

dedicated to

my husband and son

PREFACE

This Thesis is the result of work that was undertaken in order to make contributions to the synthetic chemistry of amino cyclitols and to a better understanding of epimerization reactions that occur in nitrocyclitols. In the introduction, therefore, the significance of amino cyclitols in the context of antibiotics chemistry will be outlined and the methods available for their synthesis will be reviewed. Similarly, background will be given concerning the problem of epimerizations in nitroinositols.

In the main part of the thesis is described the synthesis of some new aminodeoxyinositol derivatives. In the second part, a study of the epimerization of stereoisomeric deoxynitroinositol monomethyl ethers at different pH values is recorded. Kinetic and thermodynamic aspects of the epimerization are considered.

The candidate wishes to express her deepest gratitude to Professor Hans H. Baer for his stimulating guidance, his valuable instructions and his patience and encouragement throughout the whole process of this research. The candidate also wishes to express sincere thanks to Dr. Jan Kovar for his kind suggestion and his continuous help.

I INTRODUCTION

1 On Antibiotics	1
2 Amino Cyclitols as Constituents of Antibiotics	3
a- <u>Scyllo</u> Inosamine in Bluensomycin	3
b- <u>neo</u> -Inosamine-2 in Hygromycin	4
c- Actinamine in Actinospectacin	4
d- Streptamine in Streptomycins	6
e- 2-Deoxy Streptamine in various Antibiotics	8
3 Methods for the Synthesis of Amino Cyclitols	11
a- Reduction of Oximes or Phenylhydrazones	11
b- Reduction of Deoxynitroinositols obtained by Cyclization with Nitromethane	14
c- Displacement of Sulfonyloxy Groups or Bromine by Azides.	21
d- Ammonolysis of Epoxides and Halohydrins	24
4 Epimerization of Nitroinositols	28
II SPECIFIC AIMS OF THIS THESIS	35
III RESULTS AND DISCUSSION	36
1 Synthesis of Amino Cyclitols	36
2 Epimerization of Nitro Cyclitols	39
IV RESEARCH EQUIPMENT USED	51

V EXPERIMENTAL

Ia	Deoxynitroinositol monomethyl ethers	52
Ib	Preparation of 2,3,5,6 tetra- <u>0</u> -acetyl-1-deoxy-4- <u>0</u> -methyl- <u>1</u> -nitro-L- <u>myo</u> -inositol (<u>37c</u>)	53
II	Hydrogenation of nitroinositols	53
a-	Preparation of 1-amino-1-deoxy-4- <u>0</u> -methyl- <u>scyllo</u> -inositol hydrochloride (<u>36a</u>)	53
b-	Preparation of 1-amino-1-deoxy-4- <u>0</u> -methyl-L- <u>myo</u> -inositol hydrochloride (<u>37a</u>)	54
c-	Preparation of 3-amino-3-deoxy-6- <u>0</u> -methyl- <u>muco</u> -inositol hydrochloride (<u>38a</u>)	55
III	N-Acetyl Derivatives of Inosamines	56
a-	Preparation of 1-acetamido-1-deoxy-4- <u>0</u> -methyl- <u>scyllo</u> -inositol (<u>36b</u>)	56
b-	Preparation of 1-acetamido-1-deoxy-4- <u>0</u> -methyl-L- <u>myo</u> -inositol (<u>37b</u>)	57
c-	Preparation of 3-acetamid-3-deoxy-6- <u>0</u> -methyl- <u>muco</u> -inositol (<u>38b</u>)	57
IV	Preparation of 3-acetamido-3-deoxy-6- <u>0</u> -methyl-1,2,4,5-tetra- <u>0</u> -methylsulfonyl- <u>muco</u> -inositol (<u>38c</u>)	58
V	Attempted partial demesylation of <u>38c</u>	59

VI Epimerization of Deoxynitroinositols	60
a- Kinetic study of the epimerization, of 1-deoxy-4- <u>0</u> -methyl-1-nitro-L- <u>myo</u> -inositol (37) at different pH values	60
b- Thermodynamic equilibria of deoxynitroinositols <u>36,37</u> and <u>38</u> at different pH values	61
c- Determination of the pK_a of 1-deoxy-4- <u>0</u> - methyl-1-nitro-L- <u>myo</u> inositol (37) from ultraviolet spectrum.	62

v -

LIST OF TABLES

Table	1	Amino cyclitols found in antibiotics	2
	2	Kinetics of epimerization of L-myoinositol <u>37</u> at different pH values.	43
	3	Results of epimerization of nitroinositols <u>36</u> , <u>37</u> and <u>38</u> at pH 9.4	47
	4	Results of epimerization of nitroinositols <u>36</u> , <u>37</u> and <u>38</u> at pH 10.0	47
	5	Results of epimerization of nitroinositols <u>36</u> , <u>37</u> and <u>38</u> at pH 11.0	48
	6	Results of epimerization of nitroinositols <u>36</u> , <u>37</u> and <u>38</u> at pH 12.0	48
	7-8	Comparison between the calculated and experimental results of the epimerization of nitroinositols <u>36</u> , <u>37</u> and <u>38</u>	49-50
	9	Optical densities of <u>37</u> in buffers.	62

LIST OF FIGURES

Figure 1	Identification of neoinosamine-2.	5
2	Structure of streptomycin	7
3	Structure of neomycins and paromomycins	9
4	Structure of kanamycins	9
5	Structure of gentamicin	10
6	Mechanism of cyclization of 6-deoxy-6-nitro-aldose to deoxy nitroinositols	16
7	Kinetic study of epimerization of L-myoinositol (37) at pH 8.3	63
8	Kinetic study of epimerization of L-myoinositol (37) at pH 9.2	64
9	Kinetic study of epimerization of L-myoinositol (37) at pH 9.5	64
10	Kinetic study of epimerization of L-myoinositol (37) at pH 10.2	65
11	Kinetic study of epimerization of L-myoinositol (37) at pH 10.65	65
12	N.m.r. spectrum of 2,3,5,6-tetra-O-acetyl-1-deoxy-4-methyl-1-nitro-L-myoinositol (37c)	66
13	Infrared spectra of scyllo-inosamine-1 (36a)	67
14	Infrared spectra of L-myoinosamine-1 (37a)	68
15	Infrared spectra of muco-inosamine-3 (38a)	69

- 16 Infrared spectra of N-acetyl derivative of scyllo-inosamine (36b) 70
- 17 Infrared spectra of N-acetyl derivative of L-myo-inosamine (37b) 71
- 18 Infrared spectra of N-acetyl derivative of muco-inosamine (38b) 72
- 19 Infrared spectra of 3-acetamido-3-deoxy-6-0-methyl-1,2,4,5-tetra-0-methylsulfonyl-muco-inositol (38c) 73
- 20 N.m.r. spectrum of 3-acetamido-3-deoxy-6-0-methyl-1,2,4,5-tetra-0-methylsulfonyl-muco-inositol (38c). 74

I. INTRODUCTION.

1. On Antibiotics

The word "antibiotic" was first used by Waksman¹ to refer to "chemical substances that are produced by microorganisms and that have the capacity to inhibit and even destroy other microorganisms".

Benedict and Langlykke² suggested that the definition should include inhibitory substances of plant or animal origin. Vonderbank³ proposed that antibiotics be defined as compounds possessing inhibitory, i.e. static, degenerative, lyzing or killing activities for plant or animal microorganisms, such as viruses, rickettsia, bacteria, fungi, algae or protozoa. Such a broad definition takes into account a number of more recent developments in the field. To date, more than 600 antibiotics have been discovered in nature, and although the majority of them are of limited or no medical value, many have found clinical application, and several of these have proved beneficial in the combat of a great variety of infections. From the chemical point of view, antibiotics are not easily classified because of the great variety of structures that are encountered. However, many of the more important antibiotics are built of, or contain, carbohydrate moieties. Prominent among the carbohydrate units found in these compounds are branched-chain sugars, amino sugars and amino cyclitols. As a background review, some aspects of the chemistry of those antibiotics which contain amino cyclitols

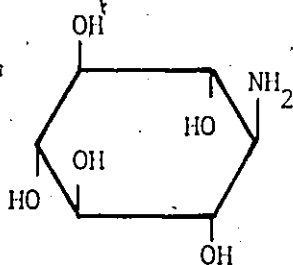
Table 1. Amino Cyclitols found in Antibiotics

Amino cyclitol

structure

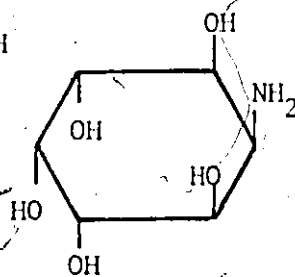
Antibiotic

scyllo-inosamine



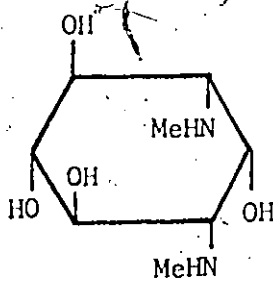
bluensomycin

neo-inosamine-2



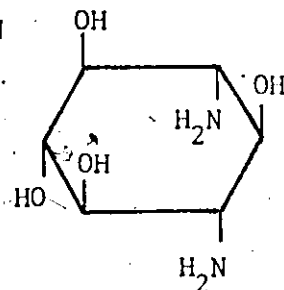
hygromycin

actinamine



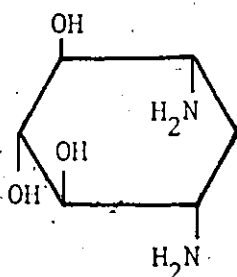
actinospectacin

streptamine



streptomycin
dihydroxy-streptomycin
mannosidostreptomycin
hydroxystreptomycin

2-deoxystreptamine



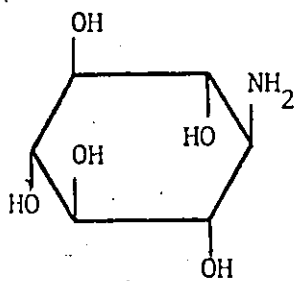
neomycins
paromycins
kanamycins
gentamicins

(Table 1, p. 2.) will be discussed in the following section.

2. Amino Cyclitols as Constituents of Antibiotics

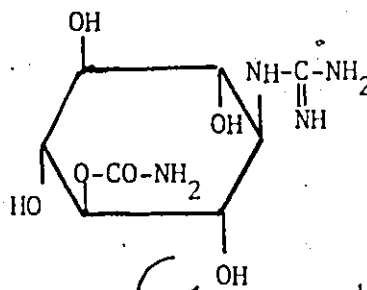
a- Scyllo-Inosamine in Bluensomycin

Bluensomycin is an antibiotic which has been isolated in the Upjohn Co. Laboratories; structural studies on this antibiotic were undertaken by Bannister and Argoudelis⁴ who obtained a degradation product, bluensidine, on mild methanolysis at room temperature. Hydrolysis of bluensidine under reflux with saturated barium hydroxide gave two moles of barium carbonate, three moles of ammonia, and an optically inactive amino cyclitol. This amino cyclitol consumed six moles of periodate and was found to be identical with scyllo-inosamine (1), by comparison of the N-acetyl and hexaacetyl derivatives with authentic samples⁵. Bluensidine is 3-O-carbamoyl-1-deoxy-1-guanidino-scyllo-inositol (2).



scyllo-inosamine

1



bluensidine

2

b- neo-Inosamine-2 in Hygromycin

Hygromycin is an antibiotic having activity against Gram-negative and Gram-positive bacteria and actinomycetes. It was extracted from fermentation broths of Streptomyces hygroscopicus. The main structural features of hygromycin were established by Mann and Woolf⁶. When hygromycin was refluxed in hydrochloric acid, an inosamine hydrochloride was isolated. The amine and hexaacetyl and N-acetyl derivatives showed no optical activity. On deamination with nitrous acid, the inosamine yielded myo-inositol (5). Posternak⁷ had shown that deamination of both myo-inosamine-2 (4) and scyllo-inosamine (1) takes place with Walden inversion leading to the formation of scyllo-inositol (6) and myo-inositol (5), respectively (Fig. 1). Assuming that Walden inversion also occurred in the case of the aminoinositol derivative from hygromycin, Mann and Woolf⁶ suggested that the formation of 5 would imply formula 3 or 1 for their compound. Only these two of the eight possible, optically inactive inosamines should yield 5 by this mechanism. The infrared spectrum and X-ray diffraction pattern of the inosamine differed from those of an authentic sample of 1. Therefore, the compound was tentatively identified as the previously unknown neo-inosamine-2 (3).

c- Actinamine in Actinospectacin

Wiley⁸ in 1962 isolated a base called actinamine from the hydrolysis products of a new antibiotic, actinospectacin, which originated

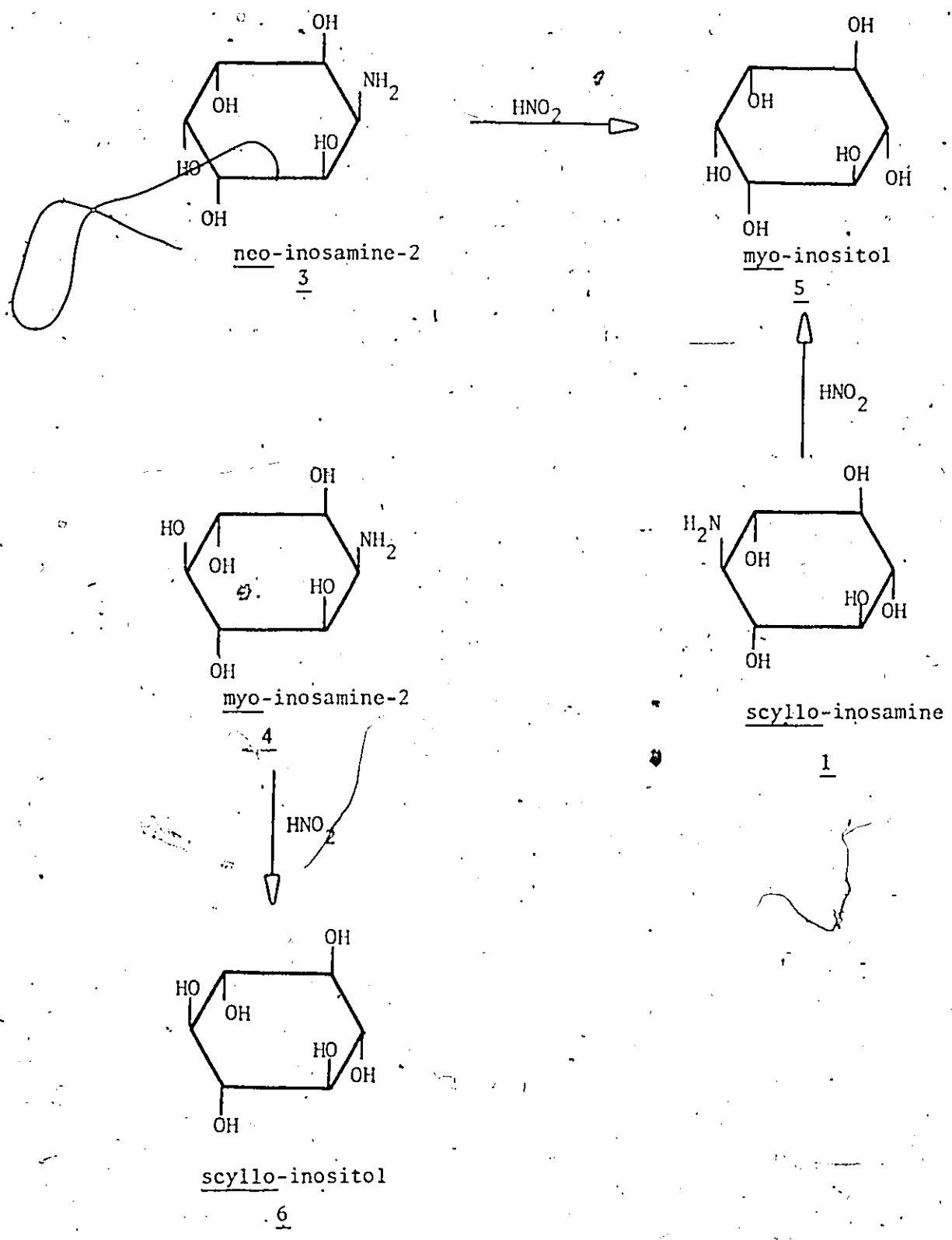
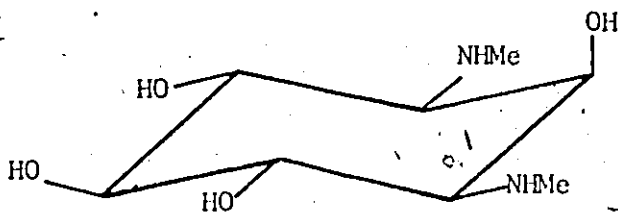


Figure 1. Identification of neo-inosamine-2.

from Streptomyces spectabilis. Actinamine was found to be an inositol derivative bearing two methylamino groups. The elucidation of its structure and configuration, which was done largely by n.m.r. spectroscopy⁹, led to formula 7.



d- Streptomine in the Streptomycins

The antibiotic streptomycin was obtained in 1944 from cultures of Streptomyces griseus by Waksman, Schatz and Bugie¹⁰. The substance has a low toxicity and is active against Gram-positive and Gram-negative organisms. It is used in treatment of many infections which are resistant to penicillin, such as typhoid fever, tularemia and brucellosis. Dihydrostreptomycin, mannosidostreptomycin and hydroxystreptomycin are closely related to streptomycin; in particular, they contain the same amino cyclitol moiety, namely, scyllo-inosadamine-1,3 (1,3-diamino-1,3-dideoxy-scyllo-inositol; streptomine) (8). The complete structure^{11,12,13} of streptomycin is shown in Fig. 2, p. 7.

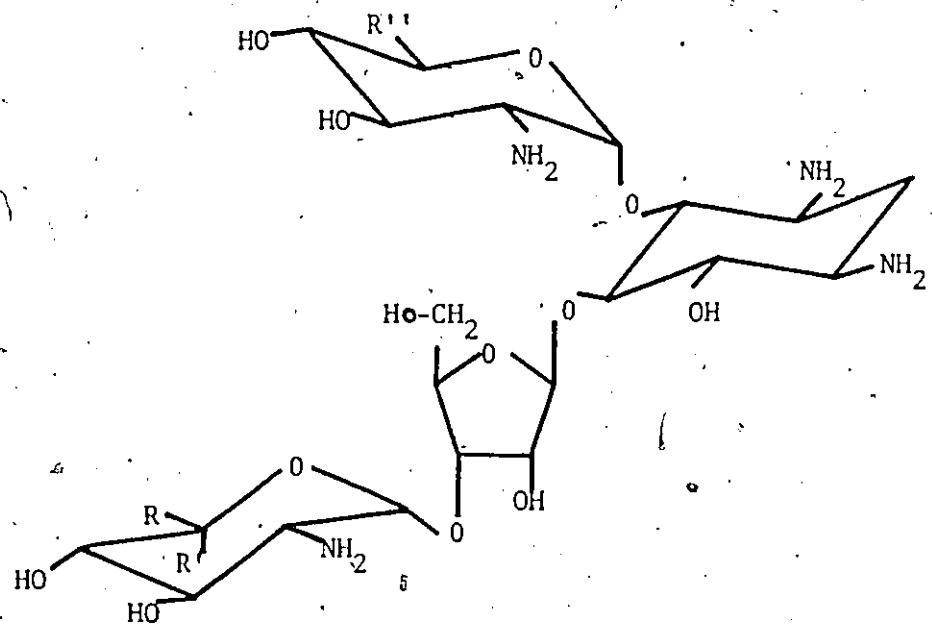
e- 2-Deoxystreptamine in Various Antibiotics

A number of antibiotics isolated from various streptomyces strains contain the amino cyclitol 2-deoxystreptamine (9), p. 10 . The neomycins are the most important in this group. The antibiotic, neomycin, was discovered by Waksman and Lechevalier¹⁴ in 1949. It was found active against Gram-positive, Gram-negative, and acid-fast bacteria. Studies by Waksman and co-workers¹⁵ revealed that the antibiotic consisted of at least three active components subsequently designated as neomycins A, B and C (Fig. 3). These active components were isolated by Folkers et al.¹⁶, Regna et al.¹⁷, and Wintersteiner et al.¹⁸, respectively. The compound originally described as neomycin A was recognised later to be a fragment of both neomycin B and C rather than a true neomycin¹⁹. The structure of the neomycins was determined chiefly by Rinehart²⁰.

The paromomycins, which possess a high degree of effectiveness in the treatment of intestinal amebiasis, are structurally²⁰ related to the neomycins; see Fig. 3.

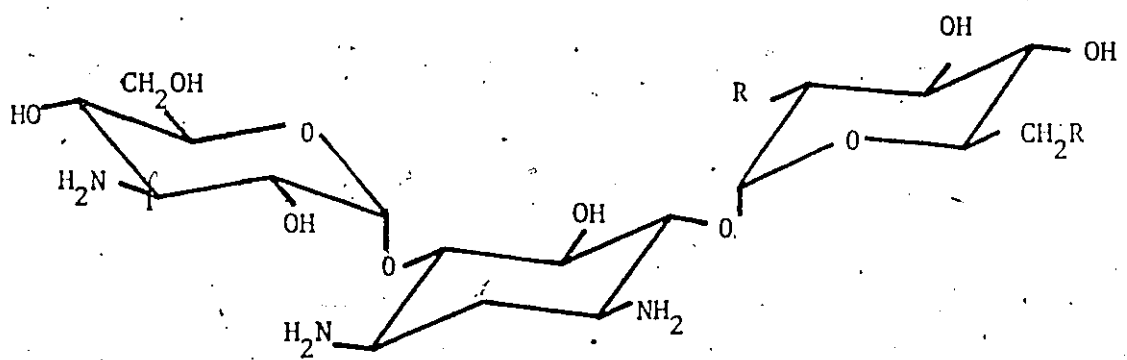
Kanamycin was isolated from cultures of Streptomyces kanamyceticus. It shows a fairly low toxicity and is very active against Gram-positive and Gram-negative bacteria including streptomycin-resistant strains. The crude antibiotic was separated into a major component, kanamycin A, and a minor component, kanamycin B²¹ (fig. 4).

Gentamicin²² was isolated from Micromonospora species, which are micro-organisms that differ from streptomycetes. The structure of



- Neomycin B: $R', R'' = CH_2NH_2$; $R = H$
- Neomycin C: $R, R'' = CH_2NH_2$; $R' = H$
- Paromomycin I: $R' = OH_2NH_2$; $R = H$; $R'' = CH_2OH$
- Paromomycin II: $R = CH_2NH_2$; $R' = H$; $R'' = CH_2OH$

Figure 3 Structure of the neomycins and paromomycins



- Kanamycin A: $R = OH$; $R' = NH_2$
- Kanamycin B: $R = R' = NH_2$

Figure 4 Structure of the Kanamycins

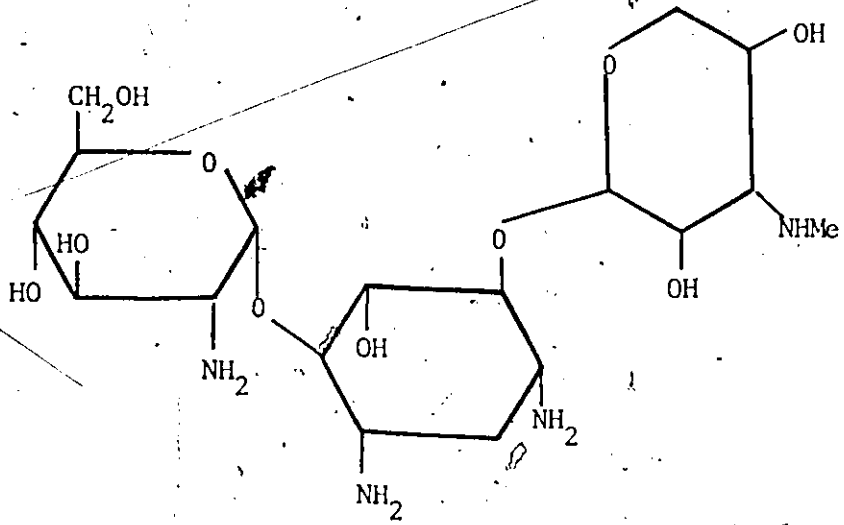
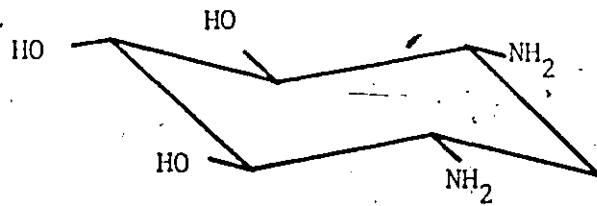


Figure 5 Gentamicin



2-deoxystreptamine

(9)

gentamicin is shown in Fig. 5.

