

*N*-[(*D*-Glucos-3-*O*-yl)acetyl]-*L*-alanine

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*N*-[2-(*D*-Glucos-3-*O*-yl)propionyl]-*L*-alanine (**7a**) was synthesized, which has a glucose residue instead of *N*-acetylglucosamine residue in the muramyl peptide. *N*-[(*D*-Glucos-3-*O*-yl)acetyl]-*L*-alanine (**7b**) was also synthesized as a glycolyl analog of **7a** in order to determine the relationship between the structure of propionyl moiety in the carbohydrate analog (**7a**) and the adjuvant activity. These simple analogs are compounds prepared for the purpose of introduction into a synthetic polymer with the view of producing polymeric drugs.

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Since it has been discovered by Shiba *et al.*, (1,2) and Merseur *et al.*, (3) that *N*-acetylmuramyl-*L*-alanyl-*D*-isoglutamine is the minimal structure required for the immunoadjuvant activity of bacterial cell-wall peptidoglycans, a number of studies on the synthesis of a variety of carbohydrate analogs has been undertaken in order to investigate the role of the carbohydrate moiety in muramyl dipeptides (4-10). In these studies, it has been found that only the *N*-acetylglucosamine moiety in muramyl dipeptides is not essential for the adjuvant activity as a carbohydrate moiety. More recently it was shown by the chemical modification of the C-2 substituent that the *D*-glucose analog has strong activity comparable to muramyl dipeptides (11).

We have also synthesized *N*-[2-(*D*-glucos-3-*O*-yl)propionyl]-*L*-alanine (**7a**) as one of the carbohydrate analogs for the same purpose. Furthermore we were interested in investigating the relationship between the structure of the propionyl moiety in the carbohydrate analog and the activity. In this paper, we deal with the synthesis of *N*-[2-(*D*-

glucos-3-*O*-yl)propionyl]-*L*-alanine (**7a**) and *N*-[(*D*-glucos-3-*O*-yl)acetyl]-*L*-alanine (**7b**) as a glycolyl analog of **7a** using a similar procedure.

The starting material is 1,2:5,6-di-*O*-isopropylidene- $\alpha$ -*D*-glucofuranose (**1**) which was synthesized by the conventional method using acetone-sulfuric acid (12). The ethyl propionate residue or ethyl acetate residue was first introduced to this furan derivative by using ethyl 2-bromopropionate and ethyl 2-bromoacetate, respectively, in the presence of sodium hydride. Hydrolysis of these compounds (**2a,b**) with 0.5 *N* sodium hydroxide gave the corresponding 3-*O*-(1-carboxyethyl) and 3-*O*-carboxymethyl derivatives (**3a,b**). Coupling of **3a** and **3b** with *L*-alanine benzyl ester was conducted with dicyclohexylcarbodiimide and *N*-hydroxysuccinimide as activating agents, to afford the corresponding propionyl and acetyl peptide derivatives (**5a,b**). These compounds were treated with 0.2 *N* sulfuric acid to convert them into the pyranoid structure until thin-layer chromatography showed the complete conversion of the starting material into the required material (**6a,b**) together with degradation products. The shorter treatment causes difficulty in the isolation by column chromatography because the 1,2-*O*-isopropylidene derivative coexists with the required material. Catalytic hydrogenolysis of **6a** and **6b** with palladium black in methanol afforded the final products (**7a,b**) in high yield.

