The Synthesis of 6-Deoxy Homologs of Muramic Acid

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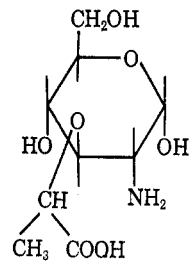
Received September 24, 1969

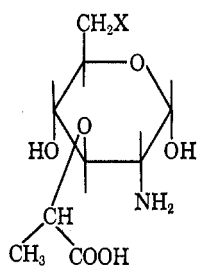
2-Amino-2,6-dideoxy-3-*O*-(D-1-carboxyethyl)-D-glucopyranose and the 6-fluoro derivative have been synthesized. Fluorine was introduced *via* displacement of the corresponding tosylate with tetrabutylammonium fluoride.

Muramic acid (I), being a constituent of the cell walls of both gram-positive and gram-negative bacteria



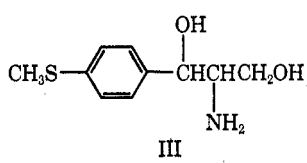
I

and vital to cell-wall synthesis, presents an interesting point of attack for the inhibition of cell-wall synthesis.[1] Lindberg[2] prepared several homologs of muramic acid with variations of the lactic acid side chain. These compounds exhibited no antibacterial activity when tested *in vitro*. Two modifications, which appeared interesting both chemically and biologically, were those which involved the replacement of the 6-hydroxyl group, shown in structure II.



IIa, X = H
b, X = F

This paper describes the synthesis of (+)-6-deoxy- and 6-fluoro-6-deoxymuramic acid. The intermediate (**1**) was prepared according to the method of Gigg and Carrol.[3] In order to separate the requisite D isomer from the isomeric mixture, the salt of (+)-(1*R*,2*S*)-2-amino-1-[4-(methylthio)phenyl]-1,3-propanediol (III)[4]



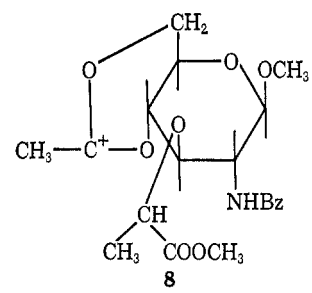
III

(1) M. R. Salton, "The Bacterial Cell Wall," Elsevier Publishing Co., New York, N. Y., 1964.
(2) B. Lindberg and H. Agbach, *Acta Chem. Scand.*, **18**, 185 (1964).
(3) R. H. Gigg and P. M. Carrol, *Nature*, **191**, 495 (1961).
(4) R. A. Cutler, R. J. Stenger, and C. M. Suter, *J. Amer. Chem. Soc.*, **74**, 5475 (1952).

was prepared. This method of separation proved far superior to the one utilized by Gigg, which involved the separation of the S-benzylpseudothiourea salt.

The opening of the oxazolidine ring in **1b** with subsequent rearrangement to the glucopyranose has been performed under a variety of conditions. Since the proposed synthetic approach required the protection of the glycosidic hydroxyl group, the conditions of methanolic hydrogen chloride utilized by Lindberg[2] were chosen. In this fashion, the opening of the oxazolidine ring and introduction of the methoxy group at the 1 position could be achieved simultaneously. The product of this rearrangement was reported as the corresponding acid.[5] However, analytical as well as nmr data confirmed the structure as being the ester **2a**. Tritylation of **2a** with subsequent acetylation produced **2b**, which was heated with glacial acetic acid to give **2c**.

A search of the literature indicates that no satisfactory method exists for the introduction of fluorine into a carbohydrate molecule under mild conditions. Recently, Gubitz[6] examined and successfully employed tetrabutylammonium fluoride (TBAF) for this purpose. Originally it was planned to activate the 6 position of **2c** by formation of the mesylate **2d** followed by displacement with fluoride. When **2d** was treated with tetrabutylammonium fluoride, the desired compound **3a** was not obtained; instead a mixture of **2c** and **2e** was produced, probably *via* the intermediate **8** formed by internal displacement of the mesylate.



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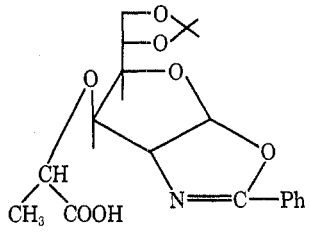
In view of this failure, attention was turned to the more reactive tosylate **2f**, which was readily prepared from **2a**. Treatment of **2f** with a slight excess of TBAF produced **3b** in good yield. Hydrolysis of **3b** in 3 *N* HCl gave **4** as a hygroscopic solid.

