Chemistry of the Neomycins. XIII. Synthesis of Aminocyclitols and Amino Sugars *via* Nitromethane Condensations[1,2]

Seiichiro Ogawa, Kenneth L. Rinehart Jr.,* Goro Kimura, and Robert P. Johnson[3]

Department of Chemistry, University of Illinois, Urbana, Illinois 61801

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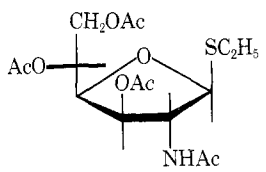
Base-catalyzed cylizations of 2-acetamido-2,6-dideoxy-6-nitro-α-D-gluco- (and -L-ido-) thiofuranosides with subsequent hydrogenations have given two known inosadiamines—streptamine and *myo*-inosadiamine-1,3—and two previously unknown optically active inosdiamines—1L-*myo*-inosadiamine-1,5 and 1L-*epi*-inosadiamine-1,3. Starting from *myo*-inosadiamine-1,3, 2-deoxystreptamine was synthesized in three steps. Neosamines B and C have also been prepared. The structures of all new compounds were determined by their nmr spectra and the reaction sequences.

Neomycin remains one of the clinically important antibiotics.[4] When structural studies on this antibiotic, actually a complex of antibiotics including neomycins B and C, neamine (neomycin A),[4] and others,[5] were nearly completed, we commenced an investigation of the mode of biosynthesis of these antibiotics.[6] Although commercially available labeled compounds (glucose-*1*-^{14}C, glucose-*6*-^{14}C, glucosamine-*1*-^{14}C, ribose-*1*-^{14}C) sufficed for studies of early steps in the biosynthesis, specifically labeled units from the antibiotics themselves were desirable for studies of later steps. These units include deoxystreptamine (**28**), neosamine C (**9b**), and neosamine B (**24**). The synthesis of these three moieties of neomycin B, again specifically labeled but with stable isotopes, was also desirable for our study of their mass spectra.

A potentially useful method for introducing label into the three units would involve the condensation of nitromethane-^{14}C with an aldehyde generated by cleavage between C-5 and C-6 of glucosamine in the furanose form. This route could lead to neosamines B and C labeled at C-6 and, after cyclization, to deoxystreptamine labeled at a ring carbon bearing an amino group. Some of the synthetic operations described have, in fact, been reported earlier by the Wolfrom group in their synthesis of streptamine.[7] In the present report we describe our investigation of the nitromethane condensation route. This report includes the syntheses of neosamine C-6-^{14}C and deoxystreptamine-*1*-^{14}C, of the unequivocal synthesis of neosamine B, and of the preparation of a number of new diaminocyclitols of potential utility as substrates for incorporation into hybrimycins.[8,9]

Synthesis of 6-Nitro Sugar Derivatives. Since the yields reported by Wolfrom in his synthesis of streptamine, especially in the base-catalyzed cyclization of the nitro sugar, were too low to use those procedures directly for our purpose, our first goal was to obtain the aldehydothiofuranoside intermediate **4a** in a pure crystalline state, for the purpose of improving the yield in the base-catalyzed condensation reaction with nitromethane. Following Wolfrom's procedure,[7,10] 2-acetamido-2-deoxy-D-glucose diethyl dithioacetal (**1**) was converted by treatment with mercuric chloride followed by acetylation to ethyl 2-acetamido-3,5,6-tri-*O*-acetyl-2-deoxy-1-thio-α-D-glucofuranoside (**2a**) in 55% yield (Figure 1). A second isomer was isolated in pure form by fractional crystallization and chromatography from this reaction mixture. This was assigned the structure ethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranoside (**3b**) (6% yield). In addition, a mixture of **3b** and the corresponding α-D-glucopyranoside (**3a**) was obtained in 7% yield.

The three compounds (**2a**, **3a**, and **3b**) were indicated to be isomeric by elemental analyses and mass spectral behavior. Both **2a** and **3a** gave molecular ions at *m/e* 391, that for **3a** being inferred from the spectrum of the mixture of **3a** and **3b** since the latter compound did not give a molecular ion peak. The highest mass ion in the spectrum of **3b** was at *m/e* 332 (M − OAc), but its trimethylsilyl derivative gave a strong ion at *m/e* 448 (M − CH_3). The melting point, 184–186°, and rotation, $[\alpha]^{28}D$ −56° (*c* 1, $CHCl_3$), of **3b** identify it as the second isomer reported by Wolfrom, *et al.* [lit. mp 179–180°, $[\alpha]^{22}D$ −42° (*c* 2, $CHCl_3$)],[7] who assigned to it the β-D-glucofuranoside structure (**2b**). However, the nmr spectrum of **3b** (see Experimental Section) identifies it as a glucopyranose derivative, with trans-diaxial coupling of all ring protons. The chemical shifts and coupling patterns of the protons of **2a** are quite different (see Experimental Section), in agreement with those expected for a furanoside. The purity of **3a** did not allow complete assignment of chemical shifts and coupling constants to its protons, but the anomeric proton was apparent, at δ 5.75 (J = 5.7 Hz).



2b

O-Deacetylation of **2a** with sodium methoxide in absolute methanol followed by oxidative cleavage between C-5 and C-6 with sodium metaperiodate[11a] gave ethyl 2-acetamido-2-deoxy-1-thio-α-D-*xylo*-pentodialdo-1,4-furanoside (**4a**), which was crystallized from ethanol to give its crystalline ethanol solvate, **4b**. Both the solvate and **4a** apparently exist as a hemiacetal, perhaps a dimer of the type described by Schaffer and Isbell for 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanoside.[11b] No aldehyde carbonyl absorption was found in their infrared spectra, and both the nmr spectra contained little or no aldehyde absorption but displayed absorption for an extra hemiacetal proton at δ 5.4 (one proton). The solvate **4b** contained ethoxyl group absorption at δ 3.72 (q) and 1.21 (t). Reduction of **4b** with sodium borohydride in aqueous solution gave ethyl 2-acetamido-2-deoxy-1-thio-α-D-xylofuranoside (**5**).[11a] The yields of **1**, **2a**, and **4b** were 60, 65, and 71%, respectively, in their individual preparative steps. Thus, the overall yield of **4b** from *N*-acetylglucosamine was 29%.

Treatment of **4b** with an equimolar amount of nitromethane in the presence of sodium methoxide catalyst at 0–5° yielded two crystalline condensation products which could be separated either by fractional crystallization from absolute ethanol or on a specially prepared silica gel column.[12a] Compound **7**, shown below to be the D-gluco isomer, was isolated in 23% yield from **4b** (16% from **2a**) and had mp 115–118°; compound **6**, the L-ido isomer, was

Table I
Nmr Methyl Absorptions of Peracetyl-1,3-inosadiamines

Configuration	Chemical shift, δ[a]	Solvent[b]	Ref
scyllo-1,3 (**11**)	1.94 (4), 1.75 (2)	D	This work
	1.90 (4), 1.70 (2)	D	43
	2.03 (2), 1.98 (2), 1.91 (2)	C	43
	2.06 (4), 1.92 (2)	W	43
myo-1,3 (**12**)	2.19, 1.96 (2), 1.94, 1.78 (2)	D	This work
	2.14, 1.92 (2), 1.89, 1.74 (2)	D	43
	2.26, 2.06 (2), 2.03, 1.90 (2)	C	43
13	2.32, 2.15, 1.93 (4), 1.73	D	This work
	2.38, 2.33, 2.03 (2), 2.00 (2), 1.92	C	This work
	2.37, 2.32, 2.04 (2), 2.00 (2), 1.93	C	12b
myo-1,5 (**18**)	2.13, 1.93, 1.89 (2), 1.76, 1.71	D	This work
epi-1,3 (**19**)	2.16, 2.12, 1.97, 1.88, 1.80, 1.77	D	This work
	2.19, 2.14, 2.04, 1.97, 1.92, 1.89	C	This work
muco-1,3	2.08, 2.05, 2.00 (3), 1.96	C	16
myo-2,4	2.10, 2.05 (2), 2.01 (2), 1.93	W	42
chiro-1,3	2.16, 2.04 (3), 1.98 (2)	C	16
chiro-1,5	2.18, 2.01 (3), 1.98 (2)	C	16

[a] Number of methyl groups indicated in parentheses; one except as noted. [b] D = DMSO-d_6; C = $CDCl_3$; W = D_2O.

isolated in 32% yield from **4b** (23% from **2a**) and had mp 206–208°.

Since isolation and purification of the low melting isomer (**7**) lowered its yield, an alternative sequence was employed in which fractional crystallization from ethanol gave the chromatographically pure high melting isomer **6** in 15% yield from **4b**, and the syrupy product obtained on evaporating the mother liquor, rich in **7**, was subjected directly to the next hydrolysis, involving a slight excess of mercuric chloride in hot water. This afforded the crystalline 2-acetamido-2,6-dideoxy-6-nitro-D-glucopyranose (**8**) in 35% yield. Compound **8**, identified by elemental analyses and infrared spectrum, was hydrogenated over platinum in acidic solution to give an oil (**9a**), which was hydrolyzed with 6 *N* hydrochloric acid to afford (almost quantitatively) 2,6-diamino-2,6-dideoxy-D-glucose (**9b**), also identified as its crystalline di-*N*-acetyl derivative (**9c**). The synthesis of diaminoglucose assigns the low melting isomer as ethyl 2-acetamido-2,6-dideoxy-6-nitro-1-thio-α-D-glucofuranoside (**7**); thus, the high melting isomer is the β-L-idofuranoside (**6**). Earlier work[7] had not assigned the stereochemistry of the two isomers.