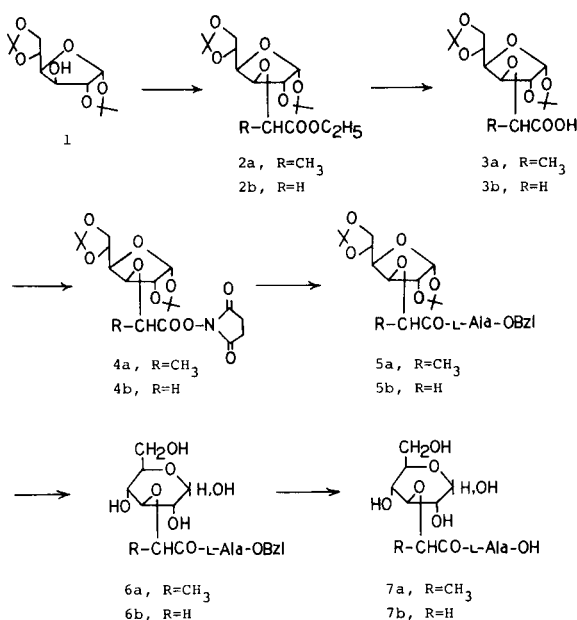
## EXPERIMENTAL

Melting points were determined in capillary tubes on a warm plate and are uncorrected. Optical rotations were determined with a Union Digital PM-101 polarimeter. The ir spectra were recorded on a JASCO A-202 spectrophotometer. The nmr spectra were measured with a JEOL PMX-60 spectrometer at 60 MHz using tetramethylsilane as the internal reference. Mass spectra were obtained on a JEOL JMS-01SG double-focusing mass spectrometer at 75 eV. The reactions were monitored on tlc with Merck F<sub>254</sub> silica gel plates, which were developed with petroleum ether-1-butanol (9:1 v/v) for compounds **2** and **3** and 1-butanol-ethanol-water (4:1:1 v/v) for compounds **4-7**.

1,2:5,6-Di-*O*-isopropylidene- $\alpha$ -*D*-glucofuranose (**1**).

This compound was prepared by the conventional method using acetone-sulfuric acid, yield 54%, mp 109.5-110.5°, lit (12) 110°.

*Anal.* Calcd. for C<sub>12</sub>H<sub>20</sub>O<sub>6</sub>: C, 55.37; H, 7.74. Found: C, 55.32; H, 7.76.



1,2:5,6-Di-*O*-isopropylidene-3-*O*-[1-(ethoxycarbonyl)ethyl]- $\alpha$ -D-glucopyranose (**2a**).

Sodium hydride (1.6 g, 34 mmoles, fine powder dispersed in mineral oil at 50% concentration) was added in portions to a solution of 1,2:5,6-di-*O*-isopropylidene- $\alpha$ -D-glucopyranose (**1**) (6.0 g, 23 mmoles) in dry THF (40 ml) under dry nitrogen. When the initial vigorous reaction had subsided, the mixture was refluxed with stirring for 30 minutes. After ethyl 2-bromopropionate (15 ml, 120 mmoles) was added to the solution cooled to room temperature, the reaction mixture was again refluxed for 6 hours. Methanol was added to decompose the excess sodium hydride and THF was evaporated under reduced pressure. The residual suspension was washed thoroughly with water and the organic layer was distilled to give the oily product, 7.9 g (95%), bp 154-154.5°/2.0 mm; ir (neat): 1742  $\text{cm}^{-1}$  (C=O); nmr (deuteriochloroform):  $\delta$  1.28-1.41 (m, 18H, 6CH<sub>3</sub>), 4.67 (d, 1H, J = 4 Hz), 5.77 (t, 1H, H-1).

Anal. Calcd. for C<sub>17</sub>H<sub>28</sub>O<sub>6</sub>: C, 56.65; H, 7.83. Found: C, 56.48; H, 7.91.

1,2:5,6-Di-*O*-isopropylidene-3-*O*-(1-carboxyethyl)- $\alpha$ -D-glucopyranose (**3a**).

Compound **2a** (2.1 g, 5.7 mmoles) was dispersed in 0.5 *N* sodium hydroxide solution (21 ml) and refluxed for 30 minutes. After cooling, the clear solution was acidified to pH 1.5 by the careful addition of 1 *N* hydrochloric acid with stirring under ice water cooling. The aqueous solution was extracted with chloroform and the chloroform extract was dried over anhydrous sodium sulfate. The drying agent was filtered off and the filtrate was evaporated under reduced pressure. The residue was lyophilized to give the crude product as a heavy sirup, 1.8 g (95%); ir (neat): 1735  $\text{cm}^{-1}$  (C=O); nmr (deuteriochloroform):  $\delta$  1.28-1.41 (m, 15H, 5CH<sub>3</sub>), 4.69 (d, 1H, J = 4 Hz), 5.80 (t, 1H, H-1), 8.90 (s, 1H, COOH).

This compound was not pure, but was satisfactory for the next synthetic step.