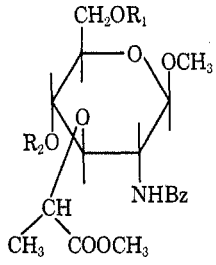
The deoxy homolog **7** was obtained from **2f** by treatment with sodium iodide in acetone, which produced the iodo lactone **5a**. This compound exhibited a molecular ion peak at *m/e* 235, and the nmr spectrum supported this structure. Further confirmation was obtained by conversion of **5a** with sodium methoxide in

(5) Lindberg reports this as the acid (see ref 2).
(6) F. W. Gubitz, to be published.

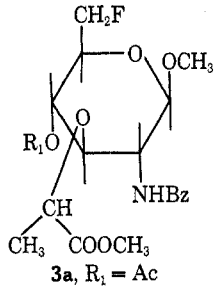
methanol into the ester **6**, whose nmr spectra exhibited six methoxy hydrogens at 165 and 170 Hz. Hydrogenation of **5a** in methanol with Raney nickel produced the 6-deoxy compound **5b**. Hydrolysis of **5b** with 3 *N* HCl produced **7** as a crystalline solid.



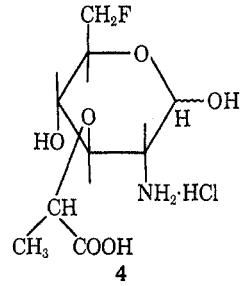
1a, (±)
b, $[\alpha]^{25}D + 66.7°$



2a, $R_1 = H$; $R_2 = H$
b, $R_1 = Tr$; $R_2 = Ac$
c, $R_1 = H$; $R_2 = Ac$
d, $R_1 = Mes$; $R_2 = Ac$
e, $R_1 = Ac$; $R_2 = H$
f, $R_1 = Ts$; $R_2 = H$



3a, $R_1 = Ac$
b, $R_1 = H$



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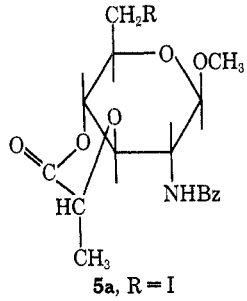
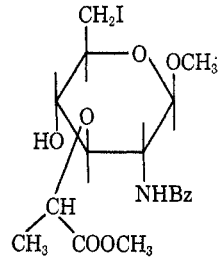


5a, R = I
b, R = H



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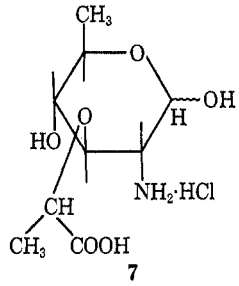

7

Experimental Section[7]

2-Phenyl-4,5-[3-*O*-(D-1-carboxyethyl)-5,6-isopropylidene-D-glucofurano]-Δ^2-oxazoline (1b).[8]—A mixture of 94 g (0.261 mol) of the DL acid **1a** and 55 g (0.261 mol) of the amine III in 1 l. of isopropyl alcohol was heated to boiling on a hot plate. After all of the solid had dissolved, the solution was filtered and the filtrate was cooled at room temperature for 5 hr and then at 0° overnight. This cooling procedure aided crystallization of the product. The solid was collected and washed with cold isopropyl alcohol: yield 100 g; mp 165–166°.

Anal. Calcd for $C_{29}H_{37}N_2O_9S$: C, 59.20; H, 6.27; N, 4.75. Found: C, 59.07; H, 6.96; N, 4.24.

To a suspension of 60.4 g (0.105 mol) of the amine salt of **1a** in 250 ml of water and 1200 ml of ether was added dropwise 101 ml of 1 *N* HCl over a 30-min period. After the addition was complete, the ether layer was collected and dried and the ether was removed. The residual white solid **1b** was recrystallized from ethyl acetate: yield 26.5 g; mp 166–167°; $[\alpha]^{25}D$ +66.7° (*c* 1, $CHCl_3$) (lit.[8] mp 163–164°; $[\alpha]^{25}D$ +65°).