3. Methods of Synthesis of Amino Cyclitols

a- Reduction of Oximes or Phenylhydrazones of Inososes

Reduction of $C = N$ bonds in phenylhydrazones and oximes of inososes has been employed extensively in partial syntheses of inosamines, and less frequently in those of inosadiazines. Carter and co-workers²³ investigated the catalytic reduction, with Raney nickel, of the phenylhydrazone 10 and the oxime 11 of ketodeoxy-scyllo-inositol^{24,25} and of the phenylhydrazone 12 of DL-2-keto-2-deoxy-epi-inositol²⁶. The phenylhydrazone 10 and oxime 11 gave the same mixture of myo-inosamine -2 (13) and scyllo-inosamine (14). Under the same conditions, the phenylhydrazone 12 yielded only one of the two possible epimers, 15 or 16; it was later shown to be 15.

May and Mosettig²⁷ reported that hydrogenation products of the oxime 17 of DL-2-keto-2-deoxy-epi-inositol varied with the reaction conditions employed. Thus catalytic hydrogenation with platinum oxide in 70% methanol gave one of two epimers, 15 or 16, in a yield of 70%. When hydrogenation was conducted in dilute acetic acid, a mixture consisting of epi-inositol 18 (yield 15%) and a small proportion of 15 (or 16) was obtained. When the catalytic hydrogenation was performed in dilute hydrochloric acid, no nitrogenous products but only deoxy-inositol 19 and a very small amount of epi-inositol 18 were formed.

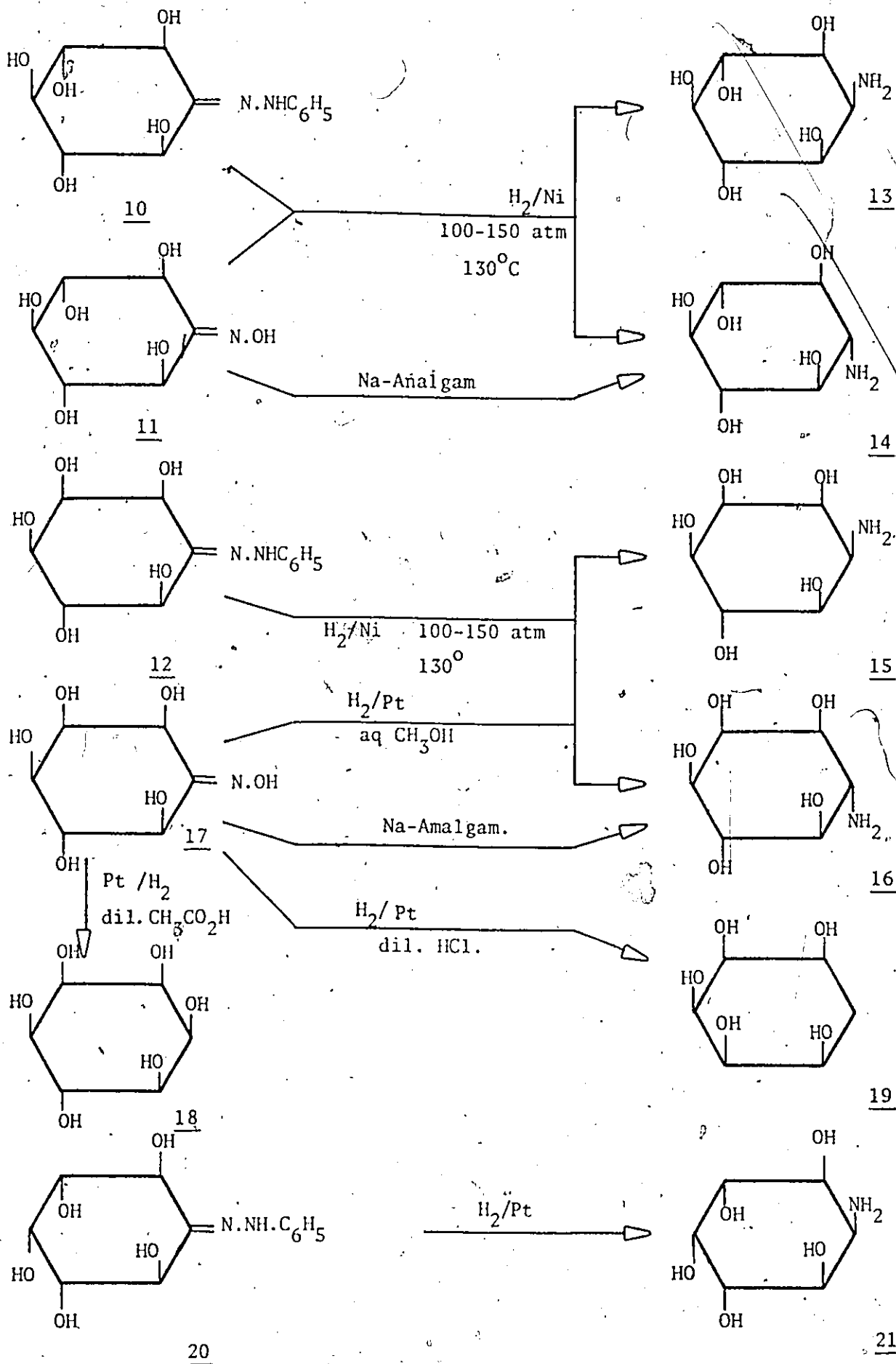
Anderson and Lardy²⁸ showed that the phenylhydrazone 10 and oxime 11 gave almost exclusively a single isomer (13) in high yields when platinum oxide in glacial acetic acid was used. However, when oxime 11 was reduced with sodium amalgam, scyllo-inosamine (14) was produced.

Latham, May and Mosettig²⁹ also investigated the catalytic hydrogenation of oxime 11. Using platinum oxide in 50% aqueous methanol, they obtained only one product, myo-inosamine-2 (13), in a yield of 55%.

The configuration of the inosamine (15 or 16) which had been prepared by Carter et al.²³ from the phenylhydrazone 12 and by May and Mosettig²⁷ from the oxime 17 was not established until Anderson and co-workers³⁰ obtained, by sodium amalgam reduction of the same oxime 17, a second inosamine. They were then able to assign to their new product the configuration of DL-myo-inosamine-4 (16) and to the previous product, the configuration of DL-epi-inosamine-2 (15).

Allen³¹ performed the synthesis of neo-inosamine-2 (21), a component of the antibiotic hygromycin. The compound was obtained in a yield of 53% by platinum-catalyzed hydrogenation of phenylhydrazone 20.

The steric direction of an amino group formed by reduction of an oxime or a phenylhydrazone is specifically influenced by the reducing agent used and the conditions employed. The experiments cited above show that catalytic reduction tends to direct the new group into cis positions with respect to its neighbouring substituents, whereas sodium amalgam reduction favours the trans configuration.



b- Reduction of Deoxynitroinositols obtained by Cyclization

Reaction with Nitromethane

Another principle for the synthesis of nitrogenous inositol derivatives was elaborated by H. O. L. Fischer and his associates. When a 6-deoxy-6-nitro-aldose is exposed to mild alkali, it cyclizes to a mixture of stereoisomeric deoxy-nitroinositols, e.g. 22→23. In this reaction, two new asymmetric centers are generated. Moreover, epimerizations may occur at the carbon atom adjacent to the nitromethyl and nitromethylene groups. Consequently, a total of 8 stereoisomers can theoretically arise. In practice, however, this number is reduced to two or three because of conformational preferences. Thus, Grosheintz and Fischer³¹ obtained three deoxynitroinositols upon cyclization of 6-deoxy-6-nitro-D-glucose (22), and the same product mixture arose from 6-deoxy-6-nitro-L-idose (24); the 5-epimer of 22. The configurations of the products were not determined at the time, nor was reduction to the amino stage one of the main objectives of the work although such reduction was shown to be feasible. Later, the configurations were established³³ to be scylló (26), myo-1 (27), and muco-3 (28). The corresponding inosamines are now available through this route. The starting compounds for the above cyclization, 6-deoxy-6-nitro-D-glucose (22) and L-idose (24), were obtained³⁴ by condensation of 1,2-O-isopropylidene-D-xylo-dialdose (25) with nitromethane followed by acid removal of the isopropylidene blocking group. It has subsequently been shown that the free xylo-dialdose (xylo-trihydroxy-glutaric dialdehyde) can be cyclized with

nitromethane, directly and without isolation of intermediates, to yield the scyllo and myo-1 deoxynitroinositols 26 and 27³⁵.

Baer and Rank³⁶ reported that the methylpyranosides of 6-deoxy-6-nitro-hexoses readily undergo base-catalysed deglycosidation to the free sugars, which do not accumulate but cyclize rapidly to inositol derivatives. The process is visualized to proceed via a nitroolefin hemiacetal (a) (fig. 6, p. 16) which instantly loses methoxide ion to furnish a nitroolefin aldose (b). The latter is attacked by hydroxyl ion, giving hexose nitronate (c) predisposed to Fischer cyclization.

Using the same pattern that Grosheintz and Fischer^{32,34} have used, Kovar and Baer³⁷ in 1973 reported the synthesis of four deoxy-nitro inositol monomethyl ethers. The first step in this synthesis was the methylation of 1,2:5,6-di-0-isopropylidene- α -D-glucofuranose (29). Partial hydrolysis of 29 with acetic acid gave 1,2-0-isopropylidene-3-0-methyl- α -D-glucofuranose (30), and treatment of 30 with sodium periodate afforded 1,2-0-isopropylidene-3-0-methyl- α -D-xylo-pentodialdo-1,4-furanose (31). Addition of nitromethane to 31 afforded a mixture of 6-deoxy-1,2-0-isopropylidene-3-0-methyl-6-nitro- α -D-glucofuranose (32) and - β -L-idofuranose (33). Removal of 1,2-ketal groups led to a mixture of 6-deoxy-3-0-methyl-6-nitro-D-glucose (34) and -L-idose (35). The β -anomer of 34 could be isolated in crystalline form. Cyclization of these nitrohexoses furnished four stereoisomeric deoxynitroinositol monomethyl ethers, namely 1-deoxy-4-0-methyl-1-nitro-scyllo-inositol (36), 1-deoxy-4-0-methyl-1-nitro-DL-myo-inositol (37), 3-deoxy-6-0-methyl-3-

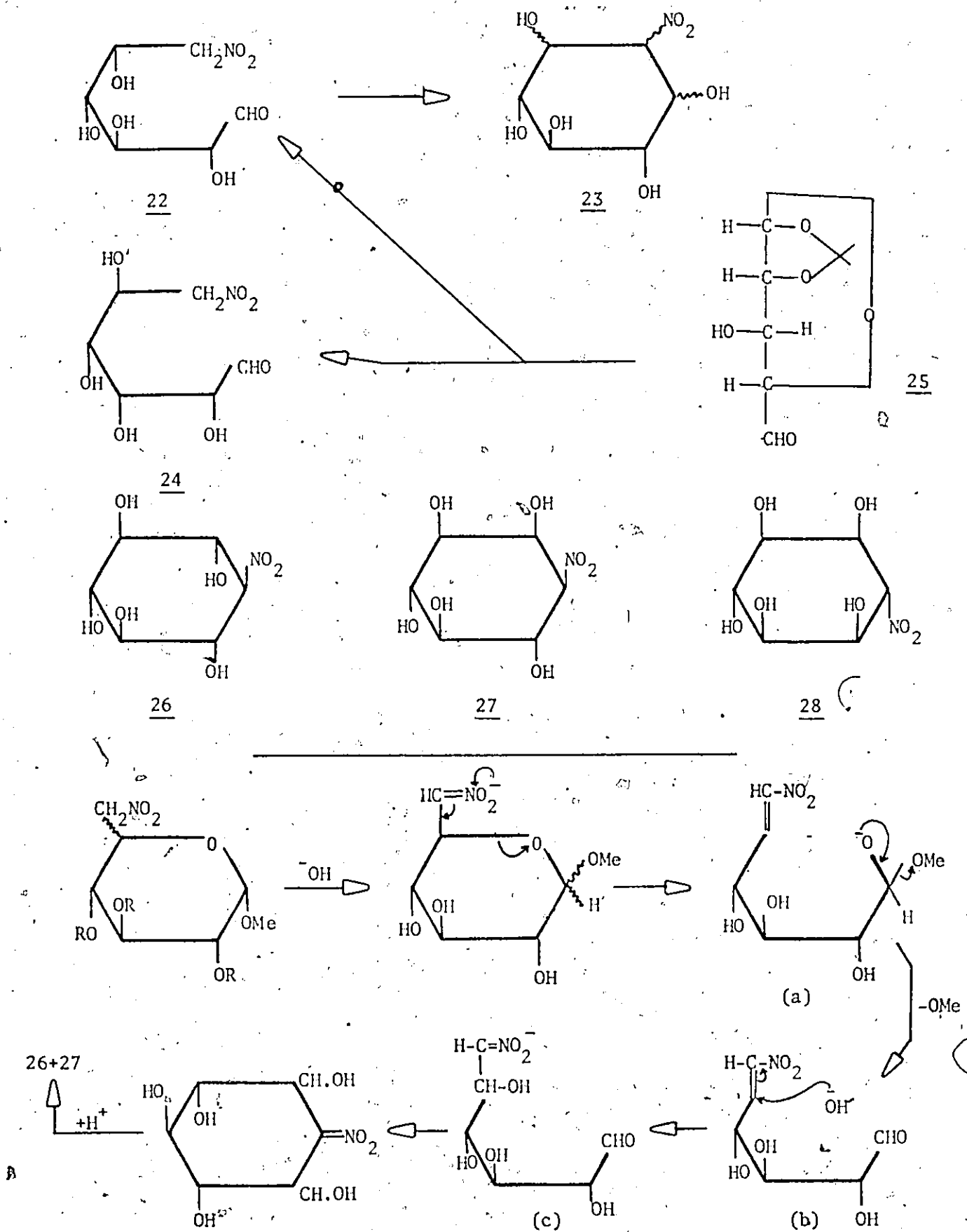
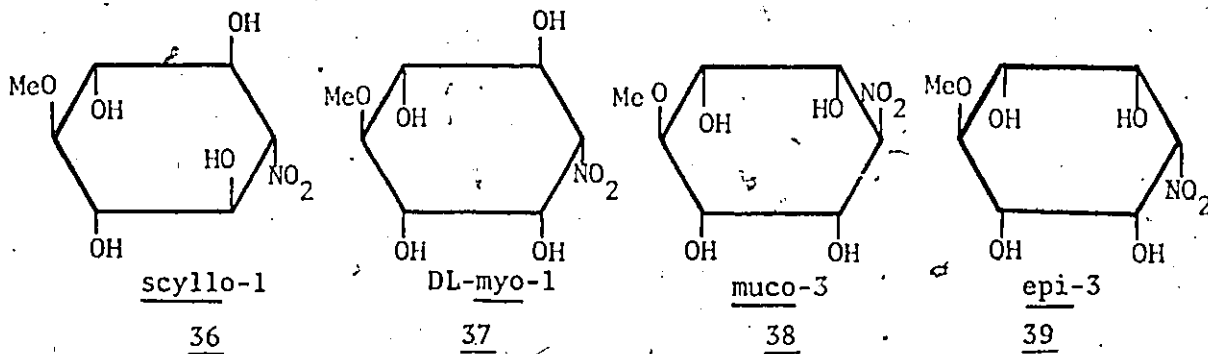
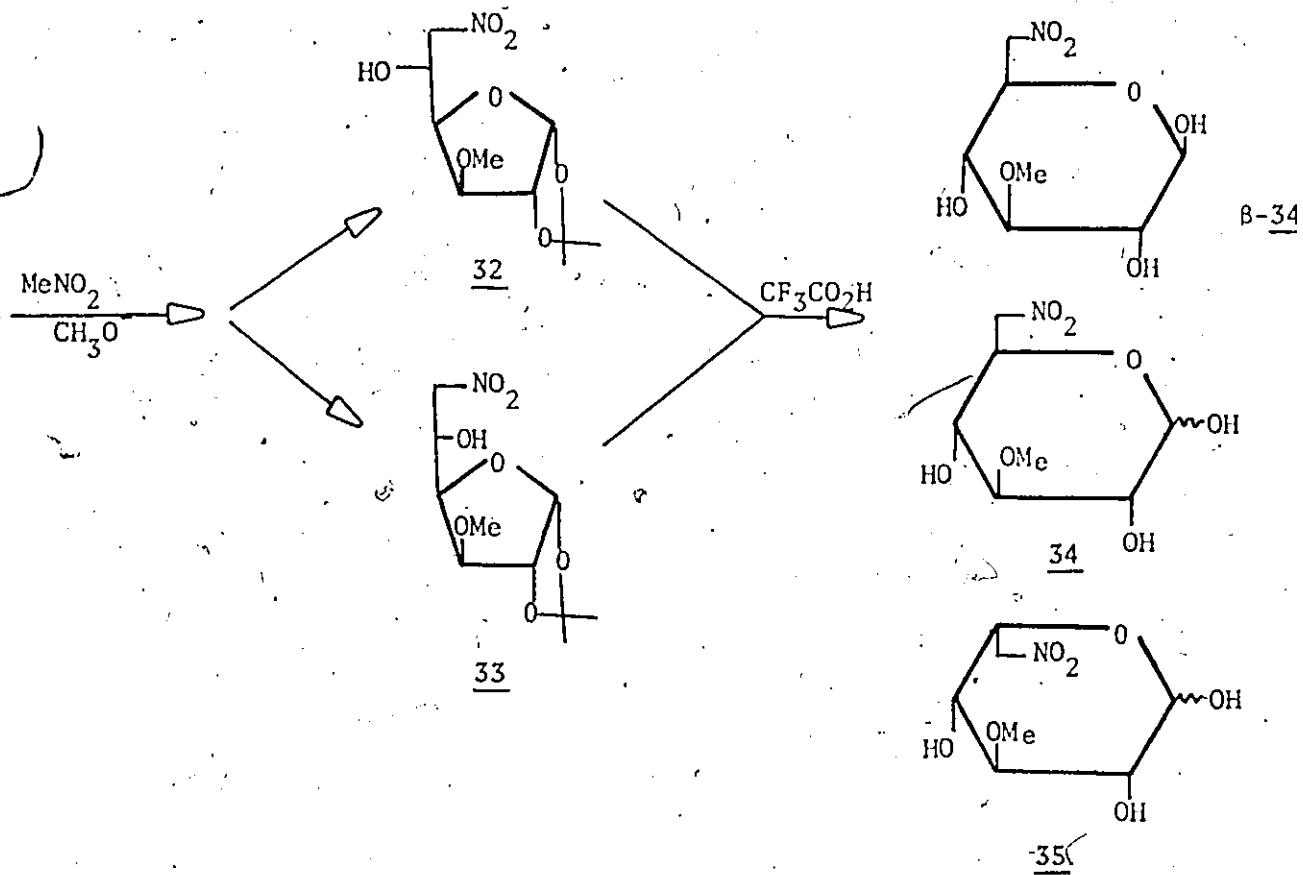
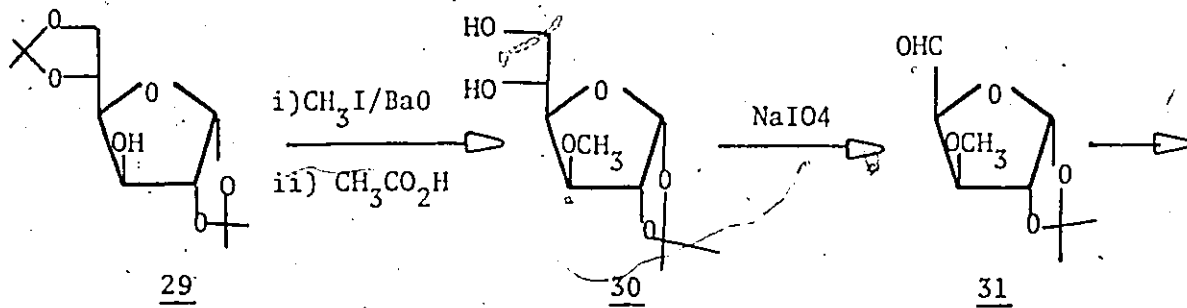


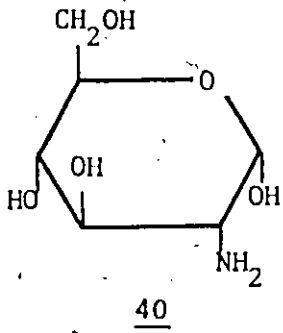
Figure 6. Mechanism of cyclization of 6-deoxy-6-nitro-aldose to deoxy nitroinositols.



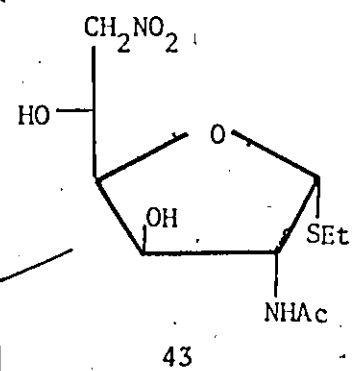
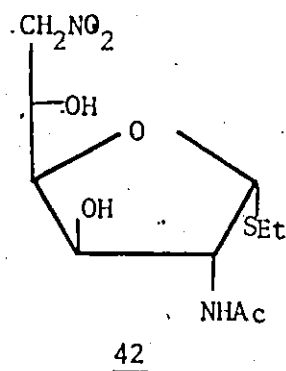
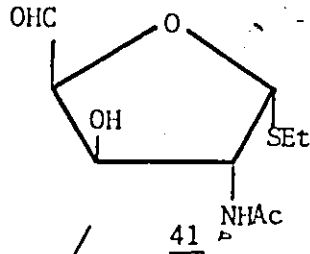
nitro-muco-inositol (38) and 3-deoxy-6-O-methyl-3-nitro-epi-inositol (39). Subsequently, by cyclization of 34 and 35 under condition of kinetic control, the optically active D- and L- enantiomers of the myo derivative were isolated³⁸.

