usually for 30 min but occasionally for 1-2 h, **1** (R_F 0.9) was absent and the corresponding 6-amino derivative **2** (R_F 0.2) was seen in t.l.c. (EtOAc). The catalyst was filtered off and washed with MeOH, and to the filtrate was added 1 mL of formyl acetate*. N-Formylation was complete at room temperature after 30 min when t.l.c. (EtOAc) showed **3** as a single spot,

R_F 0.65. The solvent was evaporated, and several portions of added EtOH were evaporated from the syrupy residue to remove acid. The slightly turbid solution of the residue in EtOAc was clarified with Celite, and **3** was then obtained crystalline (470 mg, 90%) from EtOAc-CHCl₃-Et₂O; m.p. 167-168°C (unchanged after recrystallization from 2-propanol), [α]_D + 31.3° (c 0.6, MeOH); ν_{max} 3350 (broad, OH and NH), 1675 (amide CO), and 1550 (NO₂) cm⁻¹; m/z 261 (M⁺ + 1, base peak), 245 (M⁺ + 1 - O), 243 (M⁺ + 1 - H₂O), 203 (M⁺ + 1 - Me₂CO), 185 (M⁺ + 1 - H₂O - Me₂CO). ¹H-n.m.r.: δ 8.18 (s, slightly broadened, formyl CH), 4.73 (broad s, NH), 4.51 (m, H-4), 4.41 (t, J_{2,3} = J_{3,4} = 9.2 Hz, H-3), 4.18 (m, H-6), 3.56 (t, J_{1,2} = J_{1,6} =

*Formyl acetate was generated^{35a} by heating a 1:2 mixture (v/v) of formic acid and acetic anhydride at 65°C for 1 h. For later experiments a procedure^{35b} giving a reagent of higher purity was used. Acetyl chloride was first purified by refluxing it over PCl₅ for 5 h, distilling it, and redistilling it from added quinoline (one-tenth volume). Sodium formate (1.2 equiv., dried at 130°C overnight) and acetyl chloride (1 equiv.) were then allowed to react in anhydrous ether for 5 h at room temperature, the sodium chloride was filtered off, and the solvent removed in a rotary evaporator.

9.2 Hz, H-1), 3.48 (t, $J_{1,2} = J_{2,3} = 9.2$ Hz, H-2), 2.91 (dt, $J_{4,5e} \approx J_{5e,6} \approx 4.8$, $J_{5a,5e}$ 13.3 Hz, H-5e), 2.78 (broad s, OH), 1.87 (dt, $J_{4,5a} = J_{5a,6} = 12.2$, $J_{5a,5e}$ 13.3 Hz, H-5a), 1.46 (s, 6 H, CMe₂).

Anal. Calc. for C₁₀H₁₆N₂O₆ (260.2): C 46.15, H 6.20, N 10.77.

Found: C 46.27, H 6.29, N 11.04.

A.2. 1L-(1,3/2,4,6)-6-Acetamido-1,2-O-isopropylidene-4-nitro-1,2,3-cyclohexanetriol (4)

To a solution of azidonitro compound **1** (258 mg, 1 mmol) in MeOH (10 mL) was added AcOH (0.18 mL, 3 mmol) and acetic anhydride (0.4 mL, 0.42 mmol), followed by a slurry of 10% Pd-C (200 mg) in MeOH (~ 2mL). 1,4-Cyclohexadiene (0.8 g, 10 mmol) was then added, the reaction vessel was flushed with N₂, fitted with an N₂ balloon, and immersed in an ultrasonic bath at 45°C. The mixture was sonicated for 30 min. Monitoring by t.l.c. (solvent C) indicated the conversion of **1** (R_F 0.7) into **4** (R_F 0.3) was very clean and had been virtually complete after 20 min. The catalyst was filtered off and washed well with MeOH, and the filtrate was concentrated, with addition of two portions of fresh MeOH, and then brought to dryness. Added EtOH and toluene were evaporated from the residue until a smell of acetic acid was no longer noticeable. The residue was taken up in EtOAc, and the cloudy solution was clarified by passage through

a plug of Celite/activated carbon. The clear filtrate was concentrated, and **4** then crystallized from a small amount of EtOAc upon addition of some CHCl_3 and, eventually, some hexane. Isolated and washed with a cold mixture of the same solvents, the crystals weighed 266 mg (97%); m.p. 175-177°C, raised to 185-186°C by recrystallization from 2-propanol; $[\alpha]_D + 40.3^\circ$ (c 0.5, MeOH); ν_{max} 3390 (sharp, NH), 3260 (broad, OH), 1650 (amide CO), and 1545 (NO_2) cm^{-1} ; m/z 275 ($\text{M}^+ + 1$, base peak), 217 ($\text{M}^+ + 1 - \text{Me}_2\text{CO}$), 199 ($\text{M}^+ + 1 - \text{H}_2\text{O} - \text{Me}_2\text{CO}$). $^1\text{H-n.m.r.}$: δ 5.58 (broad d, $J_{6,\text{NH}}$ 6.3 Hz, NH), 4.50 (m, H-4), 4.39 (t, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3), 4.10 (m, H-6), 3.49 (AB-m, 2 H, $J_{1,2} = J_{1,6} = J_{2,3} = 9$ Hz, H-1,2), 2.92 (dt, $J_{4,5e} = J_{5e,6} = 5.1$, $J_{5a,5e}$ 13.3 Hz, H-5e), 2.75 (broad s, OH), 1.99 (s, 3 H, Ac), 1.77 (dt, $J_{4,5a} = J_{5a,6} = 12.3$, $J_{5a,5e}$ 13.3 Hz, H-5a), 1.44 and 1.45 (2 s, 6 H, CMe₂).

Anal. Calc. for $\text{C}_{11}\text{H}_{18}\text{N}_2\text{O}_6$ (274.3): C 48.17, H 6.62, N 10.21.

Found: C 48.28, H 6.65, N 10.26.

A.3. 1L-(1,3/2,4,6)-6-Ethoxycarbonylamido-1,2-O-isopropylidene-4-nitro-1,2,3-cyclohexanetriol (5) and derivatives (5a - 5c)

Compound **1** (516 mg, 2 mmol) was converted by catalytic transfer hydrogenation into **2**, as described for the preparation of **3**. The catalyst was filtered off and washed well with MeOH, and the filtrate was evaporated to a

syrup that was taken up in water (20 mL). Pyridine (0.23 mL, 1.44 mol. equiv.) was added and then, dropwise, ethyl chloroformate (0.23 mL, 1.2 mol. equiv.), and the mixture was stirred at room temperature for 1 h, after which t.l.c. (solvent C) indicated the completion of the conversion of **2** (R_F 0.2) into **5** (R_F 0.6). The reaction mixture was diluted with an equal volume of water and extracted three times with 20 mL of EtOAc, with addition of some conc. NaCl solution in order to destabilize the emulsion that tended to form. The extract was dried (Na_2SO_4) and evaporated to a syrupy residue that was passed through a column of SiO_2 (2.3×20 cm) by means of solvent I, to give crystalline **5** (240 mg, 39.5%). A 57% yield was achieved in a similar experiment in which the ethoxycarbonylation was performed at 0°C in the presence of NaHCO_3 (1.2 mol. equiv.) instead of pyridine. Usually the crude product contained small amounts of by-products migrating more slowly in t.l.c.. Crystalline **5** had m. p. $76\text{-}78^\circ\text{C}$, $[\alpha]_D +24.1^\circ$ (c 1.1, CHCl_3); ν_{max} 3350 (broad, NH and OH), 1700-1680 (urethane CO). 1545-1530 (NO_2 and urethane NH) cm^{-1} ; m/z 305 ($\text{M}^+ + 1$), 260 ($\text{M}^+ + 1 - \text{OEt}$), 247 ($\text{M}^+ + 1 - \text{Me}_2\text{CO}$). ^1H n.m.r. ($\text{DMSO-}d_6$ at 55°C ; assignments confirmed by COSY experiment): δ 7.14 (d, $J_{6,\text{NH}}$ 8.8 Hz, NH), 5.95 (d, $J_{3,\text{OH}}$ 5.8 Hz, OH-3), 4.71 (ddd, $J_{3,4}$ 9.4, $J_{4,5e}$ 5.0, $J_{4,5a}$ 12.6 Hz, H-4), 4.08-3.96 (m, 3 H, H-3 and OCH_2Me), 3.88 (crude octet with broadened center peaks, \underline{W} 35 Hz, H-6), 3.47 (sym. septet, 2 H, \underline{W} 34.3 Hz with line separation of 9.15, 6.25, and 1.75 Hz, H-1,2), 2.34 (dt, $J_{4,5e} = J_{5e,6} = 4.8$, $J_{5a,5e}$ 12.7 Hz, H-5e),

1.86 (apparent q, W 36.6 Hz, with splittings of 12.6, 11.4, and 12.6 Hz, consistent with $J_{4,5a} \approx J_{5a,5e} \approx 12.6$ and $J_{5a,6} \approx 11.4$ Hz, H-5a), 1.38 and 1.37 (2 s, 3 H each, Me₂C), 1.17 (t, 3 H, J 7.1 Hz, CH₂CH₃).

