Syntheses with Azido-sugars[†]

Part II. Conversion of D-Glucose to (+)-Desthiobiotin^{††}

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(+)-Desthiobiotin (IIa) was prepared from D-glucose *via* several derivatives of azido-sugars. The fourth and the fifth carbons of D-glucose were changed into two asymmetric carbons of IIa through the reaction.

Although many asymmetric carbons exist in a carbohydrate molecule, few of them have been utilized for the preparation of optically active compounds. From this view-point, we planned to prepare two compounds related to (+)-biotin from some carbohydrate derivatives. One was (+)-oxybiotin and its synthesis from D-glucose was already achieved.¹⁾ This paper deals with the preparation of the other compound, (+)-desthiobiotin, from D-glucose.

(+)-Desthiobiotin was first prepared as its methyl ester by removal of sulfur from (+)-biotin methyl ester (Ib) with Raney nickel in the course of the structural elucidation of (+)-biotin (Ia)²⁾ and was later found to be biologically as active as Ia for some microorganisms.³⁾

(Ia) R=H
(Ib) R=CH₃

(IIa) R=H
(IIb) R=CH₃

FIG. 1.

[†] The paper cited in footnote 1 is regarded as consisting Part I of this series.

^{††} Presented at the Annual Meeting of the Agricultural Chemical Society of Japan, Fukuoka, April 1970; Preliminary communicated. H. Kuzuhara, H. Ohruai and S. Emoto, *Tetrahedron Letters*, **1970**, 1185.

1) Presented at the Annual Meeting of the Agricultural Chemical Society of Japan, Tokyo, April 1969. Published in part, H. Ohruai, H. Kuzuhara and S. Emoto, *Agr. Biol. Chem.*, **34**, 375 (1970).

2) V. du Vigneaud, D. B. Melville, K. Folkers, D. E. Wolf, R. Mozingo, J. C. Keresztesy and S. A. Harris, *J. Biol. Chem.*, **146**, 475 (1942).

3) S. F. Dyke, "The Chemistry of Natural Products," Vol. 6, Interscience Publishers (London), 1965, pp. 176; L. H. Sternback "Comprehensive Biochemistry," Vol. 11, Elsevier Publishing Co. (Amsterdam), 1963, pp. 74.

The preparation of (+)-desthiobiotin from other sources has never been reported although the racemic compound was synthesized by three groups.⁴⁾ Absolute configuration of Ia was recently proposed by Trotter and Hamilton on the basis of the result of X-ray analysis (anomalous diffraction) of a (+)-biotin de-

4) J. L. Wood and V. du Vigneaud, *J. Am. Chem. Soc.*, **67**, 210 (1945); J. P. Bourquin, O. Schnider and A. Grussner, *Helv. Chim. Acta*, **28**, 528 (1945); R. Duschinsky and L. A. Dolan, *J. Am. Chem. Soc.*, **67**, 2079 (1945).

FIG. 2.

rivative.⁵¹ Consequently, the absolute configuration of (+)-desthiobiotin may be justifiably assigned as shown in IIa if it is assumed that desulfurization of Ib does not cause configurational changes of the amino groups. In order to ascertain this point and determine

the absolute configuration of (+)-desthiobiotin, it will be significant to prepare the compound having the structure IIa from a compound with a well established configuration.

The preparation of two amino groups on contiguous carbons in *erythro* orientation is a key point in the synthesis of IIa. This was conducted by introducing two azide groups into a sugar intermediate in such an orientation

5) J. Trotter and J. A. Hamilton, *Biochem.*, **5**, 713 (1966).

TABLE I. NMR PARAMETERS²³⁾ FOR COMPOUNDS, VIIIb AND IXa

Compounds		Protons					
		H-1	H-2	H-3	H-4	H-5	H-6
VIIIb	Chemical shift (τ)	4.65~5.20		7.30~7.75	5.57	6.18	8.66
	Number of protons	2		2	1	1	3
	Splitting	multiplet		multiplet	multiplet	octet ^{a)}	doublet
	Coupling constant (Hz)					$J_{4,5}=10.2$ $J_{5,6}=6.0$	$J_{5,6}=6.0$
IXa	Chemical shift (τ)	5.05	4.73	7.50~7.82	6.26	5.90	8.70
	Number of protons	1	1	2	1	1	3
	Splitting	doublet	octet	multiplet	multiplet	octet ^{b)}	doublet
	Coupling constant (Hz)	$J_{1,2}=3.6$	$J_{1,2}=3.6$			$J_{4,5}=1.8$	$J_{5,6}=6.6$
			$J_{2,3e}=7.2$ $J_{2,3a}=13.2$			$J_{5,6}=6.6$	

^{a)} This collapsed to a doublet on irradiation at τ 8.66.

^{b)} This collapsed to a doublet on irradiation at τ 8.70.

and by reducing them subsequently.

Methyl 4,6-O-benzylidene-3-deoxy- α -D-*ribo*-hexopyranoside (IIIa) had been prepared from D-glucose by Vis and Karrer⁶⁾ and employed as a starting material for the preparation of IIa.