Benzyl *N*-[2-(1,2:5,6-Di-*O*-isopropylidene- $\alpha$ -D-glucopyranos-3-*O*-yl)propionyl]-L-alaninate (**5a**).

Dicyclohexylcarbodiimide (0.92 g, 4.5 mmoles) was added to an ice-cooled solution of **3a** (1.5 g, 4.5 mmoles) and *N*-hydroxysuccinimide (0.64 g, 5.6 mmoles) in THF (45 ml). The reaction mixture was stirred in an ice bath for 3 hours and at room temperature for 1 hour. The *N,N'*-Dicyclohexylurea formed was filtered off and washed with THF. After the combined filtrate and washings were again cooled in an ice bath, benzyl L-alaninate hydrochloride (1.07 g, 5 mmoles) and triethylamine (0.70 ml, 5.0 mmoles) were added to this solution. The mixture was stirred for 15 hours at room temperature. The insoluble material was filtered off and the solvent was evaporated under reduced pressure. The residual sirup was extracted with diethyl ether (70 ml) and the insoluble material was decanted. After evaporation of ether, the sirupy residue was subjected to column chromatography on silica gel. Elution with hexane-benzene-methanol (24:16:1) afforded a product as a sirup. This product was dissolved in a minimum quantity of hexane and cooled to crystallize in needles, 1.0 g (45%), mp 92.5-93.0°; ir (potassium bromide): 3300 (NH), 1730 (ester C=O), 1656 (amide C=O)  $\text{cm}^{-1}$ ; nmr (deuteriochloroform):  $\delta$  1.25-1.49 (m, 18H, 6CH<sub>3</sub>), 4.43 (d, 1H, J = 4 Hz), 5.13 (s, 2H, CH<sub>2</sub>Ph), 5.74 (d, 1H, H-1, J = 4 Hz), 7.83 (s, 5H, Ph); ms: *m/e* 493 (M<sup>+</sup>);  $[\alpha]_D^{20}$  -16.4° (c 0.50, methanol).

Anal. Calcd. for C<sub>25</sub>H<sub>35</sub>NO<sub>6</sub>: C, 60.84; H, 7.15; N, 2.84. Found: C, 60.43; H, 7.08; N, 2.87.

*N*-[2-(D-Glucos-3-*O*-yl)propionyl]-L-alanine Benzyl Ester (**6a**).

Compound **5a** (0.43 g, 0.87 mmole) was dispersed with stirring in 0.2 *N* sulfuric acid (10 ml) and warmed at 55°. The mixture became homogeneous after 3 hours and the stirring was continued for 20 hours at the same temperature. After cooling, the solution was neutralized with barium carbonate and filtered. The filtrate was evaporated under reduced pressure. The residual sirup was subjected to column chromatography on silica gel. Elution with hexane-benzene-methanol (24:16:3) afforded a product as a colorless powder. Recrystallization was carried out from methanol-diethyl ether-petroleum ether, 0.1 g (27%), mp 155.5-156.5°; ir (potassium bromide): 1750 (ester C=O), 1640, 1620 (amide C=O)  $\text{cm}^{-1}$ ; nmr (DMSO-*d*<sub>6</sub>):  $\delta$  1.32 and 1.37 (d, each 3H, CH<sub>3</sub>, J = 6, J = 7.8 Hz),

5.12 (s, 2H, CH<sub>2</sub>Ph), 7.33 (s, 5H, Ph), 8.82 (m, 1H, NH);  $[\alpha]_D^{21}$  +15.6° (c 0.09, water, after 24 hours).

Anal. Calcd. for C<sub>15</sub>H<sub>27</sub>NO<sub>6</sub>·½H<sub>2</sub>O: C, 54.02; H, 6.68; N, 3.31. Found: C, 54.36; H, 6.56; N, 3.41.

*N*-[2-(D-Glucos-3-*O*-yl)propionyl]-L-alanine (**7a**).