Wolfrom and associates³⁹ had previously utilized the principle of Fischer's nitroinositol synthesis for the first synthesis of streptomine (scyllo-inosadiazine-1,3). D-Glucosamine (40) was converted, in a number of steps, into ethylthio 2-acetamido-2-deoxy-5-aldo-D-xylo-furanoside (41), which was condensed with nitromethane to give a mixture of ethylthio 2-acetamido-2,6-dideoxy-6-nitro-D-glucofuranoside (43) and -L-idofuranoside (42). Hydrolysis of these thioglycosides with mercuric chloride was followed by alkalization whereby ring closure occurred and a mixture of 1,3-dideoxy-1-acetamido-3-nitroinositols resulted. Reduction with nickel and acetylation gave a readily separable mixture of hexaacetylstreptomine (44, major component) and of a minor stereoisomer to which formula 45 was tentatively assigned. However, in 1974 Rinehart and his co-workers⁴⁰ re-investigated some aspects of that work and reported that the minor component suggested by Wolfrom to be muco-inosadiazine (45) is in fact myo-inosadiazine-1,3 (46). The synthesis of two new, optically active inosadiazines 1L-myo-inosadiazine-1,5 (47), and 1L-epi-inosadiazine-1,3 (48), was reported⁴⁰ in this connection.

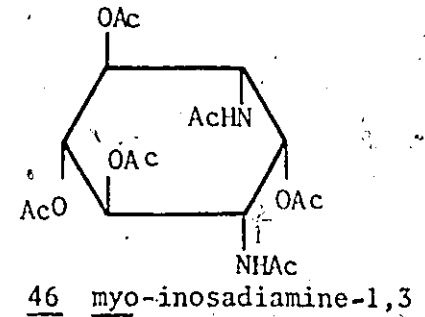
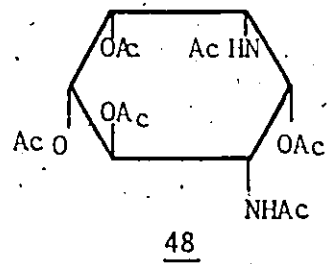
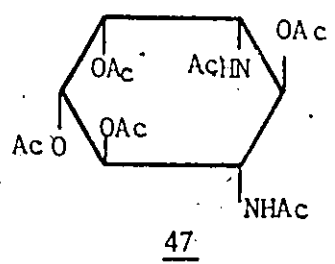
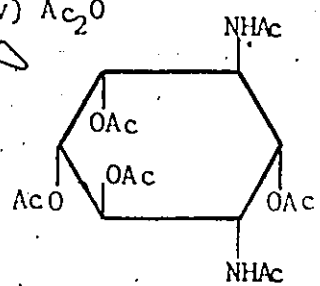
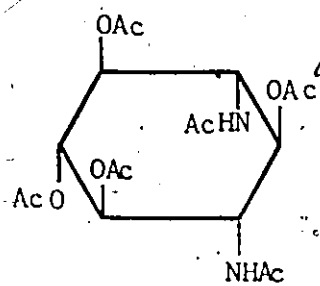
Peracetylated streptomine and several of its stereoisomers were also obtained by Hasegawa and Sable⁴¹ who subjected 1-acetamido-2,3-dihydroxyglutaraldehyde derivatives of different configurations to nitro-



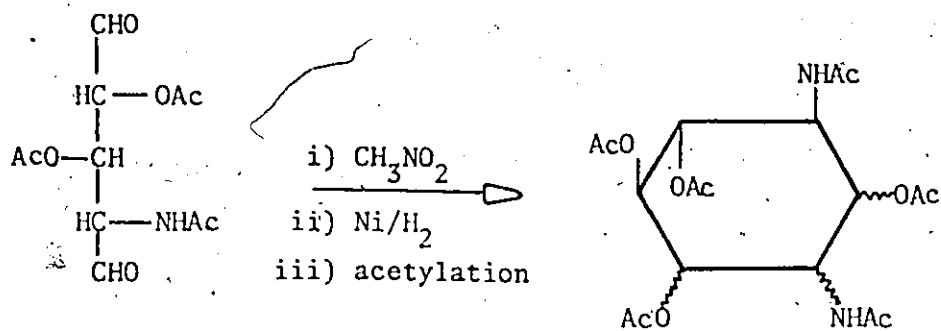
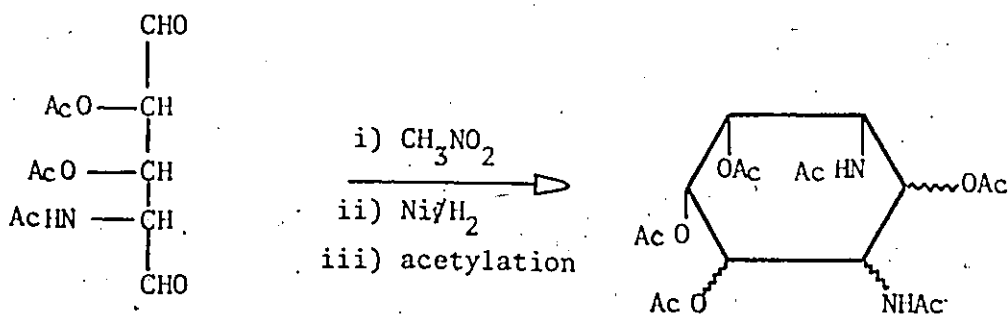
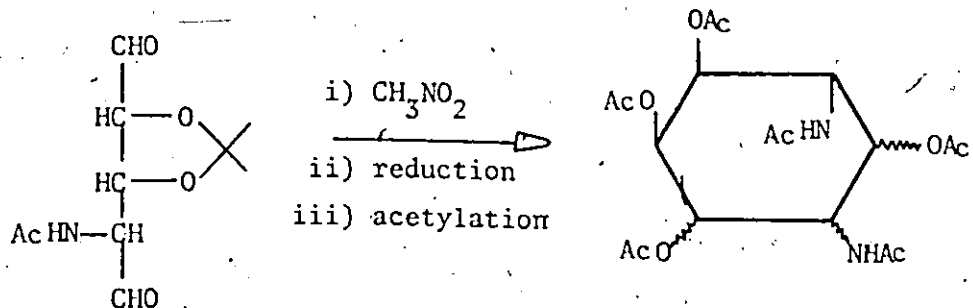
i) Ac_2O
ii) $EtSH:HCl$
iii) $HgCl_2;HgO, H_2O$
iv) $Pb(OAc)_4$



i) $HgCl_2/H_2O$
ii) $Ba(OH)_2$
iii) H_2/Ni
iv) Ac_2O



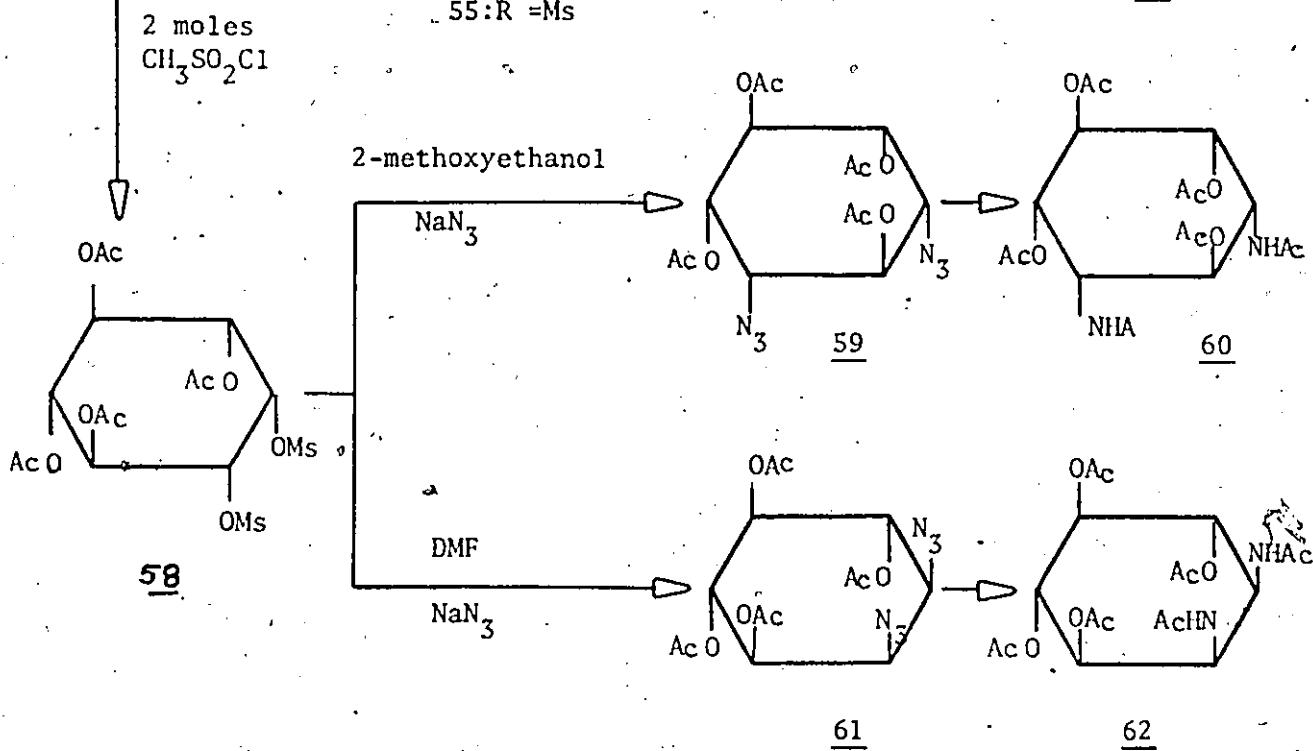
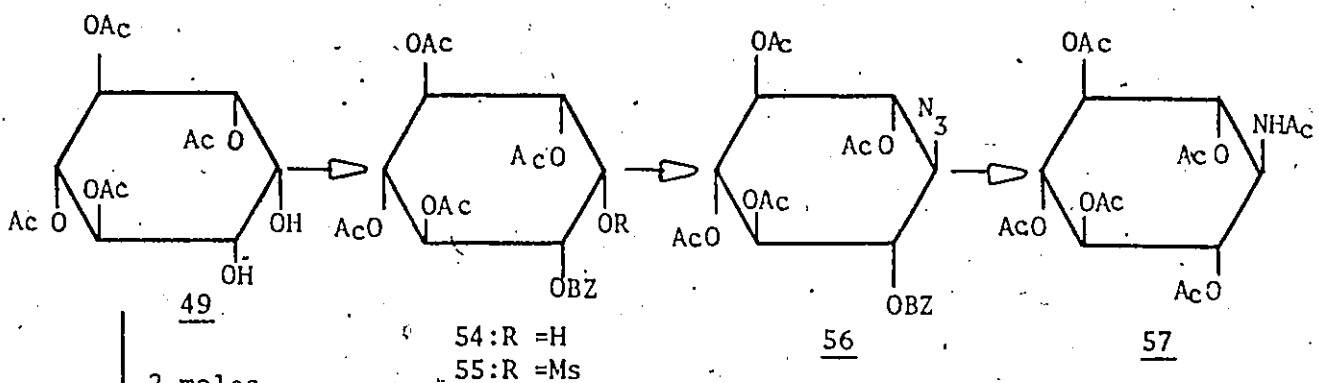
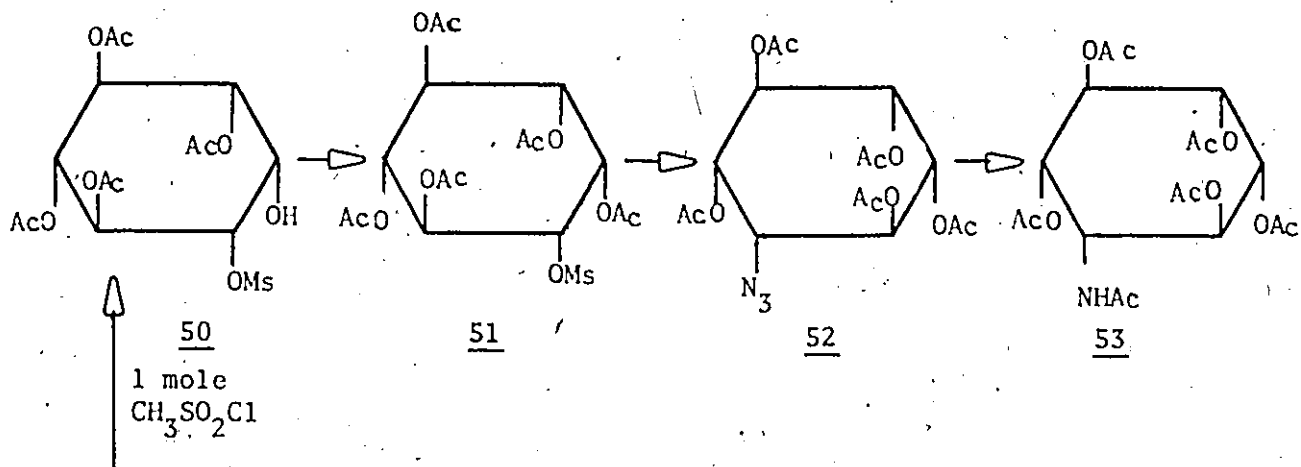
methane cyclization followed by reduction and acetylation:



c- Displacement of Sulfonyloxy Groups or Bromine by Azide

Displacement of sulfonyloxy groups by azide has been employed extensively in the synthesis of inosamines as well as inosadiazines, especially by Suami and co-workers^{42,43,44}. In 1966, they reported⁴² the synthesis of muco-inosamine-1, scyllo-inosamine, muco-inosadiazine-1,5 and myo-inosadiazine-1,2 from myo-inositol. Monomesylation of 1,4,5,6-tetra-0-acetyl-myo-inositol (49) gave 1,4,5,6,-tetra-0-acetyl-3-0-mesyl-myo-inositol (50) in 72% yield. Acetylation of this product gave the 3-0-mesyl pentaacetate 51. When 51 was treated with sodium azide in boiling aqueous 2-methoxyethanol and subsequently acetylated, an azide acetate 52 was obtained. Catalytic reduction of 52 followed by acetylation gave hexaacetyl-muco-inosamine-1 (53) in 32% yield. Considering the configuration of the product obtained, the demesylation of 51 seems to proceed through an intermediate formation of a dioxolane ring by participation of the vinical, trans oriented acetoxy group, which is then cleaved by nucleophilic attack of the azide ion in the manner of a diaxial opening.

Suami also reported⁴² that tetra-0-acetyl-3-0-benzyl-2-0-mesyl-myo-inositol (55), prepared from tetra-0-acetyl-myo-inositol by selective benzylation followed by mesylation, was demesyated by sodium azide in boiling aqueous 2-methoxyethanol through a direct S_N^2 substitution; the hexaacetyl scyllo-derivative 57 was obtained. This was expected because 55 does not possess a trans vicinal acetoxy group capable of participation. Upon treatment of 49 with two moles of methane-sulfonyl chloride in



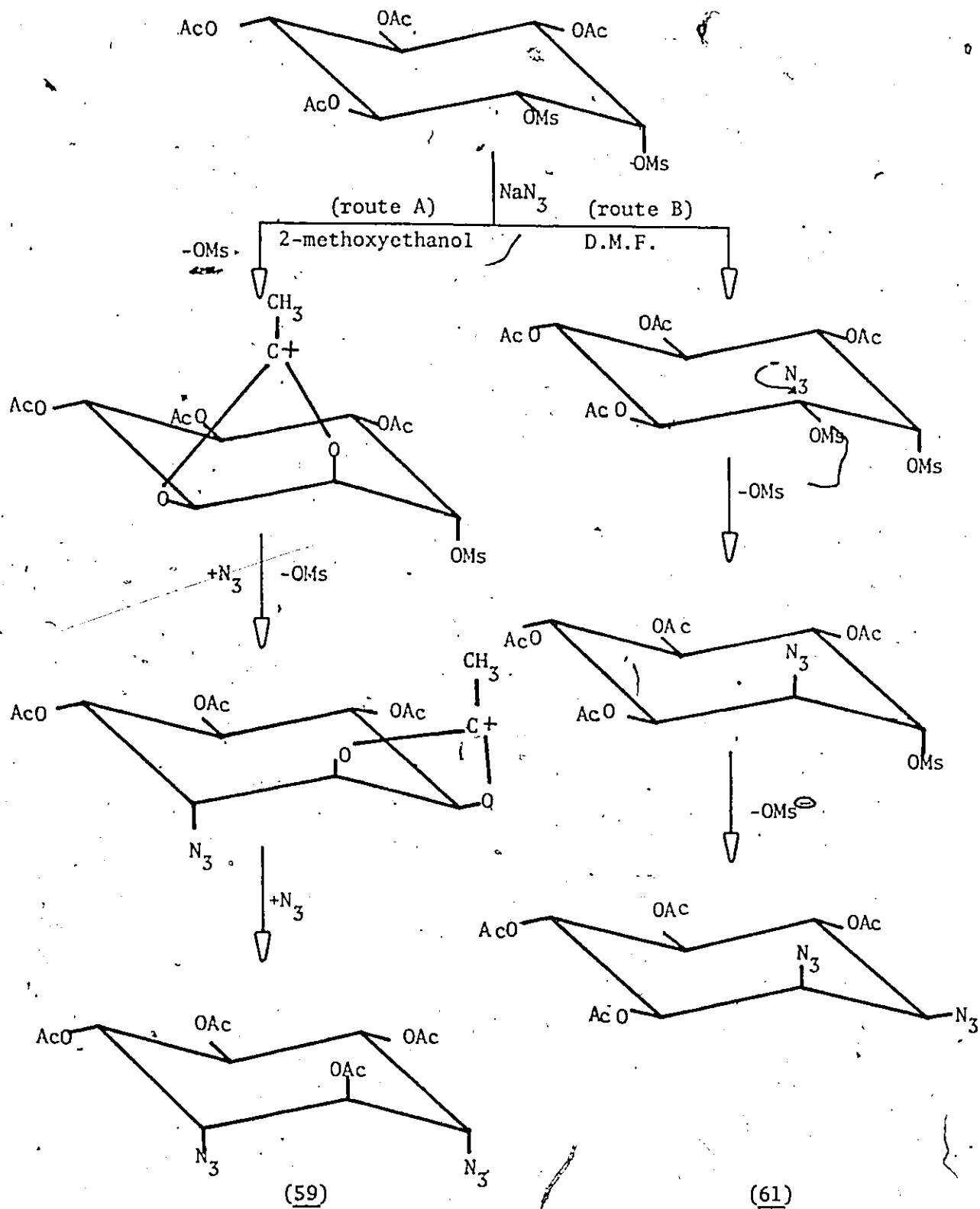


Chart 1

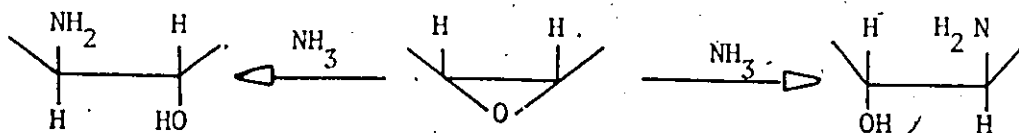
pyridine, 2,3-di-O-mesyl-myo-inositol-tetraacetate (58) was obtained in good yield. Treatment of 58 with sodium azide in aqueous 2-methoxyethanol gave a diazido derivative (59) which was converted into hexaacetyl-muco-inosadamine-1,5 (60) by hydrogenation and by subsequent acetylation. When 58 was treated with sodium azide in aqueous dimethyl-formamide instead of 2-methoxyethanol the product was the vicinal diazido compound 61 from which hexaacetyl-myo-inosadamine-1,2 (62) was subsequently obtained.

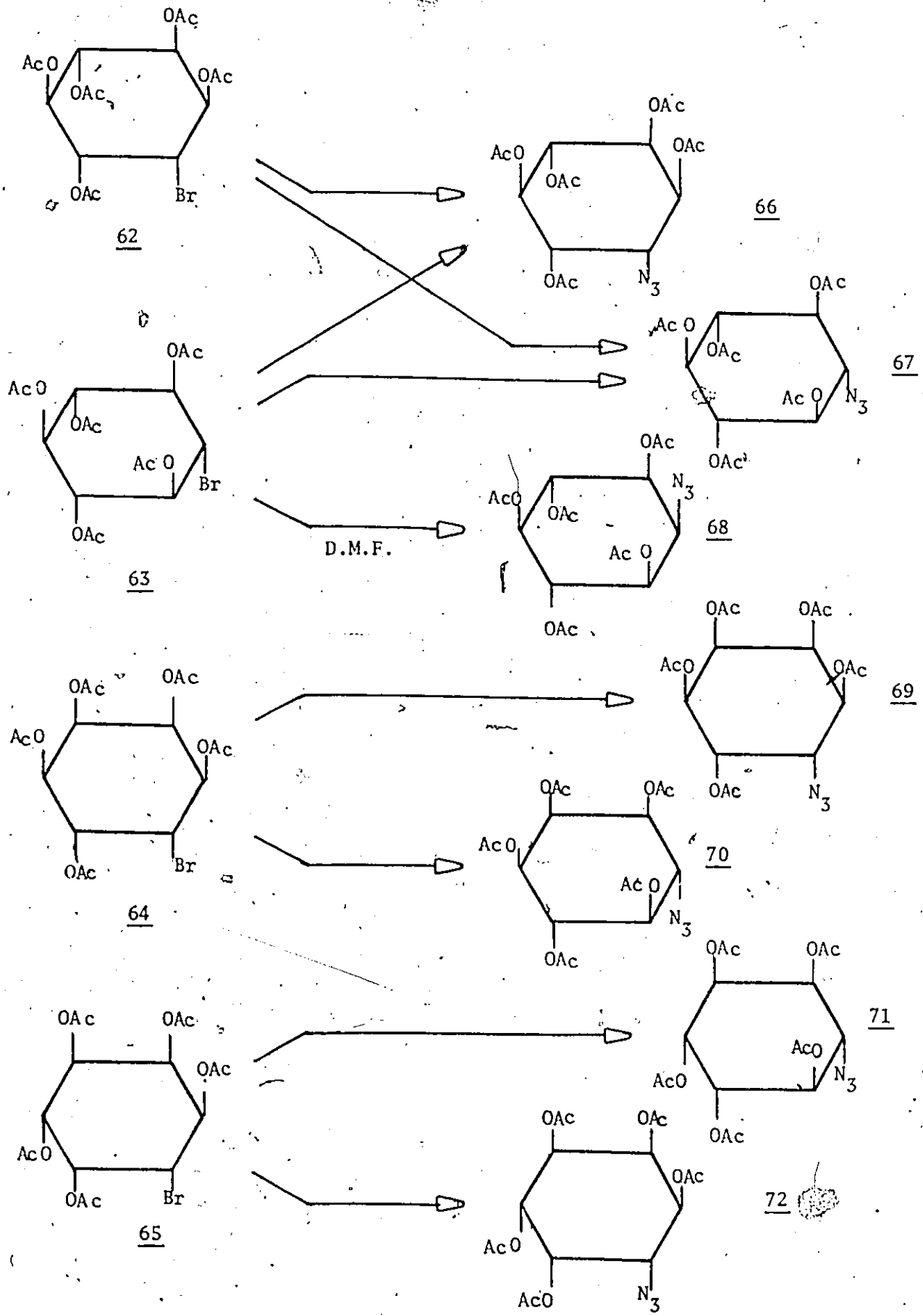
The mechanisms of formation of 59 and 61 are depicted in chart 1. In the case of production of 59 (route A), displacement with twofold anchimeric assistance of an acetoxy group is involved, whereas formation of 61 (route B) takes place by two direct displacements with Walden inversion.

In 1970, Suami and Vehida⁴⁵ carried out similar displacement reactions with sodium azide on the four bromodeoxyinositol pentaacetates 62 - 65. Again, neighbouring group participation played a role in most of these reactions which are summarized below. The seven azido inositols 66 - 67 were obtained in 60-80% yield, and each of them was transformed as before into the corresponding hexaacetyl-inosamine.

d- Ammonolysis of Epoxides and Halohydrins

Cyclitol epoxides may react with ammonia to give two isomeric amino cyclitols according to the following scheme.