Barium Hydroxide Cyclizations of 6-Nitro Sugars. Next, the base-catalyzed cyclization of the higher melting 2-acetamido-2,6-dideoxy-6-nitrohexose[7] was reexamined. The thioethyl group of **6** was hydrolyzed by mercuric chloride and the nitro sugar formed (**10**, Figure 1) was not isolated but was subjected to alkaline condensation using barium hydroxide at room temperature (Figure 2). The mixture of nitro compounds obtained was isolated as an amorphous mixture of barium salts which was hydrogenated in acidic solution over a platinum catalyst. The resultant diaminocyclitol derivatives were acetylated to give a mixture, from which three compounds were isolated by fractional crystallization from ethanol. Two of the derivatives obtained from **6** after the barium hydroxide cyclization were readily identified as hexaacetylstreptamine (**11**) and hexaacetyl-*myo*-inosadiamine-1,3 (**12**) by their melting point behavior and infrared spectra, as well as by the chemical shifts of their acetyl protons (Table I). The nmr spectra (Table I) of the third product, **13**, showed peaks for seven acetyl methyl groups at positions reported earlier for heptaacetylstreptamine,[12b] and the mass spectrum contained a protonated molecular ion at *m/e* 473.177 ($M + H)^+$ appropriate for a heptaacetylinosadiamine, with a much more intense ion at *m/e* 412 (M − HOAc). The structure of **13** was confirmed by its hydrolysis to streptamine.

Compounds **11**, **12**, and **13** were obtained in 10, 11, and 6% yields, respectively, from **6**; the total yield of *myo*-inosadiamine derivatives is thus 11% and of *scyllo*- 16%. It is of some interest to note here that the Wolfrom group isolated two products from the higher melting isomer in their streptamine synthesis, in very poor but approximately equal yields.[7] One was shown to be streptamine. From the optical inactivity of streptamine and Fischer's results,[13] which indicated that alkaline carbonyl reactions give only trans configurations, Wolfrom, Olin, and Polglase[7] deduced the configuration of streptamine as the all-trans isomer of 1,3-inosadiamine. They did not report the rotation of the second isomer and tentatively assigned to it the muco-1,3 configuration which would be the other product containing only trans configurations at the new asymmetric centers. However, had they observed that the second isomer, isolated from the reaction mixture in yield similar to that of streptamine, was also optically inactive, they could not have assigned the configuration of streptamine, although their assignment was subsequently shown to be correct by X-ray[14] and nmr[15] evidence.

The crystalline hydrolysis product **8**, from the D-gluco isomer **7**, was also subjected to barium hydroxide catalyzed cyclization, followed by reduction and acetylation (Figure 2). Two hexaacetylinosadiamines were isolated from the reaction mixture and were identified as hexaacetylstreptamine (**11**) and hexaacetyl-*myo*-inosadiamine-1,3 (**12**), respectively. The third product (**13**) was not isolated.

The isolation of **11** and **12** from **8** as well as from **6** requires an inversion of the stereochemistry found at C-5 in **8**. This could occur in the nitro sugars, but isomerization of the sugar would require loss of nitromethane and recondensation. Thus, it seems more likely that the isomerization occurs after cyclization to the nitrocylitol.

Sodium Methoxide Cyclization of 6-Nitro Sugars. The crude syrupy nitro sugar (**10**) obtained by hydrolysis of **6** with mercuric chloride was dissolved in absolute methanol and treated with an equimolar amount of sodium methoxide at 0–5° for 12 hr (Figure 2). Work-up yielded crude, crystalline **15**, which on recrystallization from methanol afforded the pure isomer in 67% yield. The structure of **15** was established by its hydrogenation to the corresponding inosadiamine, which on acetylation gave hexaacetyl-*myo*-inosadiamine-1,3 (**12**). No evidence was found for isomeric nitrocyclitols or hexaacetylinosadiamines. Thus, this base appears to be much more selective in catalyzing cyclizations than barium hydroxide. It is also of

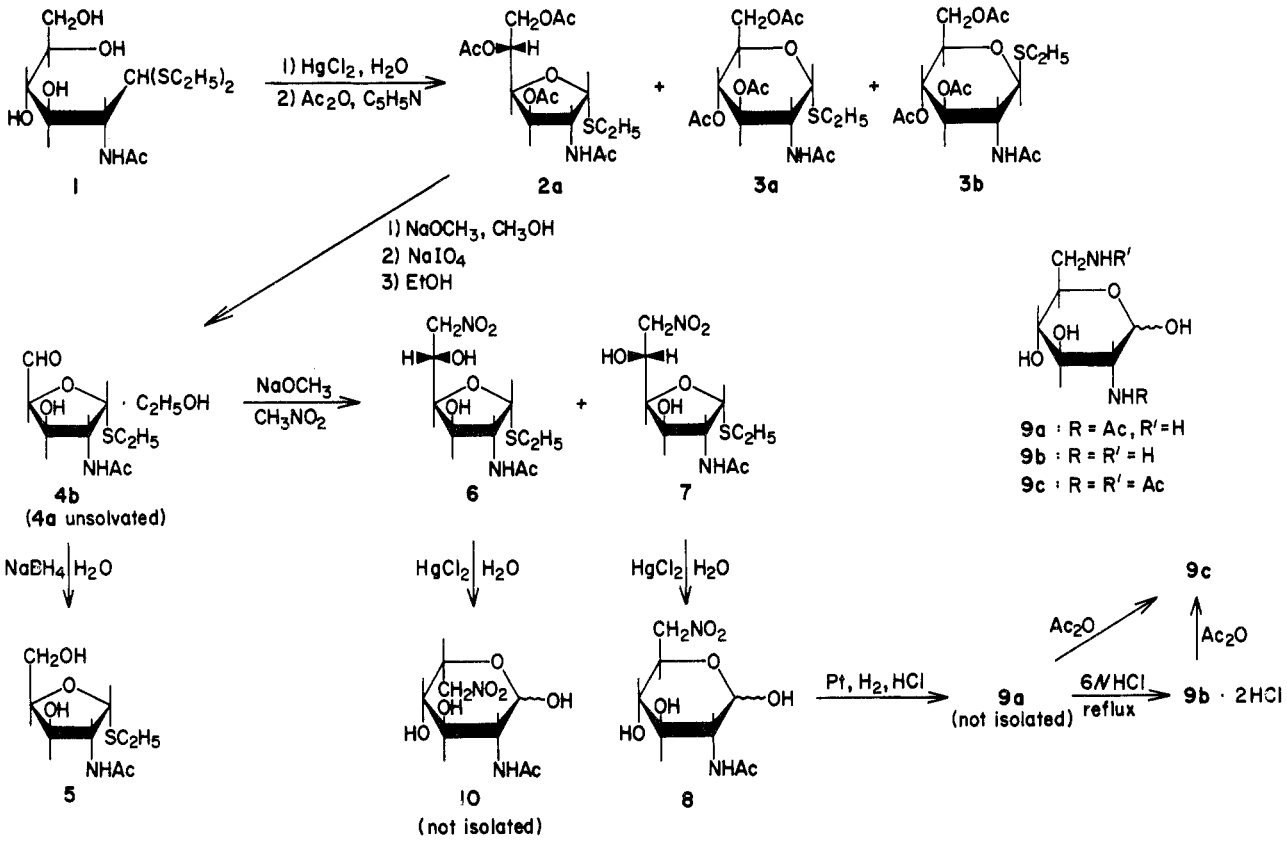


Figure 1. Synthesis of derivatives of 6-nitro-2-acetamido sugars from *N*-acetylglucosamine diethyl dithioacetal (1).

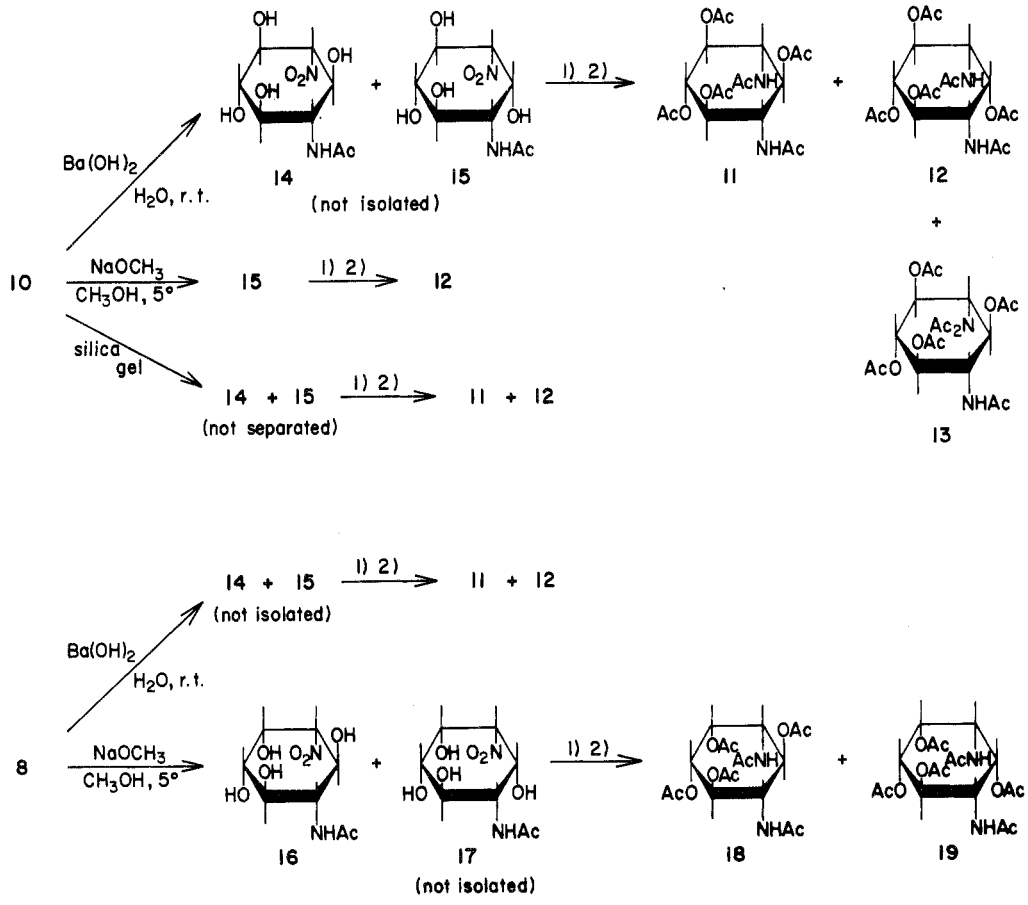


Figure 2. Preparation of diamino inositols from the D-*gluco*-6-nitro sugar derivative 8 and the L-*ido*-6-nitro sugar derivative 10. Conditions: (1) Pt, H_2, HCl; (2) Ac_2O, C_5H_5N.