When the spectrum was taken at ordinary temperature, certain differences concerning mainly the H-1,2, H-5e, and H-6 signals were observed. Thus, the approximate octet for H-6 was a broad unresolved multiplet, the H-1,2 signal was a narrower (W 23 Hz) sextet, and the H-5e signal was poorly resolved. The NH and OH-3 doublets were shifted slightly downfield to δ 7.33 and 6.10, respectively. The H-4 signal was unaffected.

Anal. Calc. for C₁₂H₂₀N₂O₇ (304.3): C 47.62, H 6.63, N 9.21.

Found: C 47.43, H 6.70, N 8.98.

In one experiment performed on a 2-mmol scale, in the presence of NaHCO₃ during N-acylation at 0°C, the slow-moving by-products seen in t.l.c. of the crude **5** appeared especially prominent, and they were isolated for examination. The column-chromatographic purification was performed using 2:3 EtOAc-hexane and, after collection of pure **5** (350 mg, 57.5%), the elution was continued and sequentially gave **5b** (R_F 0.35) and **5a** (R_F 0.1; t.l.c. with solvent C), both crystalline, in yields of 95mg (13.7%) and 55mg (10.4%), respectively.

1D-(1,3/2,4,6)-4-Ethoxycarbonylamido-6-nitro-1,2,3-cyclohexanetriol* (5a) had m.p. 202-204°C, $[\alpha]_D +22.9^\circ$ (c 0.6, MeOH); $\nu_{\max}^{\text{CHCl}_3}$ 3300 (broad, OH and NH), 1675 (carbamate CO), 1560-1540 (amide II and NO₂) cm⁻¹. The mass spectrum showed a weak M⁺ + 1 peak at m/z 265, and no species of greater mass. ¹H-n.m.r. (DMSO-d₆): δ 6.99 (d, $J_{4,\text{NH}}$ 8.5 Hz, NH), 5.62, 5.23, and 4.93 (3 d, exchangeable, J 5.6, 4.6, and 4.4 Hz, OH-1,2,3), 4.60 (ddd, $J_{5e,6}$ 4, $J_{1,6}$ 10, $J_{5a,6}$ 12 Hz H-6), 3.94 (q, 2H, J 7 Hz, OCH₂CH₃), 3.50-3.35 and 3.10 (2 m, 4 H total, H-1,2,3,4), 2.14 (dt, $J_{4,5e} \approx J_{5e,6} \approx 4$, $J_{5a,5e}$ 12 Hz, H-5e), 1.75 (~q, $J_{4,5a} \approx J_{5a,6} \approx J_{5a,5e} \approx 12$ Hz, H-5a), 1.13 (t, 3 H, J 7 Hz, CH₂CH₃). ¹³C-n.m.r. (50.32 MHz, DMSO-d₆): δ 156.4 (CO), 86.7 (C-6), 75.3, 74.1, 74.0 (C-1,2,3), 59.9 (OCH₂), 50.7 (C-4), 32.7 (C-5), 14.8 (CH₂CH₃).

Anal. Calc. for C₉H₁₆N₂O₇ (264.2): C 40.91, H 6.10, N 10.60. Found: C 41.07, H 6.08, N 10.74.

*For 5 the rules of nomenclature⁷ require that the triol system is numbered so as to give any substituted (by isopropylidene in this case) OH-groups the lowest possible locant numbers. For 5a, with unsubstituted OH-groups only, the sense of numbering is dictated by alphabetic precedence of other substituents present and must here be reversed because EtOCONH comes before NO₂. As a result, the configurational prefix is 1D.

1L-(1,3/2,4,6)-4,6-Di-(ethoxycarbonylamido)-1,2-O-isopropylidene-1,2,3-cyclohexanetriol (5b) had m.p. 188-190°C (lit.¹³ for racemate, m.p. 199-200°C), $[\alpha]_D +10.8^\circ$, $[\alpha]_{546} +13.3^\circ$, $[\alpha]_{436} +21.6^\circ$, $[\alpha]_{365} +28.4^\circ$ (c 1.2, CHCl₃) and $[\alpha]_D +1.1^\circ$, $[\alpha]_{365} -0.43^\circ$, (c 1.2, MeOH); $\nu_{\max}^{\text{CHCl}_3}$ 3300 (broad, OH and NH), 1670 (carbamate CO), 1545 (amide II) cm⁻¹; m/z 347 (M⁺ + 1, weak). ¹H-n.m.r. (200 MHz, DMSO-d₆): δ 7.26 and 7.05 (2 d, exchangeable, J 8.6 and 7.7 Hz, 2 NH), 5.12 (d, exchangeable, J 4.8 Hz, OH-3), 3.97 (q, 4 H, J 7 Hz, 2 QCH₂CH₃), 3.7-3.2 (complex m, unresolved, H-1,2,3,4,6), 1.85 (dt, $J_{5a, 5e}$ 12 Hz, H- 5e), 1.33 (s, 6 H, Me₂C), 1.27 (m, overlapped by adjacent signals, H-5a), 1.15 (t, 6 H, J 7 Hz, 2 QCH₂CH₃). In CDCl₃ solution, the dt for H-5e was at 2.24, Me₂C gave 2 separate 3-H singlets at 1.42 and 1.40, and also the OEt groups gave separate (but overlapping) quartets at 4.08 and triplets at 1.20. ¹³C-n.m.r. (50.32 MHz, DMSO-d₆): δ 156.5 and 156.0 (2 CO), 110.2 (Q₂CMe₂), 81.2, 78.5, 71.6 (C-1,2,3), 60.0 and 59.9 (2 QCH₂Me), 53.3 and 49.0 (C-4,6), 36.6 (C-5), 27.1 and 27.0 (Me₂C), 14.8 (double intensity, 2 QCH₂Me).

Anal. Calc. for C₁₅H₂₆N₂O₇ (346.4): C 52.01, H 7.57, N 8.08. Found: C 51.82, H 7.46, N 7.98.

(1,3/2,4,6)-4,6-Di-(ethoxycarbonylamido)-1,2,3-cyclohexanetriol (5c)
was obtained by overnight treatment of **5b** (95 mg) with 50% aqueous AcOH (10 mL) at room temperature, after which **5b** (R_F 0.35) was seen replaced by immobile **5c** in t.l.c. with solvent C. The acid was evaporated with additions of EtOH, and the white residue (~ 90 mg) was passed through a column of SiO₂ (1.5 × 10 cm) by means of solvent P. (With this solvent, which was also used for checking the effluent by t.l.c., **5c** is somewhat more mobile than with solvent C). There was obtained 63 mg (75%) of crystalline **5c**, m.p. 235-237°C (lit.¹³ m.p. 231-232°C); m/z 307 (98%, $M^+ + 1$), 261 (16%, $M^+ + 1 - \text{EtOH}$), 215 ($M^+ + 1 - 2 \text{EtOH}$); ¹H-n.m.r. (DMSO-d₆): δ 6.90 (d, 2 H, J 7.6 Hz, 2 NH), 4.85 (d, J 3.7 Hz, OH-2), 4.65 (d, 2 H, J 3.9 Hz, OH-1,3), 3.95 (q, 4 H, J 7.1 Hz, 2 OCH₂), 3.20 (m, 2 H, H-4,6), 3.00 (m, 3 H, H-1,2,3), 1.75 (dt, $J_{5a,5e}$ 13 Hz, H-5e), 1.15 (t, 6 H, J 7.1 Hz, 2 CH₂CH₃; superposed on m, 1 H, for H-5a). ¹³C-n.m.r. (50.29 MHz, DMSO-d₆): δ 156.3 (2 CO), 77.1 (C-2), 74.2 (C-1,3), 59.6 (2 OCH₂), 51.5 (C-4,6), 35.2 (C-5), 14.8 (2 CH₂CH₃).

Anal. Calc. for C₁₂H₂₂N₂O₇ (306.3): C 47.05, H 7.24, N 9.20.

Found: C 47.29, H 6.82, N 9.11.