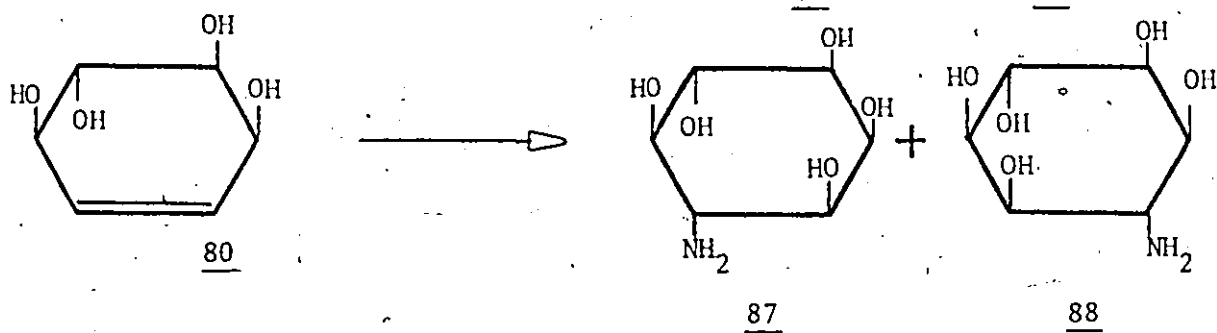
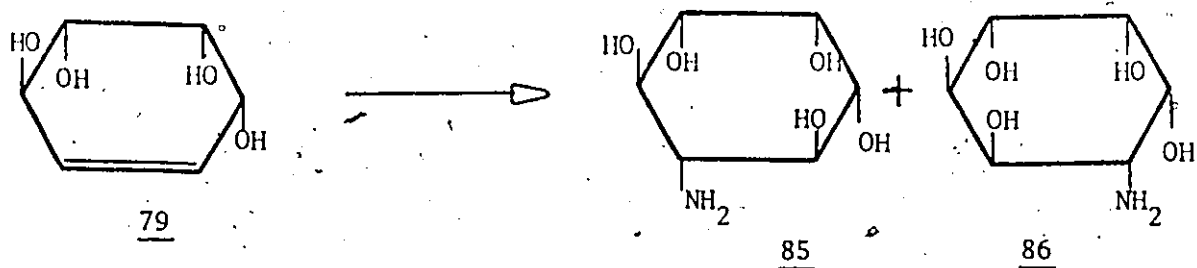
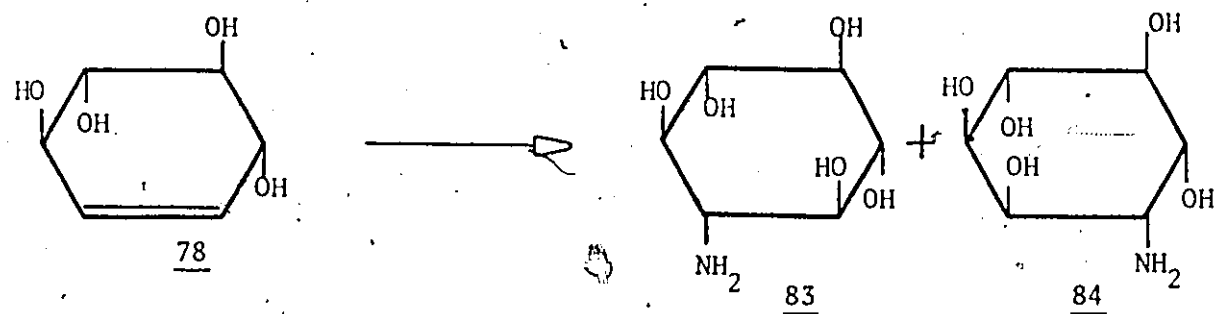
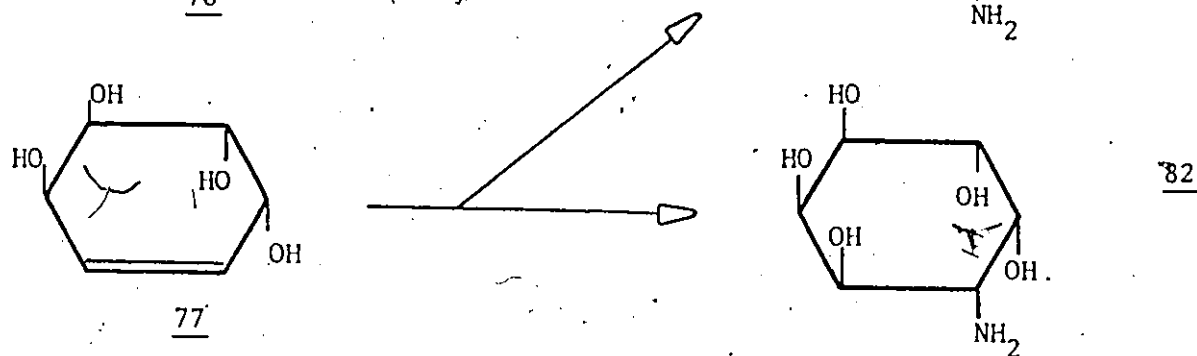
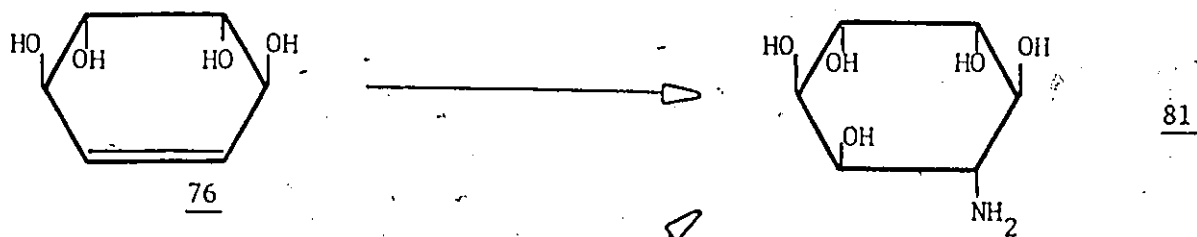
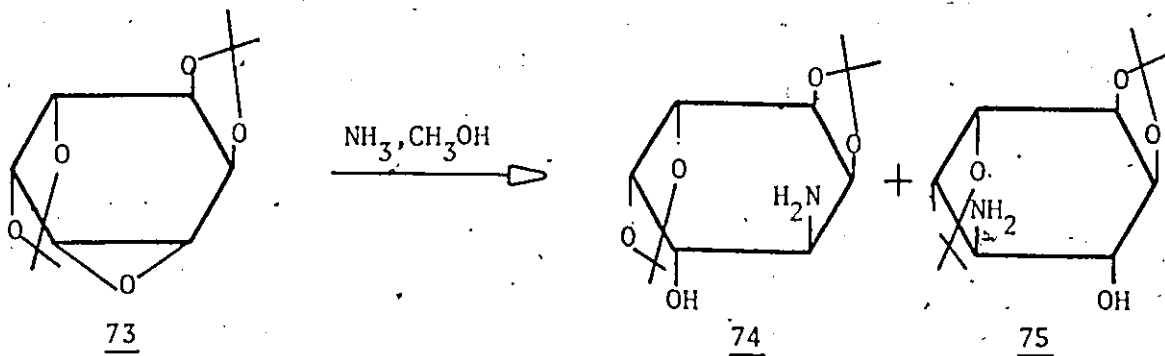


The hydroxyl and amino groups formed are trans related, and the proportion of isomers depends on the stereochemistry of the epoxide employed.

Cyclitol epoxides (1,2-anhydrocyclitols) are readily prepared by two general methods: (a), the action of bases on tosyl derivatives in which an adjacent hydroxyl group is trans oriented; and (b) treatment of cyclic olefin derivative with peracids. The former method has been used by Angyal and co-workers^{46,47}, the latter by Nakajima and co-workers⁴⁸.

Employing the above principle, Allen⁴⁹ reported a synthesis of two new inosamines. When L-1,2-anhydro-3,4;5,6,-di-0-isopropylidene-allo-inositol (73) was treated with ammoniacal methanol at 100°; a mixture of L-2,3; 4,5-di-0-isopropylidene-neo-inosamine-1 (74) and 1,2; 3,4-di-0-isopropylidene-L-inosamine-3 (75) was obtained. Upon removal of the isopropylidene groups the inosamines were separated by fractional crystallization and characterized by N-acetylation.

Starting from conduritols (tetra-hydroxycyclohexenes), Nakajima, Kurihara and Hasegawa⁵⁰ performed the synthesis of eight different inosamines, three of which had been unknown. The conduritols⁴ 76, 77, 78, 79 and 80 were first converted into epoxides⁴⁷. The latter were then treated with ammoniacal methanol and the products were acetylated without prior isolations. Fractional crystallization afforded the eight isomers of which 81, 82⁴⁹, 83²³, 87²⁷ and 84⁵¹ had been isolated and characterized previously. The new inosamines isolated were myo-inosamine-5 (85), allo-inosamine-1 (86) and muco-inosamine-3 (88).

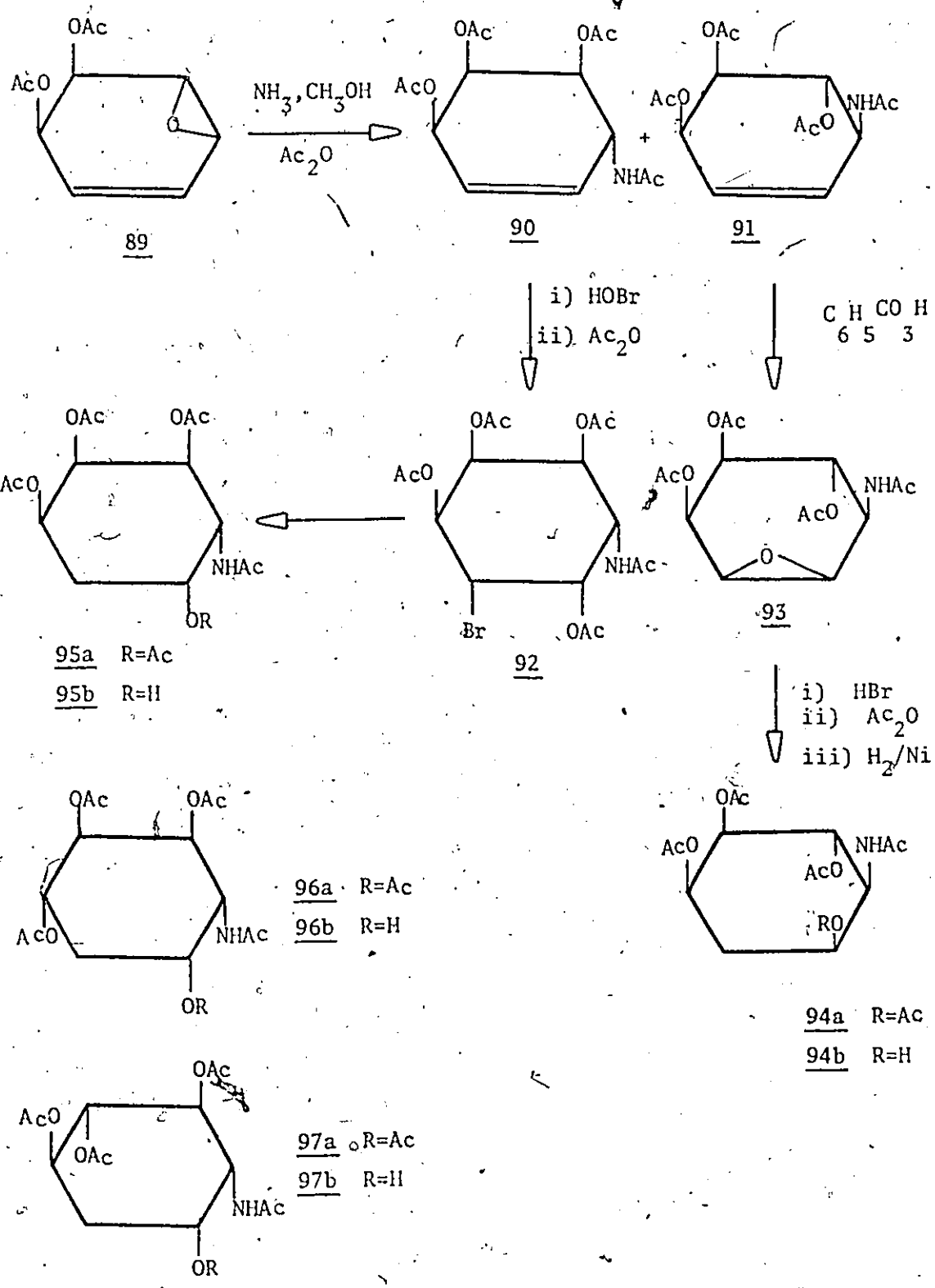


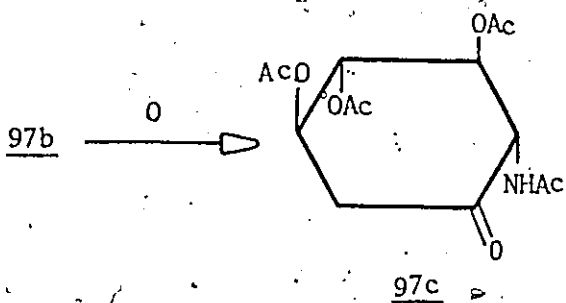
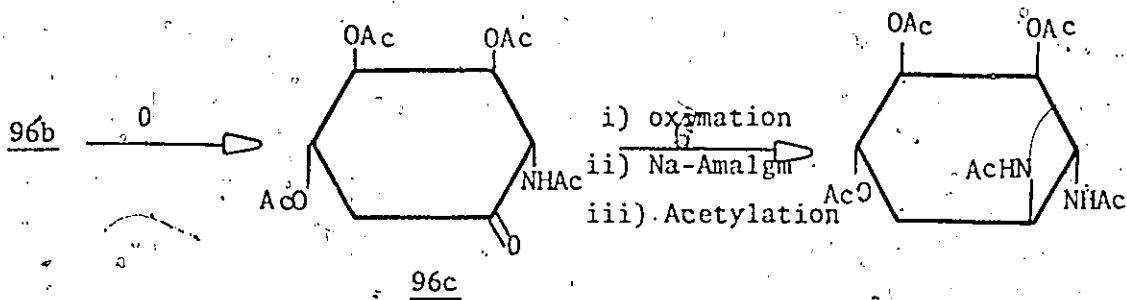
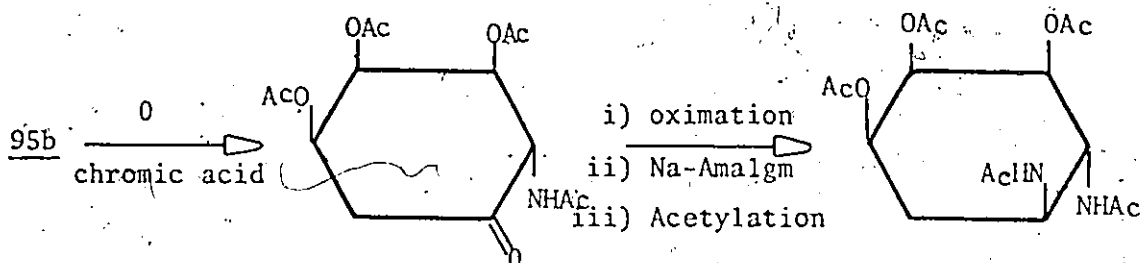
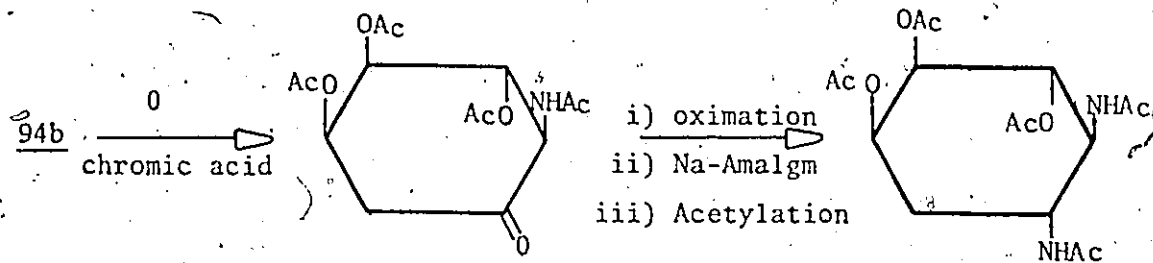
The principle of introducing an amino group by ammonolysis of epoxides and the principle of reducing a bromohydrin to generate a deoxy function⁵² were combined by Nakajima et al.⁵³ to prepare several deoxyinosamines⁵³ and deoxyinosadiazines⁵⁴.

The diacetoxy-epoxy-cyclohexene⁸⁹, for instance, gave upon ammonolysis and subsequent acetylation the two stereoisomeric acetamidotriacetoxycyclohexenes 90 and 91. Reaction of 90 with hypobromous acid followed by acetylation afforded the bromo compound 92 which was debrominated with Raney nickel to give pentaacetyl-D-5-amino-1,5-dideoxy-allo-inositol (95a). The cyclohexene 91 was converted with peroxy benzoic acid into the epoxide 93 from which pentaacetyl-DL-1-amino-1,3-dideoxy-epi-inositol (94a) was obtained by successive reaction with hydrobromic acid, acetylation and reductive debromination. Similar reaction sequences starting from stereoisomers of 89 led to the pentaacetates of DL-1-amino-1,3-dideoxy-allo-inositol (96a) and DL-1-amino-1,3-dideoxy-myo-inositol (97a). The four stereoisomeric acetamido-tri-O-acetylinosamines 94b, 95b, 96b and 97b on oxidation gave the corresponding deoxyinosamines 94c, 95c, 96c and 97c. Three of these were converted into deoxyinosadiazines⁵⁴ via their oximes.

4 Epimerization in Nitro Cyclitols

It has been demonstrated by Grosheintz and Fischer³² that 6-deoxy-6-nitro-D-glucose and -L-idose both undergo base-catalyzed cyclization



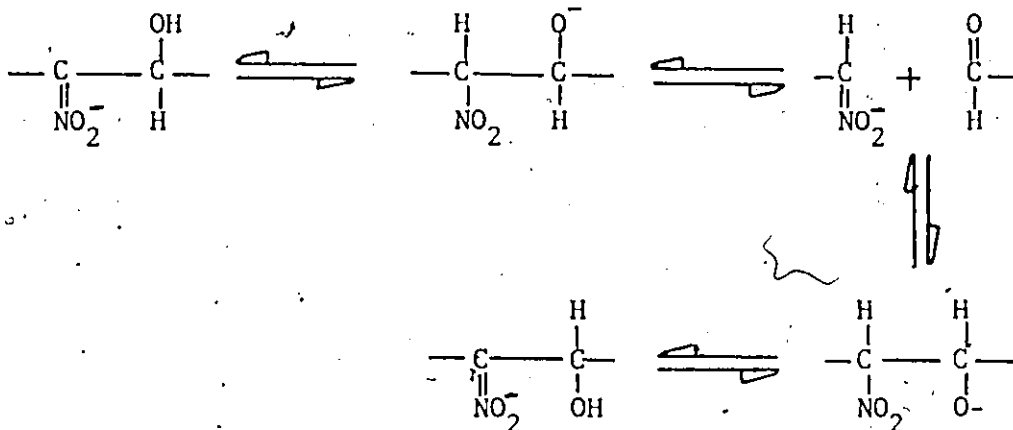


yielding the same mixture of three stereoisomeric deoxynitroinositols which were later shown³³ to possess the scyllo (26), DL-myo-1 (27), and muco-3 (28) configurations, p. 16. Lichtenthaler's³³ studies suggested that the muco isomer arises by kinetic control; when allowed to remain in alkaline medium it was said to epimerize to an equilibrium mixture of the scyllo and myo compounds.

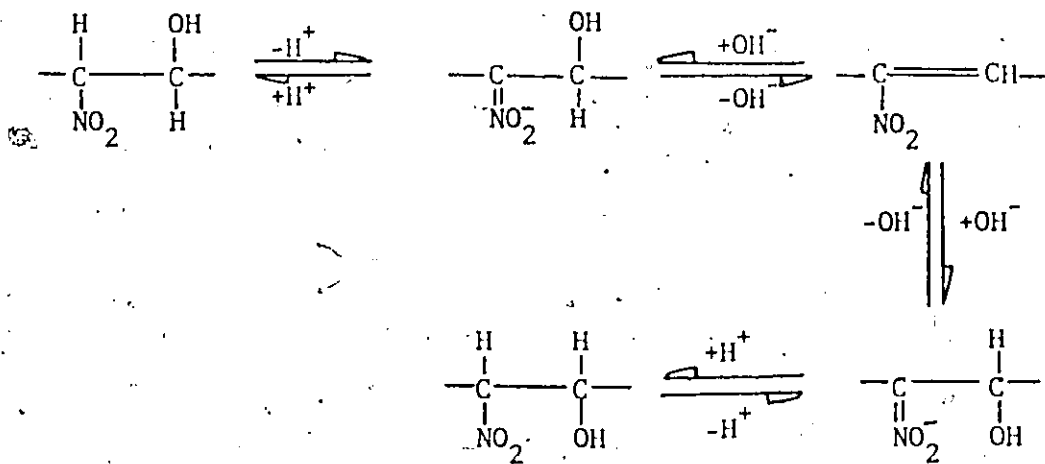
Recently, the thermodynamically controlled epimerization of such nitroinositols was investigated in greater detail by Kovar and Baer⁵⁵. These authors studied the behaviour of four stereoisomeric monomethyl ethers, namely, 1-deoxy-4-0-methyl-1-nitro-scyllo-inositol (36), 1-deoxy-4-0-methyl-1-nitro-D,L-myo-inositol (37), 3-deoxy-6-0-methyl-3-nitro-muco-inositol (38), 3-deoxy-6-0-methyl-3-nitro-epi-inositol (39) (p.17). The compounds were found to be mutually interconvertible by base, and the resulting thermodynamic equilibria were determined. In the presence of excess base, the compounds exist as nitronate salts 36, 37, and 38 (the latter being the salt common to 38 and 39 which are epimeric at the carbon bearing the nitro group), and the thermodynamic stability of the salts was demonstrated to increase in that order. On the other hand, when epimerization is allowed to take place in the presence of a catalytic amount of base, a different equilibrium is reached which is determined by the thermodynamic stabilities of the free nitro compounds. These follow the order of 36 > 37 > 38 >> 39. The results were accounted for by conformational analysis⁵⁵.