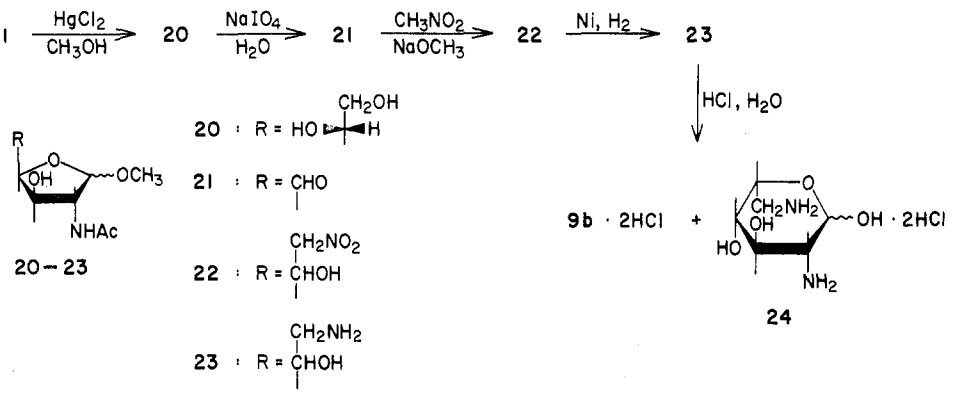


Figure 3. Synthesis of neosamine B (24) from *N*-acetylglucosamine diethyl dithioacetal (1).

interest that the preferred isomer is not that which is presumably most stable, the all-trans scyllo isomer **14**. Apparently some degree of kinetic control *obtains*.

When compound **8** was dissolved in absolute methanol and treated with equimolar sodium methoxide at 0–5° for 12 hr (Figure 2), work-up afforded a mixture of *N*-acetylnitrodeoxyinosamines—1L-5-acetamido-1,5-dideoxy-1-nitro-*myo*-inositol (**16**) and 1L-1-acetamido-1,3-dideoxy-3-nitro-*epi*-inositol (**17**)—in 72% combined yield. The mixture of **16** and **17** was hydrogenated catalytically and subsequent acetylation gave a mixture of hexaacetylinosadiamines, which could be separated by fractional crystallization from ethanol to afford 1L-hexa-*N,O*-acetyl-*myo*-inosadiamine-1,5 (**18**) and 1L-hexa-*N,O*-acetyl-*epi*-inosadiamine-1,3 (**19**) in 67 and 15% yields, respectively, from the mixture of **16** and **17**. In a separate experiment nitroinositol **16** was purified and converted separately to **18**.

In assigning the stereochemistry of **18** and **19** it was first assumed that the configurations at C-2, C-3, and C-4 of D-glucosamine must be retained and substituents at these positions of the inosadiamines must all be trans to one another. With that restriction there are eight theoretically possible isomers obtainable from the reaction,[13] six optically active forms—1L-myo-1,5, 1L-myo-2,4, 1L-epi-1,3, 1L-chiro-1,3, 1D-chiro-1,5, and 1L-muco-1,3—and two meso forms—scyllo-1,3 and myo-1,3. Six isomers have been previously reported, at least as racemates.[16-20]

Nmr spectra of **18** and **19** were determined in dimethyl sulfoxide-d_6, the solvent which gives the most reliable information relative to axial *vs.* equatorial acetoxyl and acetamido groups.[43] The nmr spectrum (Table I) of **18** in DMSO-d_6 showed five sharp signals with relative intensities of 1:1:2:1:1 at δ 2.13, 1.93, 1.89, 1.76, and 1.71, respectively. These can be ascribed to one axial acetoxyl group, three equatorial acetoxyl groups, and two equatorial acetamido groups, respectively.[19] Of the six optically active inosadiamines above, only the myo-1,5 configuration would satisfy these spectral data.

The nmr spectrum (Table I) of **19** in DMSO-d_6 showed six signals with relative intensities of 1:1:1:1:1:1 at δ 2.16, 2.12, 1.97, 1.88, 1.80, and 1.77, respectively. These can be assigned as two axial acetoxyl groups, two equatorial acetoxyl groups, and two equatorial acetamido groups, respectively.[19] Therefore, compound **19** is assigned the epi-1,3 configuration.

The configurations of the inosadiamines obtained from the sodium methoxide cyclizations at low temperature (0–5°) indicate that in this reaction, in contrast to the barium hydroxide reactions, isomerization of the nitroinositols (or nitro sugars) does not take place and that the original configuration at C-5 of the sugars is preserved during the reaction.

Synthesis of Neosamine B. In an attempt to prepare neosamine B, compound **6** was hydrolyzed in the presence of mercuric chloride. A thin layer chromatogram of the crude hydrolyzate showed a single major spot, along with a trace of starting material. An attempt was made to purify the nitro sugar (**10**) on a silica gel column; elution with chloroform containing 20% methanol gave homogeneous crystals accounting for a 77% yield. Although elemental analyses and an infrared spectrum were satisfactory for a nitroacetamido sugar, the compound's mobility in tlc was quite different from that of the original crude product. When the crystalline material was hydrogenated catalytically, compounds **11** and **12** were obtained after subsequent acetylation, in 9 and 78% yields, respectively. Apparently the crude L-ido nitro sugar had cyclized on the chromatographic column, to afford cyclitols, mainly of the myo-1,3 configuration.[22] The crystals isolated must then have been composed of 1L-1-acetamido-1,3-dideoxy-3-nitro-*scyllo*-inositol (**14**) and 1L-1-acetamido-1,3-dideoxy-3-nitro-*myo*-inositol (**15**) in the approximate ratio of 1:9.

Several additional attempts were made to prepare neosamine B from the 1-thio-L-idofuranoside. For instance, on hydrogenation of the crude nitro sugar (**10**) in acidic medium or on direct methanolysis of **6** in the presence of acidic catalyst and subsequent hydrogenation, neosamine B was sometimes detected by paper chromatography. However, many by-products, usually aminocyclitols, were also formed and the results were inconclusive.

In an effort to find an alternative route to neosamine B, that shown in Figure 3 was devised. Whitehouse and Kent[23] have reported the synthesis of methyl 2-acetamido-2-deoxy-β-D-glucofuranoside by treatment of **1** with mercuric chloride in anhydrous methanol. When this preparation was repeated in the present study, the product could not be crystallized and paper chromatography revealed the presence of two major components and traces of four NH-containing impurities. This mixture was separated by chromatography over charcoal; the major component was isolated in 30% yield, but could not be crystallized, although it was homogeneous by paper chromatography. Presumably it was a mixture of α and β anomers (**20**), not distinguishable by paper chromatographic techniques. Analytical periodate oxidation demonstrated a very rapid 1-mol uptake of oxidant, with formation of formaldehyde as expected for the furanoside structure.[23]

Glycol cleavage was accomplished on a preparative scale with a slight excess of sodium metaperiodate in aqueous solution. The deionized reaction mixture was strongly reducing to AHP, indicating the presence of the intermediate aldehyde (**21**). No attempt was made to isolate this aldehyde; rather, it was immediately condensed with excess nitromethane under basic catalysis to give the

Table II
Comparison of Natural and Synthetic 2,6-Diamino-2,6-dideoxy-L-idose

	Synthetic	Neosamine B	Ref
Dihydrochloride (**24**), $[\alpha]_D$	+23.9° (*c* 1.9, H_2O)	+17.5° (*c* 0.9, H_2O)	24
N-Acetyl derivative			
$[\alpha]_D$	+7.0° (*c* 1.9, H_2O)	+5.0°, +6.0° (*c* 1.0, H_2O)	25
R_t, PEaAW[a]	0.59	0.59	
R_t, BAW 415[a]	0.44	0.45	
M_g, borate electrophoresis[a]	0.42	0.42	
Mp, *p*-nitrophenylhydrazone	211–215°[b]	215–218°[b]	25

[a] See Experimental Section for details. [b] Mmp 212–217°.

crude mixture of epimers (**22**), in 86% yield. In view of the possible instability of the 6-nitro isomers and the facile separation procedure available for the final product diamines, it was decided to postpone epimer separation until the last stages of the synthesis. Accordingly, half of the crude mixture was immediately hydrogenated over Raney nickel. The crude primary amine mixture (**23**) gave a very strongly positive test with ninhydrin and on vigorous acidic hydrolysis gave a mixture of mono- and diamino sugars, which was separated into three diamine components (peaks I, II, III) by ion-exchange chromatography. The first (and major) diamine component was chromatographed over cellulose as its *N*-acetyl derivative to give two fractions. One of these was identified as 2,6-diacetamido-2,6-dideoxy-D-glucose (**9c**) by comparison of optical activity, melting point, and paper chromatographic behavior with those of an authentic sample of diacetylneosamine C (**9c**). This was isolated in 9% crude yield, with 3% obtained in crystalline form, based on the starting methyl furanoside **20**. The other N-acetylated component of peak I, isolated as a glass in approximately 7% yield, has not been identified. It resembled a diacetamidohexitol in its paper chromatographic mobility and lack of reaction with AHP, but was different from 2,6-diacetamido-2,6-dideoxy-D-glucitol in optical activity.

The second diamino component from the ion-exchange column (peak II), isolated as a glass in less than 1% yield, also behaved after N-acetylation as a diacetamidohexitol in paper chromatography and color tests. Insufficient material was available for further purification or comparison with known diacetamidohexitols.