Two schemes were undertaken to synthesize methyl 2-O-benzoyl-3,6-dideoxy- α -D-*ribo*-hexopyranoside (VIIIa) which was one of the important intermediates for the preparation of IIa. In the first attempt, IIIa was treated with N-bromosuccinimide, giving syrupy methyl 4-O-benzoyl-6-bromo-3,6-dideoxy- α -D-*ribo*-hexopyranoside (IV).⁷⁾ Compound IV was reduced catalytically with Raney nickel in the presence of potassium hydroxide to afford methyl 3,6-dideoxy- α -D-*ribo*-hexopyranoside (V). An attempt to get VIIIa by partial benzoylation of V was not successful although a hydrogen bond was expected to exist in the molecule of V between the hydroxyl group at C-2 and the oxygen atom of

the methoxy group at C-1.⁸⁾ Therefore, an alternative pathway, a more conventional one, was adopted to prepare VIIIa. Compound IIIa was benzoylated to methyl 2-O-benzoyl-4,6-O-benzylidene-3-deoxy- α -D-*ribo*-hexopyranoside (IIIb) and the benzylidene group of IIIb was removed by acid in *p*-dioxane,⁹⁾ giving methyl 2-O-benzoyl-3-deoxy- α -D-*ribo*-hexopyranoside (VIa). Partial tosylation of VIa afforded 6-tosylate (VIb) which was treated with sodium iodide in 2-butanone under reflux. The resulting methyl 2-O-benzoyl-3,6-dideoxy-6-iodo- α -D-*ribo*-hexopyranoside (VII) was reduced catalytically in the presence of sodium acetate to give VIIIa. The over-all yield of VIIIa was 45% from IIIa.

Compound VIIIa was then methylsulfonylated in the usual manner to give 4-mesylate (VIIIb). The methylsulfonyloxy group of VIIIb was replaced with an azide group by treatment of VIIIb with sodium azide in N, N-dimethylformamide (DMF) and methyl 4-

6) E. Vis and P. Karrer, *Helv. Chim. Acta*, **37**, 378 (1954).

7) The identification of this compound was based on the result described in the literature shown below: S. Hannessian and N. P. Plessas, *J. Org. Chem.*, **34**, 1035 (1969).

8) Generally a hydroxyl group forming a hydrogen bond is more easily acylated than non-bonded one. For example, see *Coll. Czech. Chem. Comm.*, **35**, 321 (1970).

9) H. Kuzuhara, H. Ohrui and S. Emoto, *Carbohydrate Research*, **11**, 9 (1969).

azido-2-O-benzoyl-3,4,6-trideoxy- α -D-xylo-hexopyranoside (IXa) was obtained in 93% yield. This substitution accompanied a configurational inversion at C-4 as a result of the S_N2 type of replacement,¹⁰ which was proved by comparing the NMR spectrum of IXa with that of VIIIb (Table I). Although the splittings of H-4 signals were not well resolved in the both spectra, H-5 signals which were assigned by double resonance with methyl protons at C-6 were well analysed. The change of $J_{4,5}$ value (10.2 Hz $\rightarrow$ 1.8 Hz) during the substitution reaction shows that the relative configuration of H-4 and H-5 changes from axial-axial to equatorial-axial.

In connection with the configurational and the conformational analyses of IXa, it is interesting to measure the circular dichroism (CD) of this type of compounds since the sign of the cotton effect of 4-azido-hexose cannot be predicted from the "azide octant rule"¹¹ which was recently extended to the field of sugar studies.¹² After the benzoyl group which might interfere the cotton effect due to the azide group was removed from IXa with a base, the CD curve of the resulting methyl 4-azido-3,4,6-trideoxy- α -D-xylo-hexopyranoside (IXb) was observed and found to have a positive cotton effect ($[\theta]_{286} + 188$).

The next step towards IIa was hydrolysis of the glycosidic linkage of IXa. Thus, acetolysis of IXa gave an anomeric mixture of 1-acetate and subsequent hydrolysis of the mixture with a base afforded a sole product, crystalline 4-azido-3,4,6-trideoxy- α -D-xylo-hexopyranose (X). The anomeric configuration of X was determined by measuring mutarotation, $[\alpha]_D - 15^\circ \rightarrow -43^\circ$ (H₂O) and NMR spectrum in dimethylsulfoxide-d₆¹³ (H-1, τ 5.12, $J_{1,2} =$

3.6 Hz). For further identification of X its *p*-tolylsulfonylhydrazone (XI) was prepared.

When X was treated with sodium borohydride at 0°C for 30 min, only hemiacetal was reduced leaving the azide group unaffected and syrupy 4-azido-3,4,6-trideoxy-D-xylo-hexitol (XII) was obtained in more than 90% yield. For protecting the hydroxyl groups at C-1 and C-2 XII was condensed with acetone in the presence of acidic ion-exchange resin to afford 4-azido-1, 2-O-isopropylidene-3, 4, 6-trideoxy-D-xylo-hexitol (XIIIa). The second azide group was introduced to the carbohydrate intermediate in the same way as for the first one. Thus, XIIIa was methylsulfonylated and the resulting 4-methylsulfonate (XIIIb) was treated with sodium azide in DMF to yield a syrupy compound which has two azide groups. The diazido-compound may reasonably be assigned to be 4,5-diazido-1,2-O-isopropylidene-3,4,5,6-tetradecoxy-L-arabino-hexitol (XIV), since the azide group at C-4 does not participate in the substitution at C-5.¹⁴

Catalytic reduction of the azide groups of XIV with Adams platinum was found to proceed incompletely.¹⁵ When Raney nickel was used under a slightly high pressure (ca. 4 kg/cm²) of hydrogen, however, XIV was reduced smoothly to yield the corresponding diamine (XV). Without further purification XV was treated with carbonyl chloride in water in the presence of sodium carbonate to give crystalline 4,5-N-carbonyl-4,5-diamino-1,2-O-isopropylidene-3,4,5,6-tetradecoxy-L-arabino-hexitol (XVI) in 33% yield from XIV. The isopropylidene group of XVI was removed with an acidic ion-exchange resin in water at

13) The conformation and the anomeric configuration of a sugar do not change in dimethylsulfoxide. See B. Casu, M. Reggiani, G. G. Gallo and A. Vigevani, *Tetrahedron Letters*, **1964**, 2839.