Concerning the mechanism of these epimerizations, Grosheintz and Fischer in their original work³² have proposed that a retro Henry reaction

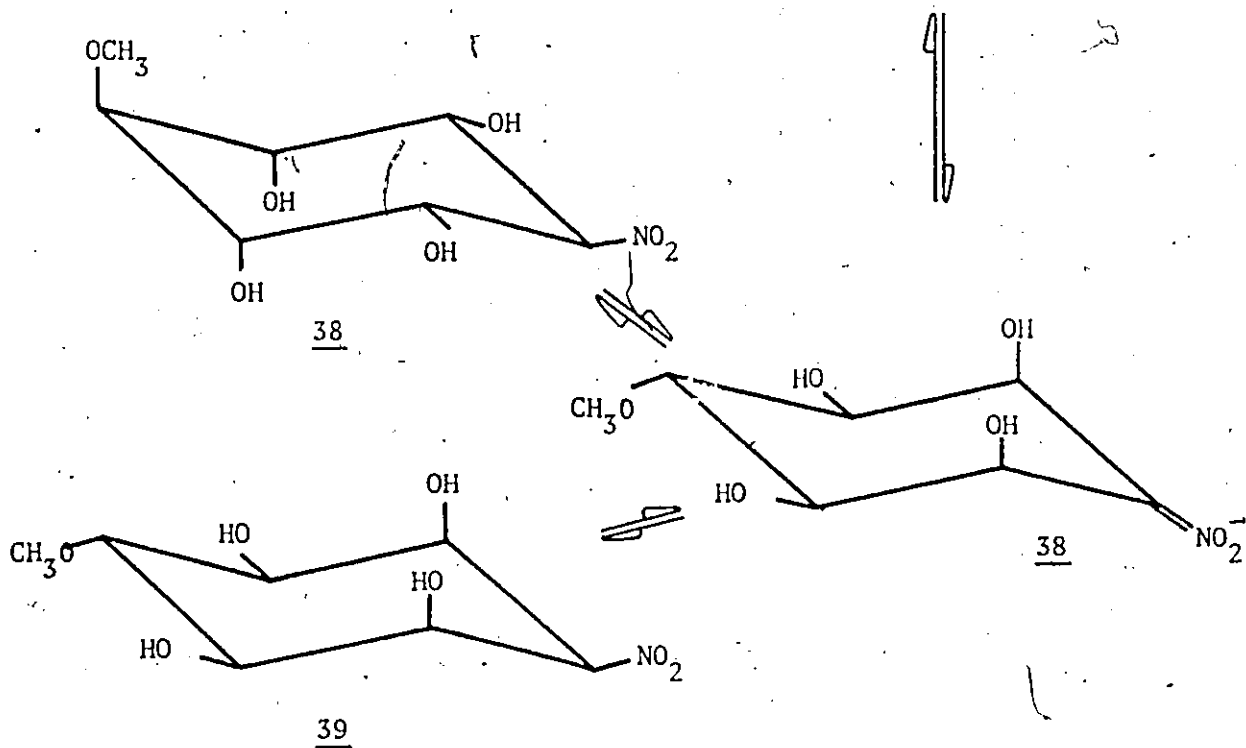
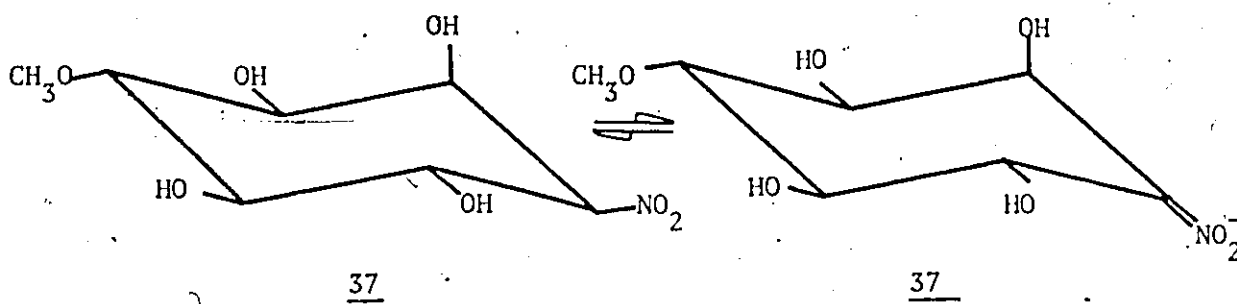
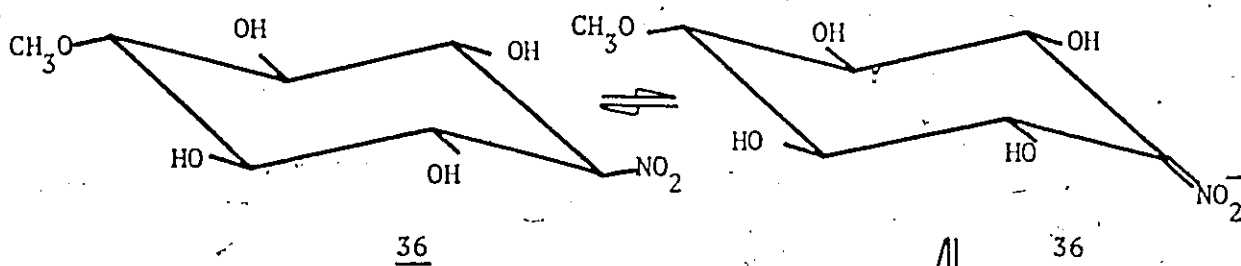
(i.e., a reversal of nitromethane-aldehyde addition) is responsible:



However, the authors did not support this proposal by experiments. By later work in nitro sugars, a considerable amount of evidence accumulated to suggest that epimerization at a position vicinal to the nitro group may proceed through nitroolefin intermediates⁵⁶⁻⁵⁹:



On the basis of more recent work, it is now believed that both mechanisms may apply, depending on the constitution and stereochemistry of the particular substrate⁶⁰⁻⁶². Thus, it was shown that the nitro-olefin mechanism does not operate, but that the reverse Henry mechanism applies, in cases where the nitro compound possesses a particularly low acidity. In nitro sugars⁶⁰⁻⁶² and nitroinositols⁵⁴, pK_a -values have been found to vary in the range of 8.0 - 11.0 and to be quite sensitive to the steric environment of the nitro group.



II. SPECIFIC AIMS OF THIS THESIS

As has been detailed in the Introduction, amino cyclitols play an important role as constituents of antibiotics. Although amino cyclitols themselves have not been found to possess antibiotic activity, they are of interest as building stones for synthetic antibiotics or as analogs that may potentially be useful in biochemical or medical research. One purpose of the present work, therefore, was to synthesize some new members of this family of compounds. Specifically, the nitroinositol monomethyl ethers recently obtained in this laboratory were to be converted into amines and, if possible, into partially blocked derivatives that might serve as starting materials for future syntheses. Secondly, a study was to be undertaken to demonstrate the relation between the acidity of nitroinositols and kinetic as well as thermodynamic aspects of their epimerization.

III. RESULTS AND DISCUSSION

I. Synthesis of Aminocyclitols

The first task was the preparation of partially blocked nitro inositols to be used for the synthesis of aminoinositols and also for epimerization studies. Following the procedure of Kovar and Baer³⁷, 1,2:5,6-di-0-isopropylidene-3-0-methyl- α -D-glucofuranose was converted, in four steps, into a mixture of 6-deoxy-3-0-methyl-6-nitro-D-glucose (34) and -L-idose (35), from which the crystalline β -D-gluco-anomer (β -34) could be isolated (see scheme, p.17).

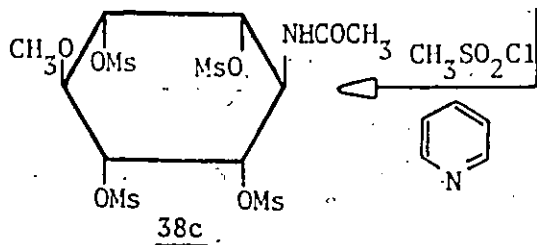
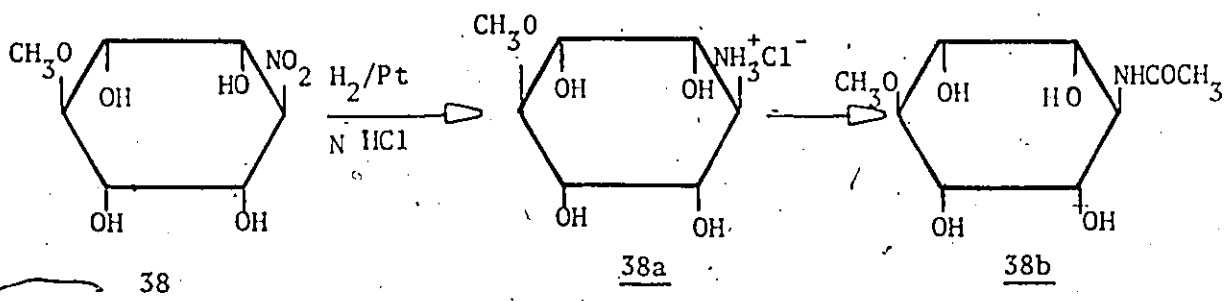
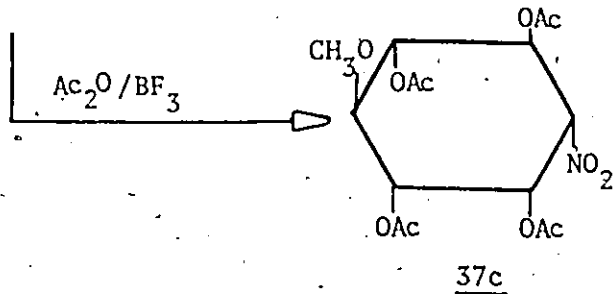
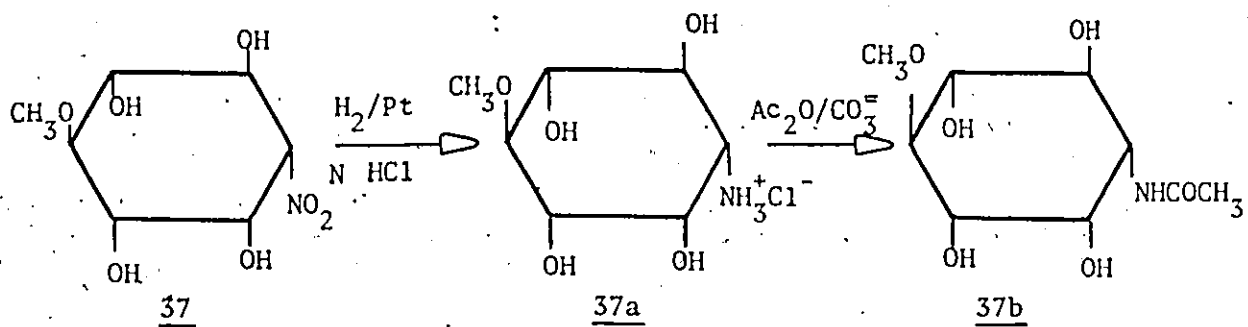
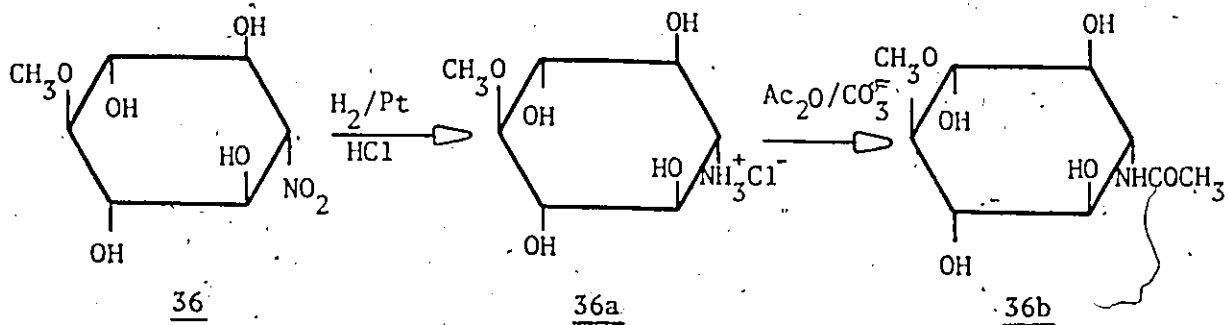
Base-catalyzed cyclization of the 6-deoxy-3-0-methyl-6-nitro-L-idose (35) furnished a mixture of two nitroinositol monomethyl ethers, which were separated after acetylation. Deacetylation of the tetra-acetylated derivatives gave pure, crystalline 1-deoxy-4-0-methyl-1-nitro-DL-myo inositol and 1-deoxy-4-0-methyl-1-nitro-scyllo-inositol (36).

Cyclization of 6-deoxy-3-0-methyl-6-nitro- β -D-glucose (β -34) under conditions of kinetic control³⁸ furnished a mixture of mainly two nitroinositol monomethyl ethers which were separated, by crystallization to give optically active 1-deoxy-4-0-methyl-1-nitro-L-myo-inositol (37) and 3-deoxy-6-0-methyl-3-nitro-muco-inositol (38).

Acetylation of the L-myo-nitroinositol 37 gave optically active 2,3,5,6-tetra-0-acetyl-1-deoxy-4-0-methyl-1-nitro-L-myo-inositol (37c) in pure crystalline form. This tetraacetate had not been prepared previously.

Catalytic hydrogenation of the nitroinositols 36, 37 and 38 in the presence of platinum and 1-2 molar equivalents of hydrochloric acid was performed at room temperature and at atmospheric pressure. The corresponding aminoinositol hydrochlorides (36a, 37a, and 38a) were obtained as colorless, high-melting, chromatographically homogeneous crystals in yields of 78-85%. The structure of the products was confirmed by elemental analysis and by infrared spectra showing medium strong bands near 1600 cm^{-1} which were due to the amine salt function (figs. 13-15). N.m.r. spectra of the products were poorly resolved, but they did at least confirm the presence of the methoxyl groups by exhibiting three-proton singlets in the expected region (1.2 - 1.35 p.p.m. downfield from acetone reference signal, in D_2O solution). Compound 37a was optically active whereas 36a and 38a were inactive. Since the hydrogenations were performed in an acidic medium it is safe to assume that no configurational changes occurred during the reaction.

The aminoinositol hydrochlorides were then N-acetylated by treatment with acetic anhydride in an aqueous-methanolic medium in the presence of an anion exchange resin. The corresponding N-acetyl derivatives 36b, 37b, and 38b were obtained crystalline in yields of 85-90%. The products gave correct microanalytical data, and their infrared spectra (figs. 16-18) showed characteristic amide-I and amide-II bands in the $1650\text{-}1620$ and $1560\text{-}1550\text{ cm}^{-1}$ regions, replacing the amine hydrochloride bands of the starting materials. The n.m.r. spectrum of each product contained a three-proton singlet attributable to the N-acetyl group, in



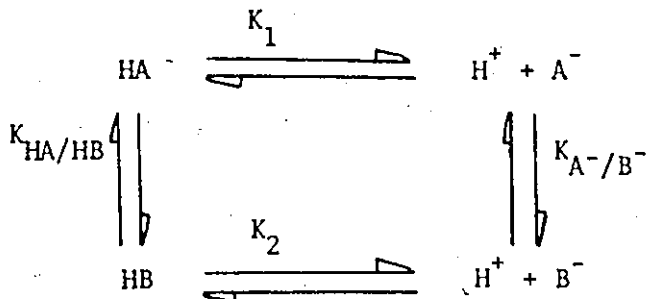
addition to the methoxyl singlet.

3-Acetamido-3-deoxy-6-0-methyl-muco-inositol (38b) was converted into crystalline 1,2,4,5-tetra-0-mesyl derivative (38c) by treatment with methanesulfonyl chloride in pyridine. It was hoped that the mesyl groups in positions 2 and 4 in this tetramesylate could be selectively removed through neighbouring group participation of the acetamido function at C-3. The expected, partially blocked product could serve useful purposes in the design of future syntheses. Unfortunately, several attempts at achieving such partial demesylation, by refluxing 38c with sodium acetate in 80% ethanol, did not lead to useful results. Mixtures showing five spots in t.l.c. were obtained (with different intensities depending on reaction times which were varied between 3 and 24 h), and no crystalline product except for some unreacted starting material could be isolated.

2 Epimerization of Nitro Cyclitols

It has been mentioned in the Introduction that compounds 36, 37, 38 and 39 are epimerized by base, and that the epimeric equilibrium established in the presence of excess base differs from that produced by a catalytic amount of base. In the former case, equilibrium exists between nitronate salts, and in the latter case, largely between free nitro compounds. However, equilibration between free nitro compounds must proceed through their anions (formed by the action of the catalyst), and therefore, for a pair of free nitro compounds HA and HB, the equilibrium constant $K_{HA/HB}$

and the equilibrium constant of their salts, K_{A^-/B^-} , must be interrelated with their acidity constants K_1 and K_2 according to the following scheme:



It follows that the ratio of equilibrium constants should be equal to the ratio of acidity constants (equation 1)

$$\frac{K_{\text{A}^-/\text{B}^-}}{K_{\text{HA}/\text{HB}}} = \frac{K_1}{K_2} \quad (1)$$

Kovar and Baer⁵⁵ obtained experimental values which were in reasonable agreement with equation 1. However, in order to test the validity of the data it was considered that several series of additional measurements should be made under different conditions. In particular, epimerizations should be studied in buffer solutions over a range of alkalinity intermediate between the previously used pH-values of 7 and ~13.

Before embarking on the investigation of equilibrium compositions at various pH-values it was desirable to determine the time required for equilibrium to occur. Best suited for kinetic experiments was the optically active L-myco compound (37) as the course of its reaction could easily be followed by polarimetry. The change in specific rotation of 37 at room temperature in buffer solutions of pH 8.3, 9.2, 9.5 10.2, and 10.65 was

recorded. In Figs. 7 - 11, the logarithms of optical rotation values are plotted against time, and it is seen that a linear relationship exists, indicating first-order kinetics. The reaction half-times ($t_{1/2}$) which were determined from the graphs are given in column 2 of Table 2. From the reaction half-time observed at each pH-value was calculated the rate constant, K_{obs} that applied to the reaction at that particular pH-value. The values for K_{obs} are given in column 2 of Table 2, and they were obtained according to equation 2:

$$K_{obs} t = \ln \frac{C_o}{C_t} \quad (2)$$

where

C_o = initial concentration of nitro compound

C_t = concentration at time t.

The reaction half-time being defined as the point where $C_t = \frac{1}{2}C_o$, it follows that

$$K_{obs} t_{1/2} = \ln 2, \text{ hence } K_{obs} = \frac{0.69}{t_{1/2}}$$

Clearly, the observed reaction rate is dependent on the pH of the medium. Now the assumption is that the epimerizing species is the nitronate anion rather than the nitroinositol molecule. The observed rate should therefore be proportional to the anion concentration, $[A^-]$, and the proportionality factor (K_{true}) should be characteristic of the structure of the anion and independent of hydrogen ion concentration.

Thus,

$$\text{rate} = K_{\text{obs}} C = K_{\text{true}} [A^-], \text{ consequently}$$

$$K_{\text{obs}} = K_{\text{true}} \frac{[A^-]}{C} \quad (3)$$

We note that $\frac{[A^-]}{C}$ is the mole fraction (N) of anion present in the nitroinositol solution. The mole fraction N is, of course, dependent on the hydrogen ion concentration of the medium but not proportional to it. It can be calculated for a given pH value from the acidity constant K_a using equation 4.

$$-pK_a + \text{pH} = \log \frac{[A^-]}{C - [A^-]} \quad (4)$$

The pK_a -value for the nitro compound in question (37) has been determined⁵⁵ to be 9.9, and with this, the values of N shown in column 4 of Table 2 were obtained. If the assumption leading to equation 3 is correct, it follows that K_{obs}/N must be constant. Column 5 (Table 2) shows reasonable constancy of this parameter. If, on the other hand, the rate would be directly proportional to the hydroxyl ion concentration, the ratio $K_{\text{obs}}/[OH^-]$ should be constant. This is not the case as is clearly demonstrated by the entries in column 6 (Table 2).

Table 2. Kinetics of epimerization of L-myoinositol 37 at various pH-values

pH	$t_{1/2}$ in hr.	K_{obs} sec ⁻¹	N	$K_{obs}/N \times 10^4$	$K_{obs}/[OH^-]$
8.3	72.0	0.26×10^{-5}	0.024	1.0	1.3
9.2	9.00	2.1×10^{-5}	0.16	1.3	1.3
9.5	5.40	3.6×10^{-5}	0.28	1.3	1.1
10.2	2.50	7.7×10^{-5}	0.66	1.2	0.48
10.65	2.30	8.5×10^{-5}	0.85	1.0	0.15

Turning now to the study of thermodynamic equilibrium of the nitroinositol derivatives, one may see from the above kinetic measurement that, at least as far as the isomer 37 is concerned, a reaction time of 2 days (about 5 half-times) should be sufficient to reach equilibrium at pH 9.2 or higher. Reactions at lower pH were considered too slow to be practical for the purposes of the present study. It was a reasonable assumption that the stereoisomers 36 and 38 would epimerize at rates of comparable magnitude.

Compounds 36, 37 and 38 were therefore allowed to epimerize for 2 days at room temperature in buffer solutions of pH 9.4, 10.0, 11.0, and 12.0. For analysis of the equilibrium composition of the reaction mixtures, the solutions were acidified, deionized, and evaporated. The residues were trimethylsilylated and analyzed by vapor phase chromatography. The results (Tables 3-6) show good agreement of equilibrium compositions arrived at from any one of the three stereoisomers at a specific pH value, and the compositions differ with different pH-values.

Based on the scheme and accompanying discussion on page 40 one can derive an equation (equation 5) which relates the equilibrium ratio of any two isomers with the pH of the medium. The derivation of this equation will be presented on pages 75-76-77.

$$\frac{[A]}{[B]} = K_{HA/HB} \frac{K_1 + [H^+]}{K_2 + [H^+]} \quad (5)$$

where [A] = the equilibrium concentration of one isomer, as determined by v.p.c. (that is, the sum of the concentrations of free nitro compound HA and its anion A⁻).

[B] = the same for the other isomer

$K_{HA/HB}$ = the equilibrium constant of the pair of free nitro compounds.

K_1 = the acidity constant of HA

K_2 = the acidity constant of HB.

The acidity constants of nitroinositol 36, 37, 38 and 39 were reported⁵⁵ to be 1.99×10^{-11} , 1.25×10^{-10} , 3.02×10^{-9} and 4.47×10^{-9} respectively. From published equilibrium data⁵⁵ one can also obtain equilibrium constants $K_{HA/HB}$ for the pairs 36:37 (3.2), 36:(38+39) (24.0), and 37:(38+39) (7.5). Insertion of these data in equation 5 gave the calculated equilibrium percentage shown in Table 7. These calculations based on the previously reported constants show considerable disagreements with the present experimental results. Even if slight adjustments in the constants are made, no significantly improved fit is obtained. For example, it was tried to use a $K_{HA/HB}$ value of 30 as found at the lowest pH instead of the reported value⁵⁵ of 24 for scyllo/muco + epi pair (it follows from the equation 5 that $K_{HA/HB}$ can never be lower than any ratio of A/B found experimentally), and to adjust slightly (by not more than 0.2) the pK-values in order to gain a better fit between the pK differences and the logarithm of $K_A^-/B^-/K_{HA/HB}$ ratio (see equation 1), but agreement remained unsatisfactory. However, if an arbitrary linear shift of all pK-values by 1 unit is made, a measure which does not disturb the aforementioned requirements of equation 1, a reasonable agreement between found and calculated values is obtained (see Table 8). This would imply that the published⁵⁵ pK-values, which were determined by half-titration, were all in error by 1 unit, or alternatively that a nitro compound may differ in its pK_a by 1 unit depending on whether it applies to a buffer medium as in the present experiments or to a dilute aqueous solution as in half-titration. To clarify the situation, the pK_a value of one of the nitro compounds,

namely 37, was determined by an independent method both in the sodium bicarbonate-sodium hydroxide buffer and the Sørensen (glycine) buffer that had been used in the present work. By use of ultraviolet spectroscopy for determining the nitronate concentration and thus the nitronate/nitro concentration ratio at a given pH (9.85 in both buffers), a value of 10.1 was obtained in both buffers. This value is very close to the half-titration value of 9.9 and it therefore appears that, within experimental error, the compound shows the same pK_a , independent of the medium and the method of determination. Consequently, it does not seem justified to assume shifted pK_a values as a device for bringing the observed and calculated results into agreement.