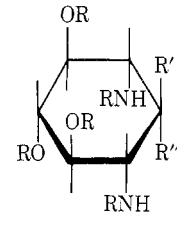
The third diamine component (peak III), 2,6-diamino-2,6-dideoxy-L-idose dihydrochloride (**24**), was obtained in 5% yield as a hygroscopic glass. This product was ninhydrin and AHP positive, indicative of the primary amine and reducing carbohydrate functions expected. Its point of elution on the gradient elution chromatography curve near 2,6-diamino-2,6-dideoxy-D-glucose indicates that there are two amino groups per molecule. L-Ido stereochemistry was adduced for this material on the basis of its mode of formation.

The synthetic material was compared with natural neosamine B as summarized in Table II: optical activity of the dihydrochloride; optical activity of the *N*-acetyl derivative; mobility of the borate complexes of the *N*-acetyl derivatives in electrophoresis, a technique previously shown[27] to be extremely sensitive to conformational differences; paper chromatographic behaviors of the *N*-acetyl derivatives; and melting point behavior of the *N*-acetyl derivatives.

Synthesis of Neosamine C-6-^{14}C and 2-Deoxystreptamine-1-^{14}C. Synthetic methods developed in previous sections of the present report appeared to lend themselves well to the preparation of specifically labeled subunits of the neomycins, and these expectations have already been realized for neosamine C and deoxystreptamine.

Neosamine C-6-^{14}C was prepared by the route shown in Figure 1 *via* compounds **4b** and **8**. Label was introduced *via* nitromethane-^{14}C. The overall yield of labeled **8** was 34% (from **4b**) and that of crude neosamine C-6-^{14}C (labeled **9b**) was 56% (from **8**).

Suami, *et al.*,[26,27] recently reported a two-step synthesis of 2-deoxystreptamine (**28**) starting from *myo*-inosadiamine 1,3-dihydrochloride (**25**) and we have followed that route in the preparation of labeled **28** from **6**. Labeled **12** was prepared as shown in Figure 2, by the silica gel cyclization. The hexaacetate **12** was easily hydrolyzed with boiling 6 *N* hydrochloric acid to give the dihydrochloride **25** in 96% yield. Under conditions slightly modified from



25, R = R′ = H; R″ = OH
26, R = Ac; R′ = Br; R″ = H
27, R = Ac; R′ = R″ = H
28, R = R′ = R″ = H

those earlier reported,[26,27] we obtained a somewhat better yield (81%) in the introduction of bromine into **25**. Debromination was effected by treating pentaacetyl-2-bromo-2-deoxystreptamine (**26**) with zinc, acetic anhydride, and water at room temperature to give pentaacetyl-2-deoxystreptamine (**27**) in 82% yield. Compound **27** was hydrolyzed with boiling 6 *N* hydrochloric acid to give deoxystreptamine dihydrochloride (**28**) in 96% yield. The overall yield (based on labeled nitromethane) was 5.0%.

Experimental Section[28]

2-Acetamido-2-deoxy-D-glucose diethyl dithioacetal (**1**) was prepared from 50.0 g of 2-acetamido-2-deoxy-D-glucose, mp 205–208° dec [lit.[34] mp 203–205° (dec)], by the procedure of Wolfrom and Anno[35] except that residual lead carbonate was removed by Amberlite MB-3 resin, and the filtrate was concentrated to yield 18.7 g of crystalline **1**, mp 128–129°, $[\alpha]^{27}D$ −30.5° (*c* 0.96, H_2O) [lit.[35] mp 130–131°; $[\alpha]^{23}D$ −35° (*c* 4.0, H_2O)]. The mother liquor yielded an additional 26.0 g of the diethyl dithioacetal, mp 124–127°. The total yield of **1** was thus 64%.

Reaction of 2-acetamido-2-deoxy-D-glucose diethyl dithioacetal (1) with mercuric chloride was carried out according to the procedure described by Wolfrom, *et al.*,[7,11a] employing 10.0 g of **1** and freshly prepared mercuric oxide.[36] The acetylated product began to crystallize when it was poured into ice and water. The crystals were filtered and washed with water; the yield was 6.88 g of ethyl 2-acetamido-3,5,6-tri-*O*-acetyl-2-deoxy-1-thio-α-D-glucofuranoside (**2a**), mp 123–125°. Recrystallization from ethanol and water gave pure needles: 6.09 g; mp 124–125°; $[\alpha]^{28}D$ +147° (*c* 3.95, $CHCl_3$) [lit.[7] mp 124.5–125.5°; $[\alpha]^{23}D$ +140° (*c* 4.0, $CHCl_3$)]. The mother liquor was evaporated and extracted with chloroform to give a second crop of crystals, 0.90 g, mp 120–122°. Recrystallization gave pure crystals, 0.63 g, mp 123–124°. The total yield of a slightly impure **2a** was thus 7.78 g (65%), that of pure crystals 6.72 g (56%). The nmr spectrum ($CDCl_3$) of **2a** contained the following absorptions, with assignments confirmed by spin decoupling: δ 1.29 (t, 3, *J* = 7.2 Hz, $SCCH_3$), 2.01 (s, 6, $COCH_3$), 2.06 (s, 6, $COCH_3$), 2.66 (q, 2, *J* = 7.2 Hz, SCH_2), 4.13 (d of d, *J* = 13.0,

5.8 Hz, H-4), 4.49 (m, 2, H-6), 4.57 (m, J = 7.8, 6.0, 4.1 Hz, H-2), 5.28 (m, H-5), 5.45 (d of d, J = 5.8, 4.1 Hz, H-3), 5.72 (d, J = 6.0 Hz, H-1), 6.70 (d, J = 7.8 Hz, NH). The infrared spectrum (Nujol) contained a strong band at 1750 cm^{-1} not found in the spectrum of **1**.

The mother liquor from the second crop of **2a** was concentrated to *ca.* 30 ml, and the mixture was kept for 3 days at room temperature. The precipitated crystals were collected by filtration and washed with a little water; yield 0.83 g (7%) of a mixture of ethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-α- (and β-) -D-glucopyranosides (**3a** and **3b**, respectively; mainly **3b**), mp 153–158°. Recrystallization from ethanol and water gave 0.62 g (5%) of colorless needles, mp 158–160°, $[\alpha]^{28}D$ −6° (*c* 1, $CDCl_3$). The nmr spectrum ($CDCl_3$) was the same as that of pure **3b** (see below) except for the following peaks due to minor amounts of **3a**: δ 1.29 (t, $SCCH_3$), 2.70 (q, SCH_2), 5.75 (d, J = 5.7 Hz, H-1).

Anal. Calcd for $C_{16}H_{25}NO_8S$: C, 49.09; H, 6.44; N, 3.58; S, 8.19; mol wt, 391. Found: C, 49.11; H, 6.47; N, 3.53; S, 8.19; mol wt, 391 (mass spectrum).

The mother liquor from the preceding crystallization was further concentrated to 3.4 g of syrup, most of which (3 g) was chromatographed over Celite employing benzene–ethanol (99:1, v/v) as development solvent, extrusion, alkaline permanganate detection,[37] and elution with acetone to yield 0.69 g (6%) of **3b**, mp 165–173°. Recrystallization from ethanol–water gave 0.43 g (4%) of **3b**, mp 184–186°, $[\alpha]^{28}D$ −56° (*c* 1, $CDCl_3$). The nmr spectrum ($CDCl_3$) of **3b** contained the following absorptions, with assignments confirmed in part by spin decoupling: δ 1.26 (t, 3, J = 7.4 Hz, $SCCH_3$), 1.93 (s, 3), 2.02 (s, 6), 2.07 (s, 3), 2.72 (q, 2, J = 7.4 Hz, SCH_2), 3.78 (m, H-5), 4.10 (m, H-2), 4.23 (m, 2, H-6), 4.75 (d, J = 10.0 Hz, H-1), 5.11 (t, J = 10.0 Hz, H-3), 5.31 (t, J = 9.0 Hz, H-4), 6.35 (d, J = 9.4 Hz, NH).

Anal. Calcd for $C_{16}H_{25}NO_8S$: C, 49.09; H, 6.44; N, 3.58; S, 8.19. Found: C, 48.93; H, 6.36; N, 3.67; S, 8.46.

A sample of the β-D-glucopyranoside **3b** (97.8 mg) was dissolved in absolute methanol (2 ml), and sodium (25 mg) was added. The solution was allowed to stand for 2 hr at room temperature, and then was neutralized with Amberlite IR-120 (H^+) cation-exchange resin, filtered, and concentrated to a syrup which crystallized on removal of the last traces of methanol under reduced pressure. Thin layer chromatography [silica gel G, benzene–methanol (8:2)] showed a single spot, R_f 0.14. A mixture of 25 mg of this de-*O*-acetylated product, 1 ml of barium oxide dried pyridine, and 0.5 ml of *N,O*-bis(trimethylsilyl)acetamide in a 5-dram vial fitted with a Teflon-lined screw cap was allowed to stand at room temperature with occasional shaking until the *N*-acetyl derivative had entirely dissolved. Excess silylating reagent and solvent were evaporated in a stream of dry nitrogen, the residue was dissolved in sodium-dried hexane, and the solution was evaporated in a stream of dry nitrogen. The residue was stored *in vacuo* over phosphorus pentoxide at about 40° for 10 hr, mp 164–168° dec. The mass spectrum contained a peak at m/e 448 ($M - CH_3$).