14) L. Goodman, "Advances in Carbohydrate Chemistry," Vol. 22, Academic Press, 1967, pp. 154.

15) After XIV was shaken for 5 hr in hydrogen which was replaced several times with fresh hydrogen, the product still showed an absorption at 2100 cm⁻¹ in IR spectrum for the azido group.

10) In this case there is no factor which inhibits a simple S_N2 substitution. See, A. C. Richardson, *Carbohydrate Research*, **10**, 395 (1969).

11) C. Djerassi, A. Moscovitz, K. Ponsold and G. Steiner, *J. Am. Chem. Soc.*, **89**, 347 (1967).

12) H. Paulsen, *Chem. Ber.*, **101**, 1571 (1968).

room temperature and crystalline 4,5-N-carbon-yl-4,5-diamino-3,4,5,6-tetra-deoxy-L-*arabino*-hexitol (XVII) was obtained in 86% yield.

The next three steps of the reactions (periodate oxidation, Wittig reaction, and catalytic hydrogenation) were carried out continuously without characterization of the products because of their quantitative limitation and instability. Compound XVII was cleaved with periodate in water between C-1 and C-2 and the resulting aldehyde (XVIII) was extracted with absolute ethanol after careful concentration by freeze-drying. Judging from the results of periodate oxidation in other sugar studies,¹⁶⁾ XVIII probably forms a dimer or an adduct with formaldehyde (by-product of the oxidation) in such a way that the aldehyde group was masked.

On the other hand, several Wittig reactions were recently reported to be successful with

FIG. 3. Thin-layer Chromatogram¹⁹⁾ of the Products of the Wittig Reaction Detected in Two Ways. Right, under UV-light; Left, with a reagent.²⁰⁾

Chloroform-methanol (94:6 v/v) was used as a developer.

FIG. 4. IR Spectra of the Prepared (+)-Desthiobiotin and of the Authentic One. (KBr)
—, From D-glucose; ---, From (+)-biotin.

¹⁶⁾ For example, see T. D. Inch, *Carbohydrate Research*, **5**, 53 (1967).

a masked aldehyde such as hemiacetal.¹⁷⁾ Therefore, two equivalents of the Wittig reagent, [3-carbomethoxy-propen-(2)-yliden-(1)]-triphenylphosphorane (XX),¹⁸⁾ was added to the alcoholic extract of XVIII and the mixture was kept at room temperature for two days. Five products (A~E) were detected on thin-layer chromatogram¹⁹⁾ of the reaction mixture under UV light (Right side of Fig. 3), and three products (D~F) were detected when a t.l.c. plate was sprayed with the reagent²⁰⁾ and charred (Left side of Fig. 3). Either D or E²¹⁾ should be methyl 7,8-N-carbonyl-7(R), 8(S)-diamino-nona-2,4-dienoate (XIX) because XIX having a conjugate system and an acid-labile ureide was to be detected by the both methods. Therefore, D and E²¹⁾ (approximate ratio, 3:1) was respectively isolated by column chromatography and subsequent preparative layer chromatography, although the separated E was not pure enough for further studies. The major product D was hydrogenated with Adams catalyst and the resulting syrup was purified by high vacuum distillation and subsequent column chromatography to give a

hemicrystalline product which agreed on t.l.c. with an authentic specimen of (+)-desthiobiotin methyl ester prepared by desulfurization of Ib. From these results, D was proved to be the desired compound XIX.

Finally the hemicrystalline reduction-product was hydrolyzed with a base to afford crystals (mp 162°C, $[\alpha]_D + 7.00$) which showed no depression of the melting point when mixed with an authentic specimen of (+)-desthiobiotin. The IR spectrum of the crystals was completely identical with that of the authentic specimen (Fig. 4).

Based on the agreement of the signs of specific rotations between IIa derived from the sugar and an authentic specimen of (+)-desthiobiotin ($[\alpha]_D + 10.4^\circ$), the absolute structure of (+)-desthiobiotin was established as 7,8-N-carbonyl-7(R), 8(S)-diamino-nonanoic acid (IIa).

This also indicates that desulfurization of Ib does not disturb the configurations of the two amino-groups.

EXPERIMENTAL

All melting points are uncorrected. Specific rotations were measured with a Perkin-Elmer 141 Polarimeter in a 1 dm tube. IR spectra were recorded with a Shimadzu IRS-27 Model D spectrometer. NMR spectra were recorded at 60 MHz with a Japan Electron Optics C-60 spectrometer. Mass spectra were obtained using a JMS-OISG spectrometer. Circular dichroism was measured with a JASCO ORD/UV-5 spectrometer. Column chromatography was performed with silica gel (0.05~0.20 mm, E. Merck) and the solvent systems specified.