A.4. 1L-(1,3/2,4,6)-6-Azido-1,2-O-isopropylidene-4-nitro-3-O-(tetrahydropyran-2-yl)-1,2,3-cyclohexanetriol (6)

Compound **1** (747 mg, 3 mmol) dissolved in 1,4-dioxane (25 mL) was treated at room temperature with 2,3-dihydropyran (1.4 ml, 15mmol) and *p*-toluenesulfonic acid monohydrate (57 mg). The conversion of **1** (R_F 0.3) into **6** (2 epimers, R_F 0.7 and 0.65) was nearly complete after 30 min (t.l.c. with solvent **J**). After 1 h, saturated aq. NaHCO_3 (3 mL) was added and the mixture evaporated. The residue was distributed between water and CHCl_3 , and the organic phase was washed with water and concentrated to a syrup of crude **6** (1.00 g, 97%) that could be used directly for preparation of **7** or **8**.

For analytical characterization, crude **6** (0.5 g) was chromatographed on a column of SiO_2 (2.3 × 8 cm) by elution with hexane followed by solvent **J**. Early fractions contained pure, faster-moving epimer which crystallized as fine needles upon concentration of the eluate and addition of hexane; yield, 261 mg; double m.p. at 97-99°C and 112-113°C; $[\alpha]_D -118.6^\circ$ (c 1, CHCl_3); m/z 343 ($M^+ + 1$, base peak), 327 ($M^+ + 1 - \text{O}$), 315 ($M^+ + 1 - \text{N}_2$), 285 ($M^+ + 1 - \text{Me}_2\text{CO}$), 268 ($M^+ - \text{O} - \text{Me}_2\text{CO}$), 242 ($M^+ + 1 - \text{C}_5\text{H}_9\text{O}_2$ [tetrahydropyranyloxy]). $^1\text{H-n.m.r.}$: δ 5.11 (t, J 2.4 Hz, H-2'), 4.57

(ddd, $J_{4,5e}$ 5.0, $J_{3,4}$ 9.35, $J_{4,5a}$ 12.3 Hz, H-4), 4.48 (t, $J_{2,3} = J_{3,4} = 9.35$ Hz, H-3), 3.67 (ddd, $J_{5e,6}$ 4.7, $J_{5a,6}$ 11.4, $J_{1,6}$ 9.9 Hz, H-6), 3.5 (m, 3 H, containing H-6', 6" signals and a ~ t, $J \sim 9.5$ Hz, for H-1), 3.78 (t, $J_{1,2} = J_{2,3} = 9.4$ Hz, H-2), 2.56 (dt, $J_{4,5e} = J_{5e,6} = 4.7$, $J_{5a,5e}$ 13.2 Hz, H-5e), 2.05 (o, $J_{4,5a}$ 12.5, $J_{5a,6}$ 11.5, $J_{5a,5e}$ 13.2 Hz H-5a), 1.7-1.5 (m, 6 H, H-3', 3", 4', 4", 5', 5"), 1.45 and 1.43 (2 s, 3 H each, Me₂C).

Anal. Calc. for C₁₄H₂₂N₄O₆ (342.3): C 49.12, H 6.48, N 16.36.

Found: C 49.01, H 6.47, N 16.47.

Further elution of the column produced mixed fractions containing both epimers as well as slower-moving by-products. The latter were separated off by repeated chromatography but the second epimer could not be obtained free from the first one, even though the product crystallized from hexane; yield, 130 mg; m.p. 95-96°C, $[\alpha]_D -65.4^\circ$ (c 1, CHCl₃). The mass spectrum showed the same peaks as for the pure first epimer, in almost the same intensity ratios. The microanalytical values found were C 49.02, H 6.43, and N 16.46. The ¹H-n.m.r. spectrum showed all the signals listed above for the first epimer (which in fact preponderated ~ 2:1), and most of the corresponding signals for the second epimer coincided with these, except

for H-2' (δ 4.67, t, J 3.7 Hz), H-3 (δ 4.40, t, J ~ 9 Hz), H-6' or H-6'' (δ 4.00, sp, J 3.5, 9, and 11 Hz), and Me₂C (δ 1.47 and 1.45, 2 s).

A.5. 1L-(1,3/2,4,6)-6-Formamido-1,2-O-isopropylidene-4-nitro-3-O-(tetrahydropyran-2-yl)-1,2,3-cyclohexanetriol (7)

A.5a From alcohol 3.—The alcohol 3 (260 mg, 1 mmol) in dry 1,4-dioxane (20 mL) was treated at room temperature with 2,3-dihydropyran (0.9 mL) in the presence of *p*-toluenesulfonic acid monohydrate (38 mg). The reaction was complete after 2 h as revealed by t.l.c. (solvent C), which showed 7 (R_F 0.5) and no more 3 (R_F 0.3). The acid catalyst was neutralized with triethylamine, the mixture concentrated under reduced pressure, and a chloroform solution of the residue was washed with water, dried (MgSO₄), and evaporated. The crude crystalline material was chromatographed on a column (2.2 × 15 cm) of SiO₂ by use of solvent C. The combined fractions containing 7 were evaporated, and added EtOH was evaporated from the residue which thereby crystallized. After trituration with EtOAc and hexane, and storage overnight in the refrigerator, the crystals of 7 (165 mg, 48%) were collected and washed with hexane; m.p. 208-209°C, $[\alpha]_D$ -47.7° (c 0.9, CHCl₃); m/z 345 (M⁺ + 1, base peak), 287 (M⁺ + 1 - Me₂CO), 261 (M⁺ + 1 - C₅H₈O [dihydropyran]), 203 (M⁺ + 1 - Me₂CO - C₅H₈O). ¹H-n.m.r.:

δ 8.18 (s, CHO), 5.70 (d, $J_{6,NH}$ 6 Hz, NH), 5.12 (t, J 2 Hz, H-2'), 4.62 (m, H-4), 4.50 (t, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 4.16 (m, H-6), 3.6-3.4 (m, 4 H, containing two triplets with J 9-10 Hz; H-1,2,6',6''), 2.83 (dt, $J_{4,5e} = J_{5e,6} = 4.7$, $J_{5a,5e} 13.1$ Hz, H-5e), 1.93 (~sx, $J_{4,5a} = J_{5a,6} = 11.5$, $J_{5a,5e} 13$ Hz, H-5a), 1.65-1.45 (m, 6 H, H-3', 3'', 4', 4'', 5', 5''), 1.42 and 1.41 (2 s, 3 H each, Me₂C).

Anal. Calc. for C₁₅H₂₄N₂O₇ (344.4): C 52.32, H 7.03, N 8.13. Found: C 52.40, H 7.06, N 7.96.

The mother liquor of the crystalline **7** was concentrated and passed once more through a column as just described. There was obtained a second crop of **7** (156 mg, 45%) which, however, did not crystallize. It gave the same mass spectrum as the crystalline material, but the ¹H-n.m.r. spectrum indicated a mixture of epimers: All signals of the crystalline epimer were present, and there was a second set of similar or identical signals which partly coincided or overlapped with the first set, except for H-2' (δ 4.68, narrow t), H-3 (δ 4.42, t, $J \sim 9$ Hz), H-6' or H-6'' (δ 4.00, m), and Me₂C (δ 1.44 and 1.43, s). Epimer proportions varied somewhat in different preparations.

A.5b From tetrahydropyranyl ether 6. – Compound **6** (342 mg, 1 mmol, epimer mixture) was dissolved in MeOH (15 mL) containing AcOH (0.06 ml, 1 mmol) and 1,4-cyclohexadiene (0.5 mL). Upon addition of 10% Pd-C, the mixture was sonicated under N₂ at 45°C for 30 min. The formation of ninhydrin-positive amine was indicated by t.l.c. (R_F 0.0 with solvent K, R_F 0.45 with solvent C). Formyl acetate (0.5 mL) was then added, and the conversion of the amine into ninhydrin-negative **7** (R_F 0.5, solvent C) was complete after 30 min. The catalyst was filtered off, the filtrate neutralized with triethylamine, and the solvent evaporated to give a crude, crystalline material. This was taken up in a little chloroform and applied to a SiO₂ column (2.3 × 10 cm). Elution with hexane followed by solvent I (100 mL) produced fast-moving impurities, and subsequent elution with solvent G (200 mL) gave **7** (295 mg, 86%) as a crystalline mixture of epimers (2:1 according to ¹H-n.m.r.). The mass-spectral data were identical with those given in section A.5a.