Ethyl 2-acetamido-2-deoxy-1-thio-α-D-*xylo*-pentodialdo-1,4-furanoside (4a) and its ethanol solvate (4b) were prepared by a modification of the procedure of Wolfrom and Winkley[11a] from 1.957 g of **2a**. Tlc [silica gel G, benzene–methanol (8:2)] showed a single spot, R_f 0.26, for the intermediate from deacetylation of **4a**. After periodate cleavage, barium chloride treatment, and filtration, the filtrate was evaporated under reduced pressure to a crude residue which was dried by codistillation with absolute ethanol several times and then dissolved in 30 ml of absolute ethanol. The solution was kept in a refrigerator for 3 hr, filtered, and evaporated to a crystalline residue. Tlc on silica gel G showed a major spot at R_f 0.63 and a minor spot at 0.31 in chloroform–methanol (17:3). The crude crystals were recrystallized from ethanol–ether to give 0.985 g (71%) of ethyl 2-acetamido-2-deoxy-1-thio-α-D-*xylo*-pentodialdo-1,4-furanoside ethanol solvate (**4b**): white needles, mp 132–133°, $[\alpha]^{24}D$ +165° (*c* 0.65, CH_3OH), tlc R_f 0.63 (silica gel G). The nmr spectrum (D_2O) of **4b** contained signals at δ 5.7 (H-1), 5.4 (H-5), 4.7–3.9 (H-2, H-3, H-4), 3.72 (q, $-OCH_2CH_3$), 2.75 (q, $-SCH_2CH_3$), 2.00 (s, $NCOCH_3$), 1.28 (t, $-SCH_2CH_3$), 1.21 (t, $-OCH_2CH_3$). Addition of ethanol to the nmr solution enhanced the signals at 3.72 and 1.21. The nmr spectra of **4b** in acetone-d_6 and dimethyl sulfoxide-d_6 both showed only a trace of aldehyde proton absorption. The total yield of **4b** was 0.985 g (71%).

Anal. Calcd for $C_9H_{15}NO_4S \cdot C_2H_5OH$: C, 47.31; H, 7.58; N, 5.02; S, 11.45; mol wt, 141. Calcd for $(C_9H_{15}NO_4S)_2 \cdot 2C_2H_5OH$: mol wt, 187. Found: C, 47.39; H, 7.08; N, 5.01; S, 11.77; mol wt, 203 (osmometric, in acetone).

When **4b** was dried at 100° (5 mm) over phosphorus pentoxide, it slowly melted to a glass, whose nmr spectrum ($CDCl_3$) lacked the signals at δ 3.72 and 1.21 but otherwise was identical with the nmr spectrum ($CDCl_3$) of **4b**.

Ethyl 2-acetamido-2-deoxy-1-thio-α-D-xylofuranoside (5) was prepared by the procedure of Wolfrom and Winkley[11a] but employing 92 mg of purified **4b**. The yield was 28 mg (35%) of **5**, mp 158–160° after sintering at 156°, $[\alpha]^{30}D$ +236° (*c* 0.60, water) [lit.[11a] mp 153–155°, $[\alpha]^{22}D$ +212 ± 3° (*c* 6.45, water)]. The mother liquor yielded 38 mg (48%) of **5**, mp 150–157° after sintering at 145°. The total yield was 83%.

Preparation of Ethyl 2-Acetamido-6-nitro-2,6-dideoxy-1-thio-β-L-ido- and -α-D-glucofuranosides (6 and 7). A. From 4b. Compound **4b** (2.22 g) was dissolved in 95% ethanol (42 ml), and equimolar nitromethane (0.555 ml) was added to the solution. The solution was cooled in an ice bath, and an equimolar amount of sodium methoxide (20% solution in absolute methanol) was added dropwise under agitation. The reaction was stirred 30 min at the same temperature and then allowed to stand in a refrigerator for 18 hr. The slightly yellow solution was neutralized with Amberlite IR-120 (H^+) and then evaporated under reduced pressure to yield a crystalline residue which showed two major spots on tlc [silica gel G, chloroform–methanol (19:1)]. One recrystallization from hot absolute ethanol gave colorless needles (**6**), mp 184–192° dec, which tlc showed to be contaminated by a small amount of the D-gluco isomer (**7**). Further recrystallization from the same solvent gave almost pure L-ido isomer (**6**) (0.925 g, 32%, based on nitromethane), mp 206–208° dec, $[\alpha]^{24}D$ +176° (*c* 2, methanol [lit.[7] mp 190–193°, $[\alpha]^{26}D$ +171° (*c* 2, methanol)].

Anal. Calcd for $C_{10}H_{18}N_2O_6S$: C, 40.80; H, 6.16; N, 9.52; S, 10.89. Found: C, 41.03; H, 6.04; N, 9.43; S, 10.91.

An oily product was obtained on evaporation of the mother liquor. Thin layer chromatography showed that it consisted mainly of the D-gluco isomer (**7**). Column chromatography (silica gel; chloroform containing 5% methanol) of the crude oily product gave pure crystalline **7** (0.670 mg, 23%), mp 115–118° (lit.[7] 114–115°).

Anal. Calcd for $C_{10}H_{18}N_2O_6S$: C, 40.80; H, 6.16; N, 9.52; S, 10.89. Found: C, 41.08; H, 6.24; N, 9.38; S, 10.76.

B. From 2a. The procedure of Wolfrom and Winkley[11a] was employed for the deacetylation of **2a** (1.957 g) and periodate oxidation as far as the removal of barium iodate by filtration. The filtrate and washings were combined and concentrated *in vacuo* to a thick oil, which was azeotroped several times with absolute ethanol, freed of sodium chloride by precipitation with ethanol, and filtered. The filtrate was concentrated *in vacuo* to a thick syrup which was dissolved in 10 ml of 95% ethanol and cooled to 0–5°. Nitromethane (2 ml) was added, followed, dropwise, by a solution of 2 *N* sodium methoxide in methanol to pH 11. The reaction mixture stood 15 hr at 0–5° and 3 hr at room temperature, Amberlite IR-120 (H^+) ion-exchange resin (~8 g) was added to remove sodium ions, and the product was concentrated to a thick yellow oil weighing 1.40 g.

The crude material was chromatographed by the method of Bhalla,[12a] employing a column 4.7 × 25 cm packed with 250 g of silica gel and developed with chloroform–methanol (17:3). Two zones, located by iodine vapor about 7–8 cm from the origin, were removed. Elution of the adsorbent from the upper region (faster moving band) yielded a yellow material which on careful crystallization from a small volume of ethanol gave 0.185 g (13%) of **6**, mp 206–208° dec, $[\alpha]^{24}D$ +176° (*c* 2, methanol).

Elution of the slower moving band gave on careful crystallization from chloroform–benzene–di-*n*-butyl ether (2:1:2, volume) 0.169 g (12%) of **7**, mp 115–116°.

Preparation of Ethyl 2-Acetamido-6-nitro-2,6-dideoxy-1-thio-β-L-idofuranoside (6) and 2-Acetamido-6-nitro-2,6-dideoxy-D-glucopyranose (8) from 4b. Nitromethane (1.335 g) was added to a solution of 6.12 g of **4b** in 95% ethanol (97 ml) at 0–5°, then 10.9 ml of 2 *N* sodium methoxide in methanol was added drop by drop during 15 min with stirring. The reaction mixture stood 16 hr in a refrigerator, then was neutralized with Dowex 50W-X8 (H^+) and evaporated under reduced pressure to give a crystalline residue. Tlc [silica gel G, chloroform–methanol (19:1)] showed two main spots running near one another, with traces of impurities. Recrystallization from absolute ethanol gave three crops weighing 0.894 g (mp 190–194° dec), 0.724 g (mp 184–192° dec), and 0.299 g (mp 180–190° dec), respectively. Tlc [silica gel G, chloroform–methanol (85:15)] showed that the first crop (except for two trace contaminants at R_f 0.53 and 0.28) was almost pure **6** but that the second and third crops were somewhat contaminated, with three spots at R_f 0.53, 0.36, and 0.28. Fractional

crystallization then gave pure **6**: 978 mg (15%); mp 206–208° dec; $[\alpha]^{24}D$ +176° (*c* 2, CH_3OH).

All the mother liquors were combined and evaporated to give a thick oily residue which was dissolved in 70 ml of water and warmed to 50–60°. When 4.0 g of mercuric chloride in 140 ml of water was added, fine white crystals of ethylmercaptomercuric chloride precipitated immediately. The reaction mixture stood at room temperature for 12 hr; then the precipitate was filtered and the filtrate was stirred with 5.0 g of fresh silver acetate for 2–3 hr. White crystals of silver chloride were removed by centrifugation. Excess silver acetate was removed by passing hydrogen sulfide through the filtrate for 25 min, the resulting black precipitate was removed by filtration through Celite, and the filtrate was evaporated under reduced pressure to afford a crystalline residue. The crystals were digested with methanol and chloroform and filtered; yield, 1.043 g. Second and third crops were obtained from the mother liquor; weight, 0.668 and 0.231 g, respectively. Tlc showed a single spot for each crop. The total yield of 8 was 1.942 g (35%). Recrystallization from methanol and chloroform gave the analytical sample, mp 193–195° dec, $[\alpha]^{18}D$ +71° (5 min) → +49° (10 hr) (*c* 1.0, water).

Anal. Calcd for $C_8H_{14}N_2O_7$: C, 38.40; H, 5.64; N, 11.20. Found: C, 38.01; H, 5.67; N, 11.32.

Ethyl 2-acetamido-2,6-dideoxy-6-nitro-1-thio-β-L-idofuranoside-6-^{14}C (6) and 6-nitro-6-deoxy-*N*-acetyl-D-glucosamine-6-^{14}C (8) were prepared as described in the preceding section, from 3.82 g of **5b** and 25.6 mg of nitromethane-^{14}C (2.38 mCi/mmol) diluted with 978.9 mg of cold nitromethane. The first and second crops of crystals from ethanol (890 mg and 449 mg, respectively) were combined and recrystallized from ethanol to give needles (707 mg, 15%). Tlc showed a single spot, for **6**. Evaporation of the mother liquors and hydrolysis of the residue with mercuric chloride (3.2 g) as in the preceding section gave crude crystals of 8 (1.387 g, 34%). The radioactivity of the recrystallized material was 52.5 μCi/mmol.