Methyl 2-O-benzoyl-4,6-O-benzylidene-3-deoxy- α -D-ribohexopyranoside (IIIb). Benzoyl chloride (1 g) was added to a solution of IIIa (1.3 g) in anhydrous pyridine (10 ml). After the mixture was kept over night at 0°C it was poured into ice-water (ca. 150 ml) and extracted with chloroform (150 ml). The extract was washed enough successively with cold aqueous sulfuric acid (2%), saturated aqueous sodium bicarbonate and water and then dried over anhydrous sodium sulfate. Evaporation *in vacuo* gave a colorless syrup which solidified soon (1.75 g, quantitative yield). Recrystal-

17) H. J. Bestman and J. Angerer, *Tetrahedron Letters*, **1969**, 3665 (1969); E. J. Corey, N. M. Weinshenker, T. K. Schaaf and W. Huber, *J. Am. Chem. Soc.*, **91**, 5675 (1969); V. A. Polenov and Yu. A. Zhdanov, *Zh. Obsch. Khim.*, **37**, 2456 (1967); Yu. A. Zhdanov and V. A. Polenov, *J. Gen. Chem. of U. S. S. R.*, **1969** 107 (1969); B. A. Dmitriev and H. K. Kochetkov, *Bull. Acad. Sci. of U. S. S. R. (Div. of Chem. Sci.)*, **1967**, 2483.

18) E. Buchta and F. Andree, *Chem. Ber.*, **92**, 3111 (1959).

19) T. l. c. was conducted on silica gel GF₂₅₄ (E. Merck, Darmstadt).

20) A mixture of methanol, concentrated sulfuric acid and *p*-methoxybenzaldehyde (8.5:1.0:0.5, v/v).

21) The assignments of other spots were speculated. Spot A might be due to the condensation product of formaldehyde with XX. Spot B corresponded to triphenyl-phosphine oxide. Spot C was observed when only XX was spotted on t. l. c. and so derived from the degradation product of XX. Spot F was due to unreacted XVIII.

22) Compounds D and E would not be *cis*, *trans*-isomers, because the hydrogenated compounds of D and E gave different spots on t. l. c.

lization from isopropyl ether was conducted as described below. After the crude syrupy IIIb was dissolved in hot isopropyl ether, the solution was evaporated on a hot plate to *ca.* 20 ml and it was allowed to stand at room temperature to give prisms.

For analyses, a part of III was chromatographed on silica gel in *n*-hexane-ethyl acetate (6:1 v/v). mp 121~123°C, $[\alpha]_D^{25} + 87.6$ ($c = 4.61$, CHCl_3), $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1710 (C=O), 1600, 1590, 1490 (benzene ring). *Anal.* Found: C, 68.38; H, 6.09. Calcd. for $\text{C}_{21}\text{H}_{22}\text{O}_6$: C, 68.09; H, 5.99%.

Methyl 4-O-benzoyl-6-bromo-3,6-dideoxy- α -D-ribo-hexopyranoside (IV). To a suspension of N-bromosuccinimide (181 mg) and barium carbonate (330 mg) in carbon tetrachloride (17 ml) was added IIIa (267 mg). The mixture was heated under reflux for 1.5 hr and cooled to 0°C. After filtration the filtrate was evaporated *in vacuo* to give a pale yellow syrup which was chromatographed on silica gel in ethyl acetate-cyclohexane (1:2 v/v); yield 304 mg (88%). $[\alpha]_D^{21} + 115^\circ$ ($c = 1.60$, CHCl_3), $\nu_{\text{max}}^{\text{H}_2\text{O}}$ cm^{-1} : 3300~3400 (OH), 1710 (C=O). *Anal.* Found: C, 48.72; H, 4.92; Br, 22.82. Calcd. for $\text{C}_{14}\text{H}_{17}\text{O}_5\text{Br}$: C, 48.69; H, 4.97; Br, 23.14%.

Methyl 3,6-dideoxy- α -D-ribo-hexopyranoside (V). To a suspension of Raney Ni (*ca.* 2 g) in 10% aqueous potassium hydroxide (10 ml) was added a solution of IV (2.2 g) in methanol (35 ml). The mixture was shaken in hydrogen for 2 hr at room temperature. After filtration of the catalyst, the filtrate was neutralized with dry ice and evaporated *in vacuo* to dryness. The resulting residue was extracted with acetone (50 ml) and the extract was evaporated *in vacuo* to give colorless syrupy V (900 mg, 87%), which was partially chromatographed for analyses. $[\alpha]_D^{18} + 159^\circ$ ($c = 2.70$, *p*-dioxane), *Anal.* Found: C, 51.31; H, 8.72. Calcd. for $\text{C}_7\text{H}_{14}\text{O}_4$: C, 51.84; H, 8.70%.

Methyl 2-O-benzoyl-3-deoxy- α -D-ribo-hexopyranoside (VIa). To a solution of IIIb (900 mg) in *p*-dioxane (18 ml) was added aqueous sulfuric acid (1.5%, 10 ml) and the mixture was heated at 55°C overnight. After the solution was neutralized at 50°C with a large excess of barium carbonate it was filtered and the resulting filtrate was evaporated *in vacuo* to give amorphous IVa (600 mg, 87%), which was dissolved in a mixture of isopropyl ether and acetone (*ca.* 3:1) and subsequently evaporated to 1/3~1/4 volume on a hot plate to afford crystalline IVa. mp 131~133°C,

$[\alpha]_D^{20} + 101^\circ$ ($c = 3.31$, CHCl_3), $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3250 (OH), 1725 (C=O). *Anal.* Found: C, 59.50; H, 6.39. Calcd. for $\text{C}_{14}\text{H}_{18}\text{O}_6$: C, 59.56; H, 6.43%.