Methyl 2-Benzamido-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-6-*O*-trityl-β-D-glucopyranoside 4-Acetate (2b).—To a solution of 30.4 g (0.0794 mol) of **2a**[2] in 520 ml of pyridine was added 27.3 g (0.098 mol) of trityl chloride.[9] The solution was stirred at room temperature for 24 hr and then heated to 100° for 3 hr. After cooling to 0°, 41.6 ml of acetic anhydride was added. The solution was left at room temperature for 24 hr. The excess solvent and acetic anhydride were removed *in vacuo*. The residue was treated with an ice–water mixture. A gum formed which gradually solidified. The solid was removed by filtration and washed with water. The material was then suspended in 1 l. of ether and 400 ml of water, and the mixture was treated dropwise with 1 *N* HCl until the pH was 4. Methylene dichloride was then added until all of the solid had dissolved. The organic layer was collected and dried, and the solvent was removed. An oily residue was obtained, which was dissolved in hot isopropyl alcohol and chilled. An oil separated which solidified on scratching. The solid **2b** was collected: yield 45 g (88%); mp 110–115°; $[\alpha]^{25}D$ +42.0° (*c* 1, DMF).

Anal. Calcd for $C_{39}H_{41}NO_9$: C, 70.18; H, 6.19; N, 2.10. Found: C, 70.41; 5.96; N, 2.39.

Methyl 2-Benzamido-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-β-D-glucopyranoside 4-Acetate (2c).—To a solution of 10 g (0.015 mol) of **2b** in 143 ml of glacial acetic acid heated to 90° was added dropwise 157 ml of water.[9] During the addition, solid began to separate. After the addition was complete (20 min), the mixture was heated for an additional 20 min at 90° and then cooled for 2 hr at 0°. The precipitated solid was collected on a filter funnel and washed with 50% acetic acid. The filtrate was then evaporated to dryness and the residue was heated with toluene *in vacuo* to remove the last traces of water. Finally, the solid was recrystallized from isopropyl alcohol, giving 3.6 g (57.3%) of **2c**: mp 206–208°; $[\alpha]^{25}D$ +39.1° (*c* 1, DMF).

Anal. Calcd for $C_{20}H_{27}NO_9$: C, 56.40; H, 6.40; N, 3.29. Found: C, 56.43; H, 6.69; N, 3.44.

Methyl 2-Benzamido-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-β-D-glucopyranoside 4-Acetate 6-Methanesulfonate (2d).—To 84 ml of dry pyridine at −10° was added dropwise 225 ml of methanesulfonyl chloride.[9] After the addition was complete, 3.9 g (0.00918 mol) of **2c** was added in five portions over a 15-min period and the solution was left at 5° overnight. The solution was then poured into 500 ml of ice–water and scratched, whereupon solid separated. After cooling for 30 min, the solid was collected, washed repeatedly with water, and dried. The material was recrystallized from ethyl alcohol, giving 3 g (71.5%) of **2d**: mp 184° dec; $[\alpha]^{25}D$ +29.9° (*c* 1, DMF).

Anal. Calcd for $C_{21}H_{29}NO_{11}S$: C, 50.20; H, 5.82; N, 2.78; S, 6.37. Found: C, 49.89; H, 5.73; N, 2.61; S, 6.37.

Reaction of 2d with Tetrabutylammonium Fluoride.—A solution of 100 mg (0.198 mmol) of **2d** and 300 mg (1.17 mmol) of tetrabutylammonium fluoride in 5 ml of methyl ethyl ketone was refluxed for 48 hr. The solvent was removed *in vacuo* and the residual oil was dissolved in 50 ml of chloroform and extracted twice with 200 ml of water. The organic layer was dried, the solvent was removed *in vacuo*, and the residue was dissolved in isopropyl alcohol. Upon cooling, a solid formed: yield 40 mg; mp 172–174°; nmr (DMSO) δ 2 and 2.07 ($2CH_3COO$). This material could not be separated into its two components.

Methyl 2-Benzamido-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-β-D-glucopyranoside 6-*p*-Toluenesulfonate (2f).—To 300

(7) All melting points were run according to the USP procedure and are uncorrected. Analyses and melting points were performed by the staff of M. E. Auerbach and K. D. Fleischer. Nmr spectra were determined on a Varian A60 spectrophotometer and the mass spectra on a Jeolco double-focusing high-resolution mass spectrometer, by R. K. Kullnig and S. Clemans.

(8) R. Gigg, P. M. Carroll, and C. D. Warren, *J. Chem. Soc.*, 2975 (1965).