Hexaacetylinosadiamines from Ethyl 2-Acetamido-6-nitro-2,6-dideoxy-1-thio-β-L-idofuranoside (6). A. Barium Hydroxide Catalyzed Cyclization. The method of Wolfrom[7] was employed directly to hydrolyze **6** (287.2 mg) with mercuric chloride (260 mg). The resultant crude oily nitro sugar (**10**) was then subjected to barium hydroxide catalyzed cyclization, again following the method of Wolfrom except that the barium hydroxide solution was neutralized with Amberlite IR-120 (H^+) after 1–2 days. The crude barium salt of mixed nitrodeoxyinosamines was hydrogenated over 200 mg of platinum catalyst, and the mixture of hexa- and heptaacetylinosadiamines was separated by multiple fractional recrystallization from ethanol: hexaacetylstreptamine (**11**), 42.5 mg (10%), mp 237–245°, identical with an authentic sample;[38] hexaacetyl-*myo*-inosadiamine-1,3 (**12**), 47.3 mg (11%), mp 287–289° (lit.[21] 283.5–285°), infrared spectrum superimposable on that of an authentic sample;[21] and heptaacetylstreptamine (**13**), 23.9 mg (6%), mp 240–252° (lit.[11b] 258–259°); the total yield was 27%.

In addition to the M + H ion, compound **13** also gave strong ions at *m/e* 455.1674 (M + H − H_2O, calcd 455.1665), *m/e* 429.1509 (M − $COCH_3$, calcd 429.1509), and *m/e* 412.1477 (M − HOAc, calcd 412.1481), as well as a weak peak at *m/e* 514.1788 (calcd 514.1798), indicative of a small amount of octaacetylinosadiamine impurity. The ir spectrum showed bands at 1750, 1700, 1680, and 1650 cm^{-1}. Hydrolysis of 10 mg of **13** with 6 *N* hydrochloric acid for 2 hr on the steam bath, followed by neutralization, gave a product which gave identical tlc behavior with that of streptamine (R_f 0.66) but differed from that of *myo*-inosadiamine-1,3 (R_f 0.58).

B. Sodium Methoxide Catalyzed Cyclization. A solution of **6** (285 mg) in water (73 ml) was hydrolyzed with mercuric chloride (275 mg) according to the Wolfrom procedure.[7] The crude hydrolyzate was dissolved in absolute methanol (35 ml) and cooled to 0–5°, then 1 ml of a 1 *N* solution of sodium methoxide in methanol was added. The solution stood in a refrigerator overnight, then was neutralized with Dowex 50W-X8 (H^+) and evaporated *in vacuo* to give a crystalline residue of L-1-acetamido-1,3-dideoxy-3-nitro-*myo*-inositol (**15**). The crystals were digested with chloroform and methanol and collected by filtration; yield 0.163 g (67%), mp 208–213° dec. The analytical sample was obtained by recrystallization from ethanol; plates, mp 207–210° dec or needles, mp 210–215° dec, $[\alpha]^{21}D$ +95° (*c* 1.0, water).

Anal. Calcd for $C_8H_{14}N_2O_7$: C, 38.40; H, 5.64; N, 11.20. Found: C, 38.60; H, 5.52; N, 11.26.

A solution of 157.9 mg of crude **15** in 15 ml of water containing 5 ml of 0.5 *N* hydrochloric acid was hydrogenated over platinum catalyst. The resultant crystalline amine hydrochloride was treated with acetic anhydride and pyridine to afford 221.5 mg (82%) of hexaacetyl-*myo*-inosadiamine-1,3 (**12**).

C. Silica Gel Catalyzed Cyclization. Compound **6** (452 mg) was dissolved in 110 ml of water and treated with 412 mg of mercuric chloride in water (27 ml) at 50–60°. The crude hydrolyzate was chromatographed on a silica gel column (30 g) packed with chloroform–methanol (4:1), eluting with chloroform–methanol (3:1). The fractions which showed a single spot on tlc (silica gel) were collected and evaporated. The major fraction, a white crystalline powder, mp 202–206°, contained a mixture of **14** and **15** (280 mg, 77% based on unrecovered **6**); the minor fraction contained 24 mg of **6**. A 106-mg portion of the mixture of **14** and **15** was dissolved in water (10 ml) containing 3 ml of 0.5 *N* hydrochloric acid and hydrogenated over platinum for 4.5 hr; hydrogen uptake was 32 ml. The catalyst was filtered and the filtrate was evaporated *in vacuo* to give a white crystalline residue. The residue was treated overnight with a mixture of 5 ml of acetic anhydride and 5 ml of pyridine. The reaction mixture was evaporated *in vacuo* and a trace of acetic anhydride and pyridine were removed by codistillation with ethanol and toluene. The partly crystallized oil was triturated with 10 ml of methanol to give 17 mg (9%) of **11**. The mother liquor was evaporated, and the residue was crystallized from ethanol to afford 143 mg (78%) of **12**, which contained a trace of **11**.

D. Silica Gel Catalyzed Cyclization to Give Hexaacetylstreptamine-*I*-^{14}C (11) and Hexaacetyl-*myo*-inosadiamine-1,3-*I*-^{14}C (12) from 6. The sample of ^{14}C-labeled **6** (707 mg) prepared above was hydrolyzed with mercuric chloride (687 mg) and chromatographed on silica gel as described in the preceding section to give a white crystalline powder (labeled **14** and **15**, 398 mg, 66%). The powder was hydrogenated (uptake 120 ml), and the product was worked up as in the preceding section to give 561 mg (54%) of crude crystals, which were recrystallized from chloroform to yield 89.3 mg (9%) of pure crystalline labeled **11**. The mother liquor was evaporated and the residue was recrystallized from ethanol to afford 354 mg (34%) of pure crystalline labeled **12**.

E. "Spontaneous Cyclization." Compound **6** was hydrolyzed with mercuric chloride to give a 30% yield of a crystalline product, mp 210–213°, single spot on tlc at the position of the 6-nitro-*N*-acetylhexose (**10**). A 38.5-mg portion of **10** was catalytically hydrogenated to an oil (single spot on tlc), which was N-acetylated to give 13 mg of a colorless, crystalline di-*N*-acetyl derivative, mp 320–323°. The mother liquor was evaporated to yield an oily product which was treated with acetic anhydride and pyridine to give a white powder, sintering 235–240°. The infrared spectrum of this compound was superimposable on that of authentic hexaacetylstreptamine (**11**).

Hexaacetylinosadiamines from 2-Acetamido-2,6-dideoxy-6-nitro-D-glucose (8). A. Barium Hydroxide Catalyzed Cyclization. A mixture of 255 mg of **8**, 5 ml of water, and 5 ml of 0.2 *N* barium hydroxide solution was allowed to stand at room temperature for 20 hr. Hydrogenation, acetylation, and work-up were as described above. The products isolated were 31 mg (7%) of hexaacetylstreptamine (**11**) and 38.3 mg (9%) of hexaacetyl-*myo*-inosadiamine-1,3 (**12**).

B. Sodium Methoxide Catalyzed Cyclization. 1. Isolation of 16 and Its Conversion to 18. A solution of 672 mg of **8** in 105 ml of absolute methanol was cooled to 0–5°, and then 2.75 ml of 1 *N* sodium methoxide in absolute methanol was added, with stirring. The reaction mixture was kept in a refrigerator overnight, neutralized with Dowex 50W-X8 (H^+) and filtered. The filtrate was evaporated under reduced pressure to give a crystalline residue of 5-acetamido-1,5-dideoxy-1-nitro-L-*myo*-inositol (**16**). Recrystallization from ethanol gave fine needles: 244 mg (36%); mp 190.5–193° dec; $[\alpha]^{24}D$ +19° (*c*, 1.05, H_2O).

Anal. Calcd for $C_8H_{14}N_2O_7$: C, 38.40; H, 5.64; N, 11.20. Found: C, 38.32; H, 5.67; N, 11.27.

The nitroinositol (**16**, 105 mg) was then hydrogenated in 11 ml of water containing 2 ml of 0.5 *N* hydrochloric acid in the presence of platinum catalyst at room temperature. Hydrogen uptake (23 ml) ceased after 3 hr. The catalyst was filtered, and the filtrate was evaporated under reduced pressure to give a white crystalline residue which was allowed to stand overnight with acetic anhydride (7 ml) and pyridine (7 ml) at room temperature and then was heated on a steam bath for 30 min. The reaction mixture was evaporated under reduced pressure; remaining traces of pyridine were removed by codistillation with toluene. The crystalline residue of L-hexaacetyl-*myo*-inosadiamine-1,5 (**18**) was digested with ethanol and the crystals were collected by filtration; yield 145 mg (81%), mp 284.5–285.5°. Recrystallization from

ethanol gave the analytical sample: mp 285.5–286.5°; $[\alpha]^{20}D$ +1° (*c* 1.0, pyridine); $[\alpha]^{20}D$ +8° (*c* 1.0, water).

Anal. Calcd for $C_{18}H_{26}N_2O_{10}$: C, 50.23; H, 6.09; N, 6.51. Found: C, 50.62; H, 6.26; N, 6.78.

2. Direct Conversion to 18 and 19. A solution of 1.52 g of **8** in 240 ml of absolute methanol was cooled by ice while 6.2 ml of 1 *N* sodium methoxide in methanol was added drop by drop, with shaking. The reaction mixture was allowed to stand in a refrigerator overnight, then was neutralized with Dowex 50W-X8 (H^+), and evaporated under reduced pressure to afford a crystalline product. The crystals were digested with 5 ml of ethanol, filtered, and washed with a mixture of ethanol and ether; needles, 1.17 g (72%), mp 169–173° dec after sintering at 140°. The crude nitrodeoxyinosamine (0.934 g) was hydrogenated and acetylated as described in run 1 to yield 842 mg (52%) of **18**, mp 282–284°, identified by the infrared spectrum.

The mother liquor was evaporated *in vacuo* and the oily residue was dissolved in chloroform and chromatographed over alumina. Elution with chloroform gave a thick oily product which crystallized gradually after 1 or 2 weeks. The crystalline mass was digested with a small amount of ethanol, filtered, and washed with a mixture of ether and ethanol to give 194 mg (7%) of L-hexa-*N,O*-acetyl-*epi*-inosadiamine-1,3 (**19**) as colorless needles. The crystals were recrystallized from ethanol–ether to afford fine needles, mp 149–152°. The crystals contain 1 mol of water of crystallization, while the anhydrous product is hygroscopic, $[\alpha]^{18}D$ +31° (*c* 1.1, chloroform).