It is known that equilibrium constants should reflect ratios of activities rather than concentrations. An activity coefficient of 1.0 has been tacitly assumed throughout the work so far. This may well be justified for pK determinations, particularly for the determination using the UV technique, because the concentrations in question are of the order of 10^{-4} mole/l. However, in the equilibrium experiments, higher concentrations were used and it is reasonable to assume that activity coefficients may then be lower and may not necessarily be the same for the anion and the undissociated nitro compound. This could account for concentrations of anion appearing to be lower than required by the pH- pK relation (equation 4), and it would be equivalent, in calculation, to shifts of pK as suggested above (Table 8). No attempts have been made to determine the activity coefficients experimentally.

Table 3- Epimerization of nitro compounds at pH 9.4

starting compounds	% of products formed		
	<u>38</u> + <u>39</u>	<u>37</u>	<u>36</u>
<u>37</u>	-	22.5	77.9
<u>38</u>	7.2	18.5	73.5
<u>36</u>	0.3	16.4	83.3
Mean	2.5	19.0	78.2

Table 4- Epimerization of nitro compounds at pH 10.

starting compounds	% of products formed		
	<u>38</u> + <u>39</u>	<u>37</u>	<u>38</u>
<u>37</u>	3.6	25.1	70.5
<u>38</u>	5.8	24.2	69.9
<u>36</u>	0.8	26.3	72.3
Mean	3.41	25.4	70.9

Table 5- Epimerization of nitro compounds at pH 11

starting compounds	% of products formed		
	<u>38</u> + <u>39</u>	<u>37</u>	<u>36</u>
<u>37</u>	16.20	34.90	48.80
<u>38</u>	18.40	29.50	51.98
<u>36</u>	20.3	56.30	43.2
Mean	18.3	33.5	47.9

Table 6- Epimerization of nitro compounds at pH 12

starting compounds	% of products formed		
	<u>38</u> + <u>39</u>	<u>37</u>	<u>36</u>
<u>37</u>	44.7	36.0	19.1
<u>38</u>	51.0	37.7	10.6
<u>36</u>	42.9	44.0	12.5
Mean	46.2	39.2	14.06

Table 7 Composition of equilibrium mixture at different pH values

pH	% scyllo <u>36</u>		% of myo <u>37</u>		% of muco + epi <u>38+39</u>	
	cal	exp.	cal.	exp.	cal.	exp.
9.4	62.0	78.0	24.4	19.1	13.7	2.5
10	44.6	70.9	26.37	25.4	29.23	3.41
11	29.46	47.9	28.8	33.5	50.76	18.3
12	15.5	14.06	29.4	39.2	55.5	46.2

Constants used: $K_{HA/HB}$ for: $\frac{\text{Scyllo}}{\text{muco} + \text{epi}} = 24.0$

$\frac{\text{myo}}{\text{muco} + \text{epi}} = 7.5$

pKa for: scyllo = 10.7

myo = 9.9

muco = 8.75

Table 8: Compositions of equilibrium mixture at different pH values.

pH	% scyllo <u>36</u>		% of myo <u>37</u>		% of muco + epi <u>38+39</u>	
	cal.	exp.	cal	exp.	cal.	exp.
9.4	76.9	78.0	19.6	19.1	3.5	2.5
10	73.6	70.9	20.6	25.4	5.8	3.41
11	50.6	47.9	26.2	33.5	23.2	18.3
12	18.9	14.06	33.9	39.2	47.2	46.2

Constants used: $K_{HA/HB}$ for: $\frac{\text{Scyllo}}{\text{muco} + \text{epi}} = 30.0$

for: $\frac{\text{myo}}{\text{muco} + \text{epi}} = 7.5$

pK_a for: scyllo = 12.0

myo = 10.9

muco + epi = 9.85

IV. RESEARCH EQUIPMENT USED

Optical rotations were measured in a 1-dm tube in a Perkin-Elmer 141 automatic polarimeter using a mercury lamp as the light source. V.p.c. was performed in a Varian Aerograph, series 1200, with hydrogen flame detector and a recorder equipped with a 224 Disc. Mod. integrator. The stainless-steel column (length, 6 ft; diameter, 1/8 in.) was packed with 3% OV17 on chromosorb W and was operated isothermally at 160-170°. The carrier gas was helium applied at a pressure of 80-90 p.s.i. N.m.r. spectra were recorded on a Varian T-60 instrument. The pH measurements were made using a glass electrode and a Philips pH meter. For infrared measurements, a Beckman IR 20 A spectrophotometer was used. T.L.C. was performed on silica gel G plates.

V. EXPERIMENTAL

Ia. Deoxynitroinositol monomethyl ethers 36, 37 and 38

Starting from 1,2:5,6 di-0-isopropylidene- α -D-glucofuranose (29), the following deoxynitroinositol monomethyl ethers were prepared as reported^{37,38}.

Nitro- inositols	Name	Found			Reported		
		mp	-OCH ₃ signal *	(α) 365	(α) 365	-OCH ₃ signal *	mp
<u>36</u>	1-deoxy-4- <u>0</u> -methyl-1-nitro- <u>scyllo</u> -inositol	251-259 ^o	δ :1.39			δ :1.41	258-260 ^o
<u>37</u>	1-deoxy-4- <u>0</u> -methyl-1-nitro- <u>L-myo</u> -inositol	176-179 ^o	δ :1.39	-182 ^o	-184 ^o	δ :1.39	178.5-179.
<u>38</u>	3-deoxy-6- <u>0</u> -methyl-3-nitro- <u>muco</u> -inositol	150-151 ^o	δ :1.15			δ :1.15	151-152 ^o

* 3-proton singlet in CF₃COOD/D₂O, with acetone as internal standard.

Ib. 2,3,5,6-Tetra-0-acetyl-1-deoxy-4-0-methyl-1-nitro-L-myo-inositol (37c)

L-myo-Nitroinositol (37) (314 mg) was acetylated with acetic anhydride (3.1 ml) in the presence of boron trifluoride etherate (12 drops) by stirring for 10 h at 0°C and another 10 h at room temperature. The reaction mixture was then evaporated in vacuo. Crystallization of the residue from ethanol furnished white crystals of 37c (373 mg, 68%), m.p. 107-110°C. The product was optically active: $[\alpha]_{578} -15^{\circ}$,

$[\alpha]_{546} -17^{\circ}$, $[\alpha]_{436} -21^{\circ}$ (C = 0.93, in MeOH).

N.m.r. data (in CDCl_3 with TMS as ref): (δ)2-2.2 (12H, 4s, -O-COCH₃), (δ)3.5 (3H, s, OCH₃), (δ)3.7 (1H, H₄), (δ)4.50-5.2 (3H, H₁, H₃, H₅), (δ)5.6-6.2 (2H, H₂, H₆); see Fig. 12, p. 66.

Anal. Calcd. for $\text{C}_{15}\text{H}_{21}\text{NO}_{11}$ (mol. wt. 391.3): C, 46.04; H, 5.41; N, 3.58. Found: C, 46.23; H, 5.44; N, 3.58.

II. Hydrogenation of the nitroinositols

a. L-Amino-1-deoxy-4-0-methyl-scyllo-inositol hydrochloride (36a)

Platinum dioxide (~ 100 gm) in 5 ml of water containing 0.6 ml of N hydrochloric acid was prehydrogenated at room temperature. After 15 minutes the hydrogen uptake ceased. The nitro compound 36 (111 mg) in 10 ml of water was then added, and the mixture was shaken under hydrogen at ordinary pressure for 7 h. The catalyst was filtered off and washed with water, and the filtrate was evaporated. The colorless

residue was triturated with absolute ethanol, and upon scratching with a glass rod and cooling with ice, gave crystals of 36a (91 mg, 78%), mp 280-282°. Recrystallization of 36a from aqueous ethanol furnished white crystals which melted at 270-272° in an evacuated capillary. T.L.C. of the crystals in 1:1 ethanol-water gave one spot (R_f 0.5). The product was optically inactive.

IR (Fig. 13): strong band at 3300 cm^{-1} (OH)

weak band at 1600 cm^{-1} (NH_3^+)

N.m.r. (solvent: D_2O , aceton as reference): δ 1.35 (3H, s, -O- CH_3).

Anal. Calcd for $\text{C}_7\text{H}_{16}\text{O}_5\text{NCl}$ (mol. wt. 229.45): C, 36.61; H, 6.97; Cl, 15.45

Found: C, 36.20; H, 6.99; Cl, 15.24.

b- 1-Amino-1-deoxy-4-0-methyl-L-myo-inositol hydrochloride (37a)

A 111-mg sample of 1-deoxy-4-0-methyl-1-nitro -L-myo-inositol (37) was hydrogenated as described in section (a). After 1.5 h shaking under hydrogen, the catalyst was filtered off and washed with water, and the solution was evaporated. Upon trituration with ethanol, the inosamine 37a was obtained as a white crystalline mass (96 mg, 82%) melting at 279-282°. Recrystallization from aqueous ethanol gave white crystals, mp 281-285°. T.L.C. of 37a in 1:1 ethanol-water gave one spot (R_f 0.43).

IR (Fig. 14): strong sharp band at 3300 cm^{-1} (OH and NH_3^+)

strong sharp band at 1600 cm^{-1} (NH_3^+)

N.m.r. (solvent, D₂O with aceton as reference): δ 1.35 (3H, s, - OCH₃).

The spectrum was not well resolved.

Rotation: optically active (α)₅₇₈ +6, (α)₅₄₆ +12, (α)₄₃₆ + 21 ($C = 0.71$, in H₂O).

Anal. Calcd. for C₇H₁₆O₅NCl (mol. wt. 229.45): C, 36.61; H, 6.97;

Cl, 15.45.

Found: C, 36.42; H, 7.24; Cl, 15.26.

c- 3-Amino-3-deoxy-6-0-methyl-muco-inositol hydrochloride (38a)

A 111-mg sample of 3-deoxy-6-0-methyl-3-nitro-muco-inositol (38) was hydrogenated for 8 h, and the reaction mixture was worked up, as described in section (a). Trituration of the residue furnished white crystals of 38a (90.5 mg, 78%) showing mp 320-322⁰ in an evaporated capillary. T.L.C. with 1:1 ethanol-water gave one spot only (R_f 0.32). The compound was optically inactive.

IR data (Fig. 15): strong broad band at 3300 cm⁻¹ (OH and NH₃⁺)

Medium band at 1600 cm⁻¹ (amine salt)

N.m.r. data (solvent: D₂O, with aceton as reference): δ 1.2 (3H, s; OCH₃)

The n.m.r. spectrum was not resolved.

Anal. Calcd. for C₇H₁₆O₅NCl (mol. wt. 229.45): C, 36.61; H, 6.97;

Cl, 15.45.

Found: C, 36.64; H, 7.17; Cl, 15.40.

III. N-Acetyl Derivatives of Inosamines

a- 1-Acetamido-1-deoxy-4-O-methyl-scyllo-inositol (36b)

A solution containing 50 mg of the scyllo aminoinositol hydrochloride (36a) in 10 ml of water and 1 ml of methanol was stirred for 90 min. at 0°-5° with 12 ml of Dowex 1-X8 (CO₃⁻ form) and 0.26 ml of acetic anhydride. The resin was filtered off and washed with water. The filtrate and washings were passed through a column containing 2 ml of Amberlite IR120 (H⁺ form) which was rinsed with water. The colorless effluent and washing were heated to boiling and then evaporated. Trituration of the residue with ethanol under ice cooling furnished white crystals of 36b (41.1 mg, 85%), mp 270-273°. Recrystallization from aqueous ethanol raised the melting point to 275-277°.

IR data (Fig. 16): strong band at 3300 cm⁻¹ (OH)
strong medium band at 1550 cm⁻¹ (amide II)
and 1620 cm⁻¹ (amide I).

N.m.r. data: (solvent, dimethyl sulfoxide, TMS as reference)

δ 1.83 (3H,s, NHC(=O)CH₃), δ 3.46 (3H,s,-OCH₃).

Anal. Calcd. for C₉H₁₇O₆N (mol. wt. 235): C, 45.90; H, 7.20;

N, 5.90.

Found: C, 45.77; H, 7.28; N, 6.04.

b- 1-Acetamido-1-deoxy-4-0-methyl-L-myo-inositol (37b)

A 50-mg sample of pure L-myo aminoinositol hydrochloride (37a) was treated as described in section II(a) to give, upon trituration of the residue with ethanol, white crystals of 37b (40 mg, 85%), mp 242-245°.

IR data (Fig. 17). strong band at 3300 cm^{-1} (OH)
strong band at 1550 cm^{-1} (amide II),
1620-1650 cm^{-1} (amide I).

N.m.r. data (solvent, dimethyl sulfoxide, TMS as reference): δ 1.88 (3H,s,NHCOCH₃)
 δ 3.40 (3H,s, OCH₃).

Anal. Calcd. for C₉H₁₇O₆N (mol. wt. 235): C, 45.90; H, 7.20; N, 5.90.

Found: C, 46.11; H, 7.22; N, 6.03.

c- 3-Acetamido-3-deoxy-6-0-methyl-muco-inositol (38b)

A 50-mg sample of the muco-aminoinositol hydrochloride (38a) was treated as described in section III(a) to give, upon trituration of the residue with ethanol, white fine crystals of 38b (42.2 mg, 90%), mp 185-188°.

IR data (Fig. 18): strong band at 3300 cm^{-1} (OH)
strong medium band at 1560 cm^{-1} (amide II)
and 1630-1650 cm^{-1} (amide I).

N.m.r. data (solvent dimethyl sulfoxide, TMS as reference): δ 1.89 (3H,s, NHCOCH₃), δ 3.37 (3H,s, OCH₃).

Anal. Calcd. for C₉H₁₇O₆N (mol. wt. 235): C, 45.90; H, 7.20; N, 5.90.

Found: C, 45.88; H, 7.31; N, 5.94.

IV. 3-Acetamido-3-deoxy-6-0-methyl-1,2,4,5,-tetra-0-methylsulfonyl-muco-inositol (38c).

To 101 mg (0.43 mM) of 3-acetamido-3-deoxy-6-0-methyl-muco-inositol (38b) in 4 ml of anhydrous pyridine, 0.16 ml of methylsulfonyl chloride was slowly added with cooling in an ice bath. The ice bath was removed and the reaction mixture stirred at room temperature for 3 days. The reaction mixture was then poured into 6 ml of cooled water. Upon scratching, yellowish crystals of 38c were formed (172.6 mg, 72%), mp 177-178°. T.L.C. of the crystals in 3:1 chloroform-methanol gave one spot (R_f 0.84). Recrystallization of 38c from acetone furnished white crystals (104 mg, 45%) of mp 187-189°.

IR data (Fig. 19): strong bands at 1654 cm^{-1} and at 3150 cm^{-1} indicating the presence of the -NH-C function.

N.m.r. data (solvent CD_3COCD_3 with TMS as reference): δ 2.0 (3H, s, NH-COCH_3); δ 2.6 (1H, NH-); δ 3.28 (6H, s, 2-OMs); δ 3.30 (6H, s, 2-OMS); δ 3.60 (3H, s, OMe); δ 3.80-4.5 (2H, H_3 , H_6); δ 5.2-5.4 (4H, H_1 , H_2 , H_4 , H_5) (Fig. 20, p. 74).

Anal. Calcd. for $\text{C}_{13}\text{H}_{25}\text{O}_{14}\text{NS}_4$ (mol. Wt. 547); C, 28.40; H, 4.50; S, 23.40.

Found: C, 28.60; H, 4.61; S, 23.21.

V. Attempt to unblock 38c by partial demesylation.

To 50 mg of 3-acetamido-3-deoxy-6-O-methyl-1,2,4,5,-tetra-O-methylsulfonyl-muco-inositol (38c) in 9 ml of 80% ethanol was added 15 mg of anhydrous sodium acetate. The reaction mixture was refluxed for 4 h.

(Experiments with reflux times of 5, 6, and 24 h. were also performed).

The solution was evaporated to dryness, and the residue was extracted twice with acetone which was evaporated to dryness. Upon addition of chloroform to the residue of evaporation, the latter partially dissolved.

A small amount of crystalline material (16 mg) remained undissolved.

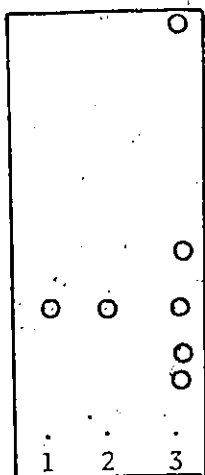
Examination by t.l.c. (in 95:5 chloroform-methanol) showed that the

chloroform solution contained a mixture of 5 compounds. The chloroform

insoluble crystals (16 mg), mp 187-189°, were examined by t.l.c. using the

same solvent (95:5 chloroform-methanol); they showed one spot (R_f 0.25)

identical with that of the starting material (38c).



1 starting material 38c

2 chloroform-insoluble crystals

3 CHCl_3 layer.

VI Epimerization of Deoxynitroinositols

a- Kinetic study of 1-deoxy-4-0-methyl-1-nitro-L-myo-inositol (37).

The kinetic study of the epimerization of the optically active L-myo inositol was conducted by following the change of optical rotation of the nitroinositol with time at different pH values. As an example for the experimental procedure, the reaction at pH 9.5 will be explained in detail. The buffer solution was prepared from 0.1M sodium bicarbonate and 0.2M sodium hydroxide⁶³ and its pH was checked with a pH meter.

Procedure: Exactly 5.65 mg of 1-deoxy-4-0-methyl-1-nitro-L-myo-inositol (37) was dissolved in 1 ml of the buffer solution (pH 9.5) at room temperature (33°). The optical rotation measurement started after 10 min. and was continued until the optical rotation remained constant.

time	α_{436}	$[\alpha]_{463}$	$\log [\alpha]_{436}$
10 min	0.198	+35	1.544
1 h	0.189	+33	1.518
2 h	0.176	+31	1.49
3 h	0.158	+27	1.44
4 h	0.134	+23.7	1.37
5 h	0.118	+20.9	1.32
6 h	0.114	+20.0	1.30
9.3 h	0.070	+12.4	1.09

The plot of $\log [\alpha]_{436}$ against time (Fig. 3) gives a straight line indicating first-order kinetics of the epimerization reaction. The half time of epimerization was obtained from the graph; $t_{1/2} = 9$ hrs.

b- Thermodynamic equilibria of deoxynitroinositols 36, 37 and 38 at different pH values

To exactly 11.15 mg of nitroinositol 36 or 37 or 38, 5 ml of buffer solution* was added at room temperature (25-27°). The solution was mixed well, closed tightly, and the tightly stoppered flask was left at room temperature for 2 days. Thereafter, 1.0 ml of the solution was neutralized by pouring it into 2 ml of 0.1N acetic acid. The resulting solution was then passed successively through 1 mL Dowex-1 X2 (or 8) (acetate form) and 1 ml Rexyn-101 (H⁺). The eluate was evaporated to dryness. The weight of the residue was ~ 2.0- 2.2 mg. The residue was dissolved by shaking in 0.15 ml of silylation reagent[†] and was left standing overnight at room temperature (27°). Then, 2 µL of the sample was injected in the vapour phase chromatograph. Each sample was measured at least twice. The V.p.c. was conducted at temperatures between 160-170°. Relative retention times T_R of the trimethylsilyl derivatives of the myo, muco and scyllo compounds were found to be 1.00, 0.80 and 1.33.

* Sørensen's glycine II⁶³

† Mixture of 17 ml pyridine plus 2 ml of 1,1,1,3,3,3-hexamethyldisilazane plus 1 ml of trimethylsilylchloride.

c- Determination of the pK_a of 1-deoxy-4-O-methyl-1-nitro-L-myoinositol (37) from ultraviolet spectrum.

The nitroinositol 37 (11.15 mg) was dissolved in 50.00 ml of 0.01N acetic acid, and 10.0 ml of the solution was diluted to 100.0 ml with the buffer solution⁶³. The pH of the resulting mixture was 9.85. The optical density of the solution was determined after 5, 7, and 15-18 minutes, no significant change being recorded within this period of time. The optical density of the pure nitronate (~ 98%) was determined in a solution prepared by diluting 5.0 ml of the stock solution of 37 to 50.00 ml with 0.10N sodium hydroxide, which resulted in pH 12.0. The results are summarized in the Table 9. Using equation 4 (p. 42), the pK_a value of L-myoinitroinositol 37 was calculated to be 10.1 for Sørensen as well as for sodium bicarbonate-sodium hydroxide buffer.