(9) P. H. Gross, K. Brendel, and H. K. Zimmerman Jr., *Justus Liebigs Ann. Chem.*, **683**, 179 (1965).

ml of pyridine which was cooled to −10° was added 8.43 g (0.00465 mol) of *p*-toluenesulfonyl chloride.[10] After the addition was complete, 15 g (0.00353 mol) of **2a** was added in five portions over a 15-min period. After stirring at −10° for 1 hr, the solution was left at 5° overnight and then poured into 2 l. of water with stirring, and the solid which separated was collected, washed with water, and dried, giving 18.6 g (71.5%) of **2f**: mp 80–83°; $[\alpha]^{25}D$ +22.4° (*c* 1, DMF).

Anal. Calcd for $C_{25}H_{31}NO_{10}S$: C, 55.85; H, 5.81; S, 5.97. Found: C, 55.89; H, 5.76; S, 5.82.

Methyl 2-Benzamido-2-6-dideoxy-6-fluoro-3-*O*-[D-1-(methoxycarbonyl)ethyl]-β-D-glucopyranoside (3b).—A solution of 2.5 g (4.65 mmol) of **2f** and 1.46 g (5.6 mmol) of tetrabutylammonium fluoride in 100 ml of dry methyl ethyl ketone was refluxed overnight. The solvent was removed and the residue was triturated with water. A gum formed (**3b**), which was collected and dried and recrystallized from isopropyl alcohol: yield 1.074 g (60%); mp 184–186°; $[\alpha]^{25}D$ +46.1° (*c* 1, DMF); nmr (DMSO) δ 1.25–1.38 (d, 3, CH_3–), 2.40 (s, 3, CH_3OC–), 3.30 (s, 3, CH_3OC–), and 3.38 (s, 2, CH_2F).

Anal. Calcd for $C_{18}H_{24}FNO_7$: N, 3.64; F, 4.94. Found: N, 3.55; F, 5.04.

2-Amino-2,6-dideoxy-6-fluoro-3-*O*-(D-1-carboxyethyl)-D-glucopyranose Hydrochloride (4).—A mixture of 2.2 g (5.72 mmol) of **3b** and 5 ml of 3 *N* HCl was heated on a steam bath with stirring for 4 hr.[8] The brown solution was cooled in an ice bath for 30 min and the benzoic acid was separated by filtration. The filtrate was decolorized with charcoal, the solution obtained by filtration was concentrated to dryness, and the residue was heated with 50 ml of acetone. The undissolved solid was removed by filtration, the gummy material obtained by removal of the solvent was triturated with ether, and the resulting solid was collected and dried. A 1.2-g yield (85.7%) of material was obtained which could not be recrystallized and was hygroscopic, $[\alpha]^{25}D$ +64.1° (*c* 1, DMF).

Anal. Calcd for $C_9H_{16}FNO_6 \cdot HCl$: N, 4.83; Cl, 12.25. Found: N, 3.47; Cl, 11.65.

Methyl 2-Benzamido-2,6-dideoxy-6-iodo-3-*O*-(D-1-carboxyethyl)-D-glucopyranoside 4-Lactone (5a).—A solution of 2.5 g (4.34 mmol) of **2f** and 5 g (33.0 mmol) of sodium iodide in 25 ml of dry methyl ethyl ketone was heated in a pressure bottle at 110° for 6 hr.[11] After cooling to room temperature, the solid was removed by filtration and the filtrate was concentrated to dryness. The residue was triturated with water and the solid **5a** which formed was collected, dried, and recrystallized from ethyl alcohol: yield 1 g (43.5%); mp 232–234°; $[\alpha]^{25}D$ +34.3° (*c* 1, DMF); mass spectrum *m/e* 461; nmr (DMSO) δ 1.25–1.62 (3, CH_3) and 3.39 (s, 3, $-OCH_3$).

Anal. Calcd for $C_{17}H_{20}NIO_6$: N, 3.04; I, 27.53. Found: N, 3.04; I, 27.62.

Methyl 2-Benzamido-2,6-dideoxy-6-iodo-3-*O*-[D-1-(methoxycarbonyl)ethyl]-β-D-glucopyranoside (6).—A solution of 300 mg (0.612 mmol) of **5a** and 33 mg (0.612 mmol) of sodium methoxide in 25 ml of dry methyl alcohol was left at room temperature overnight. The solution was made slightly acidic with glacial acetic acid and evaporated to dryness. The white solid residue was triturated with water, collected, and recrystallized from ethyl acetate. A 100-mg yield of **6** was obtained: mp 189–191°; mass spectrum *m/e* 493; nmr (DMSO) δ 2.75 and 2.84 (singlets, 3 each, 2 CH_3O–) and 1.03–1.10 (d, 3, CH_3); $[\alpha]^{25}D$ +16.4° (*c* 1, DMF).