Methyl 2-O-benzoyl-3-deoxy-6-O-tosyl- α -D-ribo-hexopyranoside (VIb). A solution of VIa (1.4 g) in dry pyridine (15 ml) was cooled in an ice-salt bath. To the solution was added *p*-tolylsulfonyl chloride (1.15 g) and the mixture was kept at -5°C for 6 days. After the mixture was poured into ice-water it was extracted with chloroform (150 ml). The extract was washed successively with aqueous sulfuric acid (3%), aqueous sodium bicarbonate and water and then dried over anhydrous sodium sulfate. It was evaporated *in vacuo* to afford syrupy VIb contaminated with a trace of 4,6-ditosylate.

The product was chromatographed on silica gel (150 g) in benzene-ether (8:1→4:1 v/v) to give pure VIb (1.5 g, 69%) which resisted to crystallization. $[\alpha]_D^{22} + 58.1$ ($c = 2.61$, CHCl_3), $\nu_{\text{max}}^{\text{H}_2\text{O}}$ cm^{-1} : 1180, 1360 (SO_2), 1720 (C=O) 3500 (OH). *Anal.* Found: C, 58.28; H, 5.53; S, 7.17. Calcd. for $\text{C}_{21}\text{H}_{24}\text{O}_8\text{S}$: C, 57.79; H, 5.54; S, 7.35%.

Methyl 2-O-benzoyl-3,6-dideoxy-6-iodo- α -D-ribo-hexopyranoside (VII). A solution of VIb (1.4 g) and sodium iodide (1.5 g) in 2-butanone was heated under reflux for 6 hr and cooled. The precipitate was filtered off and the filtrate was evaporated *in vacuo* to dryness. After the residue was extracted with chloroform (50 ml), the extract was washed with water, dried over anhydrous sodium sulfate and evaporated *in vacuo* to give a solid mass (VII, 1.3 g, 104%). Recrystallization from a mixture of ethyl acetate and *n*-hexane gave colorless needles. mp 131~132°C, $[\alpha]_D^{20} + 70.5$ ($c = 2.66$, CHCl_3), $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1700 (C=O), 3480 (OH). *Anal.* Found: C, 42.82; H, 4.43; I, 32.51. Calcd. for $\text{C}_{14}\text{H}_{17}\text{O}_5\text{I}$: C, 42.87; H, 4.37; I, 32.36%.

Methyl 2-O-benzoyl-3,6-dideoxy- α -D-ribo-hexopyranoside (VIIa). To a solution of VII (7 g) in methanol (200 ml) were added a solution of sodium acetate trihydrate (5 g) in water (20 ml) and palladium on carbon (10%, *ca.* 1 g). The mixture was shaken in hydrogen until the absorption of the gas stopped.

After filtration of the catalyst aqueous sodium bicarbonate (saturated, 30 ml) was added to the filtrate and the mixture was evaporated *in vacuo* to dryness. The resulting residue was extracted with chloroform and the extract was washed with water, dried over anhydrous sodium sulfate and evaporated *in vacuo* to

give syrupy VIIIa (3.6 g, 76%) which revealed one spot on t.l.c. (benzene-ether, 4:1 v/v). For analyses, a part of VIIIa was chromatographed on silica gel in benzene-ether (6:1 v/v). $[\alpha]_D^{20} + 96.4^\circ$ ($c=1.96$, CHCl_3). Anal. Found: C, 63.13; H, 6.57. Calcd. for $\text{C}_{14}\text{H}_{18}\text{O}_5$: C, 63.14; H, 6.81%.

Methyl 2-O-benzoyl-3,6-dideoxy-4-O-mesyl- α -D-ribo-hexopyranoside (VIIIb). Methylsulfonyl chloride (2 g) was added under cooling with ice to a solution of VIa (2.3 g). The mixture was kept over night at 5°C and poured into ice-water (ca. 100 ml) to yield a precipitate (VIIIb) which was filtered off, washed with water, dried, and recrystallized from isopropyl ether (2.8 g, 94%). mp $100\sim 103^\circ\text{C}$, $[\alpha]_D^{21} + 129.0^\circ$ ($c=1.64$, CHCl_3), $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1180, 1360 (SO_2), 1730 (C=O), τ^{23} : 8.66 (3-proton, doublet, $J_{5,6}$ 6 cps, H-6), 6.18 (1 proton, multiplet which collapsed to a doublet on irradiation at τ 8.66, $J_{4,5}=10.2$ cps, H-5). Anal. Found: C, 52.63; H, 5.84; S, 9.24. Calcd. for $\text{C}_{15}\text{H}_{20}\text{O}_7\text{S}$: C, 52.31; H, 5.85; S, 9.31%.