A partial separation of the epimers was achieved when a sample was rechromatographed on SiO₂ by use of solvent E. A nearly homogeneous epimer crystallized on concentration of some eluate fractions and, after trituration with ether-hexane and drying, melted at 196-197°C and showed [α]_D -64.7° (c 1, CHCl₃). Its ¹H-n.m.r. spectral data were identical with

those given for the crystalline **7** in section A.5a. (Both crystalline preparation did contain traces of the second epimer as indicated by very minor signals in the spectra, with the product made from **3** (section A.5a) appearing to contain slightly more of it. This may also explain the discrepancy in the $[\alpha]_D$ values; a chromatographic fraction consisting of approximately equal proportions of the two epimers – as evidenced by the n.m.r. spectrum – had $[\alpha]_D -5^\circ$, which indicates that the second epimer must be dextrorotatory.)

A.6. 1L-(1,3/2,4,6)-1,2-O-Isopropylidene-4-nitro-3-O-(tetrahydropyran-2-yl)-6-trifluoroacetamido-1,2,3-cyclohexanetriol (**8**)

Compound **1** (774 mg, 3 mmol) was tetrahydropyranylated, and the crude product **6** (epimer mixture) was subjected to phase transfer hydrogenation as described (see the preparation of **7**, section A.5b), but not subsequently treated with formyl acetate. Instead, the amine-containing hydrogenolysis solution was filtered, the catalyst washed well with MeOH, and the filtrate evaporated. The residue was dissolved in CH₂Cl₂ (25 mL), and pyridine (1 mL) followed by trifluoroacetic anhydride (1 mL) were added with ice cooling. The dark olive-colored mixture was concentrated, and several added portions of EtOH were evaporated from the crystalline

residue which showed two strong spots for epimers of **8**, R_F 0.43 and 0.35, accompanied by trace spots for **6** (R_F 0.65 and 0.55) that had resisted hydrogenation (t.l.c. with solvent I). A solution of the material in CHCl_3 was washed with water, dried (MgSO_4), and evaporated, and the product purified by chromatography on a short column of SiO_2 by use of solvent L. The eluted product having R_F 0.43-0.35 (solvent I) was recrystallized from CHCl_3 -hexane to give **8** (508 mg, 41%), m.p. 199-200°C; m/z 413 ($M^+ + 1$, base peak), 397 ($M^+ + 1 - \text{O}$), 355 ($M^+ + 1 - \text{Me}_2\text{CO}$), 329 ($M^+ + 1 - \text{C}_5\text{H}_8\text{O}$ [dihydropyran]), 313 ($M^+ + 1 - \text{O} - \text{C}_5\text{H}_8\text{O}$), 271 ($M^+ + 1 - \text{Me}_2\text{CO} - \text{C}_5\text{H}_8\text{O}$). The ^1H -n.m.r. spectrum was practically identical with the spectra of **7** (epimer mixtures), except for the absence of the low-field formyl proton singlet, a substantial downfield shift (to δ 6.42) of the NH signal, and a minor upfield shift (to δ 4.08) of the H-6 multiplet.

Anal. Calc. for $\text{C}_{16}\text{H}_{23}\text{F}_3\text{N}_2\text{O}_7$ (412.3): C 46.60, H 5.62, N 6.79.

Found: C 46.71, H 5.34, N 6.46.

B. Synthesis of (+)-Hyosamine

B.1. 1L-(1,3/2,4,6)-4-Acetamido-1,2,3-tri-O-acetyl-6-(N-methyl)acetamido-1,2,3-cyclohexanetriol (11)

A solution of the formamidonitro compound **7** (170 mg, 0.5 mmol) and LiAlH₄ (75 mg) in dry 1,4-dioxane (10 mL) was gently boiled under reflux for 1.5 h. Celite was added to the cooled mixture, and the excess of hydride was decomposed by slow addition of MeOH, followed by some water, with vigorous stirring. The solids were filtered off and washed with MeOH. The filtrate showed, in t.l.c. with solvent C, a strong, immobile, ninhydrin-positive spot attributed to the Q-protected diamine **9**, and starting **7** (R_F 0.5) was absent. Solvent evaporation gave crude **9** which was treated with 90% trifluoroacetic acid (2 mL) in MeOH (10 mL), overnight at room temperature. The reaction mixture was concentrated by evaporation in vacuo, and residual acid was removed by coevaporation with benzene. The amorphous, brown product dissolved in a small amount of water was applied to a column (1 × 10 cm) of Dowex - 50W (H⁺) cation exchange resin, which was exhaustively washed with water and then eluted with aqueous, 3.5 M ammonia. Evaporation of the eluate gave crude amine **10** as a light-brown, amorphous material, R_F 0.25 (ninhydrin-positive, with the brown,

ninhydrin-negative impurities traveling near the solvent front; t.l.c. with solvent N). 2-Deoxystreptamine (**23**) spotted for comparison had R_F 0.35. A small sample was purified by chromatography on a small column of SiO_2 with solvent Q, which readily eliminated the fast-moving impurities, and **10** was obtained as white solid; m/z 177 ($M^+ + 1$) and 161 ($M^+ - \text{Me}$) in agreement with mol. wt. 176 for $\text{C}_7\text{H}_{16}\text{N}_2\text{O}_3$. The bulk of the crude **10** was treated with acetic anhydride and pyridine (1 mL each) for 16 h at 25°C , and the reaction mixture was then processed by addition of MeOH and evaporation, with added toluene, to dryness, followed by chromatography on an SiO_2 column (1.2×25 cm) by use of solvent C. There was obtained the pentaacetyl derivative **11** (45 mg, 23.5%), which crystallized from ethanol-ether-hexane; m.p. $156\text{-}157^\circ\text{C}$ (lit.¹⁶ m.p. $156.5\text{-}157^\circ\text{C}$ for the enantiomer); $[\alpha]_D -4.4^\circ$ (c 1, MeOH) (lit.¹⁷ -7.5° in MeOH); R_F 0.4 (t.l.c., solvent C); m/z 387 ($M^+ + 1$), 343 ($M^+ - \text{Ac}$), 327 ($M^+ + 1 - \text{AcOH}$) in agreement with mol. wt. 386 for $\text{C}_{17}\text{H}_{26}\text{N}_2\text{O}_8$. The ^1H -n.m.r. spectrum showed 2 N- CH_3 signals in a $\sim 3:2$ intensity ratio at δ 2.81 (slightly broadened singlet) and δ 2.72 (sharp singlet), and there were 13-14 singlets of varying intensities in the range of δ 2.05-1.89 for COCH_3 . The combined integrals for N- CH_3 and COCH_3 were in a ratio of 1:5. Two NH doublets ($J_{4,\text{NH}} \sim 8$ Hz) integrating to one proton were at δ 5.74 and 5.66. The ring protons resonating in the range δ 5.1- 4.7 (m, 3 H, H-1,2,3) and 4.2 - 3.8 (m, 2 H, H-4,6) were ill-resolved.

B.2. Exploratory studies concerning an alternative approach from 7 to 10

B.2a Reduction of 7 with borane – To a solution of 7 (295 mg, 0.85 mmol, of an epimer mixture) in dry tetrahydrofuran (10 mL) was added borane-tetrahydrofuran complex (1.4 mL of a 1.34 M solution, 2.2 mol. equiv.) and the mixture was boiled gently under reflux for 30 min with exclusion of atmospheric moisture. Inspection by t.l.c. (solvent C) revealed a complete replacement of 7 (R_F 0.5) by two new products, R_F 0.8 and 0.45. [A separate pilot experiment had shown that continued boiling after addition of further borane did not change the t.l.c. pattern.] Excess MeOH was added to the cooled reaction mixture, the solvents were evaporated, and several portions of added MeOH were evaporated from the colorless, crystalline residue (303 mg; theoretical yield, 283 mg) which was presumed to contain the methylamino derivative 12 and probably some boron complex. The ^1H -n.m.r. spectrum was poorly resolved but it clearly indicated that the formyl group signal of 7 (s at δ 8.18) was completely removed and replaced by two doublets at δ 2.69 and 2.53 (J 5.9 and 6.1 Hz, ~ 2:1 intensity ratio) for an NH-CH₃ group. The mass spectrum showed a strong peak at m/z 331 corresponding to $M^+ + 1$ for 12 (mol. wt. 330 for $\text{C}_{15}\text{H}_{26}\text{N}_2\text{O}_6$), and lesser peaks at m/z 273 and 247, resulting from loss of acetone and dihydropyran, respectively.