Anal. Calcd for $C_{18}H_{24}INO_7$: C, 43.80; H, 492; N, 2.84; I, 25.70. Found: C, 43.72; H, 4.86; N, 2.69; I, 25.56.

Methyl-2-benzamido-2,6-dideoxy-3-*O*-(D-1-carboxyethyl)-D-glucopyranoside-4-lactone Methanolate (5b).—A solution of 6.9 g (1.48 mmol) of **5a** in 172 ml of methyl alcohol and 18 ml of triethylamine was hydrogenated with Raney nickel at 44 psi.[11] The reduction took 2 hr, after which time the catalyst was removed by filtration and the filtrate was evaporated to dryness. The solid residue was triturated with water, collected, and recrystallized from methanol, giving 3.2 g (62%) of **5b** as its methanolate: mp 199–200°; $[\alpha]^{25}D$ +15.7° (*c* 1, DMF); mass spectrum *m/e* 368.

Anal. Calcd for $C_{17}H_{21}NO_6 \cdot CH_3OH$: C, 58.85; H, 6.82; N, 3.82. Found: C, 58.84; H, 6.98; N, 3.90.

2-Amino-2,6-dideoxy-3-*O*-(D-1-carboxyethyl)-D-glucopyranose Hydrochloride (7).—The hydrolysis of **5b** was performed in the same manner as described for the preparation of **4**. A 2.2-g yield of crude product was obtained which, after recrystallization from acetone, gave 850 mg (53.5%) of **7**: mp 174–175°; $[\alpha]^{25}D$ +111.0° (*c* 1, DMF).

Anal. Calcd for $C_9H_{17}NO_6 \cdot HCl$: N, 5.14; Cl, 13.05. Found: N, 5.12; Cl, 13.26.

Registry No.—Amine (III) salt of **1a**, 23924-09-6; **1b**, 23912-19-8; **2b**, 23912-20-1; **2c**, 23912-21-2; **2d**, 23912-22-3; **2f**, 23924-03-0; **3b**, 23924-04-1; **4**, 23924-05-2; **5a**, 23924-06-3; **5b**, 23967-32-0; **6**, 23924-07-4; **7**, 23924-08-5.

(10) W. S. Cohen, D. Levy, and E. D. Bergmann, *Chem. Ind.* (London), 1802 (1964).

(11) K. Brendel, P. H. Gross, and H. K. Zimmerman, Jr., *Justus Liebigs Ann. Chem.* **691**, 192 (1966).

A Convenient Synthesis of Protected N-Methylamino Acid Derivatives

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Received November 24, 1969

Methylation of the amide nitrogen of selected N-benzyloxycarbonyl and N-*t*-butyloxycarbonylamino acids with methyl iodide and silver oxide in dimethylformamide gives the corresponding N-methylamino acid derivatives in excellent yield. An unprotected carboxyl group also is converted by methylation into the methyl ester. The methylation reaction was shown to occur without racemization of the amino acid. The methyl esters obtained were converted by saponification into the corresponding N-protected N-methylamino acids. The N-*t*-butyloxycarbonyl derivatives of cysteine and serine gave, upon methylation, unsaturated amino acid products.

N-Methylamino acids are constituents of several naturally occurring peptide and depsipeptide antibiotics.[1] Peptides that contain N-methylamino acids are also of interest in relation to studies of peptide conformations.[2] Suitable synthetic methods for the preparation of N-methylamino acids are, therefore, of importance pursuant to the synthesis of peptide antibiotics and of N-methylated peptides. We herein report a convenient one-step synthesis of N-monomethyl-α-amino acids suitably protected for further elaboration in peptide synthesis.

The method of choice for the preparation of optically pure N-methylamino acids involves a three-step sequence in which an N-benzylamino acid is methylated with formaldehyde–formic acid followed by reductive removal of the N-benzyl group.[3] The N-methylamino

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(3) P. Quitt, J. Hellerbach, and K. Vogler, *Helv. Chim. Acta*, **46**, 327 (1963).