Methyl 4-azido-2-O-benzoyl-3,4,6-trideoxy- α -D-xylo-hexopyranoside (IXa). To a solution of VIIIb (2.5 g) in DMF (80 ml) was added a solution of sodium azide (2 g) and urea (400 mg) in water (6 ml). The mixture was heated at 120°C for 7 hr, poured into water (300 ml) and extracted with ether (500 ml). After washing with water the extract was dried over sodium sulfate and evaporated *in vacuo* to give syrupy VIIa (2.0 g, 93%), which was chromatographed on silica gel (120 g) in benzene-ether (12:1 v/v). The purified IXa crystallized on standing. mp $80\sim 83^\circ\text{C}$, $[\alpha]_D^{21} + 61.2^\circ$ ($c=2.27$, CHCl_3), $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1720 (C=O); 2100 (N_3), τ^{23} : 8.70 (3 protons, doublet, H-6, $J_{5,6}$ 6.6 Hz), 6.26 (1 proton, broad multiplet, H-4), 5.90 (1 proton, octet which collapsed to a doublet on irradiation at τ 8.70, H-5, $J_{4,5}$ 1.8 Hz). Anal. Found: C, 57.48; H, 5.69; N, 14.65. Calcd. for $\text{C}_{14}\text{H}_{17}\text{O}_4\text{N}_3$: C, 57.72; H, 5.88; N, 14.43%. In another run, VIIIb (51 g) in DMF (1 liter) was treated in a similar way. In this case IXa crystallized as soon as the reaction mixture was poured into water. It could be recrystallized from cyclohexane. mp $80\sim 83^\circ\text{C}$.

Methyl 4-azido-3,4,6-trideoxy- α -D-xylo-hexopyranoside (IXb). An aqueous solution of sodium hydroxide (1%,

14 ml) was added to a solution of IXa (800 mg) in ethanol (30 ml). After the mixture was heated at 60°C for 1 hr, it was neutralized with solid carbon dioxide and evaporated to dryness. The resulting residue was extracted with chloroform (60 ml) and the extract was washed with water, dried over sodium sulfate and evaporated *in vacuo* to give syrupy IXb which solidified soon. Recrystallization from various solvents was unsuccessful, and IXb was purified by column chromatography on silica gel (cyclohexane-ethyl acetate, 2:1 v/v). mp $64\sim 5^\circ\text{C}$, $[\alpha]_D^{23} + 107.1^\circ$ ($c=4.46$, CHCl_3), $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 2100 (N_3), 3450 (OH), $[\theta]_{286}^{20} + 188$ ($c=0.0465$, ethanol). Anal. Found: C, 44.77; H, 6.94; N, 22.62. Calcd. for $\text{C}_7\text{H}_{13}\text{O}_3\text{N}_3$: C, 44.91; H, 7.00; N, 22.45%. This compound sublimes under reduced pressure.

4-Azido-3,4,6-trideoxy- α -D-xylo-hexopyranose (X). Sulfuric acid (98%, 0.3 ml) was added dropwise to a solution of IXa (1.8 g) in acetic anhydride (30 ml) under cooling in an ice-bath. The mixture was allowed to stand at room temperature for 5 days. The solution was poured into ice-water (250 ml), neutralized with an excess of sodium bicarbonate and extracted with dichloromethane (300 ml). The extract was washed with water, dried over sodium sulfate, and evaporated *in vacuo* to afford a brown syrup (1.73 g), which showed two main spots²⁴⁾ and two faint spots on t.l.c. (silica gel G, benzene-ether 8:1 v/v). Two major products (anomers of 1-acetate) were isolated in whole by column chromatography by using silica gel (100 g) and benzene-ether (10:1 v/v) as an eluent; yield 1.1 g (55%), $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1490, 1500, 1590, 1605 (benzene ring), 1720 $\sim$ 1770 (C=O of esters), 2100 (N_3).

The anomeric mixture of 1-acetate (1.1 g) was dissolved in methanol (25 ml) and cooled in an ice-bath. Aqueous sodium hydroxide (1 N, 6.8 ml) was added to the solution and the mixture was kept for 2 hr at 0°C . After neutralization with solid carbon dioxide the mixture was evaporated *in vacuo* below 45°C and the resulting residue was extracted with acetone (30 ml). The extract was evaporated *in vacuo* to afford a yellow syrup, which was kept at 0°C , allowed to crystallize overnight and recrystallized from isopropyl ether-acetone; yield 540 mg (90% from 1-acetate). mp $87\sim 90^\circ\text{C}$, $[\alpha]_D^{20} - 14.5^\circ$ (6 min) $\rightarrow -43.4^\circ$ (66 min, const.) ($c=1.43$, H_2O), $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 2100 (N_3), 3300 $\sim$ 3500 (OH), τ^{25} : 8.89 (three protons, doublet,

23) Chloroform-d and tetramethylsilane (TMS) were used as a solvent and as an internal reference, respectively.

24) They moved so close to each other.

H-6, $J_{5,6}$ 6.6 Hz), 5.90 (1 proton, quartet, H-5, $J_{4,5}$ 1.8 Hz; $J_{5,6}$ 6.6 Hz), 5.41 (1 proton, doublet which disappeared on addition of D_2O , OH-2, $J_{2,OH}$ 7.2 Hz), 5.12 (1 proton, quartet which collapsed to a doublet on irradiation at τ 3.67, H-1, $J_{1,OH}$ 4.2 Hz, $J_{1,2}$ 3.6 Hz), 3.67 (1 proton, doublet which collapsed to a singlet on irradiation at τ 5.12, OH-1, $J_{1,OH}$ 4.2 Hz). *Anal.* Found: C, 41.70; H, 6.23; N, 24.29. Calcd. for $C_6H_{11}O_3N_3$: C, 41.61; H, 6.40; N, 24.27%.