B.2b N-Acetylation of 12. – The material (300 mg from section B.2a above, plus 110 mg from a similar run) was N-acetylated by treatment with acetic anhydride in methanolic solution (2 h at room temperature). For processing, the methanol was evaporated and the residue taken up in CHCl_3 and washed with aq. NaHCO_3 and water. Concentration of the dried (MgSO_4) organic solution gave a crystalline material that was inhomogeneous in t.l.c. (solvent H), showing a fast-moving (R_F 0.75) and a slow-moving (R_F 0.2) spot, both strong, and several intermediate trace spots. Column chromatography on SiO_2 (2.3×10 cm) was performed using first solvent I (200 mL) to elute the less-polar material (120 mg), and then solvent G (300 ml) to produce crystalline material having R_F 0.2 (274 mg, 54.8%); end-fractions containing some even more-polar material (R_F 0.1, 49 mg) were discarded. The product having R_F 0.2 was 1L-(1,3/2,4,6)-1,2-O-isopropylidene-6-(N-methyl)acetamido-4-nitro-3-O-(tetrahydropyran-2-yl)-1,2,3-cyclohexanetriol (13), m.p. 189-192°C (not recrystallized); m/z 373 ($M^+ + 1$, base peak), 315 ($M^+ + 1 - \text{Me}_2\text{CO}$), 289 ($M^+ + 1 - \text{C}_5\text{H}_8\text{O}$ [dihydropyran]), 231 ($M^+ + 1 - \text{Me}_2\text{CO} - \text{C}_5\text{H}_8\text{O}$) in accord with mol. wt. 372 for $\text{C}_{17}\text{H}_{28}\text{N}_2\text{O}_7$. $^1\text{H-n.m.r.}$: δ 5.13 (narrow t H-2'), 4.68 (m, 2 H, H-4,6), 4.48 (t, with small satellite t at 4.40 for epimer, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 3.64 (dd, $J_{1,2}$ 8.8, $J_{1,6}$ 11 Hz, H-1), 3.50 (m, 3 H, H-2,6',6''), 2.94 (s,

with small satellite at 2.86, 3 H, N-Me), 2.38 (m, H-5e), 2.11(s, 3 H, Ac), 2.01 (~ q, splittings 12-13 Hz, H-5a), 1.65-1.45 (m, 6 H, H-3', 3'', 4', 4'', 5',5''), 1.42 and 1.39 (2 s, 3 H each, Me₂C).

B.2c Catalytic hydrogenation of 13.– Nitro compound **13** (268 mg, 0.72 mmol) and PtO₂ (227 mg, 1 mmol) in 99% EtOH (25 mL) were shaken overnight under H₂ at ordinary temperature and pressure. Hydrogen uptake was 94 mL [calc: (0.72 × 3 + 1 × 2) × 22.4 = 93]. The solution gave a positive ninhydrin reaction. Acetic anhydride (1.5 mL) was added, and after 15 min the ninhydrin reaction was negative. The catalyst was filtered off and washed exhaustively with EtOH, and the filtrate was evaporated to a colorless, gel-like residue. T.l.c. showed **14** as a slow-moving (solvent C) or immobile (EtOAc) spot and a contaminant migrating with the solvent front, but **13** (R_F 0.7 and 0.6, respectively) was absent. The material was passed through an SiO₂ column (2.3 × 10 cm) by means of EtOAc (100 mL, to remove the non-polar impurity) followed by solvent M to produce the desired compound as a colorless syrup that failed to crystallize and was dried in an oil-pump vacuum to a hard glass (280 mg, quantitative). It was 1L-(1,3/2,4,6)-4-acetamido-1,2-O-isopropylidene-6-(N-methyl)acetamido-3-O-(tetrahydropyran-2-yl)-1,2,3-cyclohexanetriol (14); m/z 385 (M⁺ + 1), 327 (M⁺ + 1 - Me₂CO), 301 (M⁺ + 1 - C₅H₈O, base peak), 243 (M⁺ + 1 -

Me₂CO - C₅H₈O). Like **11**, the compound displayed 2 N-CH₃ singlets (δ 2.88 and 2.80, intensity ratio 2:1) and multiple COCH₃ signals in the range δ 2.15-1.90. The intensity ratio of the former to the latter group of signals was 1:2. Each of the N-CH₃ signals was accompanied by a minor satellite at slightly lower field, probably due to tetrahydropyranyl 2'-epimers.

C. Synthesis of (-)-Hyosamine

C.1. Exploratory experiment: Attempted preparation of 1L-(1,3/2,4,6)-6-acetamido-1,2-O-isopropylidene-4-methylamino-1,2,3-cyclohexanetriol (16)

The acetamidonitro compound **4** (274 mg, 1 mmol) was dissolved in 99% EtOH (20 mL) containing AcOH (0.06 mL, ~ 1 mmol), and hydrogenated over Adams catalyst (114 mg of PtO₂, 0.5 mmol) at ambient temperature and pressure. Hydrogen uptake (90 mL, as calculated) was complete after 18 h. A strong ninhydrin-positive spot (R_F 0.3) for the amine **15** was seen, and starting **4** (R_F 1.0) was absent (t.l.c. with solvent A; double irrigation). The catalyst was removed and washed well with EtOH, and the filtrate evaporated to give syrupy **15**, which was dried in a high vacuum to a hard glass, was homogeneous in t.l.c., and characterized by its mass

spectrum [m/z 245 ($M^+ + 1$, base peak) and 187 ($M^+ + 1 - \text{Me}_2\text{CO}$)] that accorded with the structure of 1L-(1,3/2,4,6)-6-acetamido-4-amino-1,2,-O-isopropylidene-1,2,3-cyclohexanetriol ($\text{C}_{11}\text{H}_{20}\text{N}_2\text{O}_4$, mol. wt. 244). It was used as such for the preparation of **18** (see C.3).

Several attempts were made to N-methylate **15** to **16**. Treatment with paraformaldehyde in MeOH, followed by addition of NaBH_3CN , did not appear to change the compound (t.l.c.). Treatment with aqueous 37% formaldehyde followed by NaBH_3CN led to a very complex t.l.c. pattern. The approach was abandoned.

C.2. Exploratory experiment: Attempted preparation of 1 L-(1,3/2,4,6)-4-formamido-1,2-O-isopropylidene-3-O-(tetrahydropyran-2-yl)-6-trifluoroacetamido-1,2,3-cyclohexanetriol (17)

The trifluoroacetamidonitro compound **8** (0.50 g) was hydrogenated over PtO_2 (0.1 g) in 99% EtOH (25 mL) containing AcOH (0.08 mL) at ambient temperature and pressure. Reduction was extremely sluggish: After 16 h, only a trace of ninhydrin-positive product was seen in t.l.c. (R_F 0.0 in solvent I, 0.2 in solvent C). More PtO_2 (0.1 g) was added, and shaking under H_2 was continued for 4 days, after which it was still far from complete. Continuation for another 2 days with additional catalyst

(0.2 g) brought no change in the t.l.c. pattern which still showed much starting **8** (R_F 0.43 and 0.35 [small] for 2 epimers, with solvent I; or R_F 0.8 [1 spot only for both epimers] with solvent C) together with slow-moving amine spots. The catalyst was removed, the filtrate concentrated, and the hydrogenation was repeated (4 days) with fresh catalyst. However, a significant amount of **8** remained even then. Work-up gave a crystalline but inhomogeneous material that was dissolved in MeOH (15 mL) and treated with formyl acetate (1 mL) for 3 h at room temperature. The mixture was then concentrated, taken up in CHCl_3 , and washed with aq. NaHCO_3 solution and water. The dried (MgSO_4) organic phase was concentrated and chromatographed on a column (2.3×10 cm) of SiO_2 . Elution was started with solvent I (200 mL), which produced unchanged **8** (172 mg, 34% recovery), m.p. 199-200°C. Continued elution with solvents G (200 mL) and E (100 mL) and finally, with pure EtOAc gave 3 fractions of products: R_F 0.6 (31 mg), R_F 0.3 (176 mg), and R_F 0.2 (double spot; 116 mg) (t.l.c. with solvent H). It is probable that one of the products was the desired formamido derivative **17** but no attempts at identification were made. The procedure was considered impractical because of the large amounts of time and expensive catalyst it consumed, and the approach was abandoned.