Table 9 Optical densities of 37 ($M = 1.0 \times 10^{-4}$) in buffers

buffer	pH	λ_{max} m μ	Optical density
Sørensen	9.85	250	0.370
bicarbonate, NaOH	9.85	251	0.370
0.1N NaOH	12.0	250	1.055

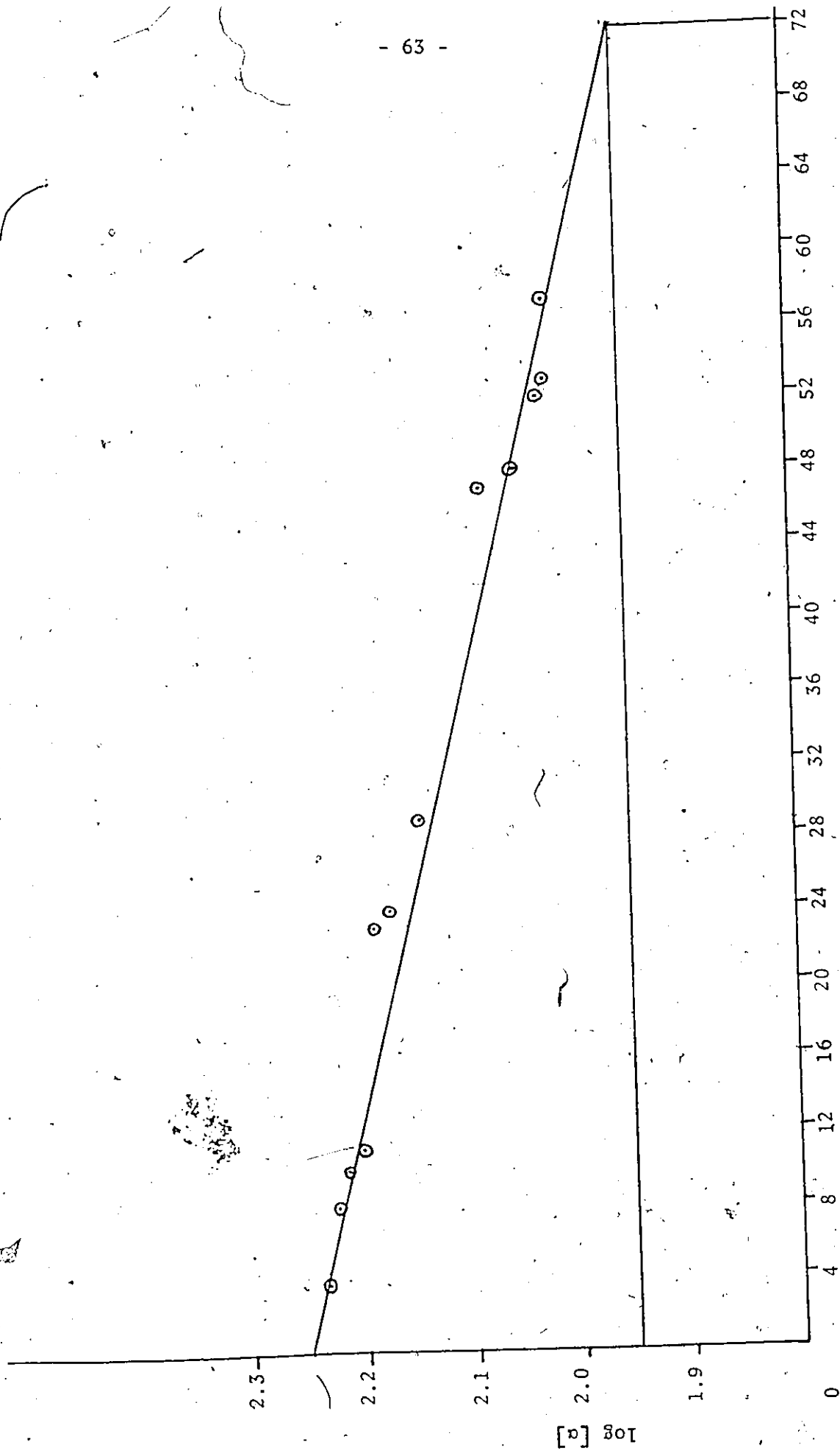


Figure 7 Kinetic study of epimerization of L-myo-nitroinositol (37) at pH 8.3

Figure 8. Kinetic study of epimerization of L-my α -nitro inositol (37) at pH 9.2

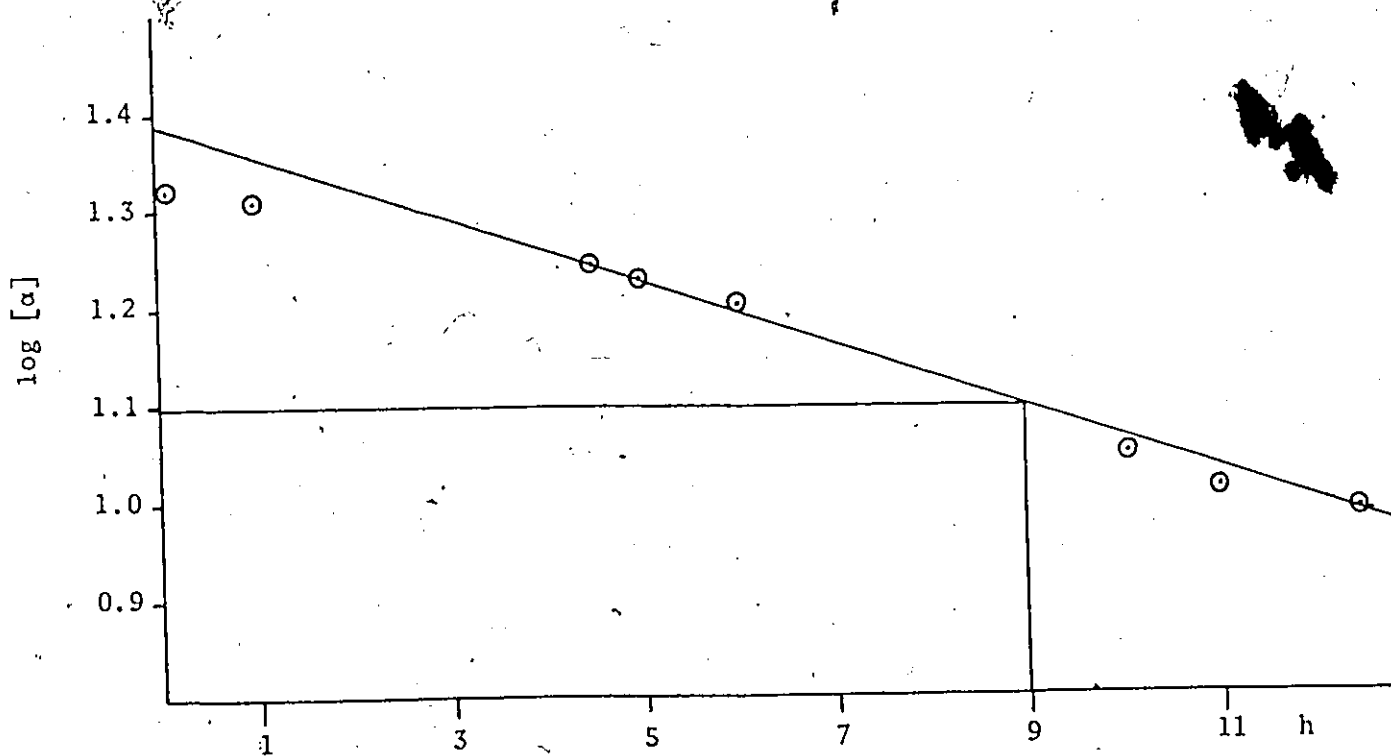


Figure 9 Kinetic study of epimerization of L-my α -nitro inositol (37) at pH 9.5

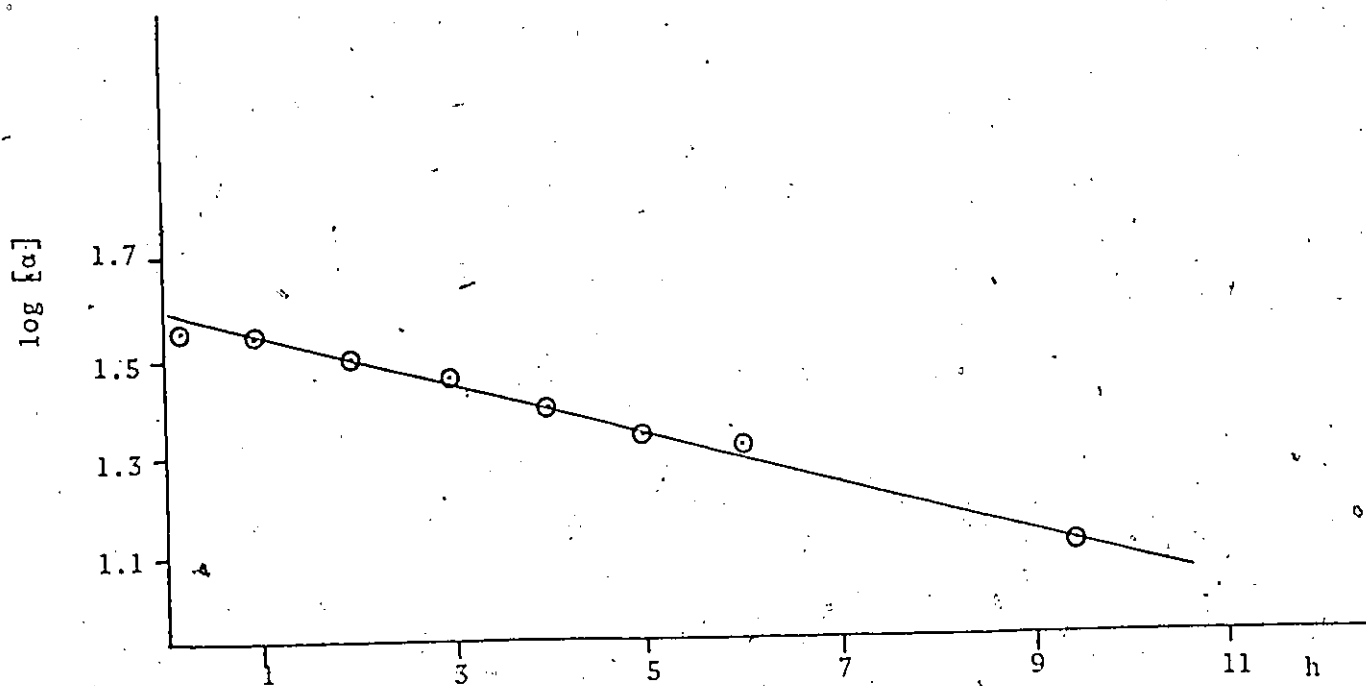


Figure 10 Kinetic study of epimerization of L-myo-nitro inositol (37) at pH 10.2

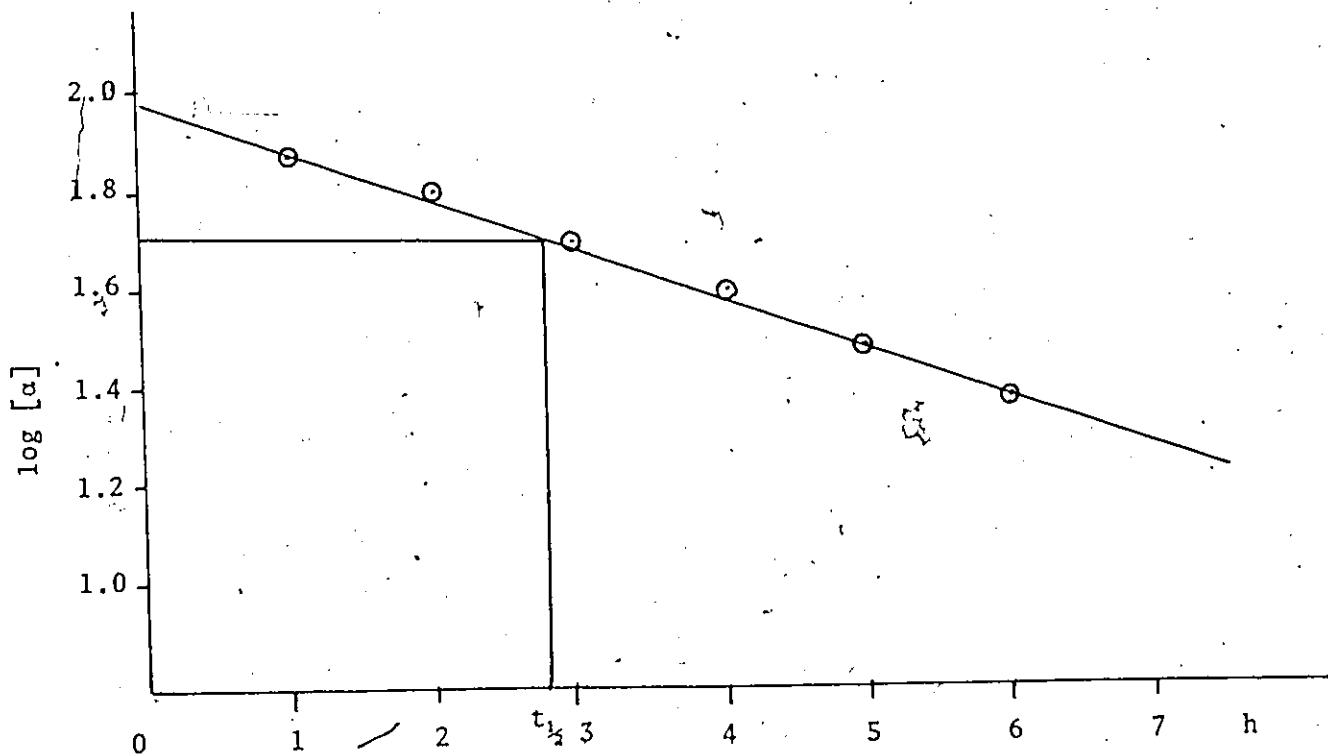
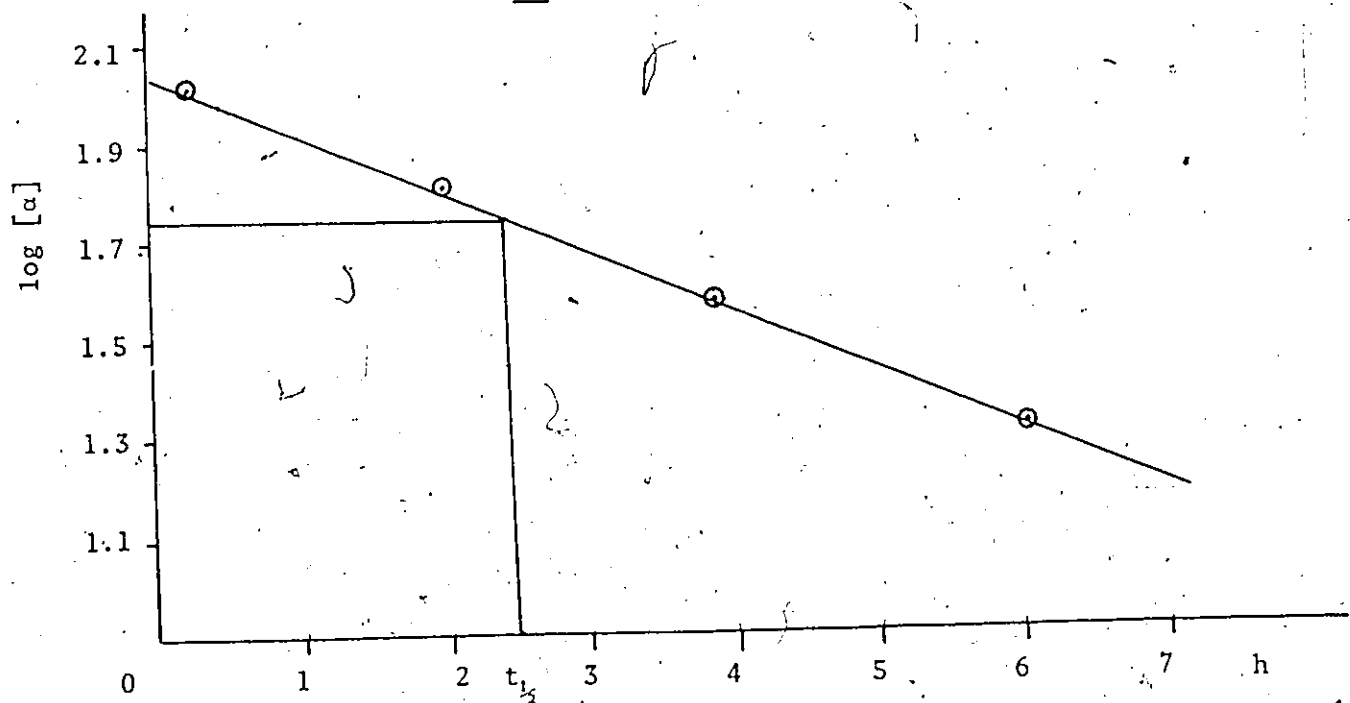


Figure 11. Kinetic study of epimerization of L-myo-nitro inositol (37) at pH 10.65



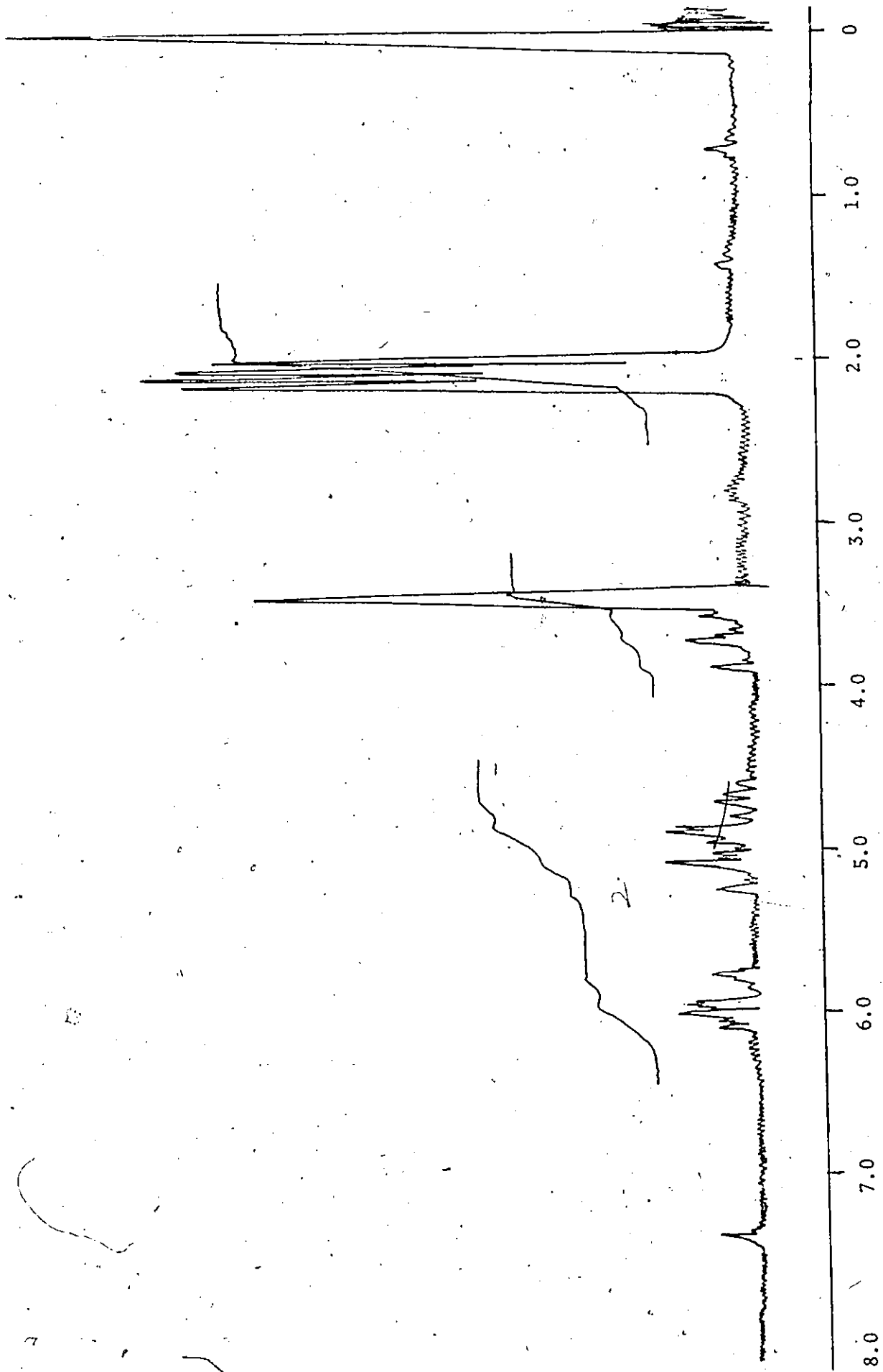


Figure 12 N.M.R. spectrum of 2,3,5,6-tetra-O-acetyl-1-deoxy-1-nitro-L-myo-inositol (37C)

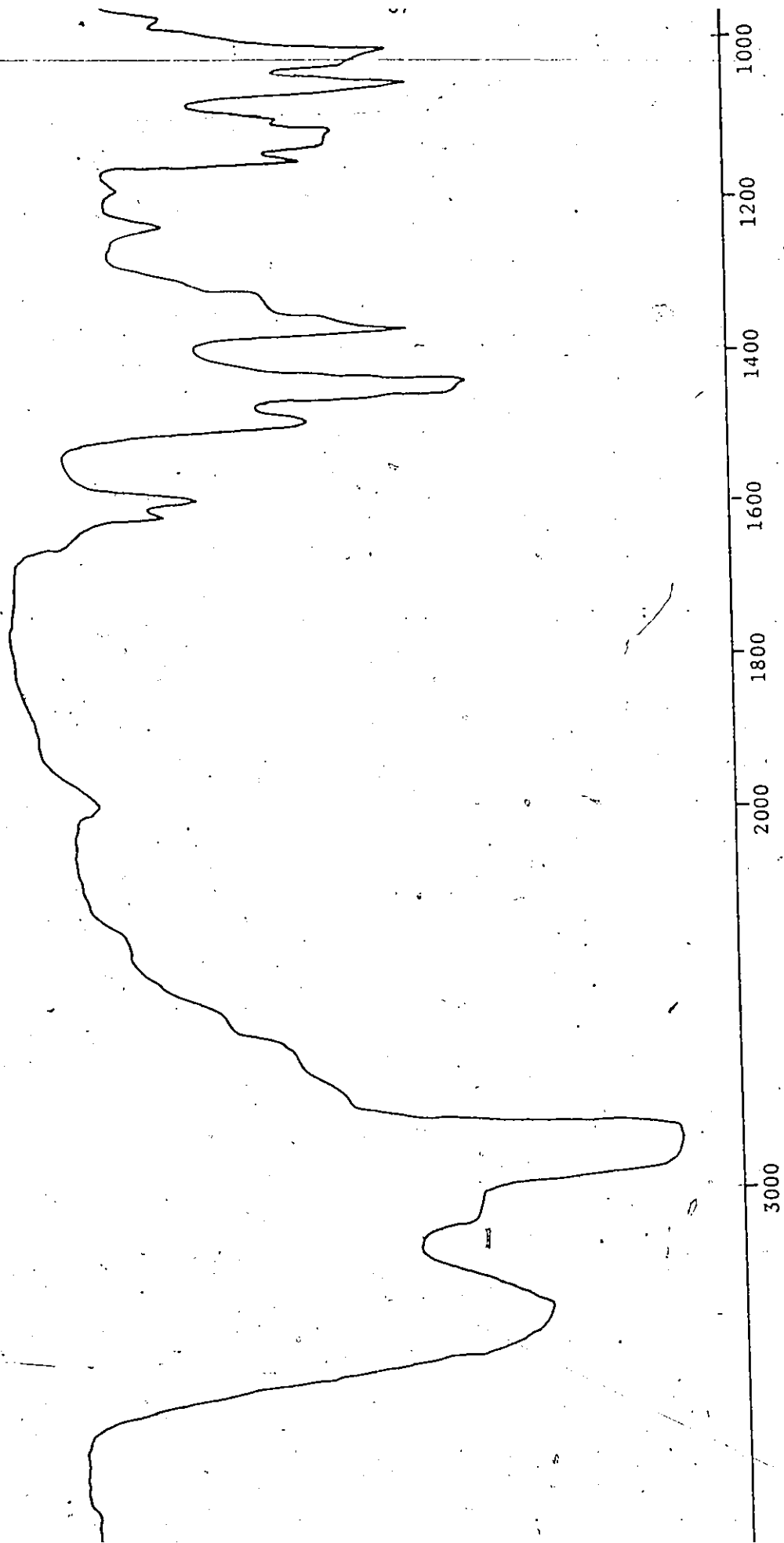


Figure 13 IR spectrum of 1-amino-1-deoxy-4-O-methyl-scyllo-inositol hydrochloride (36a).