4-Azido-3,4,6-trideoxy- α -D-xylo-hexopyranose p-tolylsulfonfylhydrazone (XI). A solution of X (96 mg) and *p*-tolylsulfonfylhydrazide (113 mg) in methanol (3 ml) was heated under reflux for 1 hr. Although several products were detected on t.l.c. (silica gel G, $CHCl_3$ - CH_3OH , 94:6 v/v), XI crystallized from the mixture on standing in an open flask for 2 days. The crude XI was recrystallized from ethanol. Yield 68 mg (35%). mp 146°C (Decompose), $[\alpha]_D^{22} - 54.0^\circ$ (5 min, $c=1.30$, *p*-dioxane). *Anal.* Found: C, 45.96; H, 5.83; N, 20.70; S, 9.37. Calcd. for $C_{13}H_{19}O_4N_3S$: C, 45.73; H, 5.61; N, 20.51; S, 9.39%.

4-Azido-3,4,6-trideoxy-D-xylo-hexitol (XIII). Sodium borohydride (58 mg) was added under ice-cooling to a solution of X (470 mg) in methanol (3.5 ml). The mixture was kept at 0°C for 30 min and then stirred with Amberlite IR-120 (H^+ , 1.5 ml). After filtration the filtrate was evaporated *in vacuo* with several additions of methanol and finally to dryness, to give syrupy XII (540 mg, 93%) which showed one spot on t.l.c. (silica gel G, $CHCl_3$ - CH_3OH , 4:1 v/v). For analyses, it was partially chromatographed on silica gel in chloroform-methanol (9:1 v/v). $[\alpha]_D^{22} + 49.0^\circ$ ($c=2.57$, *p*-dioxane) $\nu_{max}^{H_{11}m} cm^{-1}$: 2150 (N_3), 3300~3600 (OH). *Anal.* Found: C, 40.59; H, 7.24; N, 23.92. Calcd. for $C_6H_{13}O_3N_3$: C, 41.13; H, 7.48; N, 23.99%.

4-Azido-1,2-O-isopropylidene-3,4,6-trideoxy-D-xylo-hexitol (XIIIa). A mixture of XII (500 mg) and Dowex 50 W (H^+ , 400 mg) in dry acetone (20 ml) was stirred for 4 hr at room temperature. After filtration the filtrate was evaporated *in vacuo* to give syrupy XIIIa, which was chromatographed on silica gel (ethyl acetate-cyclohexane, 1:1 v/v); yield 440 mg (71%). $[\alpha]_D^{22} + 27.7^\circ$ ($c=1.52$, $CHCl_3$) $\nu_{max}^{KBr} cm^{-1}$: 2150 (N_3). *Anal.*

Found: C, 50.14; H, 8.02; N, 19.54. Calcd. for $C_9H_{17}O_3N_3$: C, 50.22; H, 7.96; N, 19.52%.

4-Azido-1, 2-O-isopropylidene-5-O-methylsulfonyl-3, 4, 6-trideoxy-D-xylo-hexitol (XIIIb). Methylsulfonyl chloride (300 mg) was added under cooling to a solution of XIIIa (280 mg) in pyridine (3 ml). After the mixture was kept at 0°C overnight, it was poured into ice-water and extracted with chloroform (30 ml). The extract was washed with cold aqueous sulfuric acid (below 3%) and water, dried over anhydrous sodium sulfate and evaporated *in vacuo* to give syrupy XIIIb (320 mg, 83%). For analyses, it was chromatographed on silica gel (cyclohexane-ethyl acetate, 3:1 v/v). $[\alpha]_D^{26} + 72.6$ ($c=1.72$, $CHCl_3$) $\nu_{max}^{H_{11}m} cm^{-1}$: 1175, 1360 (SO_2), 2100 (N_3). *Anal.* Found: C, 40.99; H, 6.53; N, 14.37; S, 10.96. Calcd. for $C_{10}H_{19}O_5N_3S$: C, 40.95; H, 6.53; N, 14.32; S, 10.93%.

4, 5-Diazido-1, 2-O-isopropylidene-3, 4, 5, 6-tetradexoxy-L-arabino-hexitol (XIV). To a solution of XIIIb (2.9 g) in DMF (100 ml) was added a solution of sodium azide (2 g) and urea (400 mg) in water (10 ml). The mixture was heated at 110°C overnight and evaporated *in vacuo*. To the resulting residue were added chloroform (150 ml) and water (100 ml). The chloroform layer was separated, washed with water (50 ml), dried over sodium sulfate and evaporated *in vacuo* to afford syrupy XIV, which was chromatographed on silica gel (cyclohexane-ethyl acetate, 7:1 v/v); yield 2.2 g (92%). $[\alpha]_D^{23} + 126.7$ ($c=1.16$, $CHCl_3$) $\nu_{max}^{H_{11}m} cm^{-1}$: 2100 (N_3), m/e : 225 (M^+-CH_3). *Anal.* Found: C, 45.03; H, 6.72; N, 34.84. Calcd. for $C_9H_{16}O_2N_6$: C, 44.99; H, 6.71; N, 34.98%.

4, 5-Diamino-1, 2-O-isopropylidene-3, 4, 5, 6-tetradexoxy-L-arabino-hexitol (XV). To a solution of XIV (800 mg) in methanol (25 ml) was added Raney Ni and the mixture was shaken in hydrogen under a pressure (4 kg/cm²) at room temperature for 3 hr. After filtration of the catalyst the filtrate was evaporated *in vacuo* to yield syrupy XV (400 mg, 63%), which was then used for the preparation of XVI without further purification.