C.3. First synthesis of (-)-hyosamine (21), characterized as pentaacetyl derivative 22

Glassy amine **15** obtained in practically quantitative yield by catalytic hydrogenation of 1.2 mmol of the acetamidonitro compound **4** (see C.1) was suspended in 1,4-dioxane (25 mL), and 2,3-dihydropyran (500 mg, 6 mmol) followed by *p*-toluenesulfonic acid monohydrate (250 mg, 1.3 mmol) were added. The mixture was stirred at room temperature, with DMF (2 mL) being added after 30 min in order to aid in dissolving the amine. After 2 h the clear solution appeared free from **15** (R_F 0.2) and showed a strong spot for its tetrahydropyranyl derivative **18** (R_F 0.8, accompanied by a weak spot for the epimer, R_F 0.7 (t.l.c. with solvent A and indication by ninhydrin). The mixture was made slightly basic with triethylamine and concentrated in vacuo, with removal of DMF eventually being completed by coevaporation with toluene. The dried residue was purified on a column of SiO₂ (2.2 × 13 cm) which was irrigated first with CHCl₃ (to remove a yellow, fast-moving impurity) and then with solvent D (100 mL) followed by solvent A (200 mL), to furnish ninhydrin-positive fractions that gave, on evaporation, glassy 1L-(1,3/2,4,6)-6-acetamido-4-amino-1,2,-O-isopropylidene-3-O-(tetrahydropyran-2-yl)-1,2,3-cyclo-hexanetriol (18) in a yield of 329 mg (83.6% based on **4**); m/z 329 ($M^+ + 1$) and 245 ($M^+ + 1 - C_5H_8O$ [dihydropyran]) in harmony with the composition C₁₆H₂₈N₂O₅ (mol. wt. 328).

The crude **18** (328 mg, 1 mmol) dissolved in CH₂Cl₂ (10 mL) was treated overnight at room temperature with N,N-dimethylformamide dimethyl acetal (0.2 mL). The solvent was evaporated to give a pale yellow syrup presumed to contain the N,N-dimethylformamidine **19**; the expected M⁺ + 1 peak was present at m/z 384 as the base peak in the mass spectrum. The material was redissolved in CH₂Cl₂ (10 mL), and treated overnight at room temperature with methyl trifluoromethanesulfonate (0.17 mL, 1.5 mmol) in order to generate the N,N,N'-trimethylformamidinium salt **20**. The reaction mixture was evaporate to a syrup that was immediately boiled under reflux for 1.5 h with 6 M HCl (10 mL) in order to effect hydrolysis of the amidinium function to a methylamino group; concomitantly, hydrolysis of all protecting groups in the molecule was expected to take place, leading to (-)-hyosamine (**21**). The hydrolyzate was a dark liquid containing some tarry decomposition products. It was cooled, diluted with some water and extracted with CHCl₃. The organic solvent took out much of the dark impurities, leaving behind a clear, yellow aqueous phase. This was evaporated to give a brownish, crystalline residue of aminocyclitol hydrochloride. However, the product was not homogeneous; two closely-spaced ninhydrin-positive spots (R_F 0.3-0.4) were seen in t.l.c. (solvent N). The faster-moving spot migrated like authentic 2-deoxystreptamine (**23**) run for comparison, and the slower spot was attributed to the target compound **21**. The material was taken up in a little

aqueous methanol and applied to a small column of Dowex-50W (H⁺) cation exchange resin (~ 10 mL), which was rinsed with MeOH and water whereby the brown impurities emerged. The column was then eluted with aqueous 5% NH₃ until all ninhydrin-positive material was recovered, as checked by t.l.c.. No separation of the two components was observed or intended. The residue obtained by evaporation of the ammoniacal eluate was dried in a desiccator and then peracetylated (16 h at room temperature) with acetic anhydride and pyridine. Processing by coevaporation of the reagents with toluene gave a syrupy mixture of peracetyl derivatives, R_F 0.4 (attributed to **22**; compare **11**) and R_F 0.3 (migrating like authentic pentaacetyl-2-deoxystreptamine **24**) in t.l.c. with solvent C. Note that the relative mobilities were reversed in comparison to unprotected **21** and **23**.

Separation of the mixture on a column (2.3 × 15 cm) of SiO₂ by use of solvent D as the eluent and collection of 50-mL fractions gave **22** (75 mg from fraction No. 6, 19.4% based on **18**) and **24** (52 mg from fractions No. 7-10, 14%). Both crystallized as fine colorless needles from ethanol-ether-hexane. The target compound **22**, 1D-(1,3/2,4,6)-4-acetamido-1,2,3-tri-O-acetyl-6-(N-methyl)acetamido-1,2,3-cyclohexanetriol, had m.p. 156-157°C as reported¹⁶; [α]_D +4.6° (c 1, MeOH), lit.¹⁷ +5.4° and¹⁶ +7.5°; m/z

387 ($M^+ + 1$, base peak), 373 ($M^+ + 2 - \text{Me}$), 345 ($M^+ + 2 - \text{Ac}$), 327 ($M^+ + 1 - \text{AcOH}$), 254 ($M^+ + 1 - \text{AcNH}_2 - \text{AcNHMe}$). The $^1\text{H-n.m.r.}$ spectrum was indistinguishable from that of enantiomer **11** described earlier.

The by-product **24**, penta-O,N-acetyl-2-deoxystreptamine, was optically inactive and had m.p. 318-321°C (lit.²⁵ m.p. 322-323°C); m/z 373 ($M^+ + 1$, base peak), 359 ($M^+ + 2 - \text{Me}$), 331 ($M^+ + 2 - \text{Ac}$), 313 ($M^+ + 1 - \text{AcOH}$), 254 ($M^+ + 1 - 2 \text{AcNH}_2$). $^1\text{H-n.m.r.}$ (2-deoxystreptamine numbering): δ 5.68 (broad d, 2 H, NH-1,3), 5.23 (t, $J_{4,5} = J_{5,6} = 9.8$ Hz, H-5), 4.88 (t, 2 H, splitting ~ 10 Hz, H-4,6), 4.17 (m, 2 H, H-1,3), 2.42 (m, H-2e), 2.03 (s, 6 H, AcO-4,6), 1.99 (s, 3 H, AcO-5), 1.90 (s, 6 H, AcN-1,3), 1.38 (m, H-2a).

C.4. Second synthesis of (-)-hyosamine

C.4a 1L-(1,3/2,4,6)-6-Ethoxycarbonylamido-4-formamido-1,2-di-O-isopropylidene-1,2,3-cyclohexanetriol (25)

Platinum dioxide (550 mg) suspended in 99% EtOH (20 mL) containing AcOH (0.17 mL) was prehydrogenated. The nitrocarbamate **5** (600 mg, 1.97 mmol) dissolved in EtOH (10 mL) was added, and the mixture vigorously shaken for 30 h under H_2 at ordinary temperature and pressure. Complete replacement of **5** (R_F 0.5) by the corresponding amine, giving an immobile, ninhydrin-positive spot, was revealed by t.l.c. (solvent

C). The flask was disconnected from the hydrogen supply and cooled in an ice bath, and formyl acetate (3 mL) was introduced. The mixture was stirred for 2 h at room temperature; t.l.c. then indicated almost complete replacement of the immobile spot by a single spot for **25** having R_F 0.15. After filtration and evaporation of the solution, and several evaporations of portions of fresh EtOH from the residue, **25** was obtained as colorless crystals (460 mg, 76%). An analytical sample was freed from a trace contaminant (immobile material) by passage through a silica gel column with CHCl_3 containing 3% of MeOH. Pure **25** had R_F 0.45 (solvent **B**), m.p. 127-129°C, $[\alpha]_D +6.1^\circ$ (c 0.9, CHCl_3); $\nu_{\text{max}}^{\text{CHCl}_3}$ 3300 (broad, OH and NH), 1678 and 1543 (amide I and II bands) cm^{-1} ; m/z 303 ($M^+ + 1$, base peak), 285 ($M^+ + 1 - \text{H}_2\text{O}$), 245 ($M^+ + 1 - \text{Me}_2\text{CO}$), 227 ($M^+ + 1 - \text{H}_2\text{O} - \text{Me}_2\text{CO}$). $^1\text{H-n.m.r.}$ (acetone- d_6): δ 8.13 and 8.03 (slightly broadened s, and d with J 12 Hz, ratio ~ 4:1, total intensity 1 H, formyl proton), ~ 7.3 and 6.7 (d with J 7 Hz and t with J 11 Hz, both broadened, ratio 4:1, total intensity 1 H, formamido NH), ~ 6.4 (broad t, carbamate NH), ~ 4.7 and 4.5 (2 d with J 4.7 Hz, ratio 1:4, total intensity 1 H, OH-3), 4.01 (q, 2 H, OCH_2CH_3), 3.9-3.8, 3.7, and 3.5-3.4 (3 m, ill resolved, 2, 1, and 2 H, H-1,2,3,4,6), 2.24 (dt, $J_{4,5e} = J_{5e,6} = 4.5$, $J_{5a,5e}$ 13.2 Hz, H-5e), 1.47 (~ q, splittings 11-13 Hz, H-5a), 1.36 and 1.33 (2 s, 3 H each, Me_2C), 1.16 (t, 3 H, J 7.1 Hz, OCH_2CH_3). Upon D_2O exchange,

the NH and OH signals disappeared and the small doublet at δ 8.03 collapsed to a singlet while the broadened singlet at δ 8.13 became a sharp singlet.