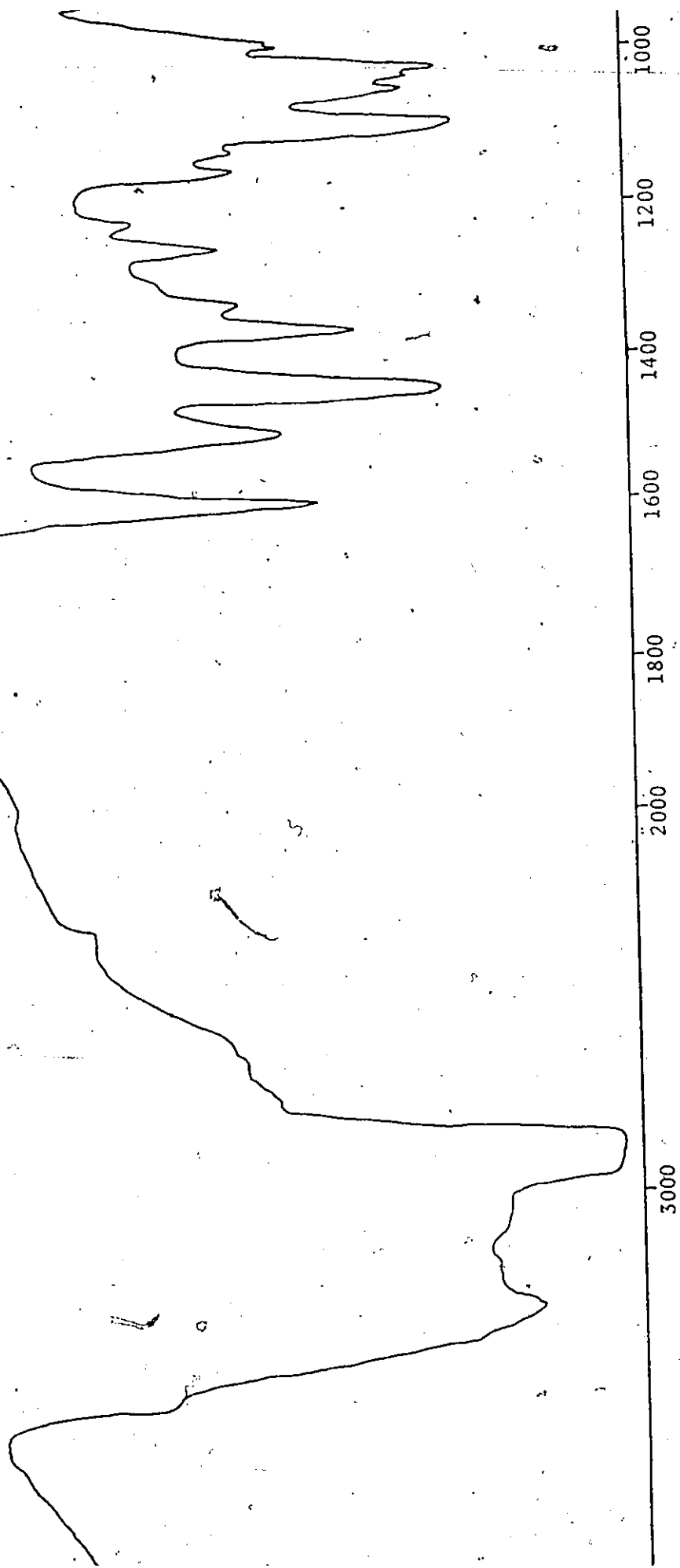


Figure 14 IR spectrum of 1-amino-1-deoxy-4-0-methyl-L-myo-inositol hydrochloride (37a)

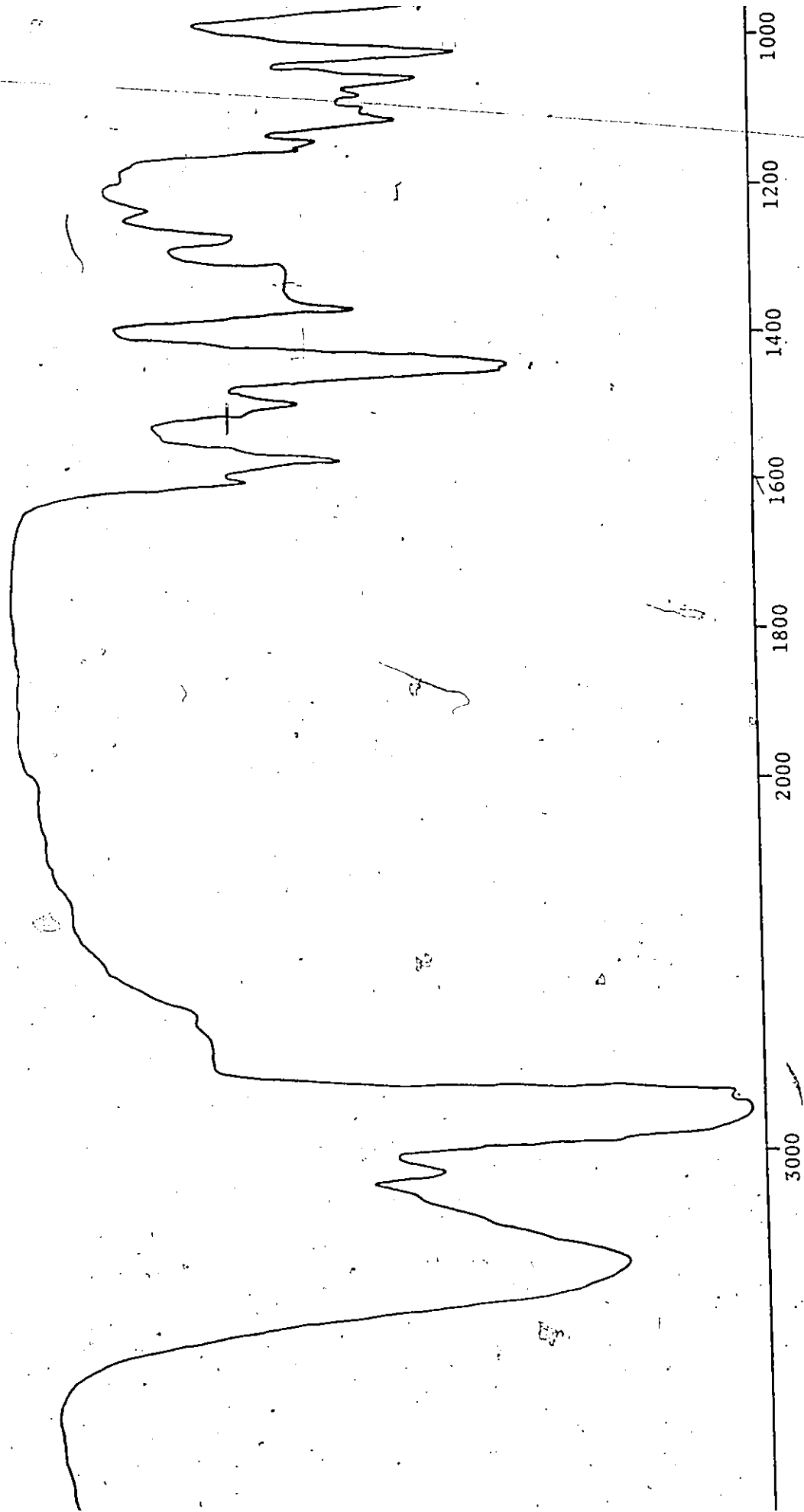


Figure 15 IR spectrum of 3-amino-3-deoxy-6-O-methyl-muco-inositol hydrochloride (38a)

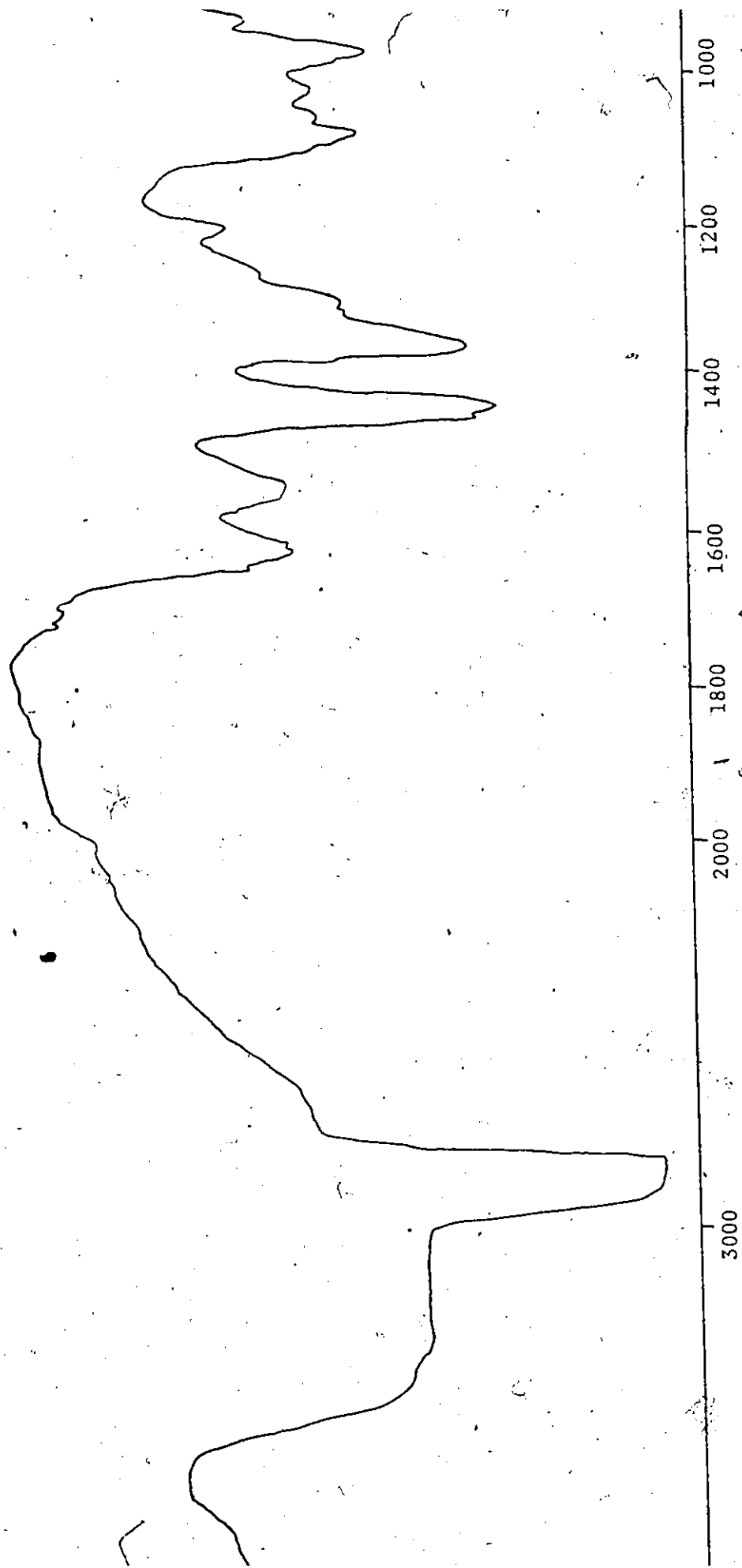


Figure 16 IR Spectrum of 1-acetamido-1-deoxy-4-O-methyl-scyllo-inositol (36b)

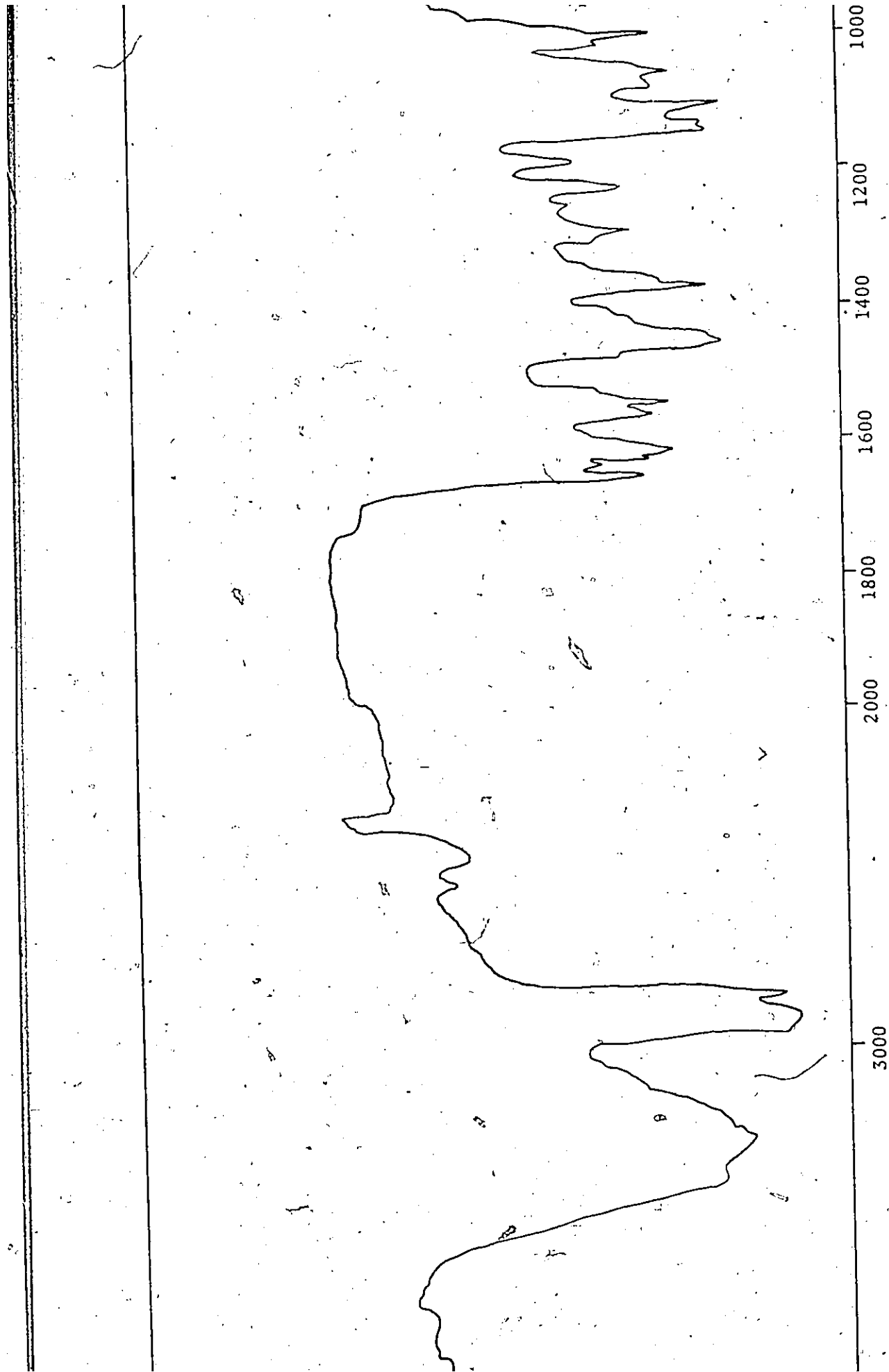


Figure 17 IR spectrum of 1-acetamido-1-deoxy-4-O-methyl-L-myo-inositol (37b).

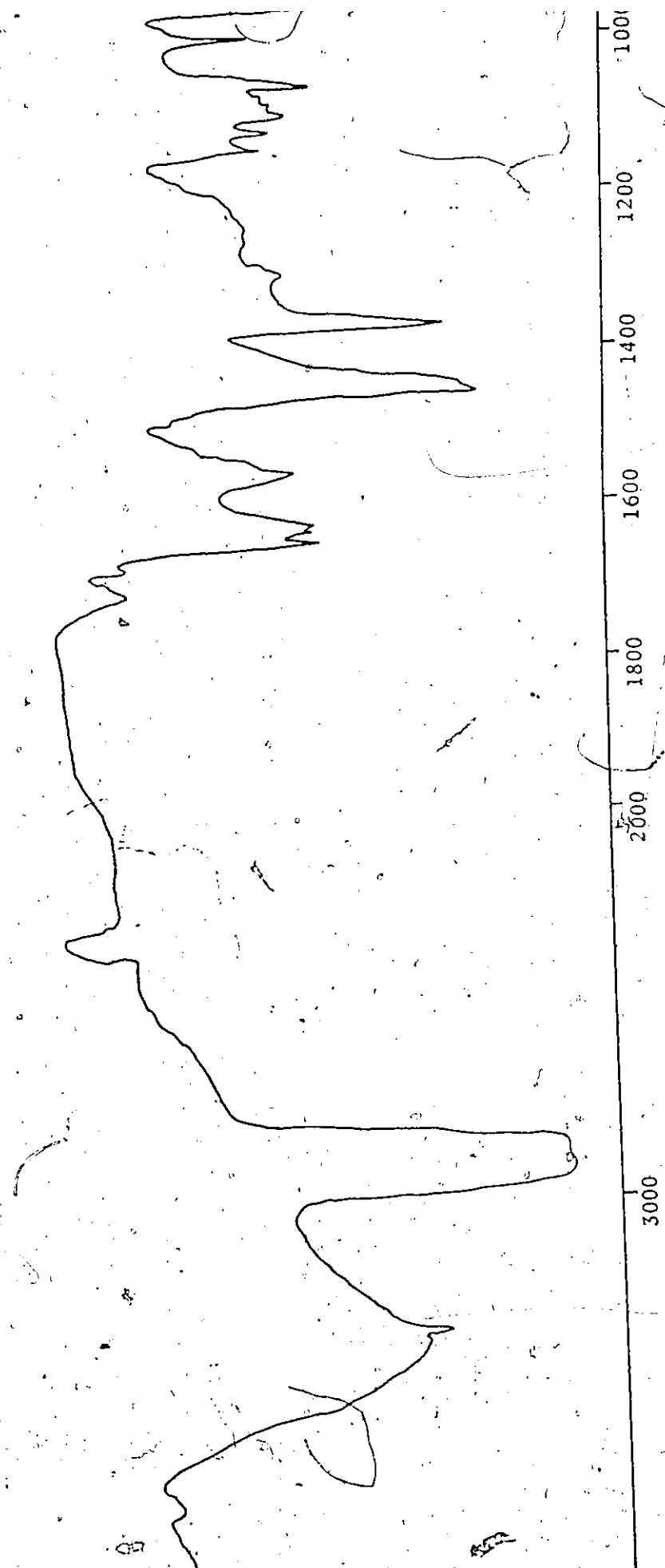


Figure 18 IR spectrum of 3-acetamido-3-deoxy-6-O-methyl-muco-inositol (38b).



1H NMR spectrum of methyl enil formyl-muco-inositol (58c)

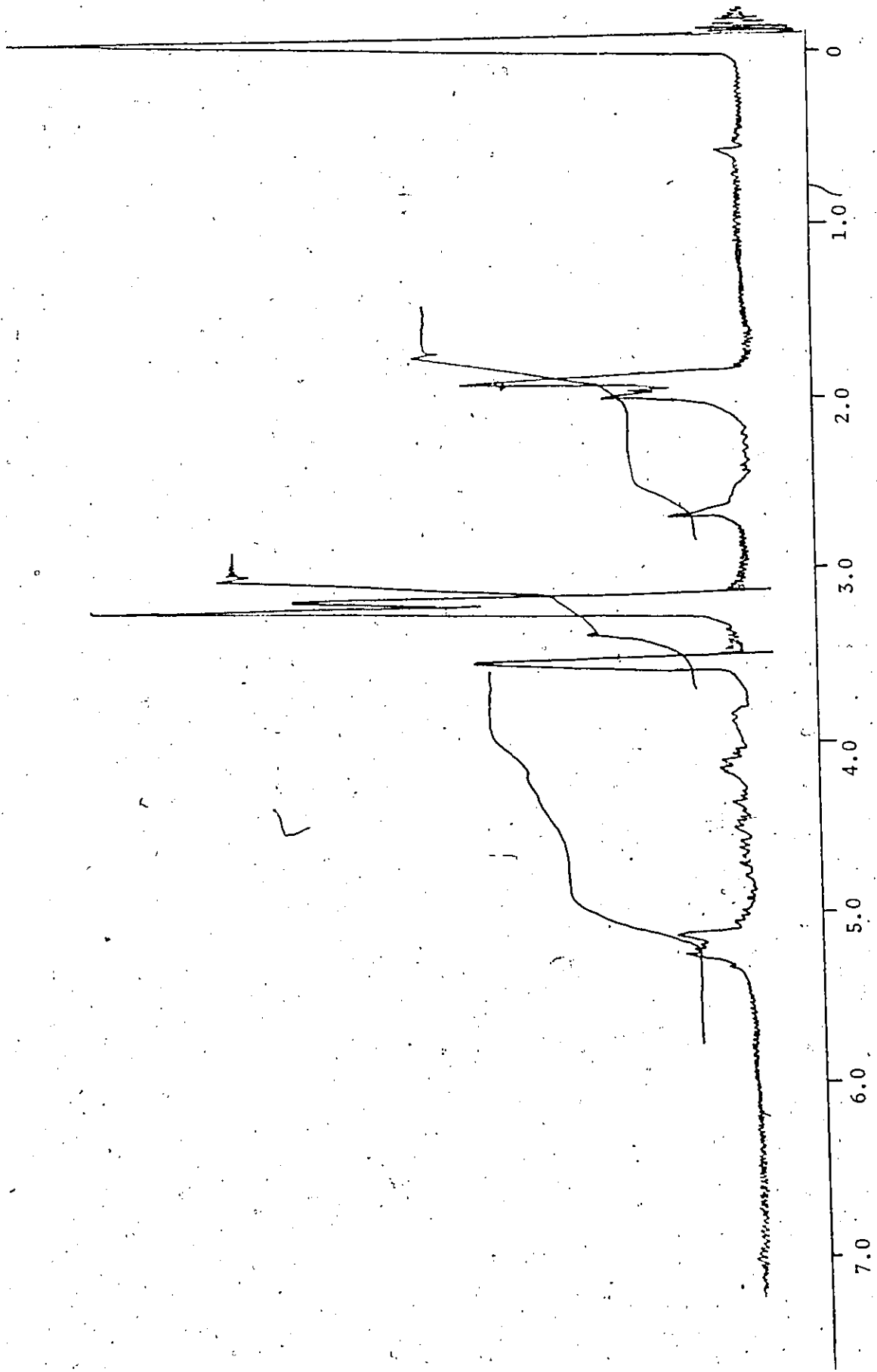


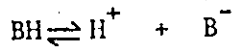
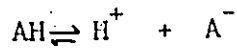
Figure 20 N.M.R. spectrum of 3-acetamido-3-deoxy-6-O-methyl 1,2,4,5-tetra-O-methylsulfonyl-muco-inositol (38c)

Derivation of Equation 5.

1. Compounds A, B, C.....(the sum of acid and base form as determined by GLC).

$$\text{i.e. } [A] = [AH] + [A^-] \quad (0)$$

2. Acid base equilibria



where from $K_A = \frac{[H^+][A^-]}{[AH]} \quad (1)$

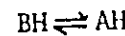
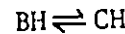
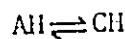
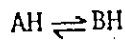
$$K_B = \frac{[H^+][B^-]}{[BH]}$$

$$K_C = \frac{[H^+][C^-]}{[CH]}$$

etc

3. Thermodynamic equilibria

a) of the acid form

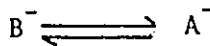
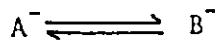


etc

where from $K_{AH/BH} = \frac{[AH]}{[BH]} \quad (3)$

$$K_{AH/CH} = \frac{[AH]}{[CH]} \quad (4)$$

b) of the nitronate form



where from
$$K_{A^-/B^-} = \frac{[A^-]}{[B^-]} \quad (5)$$

From equation (0)

$$[A^-] = [A] - [AH] \quad (5a)$$

(5a) into (1)
$$K_A = \frac{[H^+] ([A] - [AH])}{[AH]} \quad (6a)$$

also

$$K_B = \frac{[H^+] ([B] - [BH])}{[BH]} \quad (6b)$$

From (3)
$$[AH] = K_{AH/BH} [BH] \quad (7)$$

From (6b)
$$K_B [BH] + [H^+] [B] - [BH] = [H^+] [B]$$

$$K_B [BH] + [H^+] [BH] = [H^+] [B]$$

$$[BH] (K_B + [H^+]) = [H^+] [B]$$

$$[BH] = \frac{[H^+] [B]}{K_B + [H^+]} \quad (8)$$

(7) into (6a)
$$K_A = \frac{[H^+] ([A] - K_{AH/BH} [BH])}{K_{AH/BH} [BH]} \quad (9)$$

(8) into (9)

$$K_A = \frac{[H^+] ([A] \cdot K_{AH/BH} \frac{[H^+] [B]}{K_B - [H^+]})}{K_{AH/BH} \frac{[H^+] [B]}{K_B + [H^+]}} \quad (10)$$

From equation (1)

$$[A] = \frac{K_{AH/BH} K_A [B] + K_{AH/BH} [H^+] [B]}{K_B + [H^+]}$$

$$\frac{[A]}{[B]} = \frac{K_A + [H^+]}{K_B + [H^+]} \quad (6)$$

where

A = nitroinositol 36

B = nitroinositol 37

K_A = acidity constant of nitroinositol 36

K_B = acidity constant of nitroinositol 37

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