The crude XV gave a positive result in the ninhydrin test and had no absorption at 2100~2200 (N_3) in the IR spectrum.

4,5-N-Carbonyl-4,5-diamino-1,2-O-isopropylidene-3,4,5,6-tetradexoxy-L-arabino-hexitol (XVI). Carbonyl chloride was bubbled for 50 min at room temperature into a

25) Dimethylsulfoxide- d_6 and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) were used as a solvent and as an internal standard, respectively.

solution of XV (200 mg) in aqueous sodium carbonate (10%, 20 ml). A drop of methanolic phenolphthalein was added every ten minutes during the reaction in order to make sure that the solution was kept alkaline. The mixture was evaporated *in vacuo* to 1/4 volume and extracted with chloroform (150 ml). The extract was dried over sodium sulfate and evaporated *in vacuo* to give syrupy XVI (120 mg, 53%) which crystallized soon. It was recrystallized from ethyl-acetate-*n*-hexane. mp 128~129°C, $[\alpha]_D^{24} + 32.9^\circ$ ($c = 1.04$, CHCl_3) $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1710 (C=O), 3250 (NH), m/e : 214 (M^+), 199 ($\text{M}^+ - \text{CH}_3$). Anal. Found: C, 56.29; H, 8.27; N, 13.27. Calcd. for $\text{C}_{10}\text{H}_{18}\text{O}_3\text{N}_2$: C, 56.05; H, 8.47; N, 13.08%.

4,5-N-Carbonyl-4,5-diamino-3,4,5,6-tetra-deoxy-L-arabino-hexitol (XVII). A mixture of XVI (230 mg) and Dowex 50 W (H^+ , 240 mg) was stirred in water (12 ml) for 1 hr at room temperature.

After filtration the filtrate was evaporated *in vacuo* to afford crystalline XVII (160 mg, 86%), which was recrystallized from a small amount of methanol. mp 125~130°C, $[\alpha]_D^{20} + 43.9^\circ$ ($c = 2.55$, ethanol), $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1660, 1680 (shoulder) (C=O), 3300, 3400 (OH and NH). Anal. Found: C, 48.66; H, 7.86; N, 16.00. Calcd from $\text{C}_7\text{H}_{14}\text{O}_3\text{N}_2$: C, 48.26; H, 8.10; N, 16.08%.

Sodium periodate oxidation of XVII and the subsequent Wittig reaction to methyl 7,8-N-carbonyl-7(R), 8(S)-diamino-nona-2,4-dienoate (XIX). Sodium periodate (792 mg) was added to a cold solution of XVII (580 mg) in water (20 ml). After the mixture was kept for 2 hr at 0°C, it was evaporated completely by freeze-drying and extracted with absolute ethanol (90 ml).

Wittig reagent (XX, 2.4 g) was added to the extract and the mixture was stirred for two days at room temperature, protecting from a moisture. The resulting solution was evaporated *in vacuo* to dryness and the residue was suspended in ethyl acetate (30 ml). After removal of an impurity by filtration the filtrate was evaporated to a volume of ca. 20 ml and the resulting triphenylphosphine oxide (800 mg) was filtered off.

The filtrate was evaporated and chromatographed

on silica gel (130 g, $\text{CHCl}_3\text{-CH}_3\text{OH}$, 94:6 v/v). The fractions containing D and E (Fig. 3) were collected and evaporated to afford a deeply brown syrup (200 mg), from which components D and E were isolated respectively by twice repeated preparative layer chromatography (silica gel GF₂₅₄, 2 mm thick, 20 cm $\times$ 20 cm, $\text{CHCl}_3\text{-CH}_3\text{OH}$ 94:6 v/v, detected by UV-light). Almost pure D (XIX, 70 mg) and crude E E (25 mg) were obtained.

(+)-*Desthiobiotin (IIa) via its methyl ester (IIb)*. A solution of XIX (63 mg) in methanol was shaken at room temperature in hydrogen with Adams catalyst, absorbing 11 ml of hydrogen. After filtration the filtrate was evaporated *in vacuo* to give a syrup, which was distilled at 100~110°C under a high vacuum (2×10^{-5} mmHg). As the distillate was found to be contaminated with a trace of impurity according to t.l.c., it was chromatographed on silica gel (7 g) in chloroform-methanol (96:4 v/v). The resulting syrup (36 mg) crystallized²⁶⁾ on standing and was identical with an authentic specimen of Ib on t.l.c.²⁷⁾ (silica gel G, $\text{CHCl}_3\text{-CH}_3\text{OH}$, 9:1 v/v). To a solution of the crystals (36 mg) in ethanol (0.025 ml) was added a solution of aqueous sodium hydroxide (1 N, 0.25 ml). After the mixture was kept at 50°C for 30 min, it was acidified with aqueous hydrogen chloride (5 N, 0.05 ml) to afford crystalline Ia, which was filtered off, washed with water and dried; yield 20 mg (6% for XVII). mp 160~162°C. No depression when mixed with an authentic specimen. $[\alpha]_D^{19} + 7.00$ ($c = 0.91$, ethanol).²⁸⁾

Its IR spectrum completely agreed with that of an authentic sample (Fig. 4).

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26) The melting point was too low to observe.

27) Detected by spraying with 10% aqueous sulfuric acid and charring.

28) $[\alpha]_D^{17} + 10.4$ ($c = 0.58$, ethanol) for the authentic sample.