The ^{13}C -n.m.r. spectrum (50.32 MHz, acetone- d_6) also revealed two sets of signals due to hindered amido group rotation. Signals for the major species were: δ 161.8 and 156.3 (formamido and carbamate CO), 110.7 (Me_2CO_2), 81.7, 79.0, and 73.2 (C-1,2,3), 60.2 (OCH_2CH_3), 51.4 and 49.4 (C-4,6), 36.2 (C-5), 26.6 and 26.5 (CMe_2), 14.3 (OCH_2CH_3). For the minor species, small signals were present at δ 164.8 (formamido CO), 110.9 (Me_2CO_2), close to the above C-1,2,3 and C-5 signals, and at 55.0 (presumably C-4); the remaining resonances must have coincided with those of the major species.

Anal. Calc. for $\text{C}_{13}\text{H}_{22}\text{N}_2\text{O}_6$ (302.3): C 51.65, H 7.34, N 9.27. Found: C 51.48, H 7.54, N 9.14.

C.4b 1L-(1,3./2,4,6)-6-Ethoxycarbonylamido-1,2-O-isopropylidene-4-methylamino-1,2,3-cyclohexanetriol (26)

A solution of **25** (653 mg, 2.15 mmol) in THF (10 mL, dried over Na) was added dropwise during 5 min to 1M borane in THF (5 mL), maintained at 0°

under N₂ in a flask equipped with dropping funnel, reflux condenser, and gas inlet. The colorless solution was then heated at reflux for 5 h, under N₂, whereafter the complete replacement of **25** (R_F 0.2) by a ninhydrin-positive product having R_F 0.55 was indicated by t.l.c. (solvent P). The mixture was allowed to cool to 25°, the N₂ supply was disconnected, and a stream of CO₂ was bubbled through the solution for 20 min. Evaporation of the solvent followed by evaporation of 6 portions of added MeOH from the syrupy residue gave crude **26**. Chromatography of the material on a column of SiO₂ (2 × 20 cm) using solvent Q furnished pure **26** as a colorless, brittle glass (218 mg, 35%), m/z 289 (18%, M⁺ + 1). ¹H-n.m.r.: δ 4.09 and 1.22 (q, 2 H, and t, 3 H, respectively; J 7.1 Hz, QCH₂CH₃), 2.60 and 2.44 (2 d, J 6.1 and 5.9 Hz, respectively, intensity ratio 1:1.2 with total intensity 3 H, N-CH₃), 1.42 (s, 6 H, CMe₂). ¹³C-n.m.r.*: δ 156.0 (CO), 112.2 [112.3] (CMe₂), 80.5 [80.6], 78.5 [78.3], and 69.7 [69.1] (C-1,2,3), 63.1 [63.9] (C-4), 61.2 (OCH₂), 49.2 [49.1] (C-6), 42.5 [42.6] (NHMe), 27.7 [27.55] (C-5), 26.7 (CMe₂), 1.44 (OCH₂CH₃).

*Where double signals occurred, the less intense ones are listed in brackets; they had 70-95% the intensity of the stronger signals.

Anal. Calc. for $C_{13}H_{24}N_2O_5 \cdot 0.5 H_2O$ (297.4): C 52.51, H 8.47,
N 9.42. Found: C 52.40, H 8.63, N 9.19.

C.4c 1D-(1,3/2,4,6)-4-Ethoxycarbonylamido-6-(N-methyl)acetamido-1,2,3-cyclohexanetriol (27)

A sample of **26** was treated with 50% aqueous AcOH, overnight at room temperature, whereby **26** (R_F 0.55) was replaced by a product having R_F 0.1 (t.l.c. with solvent P). The acid was evaporated with several additions of toluene and the syrupy product dried in an oil pump vacuum.

Spectroscopy indicated that it was the N-methylacetamide **27**. 1H -n.m.r. (DMSO- d_6): δ 6.19 (d, J 8.5 Hz, carbamate NH), 3.96 (q, 2 H, J 7.1 Hz, O-CH₂), 3.20 (m, W ~ 35 Hz, H-4), 3.1-2.9 (m, 3 H, narrowed on D₂O exchange, H- 1,2,3), 2.29 (s, 3 H, NCH₃; superposed on m for H-6), 1.92 (dt, J_{4,5e} = J_{5e,6} = J_{5a,5e} = 12,7 Hz, H-5e), 1.87 (s, 3 H, NAc), 1.15 (t, 3 H, J 7.1 Hz, CH₂CH₃), 0.99 (~q, splittings ~12.3 Hz, H-5a). ^{13}C -n.m.r. (DMSO- d_6 , assignments aided by ADEPT experiment): δ 173.1 (amide CO), 156.2 (carbamate CO), 76.5 (C-2), 74.4 (double line seen on expansion, C-1,3), 59.8 (Q-CH₂), 58.9 (C-6), 51.5 (C-4), 32.6 (N-CH₃), 31.9 (C-5), 22.3 (COCH₃), 14.9 (CH₂CH₃).

C.4d Acetylation of impure 26

A 130-mg sample of syrupy **26** from chromatographically inhomogeneous fractions that emerged from the column after the elution of the pure compound (see the preparation of **26**) apparently contained some partially deacetonated material. It was acetylated overnight at room temperature, using 1:1 acetic anhydride–pyridine (8 mL), in an effort to obtain a crystalline derivative. Processing of the reaction mixture by dilution with CH₂Cl₂, successive washing with water, aqueous NaHCO₃ solution and water, drying (Na₂SO₄), and evaporation with added portions of toluene gave a colorless syrup which failed to crystallize. It showed 2 spots, R_F 0.8 (major) and R_F 0.95 (minor) in t.l.c. with solvent P. Spectroscopic examination led to the conclusion that the product consisted mainly of the N,Q-diacetyl derivative (**28**) of **26**, accompanied by a small proportion of the tri-O-acetyl derivative (**29**) of **27**. Thus, the mass spectrum showed prominent peaks at m/z 373 (54%, M⁺ + 1), 315 (100%, M⁺ + 1 - Me₂CO), and 269 (12%, M⁺ + 1 - Me₂CO - C₂H₅OH) for the major component **28** (C₁₇H₂₈N₂O₇, mol. wt. 372), and small peaks at m/z 417 (9%, M⁺ + 1) and 357 (2%, M⁺ + 1 - AcOH) attributable to **29** (C₁₈H₂₈N₂O₉, mol. wt. 416). The ¹H-n.m.r. spectrum (300 MHz, CDCl₃) exhibited substituent resonances for the carbamate OCH₂CH₃ group (multiplets at δ 4.09 and 1.22, intensity ratio 2 H : 3 H), the N-CH₃ group (singlets at δ 2.89, 2.83, and 2.74 in a

ratio 1:2:1, with total intensity of 3 H), the N- and O- acetyl groups (multiple singlets at δ 2.12-1.96), and the acetonide methyl groups (singlets at δ 1.43 and 1.41). Integration for the total of acetyl signals was somewhat greater than 6 H, whereas integration for the acetonide methyl signals was somewhat smaller than 6 H. The ^{13}C -r.m.r. spectrum (75.43 MHz, CDCl_3 , ADEPT plot) showed methyl resonances (δ) for $\text{CH}_3\text{CH}_2\text{O}$ (14.5), OCOCH_3 (20.9-20.4), NCQCH_3 (22.15-22.1), $\text{O}_2\text{C}(\text{CH}_3)_2$ (26.7-26.4), and N- CH_3 (32.0).

Column chromatography of the material (solvent F) did not separate the components.