A solution of **6a** (0.1 g, 0.24 mmole) in methanol (15 ml) was hydrogenolyzed in the presence of palladium black (0.02 g) at room temperature for 4 hours. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The residue was lyophilized to give the product as a colorless hygroscopic powder, 77 mg (96%); ir (potassium bromide): 1725 (carboxyl C=O), 1640 (amide C=O)  $\text{cm}^{-1}$ ; nmr (DMSO-*d*<sub>6</sub>):  $\delta$  1.31 (d, 6H, 2CH<sub>3</sub>, J = 7.2 Hz), 4.97 (d, 1H, H-1, J = 2 Hz), 8.63 (m, 1H, NH);  $[\alpha]_D^{25}$  +39.3° (c 0.061, water, after 24 hours).

Anal. Calcd. for C<sub>12</sub>H<sub>21</sub>NO<sub>6</sub>·H<sub>2</sub>O: C, 42.23; H, 6.79; N, 4.10. Found: C, 42.21; H, 6.85; N, 3.94.

1,2:5,6-Di-*O*-isopropylidene-3-*O*-(ethoxycarbonyl)methyl- $\alpha$ -D-glucopyranose (**2b**).

Sodium hydride (1.6 g, 33 mmoles, fine powder dispersed in mineral oil at 50% concentration) was added in portions to a solution of 1,2:5,6-di-*O*-isopropylidene- $\alpha$ -D-glucopyranose (**1**) (6.0 g, 23 mmoles) in dry THF (70 ml) under dry nitrogen. When the initial vigorous reaction had subsided, the mixture was refluxed with stirring for 30 minutes. The solution was cooled in an ice bath, ethyl bromoacetate (19.3 g, 115 mmoles) was added, and the solution was stirred for 6 hours at 0°. Methanol was added to decompose the excess of sodium hydride and THF was evaporated under reduced pressure. The residual suspension was sufficiently washed with water and the organic layer was distilled to give the oily product (bp 171-172°/0.5 mm). After standing at room temperature, the product was solidified and recrystallized from petroleum ether to give the pure product as prisms, 5.0 g (64%), mp 80.5-81.5°; ir (potassium bromide): 1750  $\text{cm}^{-1}$  (C=O); nmr (tetrachloromethane):  $\delta$  1.33-1.43 (m, 15H, 5CH<sub>3</sub>), 4.58 (d, 1H, J = 4 Hz), 5.73 (d, 1H, H-1, J = 4 Hz);  $[\alpha]_D^{22}$  -3.6° (c 1.0, chloroform).

Anal. Calcd. for C<sub>16</sub>H<sub>26</sub>O<sub>6</sub>: C, 55.48; H, 7.56. Found: C, 55.47; H, 7.66.

1,2:5,6-Di-*O*-isopropylidene-3-*O*-carboxymethyl- $\alpha$ -D-glucopyranose (**3b**).

Compound **2b** (3.4 g, 9.8 mmoles) was dispersed in 1 *N* sodium hydroxide solution (35 ml) and refluxed for 1 hour. After cooling, the clear solution was acidified to pH 1.5 by the careful addition of 1 *N* hydrochloric acid with stirring under ice-water cooling. The aqueous solution was extracted with chloroform and the chloroform extract was dried over anhydrous sodium sulfate. The drying agent was filtered off and the filtrate was evaporated under reduced pressure. The residue was lyophilized to give the crude product as a heavy sirup, 3.3 g (100%); ir (neat): 1760  $\text{cm}^{-1}$  (C=O); nmr (tetrachloromethane):  $\delta$  1.30, 1.37, 1.42 and 1.47 (s, each 3H, CH<sub>3</sub>), 4.59 (d, 1H, J = 4 Hz), 5.80 (d, 1H, H-1, J = 4 Hz), 8.92 (s, 1H, COOH);  $[\alpha]_D^{22}$  -61.6° (c 1.0, chloroform).

This compound was sufficiently pure to use in the next synthetic step.

Benzyl *N*-[1-(1,2:5,6-di-*O*-isopropylidene- $\alpha$ -D-glucopyranos-3-*O*-yl)acetyl]-L-alaninate (**5b**).