Anal. Calcd for $C_{18}H_{26}N_2O_{10}\cdot H_2O$: C, 48.21; H, 6.29; N, 6.28. Found: C, 48.24; H, 6.59; N, 6.38.

***myo*-Inosadiamine-1,3 Dihydrochloride.** A solution of 479 mg of hexaacetyl-*myo*-inosadiamine-1,3 (**12**) was heated in 6 *N* hydrochloric acid on a boiling water bath for 2 hr and then evaporated under reduced pressure to a crystalline residue. Crystallization from ethanol–water gave 270 mg (93%) of *myo*-inosadiamine-1,3 dihydrochloride; recrystallization yielded the analytically pure sample, mp 222–240.5° (lit.[21] 221–241.5°). The infrared spectrum was superimposable on that of an authentic sample.[21]

***myo*-Inosadiamine-1,3-*l*-^{14}C Dihydrochloride.** Hexaacetyl-*myo*-inosadiamine-1,3-*l*-^{14}C (**12**, 567 mg) was heated for 2 hr with 30 ml of refluxing 6 *N* hydrochloric acid. The reaction mixture was evaporated *in vacuo* and a trace of hydrochloric acid was removed by repeated codistillation with water. The glassy residue was triturated with water and ethanol to afford fine needles [291 mg (88%) after drying over phosphorus pentoxide *in vacuo* at 100°].

Preparation of Di-*N*-acetylneosamine C from 7. The crystalline D-*gluco*-nitrothiofuranoside (**7**) was hydrolyzed with mercuric chloride in aqueous solution to give colorless crystals (**8**), mp 180–183°, in 70% yield. The crude nitro sugar (**8**), which showed a single spot on tlc, was hydrogenated in an acidic solution in the presence of platinum catalyst to give an oily product, which showed a single spot on paper chromatography (R_f 0.19, BPW 643). The crude product was converted to its di-*N*-acetyl derivative (**9c**), which was identified as di-*N*-acetylneosamine C by its paper chromatographic behavior (R_f 0.32, BEW 415; R_f 0.51, BPW 643). A trace of di-*N*-acetylneosamine B could also be detected by paper chromatography (R_f 0.48, BEW 415; R_f 0.54, BPW 643).

Di-*N*-acetylneosamine C-*6*-^{14}C was prepared from labeled **8** by the micro N-acetylation method: mp 200–207°; R_f 0.52 (BPW); radioactivity, 53.0 μCi/mmol.

Neosamine C-*6*-^{14}C Dihydrochloride (9b). A sample of labeled **8** (298.3 mg) was hydrogenated over platinum at room temperature in 30 ml of water containing 12 ml of 0.5 *N* hydrochloric acid. Hydrogen uptake (85 ml) ceased after 3 hr, the catalyst was filtered, and the filtrate was evaporated *in vacuo* to an oily residue. The residue was heated in 6 *N* hydrochloric acid (20 ml) at reflux for 2 hr. After it had been decolorized with carbon, the solution was evaporated *in vacuo* and dried by codistillation with absolute ethanol; the crude dihydrochloride (**9b**) weighed 167 mg (56%). The product was purified by preparative thin layer chromatography (cellulose powder, 15 g, 20 × 20 cm plate, BAW 221). Spots were detected by ninhydrin spray. Radioactivity of the sample was 54.8 μCi/mmol. The purity of chromatographed **9b** was determined by paper chromatography of its di-*N*-acetyl derivative (**9a**) obtained by the microacetylation technique. Paper chromatography showed one major spot, R_f 0.51, and a minor spot, R_f 0.61, in BPW 643. By radioanalysis, the relative intensities of the spots were 91.7 and 8.3%, respectively.

Methyl 2-Acetamido-2-deoxy-D-glucofuranoside (20). A slurry of 20.1 g of mercuric oxide, 24.9 g of mercuric chloride, and 14.9 g of *N*-acetylglucosamine diethyl dithioacetal (**1**) in 150 ml of methanol was stirred at room temperature for 6 hr and then treated with 6 ml of pyridine, stirred briefly in an ice bath, and filtered through Celite. The filtrate was shaken over liquid mercury for 60 hr. After the precipitate had settled, the yellow methanolic solution was decanted and filtered. Additional methanol was used to wash the mercury and salts. Evaporation of solvent from the combined filtrates left 9.6 g of a yellow gum, which was triturated with 200 ml of boiling 2-propanol. The resultant solution was filtered while hot and then evaporated to a thick oil, which was dissolved in methanol, filtered, and assayed by paper chromatography. The major CLOR-positive component of the mixture had R_f 0.63 in PEaW (R_{NAG} 1.39), a minor component had R_f 0.54, and trace components had R_f 0.33, 0.47, 0.70, and 0.78. The respective R_f values in BEW are 0.45, 0.35, 0.08, 0.26, 0.51, and 0.62.

A portion (620 mg) of the crude methyl furanoside mixture was chromatographed over a charcoal–Celite column (30 × 420 mm), eluting with 5% ethanol in water. The 20-ml fractions were analyzed by CLOR and residue weight. The first half of the major peak (tubes 43 through 59) contained the methyl pyranoside and furanoside; the balance (tubes 60 through 100) contained nearly pure furanoside (R_f 0.63, BEW). Combination of tubes 60–100, and removal of solvent, gave 260 mg (30%, based on **1**) of methyl 2-acetamido-2-deoxy-D-glucofuranoside (**20**), homogeneous by paper chromatography. A second pure methyl furanoside peak was obtained by washing the column with 7.5% ethanol in water. All efforts to crystallize this compound were unsuccessful.

Quantitative periodate oxidation by the method of Argoudelis[39] showed a 1-mol uptake in 7 min with slight subsequent overoxidation. Chromotropic acid determination[39] of formaldehyde fromation, using mannitol as a standard, showed 0.72 to 0.80 mol of formaldehyde produced per mole of sample oxidized.

Preparation of Neosamines B and C (24 and 10) from 20. A solution of 1.47 g (6.17 mmol) of methyl 2-acetamido-2-deoxy-D-glucofuranoside (**20**) and 1.47 g (6.80 mmol) of sodium metaperiodate in 40 ml of water was kept at 0–5° for 1 hr and then dripped slowly through a column containing 20 ml (12.5 mequiv) of Amberlite MB-3 mixed bed (H^+ and OH^-) ion-exchange resin. The pale yellow eluate was strongly AHP-reducing at first, gradually decreasing in intensity as the column was washed with 1 l. of water. Removal of the water under reduced pressure at 40° left **21** as a yellow syrup which was dried by repeated evaporation from methanolic solution; weight 1.58 g.

The thick syrup of crude **21** dissolved in 20 ml of absolute ethanol and 20 ml of nitromethane was chilled; sodium methoxide in absolute ethanol was then added until the sugar solution gave an alkaline test (pH 8 to 9) when applied to moist pH test paper. The stoppered flask was refrigerated at 10° for 33 hr and then a large excess of dry Dowex-50 (H^+ form) ion-exchange resin was stirred in. The resin was removed by filtration and washed well with methanol. The pale yellow filtrate was freed of solvent and the residue was dried over phosphorus pentoxide *in vacuo* to give 1.40 g (86% based on **20**) of a yellow glass assumed to be a mixture (**22**) of C-5 epimers of the 6-nitrofuranoside.

Half of this yellow glass was hydrogenated at atmospheric pressure in aqueous solution over approximately 2 g of Raney nickel. Hydrogen uptake amounted to only 115 ml (57.5% of theory) in 40 hr. The catalyst was removed by filtration and washed liberally with water. The combined filtrate and washings, strongly ninhydrin-positive, were freed of solvent and dried, leaving 549 mg of crude **23** as a yellow-brown syrup.

This was dissolved in 40 ml of 1.5 *N* hydrochloric acid and heated 4 hr at 50°, then 2 hr at 95–100°. The dark brown solution was slurried a few minutes with charcoal and filtered. The clear, almost colorless, filtrate was freed of solvent and dried over sodium hydroxide to 450 mg of a glass which retained the odor of hydrochloric acid.

A small sample of this was N-acetylated in phosphate buffer and subjected to paper electrophoresis: M_g 0.18, 0.29, and 0.55 (in decreasing order of intensity to AHP), corresponding to *N,N'*-diacetylneosamine C (M_g 0.20), *N*-acetylglucosamine (M_g 0.30), and *N,N'*-diacetylneosamine B (M_g 0.56), respectively.

The crude amine mixture was gradient eluted (0.5 to 2.0 *N* hydrochloric acid) from a 50-ml column of Dowex-50 (H^+ form) resin. Semiquantitative NIN colorimetry showed three peaks, which were collected, freed of solvent, and assayed separately.

Peak I yielded 168 mg of a glass, $[\alpha]^{28}D$ +52.0° (*c* 1.68, water), which was N-acetylated by the phosphate method and analyzed by paper chromatography and electrophoresis. This mixture was combined with an equal amount of corresponding material from a

second run and separated by cellulose powder chromatography with BAW 415. Two CLOR-positive fractions were obtained.

Solvent removal from the first fraction gave 146 mg (9%, based on **20**) of a glass, $[\alpha]^{28}D$ +29.5° (*c* 2, water), R_f of 0.52 in PEaAW, R_f 0.40 in BAW 415 (CLOR spray). Crystallization from ethanol–ether gave, after prolonged standing, 50 mg (3%) of colorless needles: mp 211–214° dec; $[\alpha]^{25}D$ +32° (*c* 1.06, water) [lit.[40] mp 209–215° dec; $[\alpha]_D$ +36° (*c* 0.7, water)]. A mixture melting point with authentic 2,6-diacetamido-2,6-dideoxy-D-glucose (**9a**) showed no depression.

Solvent removal from the second fraction from peak I gave 121.5 mg of a glass: $[\alpha]^{28}D$ +18° (*c* 2, water), AHP-negative, R_f 0.44 in PEaAW (CLOR)[41] (7% from **20**, based on a diacetamidodideoxyhexitol structure).