C.4e 1D(1,3/2,4,6)-4-Acetamido-1,2,3,-tri-O-acetyl-6-(N-methyl)acetamido-1,2,3-cyclohexanetriol (22)

A sample (30 mg) of the mixture of acetylated products just described (28 plus a minor proportion of 29) was heated for 2 h on a steam bath with 6 M HCl (2 mL). The acid was removed by coevaporation with several portions of added water in a rotary evaporator, and the residue was dried in a desiccator over KOH. A ^{13}C -n.m.r. spectrum of the material in D_2O (1 mL) showed that the isopropylidene and all acetyl groups were removed, but that the carbamate group had resisted hydrolysis; hence, the product must

have been the hydrochloride of deacetonated **26**: δ 158.1 (CO), 74.9, 73.5, 71.5 (C-1,2,3), 61.7 (Q-CH₂), 57.2, 50.6 (C-4,6), 29.6 (N-CH₃), 27.5 (C-5), 13.6 (CH₂CH₃). The sample was transferred from the spectroscopy tube into a small flask and, after the addition of an equal volume of 4 M NaOH, heated for 3 h on a steam bath. The solution was then cooled, neutralized with acetic acid, and evaporated to dryness. After drying it overnight in a desiccator, the white crystalline mass of sodium acetate and chloride [presumed to contain (-)-hyosamine (**21**) in salt form] was covered with acetic anhydride (3 mL) and agitated overnight at room temperature. Removal of the anhydride by repeated coevaporation with excess toluene, extraction of the desiccator-dried salt residue with CHCl₃, and evaporation of the colorless extract gave syrupy **22** (18 mg, ~ 60%), $[\alpha]_D +6.8^\circ$ (c 1.8, MeOH), lit.¹⁶ $+7.5^\circ$ and¹⁷ $+5.4^\circ$ (MeOH); m/z 387 (base peak, M⁺ + 1), 373 (M⁺ + 2 - Me), 345 (M⁺ + 2 - Ac), 327 (M⁺ + 1 - AcOH). The substance crystallized from a small amount of EtOH upon careful addition of Et₂O and hexane and storage at -18°C; m.p. 157-158°C, lit.¹⁶ m.p. 156.5-157°C. The ¹H-n.m.r. spectrum (200 MHz) corresponded in every detail to the spectra of **11** and **22** obtained by the preceding syntheses, except that the two NH doublets ($J_{4,NH}$ 8 Hz, integrating to 1 proton) were slightly shifted downfield (by 0.2 p.p.m.) and the stronger of the two N-CH₃ signals, previously seen as a broadened singlet at δ 2.81, appeared as two separated peaks at δ 2.84 and 2.81. (The second, weaker N-CH₃ signal was sharp singlet at δ 2.71 as before.) These differences were probably due to concentration effects.

D. Unsymmetrical Glycosylation of 2-Deoxystreptamine: Synthesis of 1,3-Di-N-acetyl-4,5-O-isopropylidene-6-O-(2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyl)-2-deoxystreptamine (32 β).

Crystalline 1L-(1,3/2,4,6)-4,6-diacetamido-1,2-Q-isopropylidene-1,2,3-cyclohexanetriol (**30**, 1,3-di-N-acetyl-4,5-Q-isopropylidene-2-deoxy-streptamine) was prepared by platinum-catalyzed hydrogenation of the azidonitro cyclitol **1**, followed by N-acetylation, according to the published procedure⁸. The product obtained in 75% yield had m.p. 253-255 (dec.) as reported, and its infrared and ¹H-n.m.r. spectra agreed with those of an authentic sample. Mass spectral data (not previously reported) were as follows: m/z 287 (59%, $M^+ + 1$), 229 (100%, $M^+ + 1 - Me_2CO$), 211 (12%, $M^+ + 1 - Me_2CO - H_2O$), 193 (4%, $M^+ + 1 - Me_2CO - 2 H_2O$), 168 (13%, $M^+ + 1 - Me_2CO - H_2O - CH_3CO$), 113 (24%, $M^+ + 1 - Me_2CO - 2 NHCCH_3$), 110 (22%, $M^+ + 1 - Me_2CO - H_2O - CH_3CO - NHCCH_3$).

Tetra-Q-acetyl- α -D-glucopyranosyl bromide (**31**) was prepared from D-glucose by one of the standard laboratory procedures³⁶, namely, treatment of D-glucose pentaacetate with HBr in glacial acetic acid. The crystalline product gave a perfect ¹H-n.m.r. spectrum, showing no trace of impurity: δ 6.59 (d, $J_{1,2}$ 4.0 Hz, H-1), 5.54 (t, $J_{2,3} = J_{3,4} = 10$ Hz, H-3), 5.14 (t, $J_{3,4} = J_{4,5} = 10$ Hz, H-4), 4.82 (dd, $J_{1,2}$ 4.0, $J_{2,3}$ 10 Hz, H-2), 4.25 (m, 2

H, H-5,6), 4.11 (~dd, $J_{6,6'}$ 12.4 Hz, H-6'), 2.085, 2.08, 2.04, 2.02 (4 s, 3 H each, 4 OAc).

Condensation of 30 and 31. – The bromo sugar **31** (410 mg, 1 mmol) was added portionwise during 4 h to a stirred mixture of **30** (140 mg, 0.5 mmol), $\text{Hg}(\text{CN})_2$ (126 mg, 0.5 mmol), and HgBr_2 (180 mg, 0.5 mmol) in 1,2-dichloroethane (10 mL, dried over molecular sieve 4A); the reaction mixture was protected from atmospheric moisture and direct light. After the addition, stirring was continued overnight. Complete consumption of **30** (R_F 0.35) and formation of a major (R_F 0.7) and a minor (R_F 0.8) product was indicated by t.l.c. (solvent B); unreacted, excess **31** (R_F 1.0) was also present. The reaction mixture was diluted with CH_2Cl_2 and filtered, and the filtrate was washed with aqueous 1M KBr solution followed by water, dried (Na_2SO_4), and evaporated to give a syrup (~ 0.5 g). Chromatography of the material on a column of SiO_2 (20 × 2 cm) by use of solvent D gave in early fractions the fast-moving, remnant **31** and subsequently yielded a mixture (150 mg, 49.7%) of the two reaction products as a white, crystalline material, m.p. 205-210°C (with decomposition, after slight sintering from 180°C); $[\alpha]_D$ -12.3° and -9.4° (from 2 separate runs; c 0.8, CHCl_3); m/z 617 (43%, $M^+ + 1$), 559 (12%, $M^+ + 1 - \text{Me}_2\text{CO}$), 331 (100%, $\text{C}_{14}\text{H}_{19}\text{O}_9$ [tetra-O-acetylglucosyl]⁺), 287 (23%, $\text{C}_{13}\text{H}_{22}\text{N}_2\text{O}_5$ [O-isopropylidene-di-N-acetyldeoxystreptamine]⁺ + 1), 271, 211, and 151 (20, 12, and 7%, loss of 1, 2, and 3 AcOH from $\text{C}_{14}\text{H}_{19}\text{O}_9$ fragment), 229 (13%, loss of Me_2CO

from C₁₃H₂₂N₂O₅ fragment), 169 and 109 (62 and 23%, loss of 1 and 2 AcOH from fragment m/z 229).

On the basis of t.l.c. spot intensities the ratio of the major product to the minor product appeared roughly 4:1 (3:1 in a second experiment). Rechromatography using solvent Q removed traces of faster-moving impurities and the substance melted thereafter at 211-214°C without significant prior sintering, but separation of the two component was not achieved. The ¹H-n.m.r. spectrum showed a set of prominent signals attributable to the major component, which was the glycoside 32β (assignments aided by COSY plot): for the glycosyl moiety, δ 5.22 (t, J_{2,3} + J_{3,4} = 19.1 Hz, H-3), 5.04 (t, J_{3,4} + J_{4,5} = 19.5 Hz, H-4), 4.93 (dd, J_{1,2} 8.0, J_{2,3} 9.7 Hz, H-2), 4.68 (d, J_{1,2} 8.0 Hz, H-1), 4.26 (dd, J_{5,6} 4.5, J_{6,6'} 12.4 Hz, H-6), 4.14 (dd, J_{5,6'} 2.3, J_{6,6'} 12.4 Hz, H-6'), 3.70 (ddd, J_{5,6'} 2.3, J_{5,6} 4.5, J_{4,5} 10.1 Hz, H-5), 2.07, 2.01, 2.00, 1.98 (4 s, 3 H each, 4 OAc); for the cyclitol moiety, δ 6.05 and 5.62 (2 d, J 5.8 and 7.6 Hz, NH-1,3), 3.95 (t, J_{1,6} + J_{5,6} = 19.4 Hz, H-6), 3.05 (dd, J 9.2 and 10.6 Hz, H-4), 3.4 (m, H-1 or -3), 3.36 (t, J_{4,5} + J_{5,6} = 18.7 Hz, H-5), 2.57 (dt, J_{1,2e} = J_{2e,3} = 5, J_{2a,2e} 13.4 Hz, H-2e), 1.94 and 1.90 (2 s, 3 H each, 2 NHAc), 1.70 (~q, w 36 Hz, H-2a), 1.42 and 1.37 (2 s, CMe₂). The signals of the minor component were for the most part overlapped by those of 32β and could not be properly analyzed.

Anal. Calc. for C₂₇H₄₀N₂O₁₄ (616.7): C 52.58, H 6.54.

Found: C 52.49, H 6.60.

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