Dicyclohexylcarbodiimide (2.40 g, 11.7 mmoles) was added to an ice-cooled solution of **3b** (3.74 g, 11.7 mmoles) and *N*-hydroxysuccinimide (1.67 g, 14.5 mmoles) in THF (117 ml). The reaction mixture was stirred in an ice bath for 3 hours and at room temperature for 1 hour. The *N,N'*-Dicyclohexylurea formed was filtered off and washed with THF. After the combined filtrate and washings were again cooled in an ice bath, benzyl L-alaninate *p*-toluenesulfonate (4.59 g, 13.0 mmoles) and triethylamine (1.83 ml, 13.0 mmoles) were added to this solution. The mixture was stirred for 48 hours at room temperature. The insoluble material was filtered off and the solvent was evaporated under reduced pressure. The residual sirup was extracted with diethyl ether (200 ml) and the insoluble pasty material was decanted. After evaporation of ether, the sirupy residue was subjected to column chromatography on silica gel. Elution with hexane-benzene-methanol (24:16:1) afforded a product as a sirup, 2.8 g (50%); ir (neat): 3350 (NH), 1730 (ester C=O), 1675 (amide

*N*-[(D-Glucos-3-O-yl)acetyl]-L-alanine

C=O)  $\text{cm}^{-1}$ ; nmr (tetrachloromethane):  $\delta$  1.27-1.43 (m, 15H, 5CH<sub>3</sub>), 4.53 (d, 1H, J = 4 Hz), 5.08 (s, 2H, CH<sub>2</sub>Ph), 5.76 (d, 1H, H-1, J = 4 Hz), 7.25 (s, 5H, Ph).

*Anal.* Calcd. for C<sub>24</sub>H<sub>33</sub>NO<sub>9</sub>: C, 60.12; H, 7.36; N, 2.92. Found: C, 60.16; H, 7.13; N, 2.96.

*N*-[(D-Glucos-3-O-yl)acetyl]-L-alanine Benzyl Ester (**6b**).

The solution of **5b** (2.83 g, 5.90 mmoles) in chloroform (5 ml) was dispersed with stirring in 0.2 *N* sulfuric acid (70 ml) and warmed at 55°. After 10 minutes, chloroform was removed under reduced pressure. The stirring was continued for 6 hours at 55° and the mixture became homogeneous in this time. After cooling, the solution was neutralized with barium carbonate and filtered. The filtrate was evaporated under reduced pressure. The residual sirup was subjected to column chromatography on silica gel. Elution with hexane-benzene-methanol (24:16:3) afforded a product as a colorless powder. This powder was recrystallized from methanol-diethyl ether-petroleum ether as needles, 0.8 g (33%), mp 132-133°; ir (potassium bromide): 1750 (ester C=O), 1620 (amide C=O)  $\text{cm}^{-1}$ ; nmr (DMSO-d<sub>6</sub>):  $\delta$  1.42 (d, 3H, CH<sub>3</sub>, J = 7.2 Hz), 5.13 (s, 2H, CH<sub>2</sub>Ph), 7.33 (s, 5H, Ph), 8.57 (m, 1H, NH);  $[\alpha]_D^{20} + 1.1^\circ$  (c 0.35, water, after 24 hours).

*Anal.* Calcd. for C<sub>18</sub>H<sub>25</sub>NO<sub>9</sub>·½H<sub>2</sub>O: C, 52.94; H, 6.42; N, 3.43. Found: C, 52.85; H, 6.45; N, 3.32.

*N*-[(D-Glucos-3-O-yl)acetyl]-L-alanine (**7b**).

A solution of **6b** (0.1 g, 0.25 mmole) in methanol was hydrogenolyzed in the presence of palladium black (0.02 g) at room temperature for 2.5 hours. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The residue was dissolved in benzene and lyophilized to give the product as a colorless hygroscopic powder, 74 mg (95%); nmr (DMSO-d<sub>6</sub>):  $\delta$  1.33 (d, 3H, CH<sub>3</sub>, J = 6.6 Hz), 4.93 (d, 1H, H-1, J = 2 Hz), 8.50 (m, 1H, NH);  $[\alpha]_D^{20} 0^\circ$  (c 0.03, water, after 24 hours).

*Anal.* Calcd. for C<sub>11</sub>H<sub>19</sub>NO<sub>9</sub>·½H<sub>2</sub>O: C, 41.51; H, 6.33; N, 4.40. Found: C, 41.38; H, 6.38; N, 4.21.

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