Evaporation of peak III and extensive drying over sodium hydroxide at 0.2 mm left 38 mg (5%) of 2,6-diamino-2,6-dideoxy-L-idose dihydrochloride (**24**) as a glass, $[\alpha]^{28}D$ +23.9° (*c* 1.9, water) [lit.[24] $[\alpha]_D$ +17.5° (*c* 0.9, water)]. Treatment with acetic anhydride and phosphate solution gave the *N*-acetyl derivative, which was purified by preparative paper chromatography in BAW 415 to give a glass; 20.6 mg, $[\alpha]^{25}D$ +7.0° (*c* 1.9, water) [lit.[25] $[\alpha]_D$ +5° (*c* 1, water)]. Electrophoresis in borate solution of alternate spots of the synthetic material and *N,N'*-diacetylneosamine B showed no differentiation, M_g 0.42. Approximately 6 mg of the N-acetylated derivative was converted to its *p*-nitrophenylhydrazone with 6 mg of *p*-nitrophenylhydrazine in boiling methanol. Crystallization from methanol and absolute ethanol gave small yellow needles, mp 211–215° dec [lit. mp for *N,N'*-diacetylneosamine B *p*-nitrophenylhydrazone 215–218° dec[25]]. A mixture melting point with an authentic sample had mp 212–217° dec.

Pentaacetyl-2-bromo-2-deoxystreptamine (26). Anhydrous *myo*-inosadiamine-1,3 dihydrochloride (**25**, 882 mg, dried over phosphorus pentoxide *in vacuo* at 100°, obtained by hydrolysis of **12** in refluxing 6 *N* hydrochloric acid), acetyl bromide (1.2 ml), and acetic anhydride (2.6 ml) were heated in a sealed tube at 140° for 5 hr. The tube was cooled, then opened carefully and ethanol (6 ml) was added gradually with cooling in an ice bath. The mixture was allowed to stand in a refrigerator overnight, evaporated *in vacuo* to a glassy residue, then dried thoroughly in a vacuum desiccator. Acetic anhydride (15 ml) and pyridine (15 ml) were added, and the mixture stood at room temperature for 10 hr. Colorless crystals were filtered and washed with pyridine, and the filtrate and washing were combined and evaporated *in vacuo* to afford a white solid residue, which was recrystallized from ethanol to give pentaacetyl-2-bromo-2-deoxystreptamine (**26**) as colorless needles (660 mg, 42%), mp 257–258.5° dec (lit.[27] 256.5–258.5° dec). A second crop of crystals (270 mg, 17%), mp 255–256° dec, was obtained from the mother liquor. The mother liquor was finally diluted with methanol, treated with Amberlite IRA-400 (hydroxide phase), concentrated, and passed through an activated alumina column. Elution with chloroform yielded a third crop of crystals (240 mg, 15%), mp 245–252° dec. The second and third batches of crystals were combined and recrystallized from ethanol to give colorless needles (367 mg, 23%), mp 257–258.5° dec. The total yield of **21** was thus 1.027 g (65%). The ir spectra of the three crops of crystals were all superimposable on that of an authentic sample.[27]

Pentaacetyl-2-bromo-2-deoxystreptamine-*1*-^{14}C (26). Anhydrous *myo*-inosadiamine-1,3-*1*-^{14}C dihydrochloride (**25**-^{14}C, 290 mg, obtained by hydrolysis of labeled **12**) was heated in a sealed tube with acetyl bromide (0.41 ml) and acetic anhydride (0.88 ml) and worked up as described in the preceding section for the unlabeled compound. The first crop weighed 221 mg (42%), the second crop 139 (27%), and the third crop 64 mg (total yield 81%), mp 257–258.5° dec.

Pentaacetyl-2-deoxystreptamine (27). A slurry of pentaacetyl-2-bromo-2-deoxystreptamine (**26**, 440 mg) and acetic anhydride (15.4 ml) was stirred vigorously with pulverized zinc metal (7.8 g). Water (0.38 ml) was added after 1 and 2 hr, stirring was continued for 1 hr longer, and the remaining solids were filtered and washed thoroughly with acetic anhydride. The filtrate and washings were then combined and evaporated under reduced pressure to a crystalline residue which was recrystallized from ethanol to give fine crystals, 269.5 mg (74%), mp 322–323° (lit.[27] mp above 300°). The infrared spectrum was superimposable on that of an authentic sample.[27]

Pentaacetyl-2-deoxystreptamine-*1*-^{14}C (27). A slurry of labeled **26** (220 mg), acetic anhydride (7.7 ml), and pulverized zinc (3.9 g) was treated as for the unlabeled material in the preceding section. The recrystallized product weighed 149.9 mg (82%), mp 322–323°. The ir spectrum was superimposable on that of an authentic sample derived from neomycin B. Radioactivity of the sample was 53.2 μCi/mmol.

2-Deoxystreptamine Dihydrochloride (28). A mixture of pentaacetyl-2-deoxystreptamine (**27**, 129 mg) and 6 *N* hydrochloric acid (15 ml) was heated at reflux for 2 hr and then evaporated under reduced pressure to give a thick oil, which crystallized gradually during codistillation with ethanol. The crystals were digested with ethanol and collected by filtration; yield 77.9 mg (96%). The infrared spectrum was superimposable on that of an authentic sample.[27]

2-Deoxystreptamine-*1*-^{14}C Dihydrochloride (28). Labeled **27** (129 mg) was hydrolyzed with 6 *N* hydrochloric acid (15 ml) and worked up as in the preceding section; yield 79.4 mg (96%). Tlc (cellulose powder, BAW 221) showed a single spot. Radioactivity of the sample was 50.6 μCi/mmol.

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Registry No.—**1**, 6838-16-0; **2a**, 7115-40-4; **3a**, 49810-41-5; **3b**, 4239-72-9; **4a**, 49810-43-7; **5**, 7115-38-0; **6**, 49810-45-9; **7**, 49810-46-0; **8**, 49810-47-1; **9c**, 10536-74-0; **11**, 7380-63-4; **12**, 6255-71-6; **13**, 18376-9; **15**, 49810-52-8, **16**, 49810-53-9; **18**, 49810-54-0; **19**, 49810-55-1; **20**, 49810-56-2; **21**, 49810-57-3; **24**, 49810-58-4; **24** *N,N'*-diacetyl *p*-nitro-phenylhydrazone, 49810-59-5; **25**, 16656-63-6; **25** ^{14}C-labeled, 49810-61-9; **26**, 18783-89-6; **26** ^{14}C-labeled, 49775-26-0; *muco*-1,3-peracetylinosadiamine, 49810-62-0; *myo*-2,4-peracetylinosadiamine, 19046-76-5; *chiro*-1,3-peracetylinosadiamine, 16020-11-4; *chiro*-1,5-peracetylinosadiamine, 49810-65-3; 2-acetamido-2-deoxy-D-glucose, 7512-17-6.

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γ Condensation of an Allylic Phosphonium Ylide

E. J. Corey* and Bruce W. Erickson

Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138

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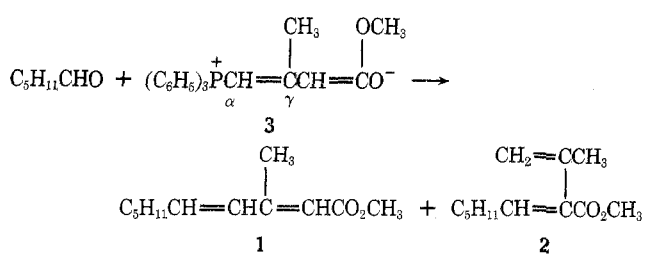
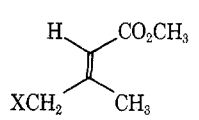
The Wittig reaction of (*E*)-3-methoxycarbonyl-2-methylallyltriphenylphosphonium bromide with *n*-hexanal furnished all four geometric isomers of methyl 3-methyl-2,4-decadienoate, the normal α-condensation product, and both geometric isomers of methyl 2-isopropenyl-2-octenoate, the unprecedented γ-condensation product. The α:γ product ratio varied from 1:9 to 9:1 in response to the tertiary amine base and the group IIB metal halide present. In contrast, the analogous trans phosphonate provided only the trans-2,trans-4 and the cis-2,trans-4 isomers of the α-condensation product in 6:1 ratio.

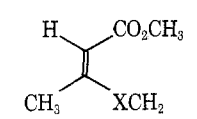
Aldehydes normally condense with allylic phosphonium ylides at the ylide α-carbon atom.[1-5] The Wittig reaction of *n*-hexanal with the stabilized allylic phosphonium ylide **3**, however, generates not only all four geometric isomers of methyl 3-methyl-2,4-decadienoate (**1**), the normal α-condensation product, but also both geometric isomers of methyl 2-isopropenyl 2-octenoate (**2**), the unprecedented γ-condensation product. Under the appropriate reaction conditions, either ester can be produced in >90% relative yield.



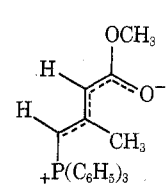
The crystalline trans phosphonium bromide[2,3] **7e** was obtained in 84% yield on heating equimolar quantities of methyl 4-bromo-3-methyl-2-butenoate (**6e:6z** = 86:14) and triphenylphosphine in acetonitrile. This salt slowly isomerized in dry dimethyl sulfoxide near 25°; the isomer ratio at equilibrium was **7e:7z** = 47:53. Treatment of the trans phosphonium salt **7e** with excess sodium hydroxide furnished the phosphonium ylide **3** as yellow crystals in 68% yield. A $CDCl_3$ solution of this ylide near 25° contained two isomeric species in 2:1 ratio. The major and minor species are assigned the structures **3z** and **3e**, respectively,



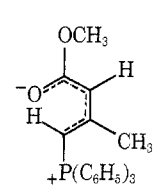
4e, X = OH
5e, X = O_3P
6e, X = Br
7e, X = $P^+(C_6H_5)_3Br^-$
8e, X = $PO(OC_2H_5)_2$



6z, X = Br
7z, X = $P^+(C_6H_5)_3Br^-$
8z, X = $PO(OC_2H_5)_2$



3e



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