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Note

Synthesis of some 2-acylamino-2-deoxy-1,3,4-tri-*O*-dodecanoyl-β-D-glucopyranose 6-phosphates

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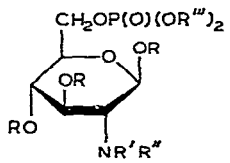
Lipid A is responsible for most of the biological activity of Gram-negative bacterial lipopolysaccharides. This glycophospholipid contains a backbone of (1→6)-linked 2-amino-2-deoxy-β-D-glucopyranose disaccharide units *N*-acylated with D-3-hydroxytetradecanoic acid and esterified with fatty and phosphoric acids. D-3-Hydroxytetradecanoic acid connected with the amino group of 2-amino-2-deoxy-D-glucose is considered[1] to be the immunodominant group of lipid A.

In our previous report[2], it was shown that the 2-deoxy-2-(DL-3-hydroxytetradecanoyl)amino- and 2-deoxy-2-tetradecanoylamino-D-glucose 6-phosphates inhibited an interaction of lipid A with a specific antiserum. This prompted the preparation of more-lipophilic haptens and an investigation of their inhibitory activity. We now report on the synthesis of derivatives of 2-amino-2-deoxy-D-glucose esterified with dodecanoic acid.



1 R = $COC_{11}H_{23}$, R'R'' = $CHC_6H_4OCH_3$ (4), R''' = Ph
2 R = $COC_{11}H_{23}$, R'R'' = $H_2 \cdot HCl$, R''' = Ph
3 R = $COC_{11}H_{23}$, R' = H, R'' = Ac, R''' = Ph
4 R = R' = $COC_{11}H_{23}$, R'' = H, R''' = Ph
5 R = $COC_{11}H_{23}$, R' = H, R'' = $COC_{13}H_{27}$, R''' = Ph
6 R = $COC_{11}H_{23}$, R' = H, R'' = $COCH_2CH(OH)C_{11}H_{23}$, R''' = Ph
7 R = $COC_{11}H_{23}$, R' = R''' = H, R'' = Ac
8 R = R' = $COC_{11}H_{23}$, R'' = R''' = H
9 R = $COC_{11}H_{23}$, R' = R''' = H, R'' = $COC_{13}H_{27}$
10 R = $COC_{11}H_{23}$, R' = R''' = H, R'' = $COCH_2CH(OH)C_{11}H_{23}$

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TABLE I

^{13}C-N.M.R. DATA FOR COMPOUNDS **3**, **11**, AND **12**

Compound	*Chemical shifts (p.p.m.)*					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
3	92.3	53.8	72.4	68.2	73.1	66.9
11	92.7	52.7	72.6	67.8	73.2	61.7
12	92.3	73.5	72.6	68.8	73.3	62.5

The route described[3] for the synthesis of 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-D-glucopyranose 6-phosphate was used. Selective phosphorylation of 2-deoxy-2-(4-methoxybenzylidene)amino-D-glucose[4] with diphenyl phosphorochloridate was followed by *O*-acylation with dodecanoyl chloride to give 2-deoxy-1,3,4-tri-*O*-dodecanoyl-2-(4-methoxybenzylidene)amino-β-D-glucopyranose (6-(diphenyl phosphate) (**1**). 2-Amino-2-deoxy-1,3,4-tri-*O*-dodecanoyl-β-D-glucopyranose 6-(diphenyl phosphate) hydrochloride (**2**) was obtained by mild, acid hydrolysis of **1**. Amidation of **2** with acetic, dodecanoic, tetradecanoic, and DL-3-hydroxytetradecanoic acids afforded **3**–**6**, from which the phenyl groups were removed by catalytic hydrogenolysis to yield the phosphates **7**–**10**.

The presence of the phosphate group at C-6 and the β-anomeric configuration of the products were confirmed by ^{13}C-n.m.r. spectroscopy. For interpretation of ^{13}C-n.m.r. data, 2-deoxy-1,3,4,6-tetra-*O*-dodecanoyl-2-dodecanoylamino-β-D-glucopyranose[5] (**11**) was prepared. Its spectrum (Table I) was similar to that[6] of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (**12**). The exception was the C-2 signal of **11**, which was shifted to 52.5 p.p.m. because of the presence of the amino group at C-2. The downfield shift of the C-6 signal of **3** by 5.2 p.p.m. was due to the phosphate group at C-6. The inhibitory activity of the new compounds is being investigated.

EXPERIMENTAL

General methods. — Melting points were determined on a Boethius table, and optical rotations were measured with a Perkin–Elmer Model 141 polarimeter. Proton-decoupled, ^{13}C-n.m.r. spectra (22.6 MHz) were recorded for solutions in $CDCl_3$ with a Bruker HX-90 E spectrometer. Chemical shifts are expressed in p.p.m. from external Me_4Si. Liquid column chromatography was carried out on Silikagel L (40–100 μm, Lachema n.p., Brno).

*2-Deoxy-1,3,4-tri-*O*-dodecanoyl-2-(4-methoxybenzylidene)amino-β-D-glucopyranose 6-(diphenyl phosphate)* (**1**). — Diphenyl phosphorochloridate (2.6 ml, 12.0 mmol) was added to a solution of 2-deoxy-2-(4-methoxybenzylidene)amino-D-glucose[4] (3 g, 10.1 mmol) in dry pyridine (100 ml) at −10°, and the mixture was kept for 12 h at −5°. A solution of dodecanoyl chloride (8 g, 36.4 mmol) in dry chloroform

(40 ml) was then slowly added dropwise at 70°. The mixture was stored for 1.5 h at 70° and then concentrated *in vacuo*. A solution of the residue in chloroform (250 ml) was washed with saturated, aqueous sodium hydrogencarbonate (2 × 300 ml) and water (4 × 300 ml), dried, and concentrated *in vacuo*. Traces of pyridine were removed by distillation of toluene (3 × 5 ml) from the residue, which was crystallised from ethanol–hexane to yield **1** (7.6 g, 70%), m.p. 70.5–71°, $[\alpha]_D^{25}$ +55° (*c* 0.5, chloroform).

Anal. Calc. for $C_{62}H_{94}NO_{12}P$: C, 69.18; H, 8.80; N, 1.30. Found: C, 68.69; H, 8.91; N, 1.34.

*2-Amino-2-deoxy-1,3,4-tri-*O*-dodecanoyl-β-*D*-glucopyranose 6-(diphenyl phosphate) hydrochloride* (**2**). — A solution of **1** (2 g, 1.86 mmol) in acetone (20 ml) containing 6M hydrochloric acid (0.31 ml, 1.86 mmol) was boiled for 1 min. The precipitate obtained after 20 h at 5° was collected, washed with cold acetone, and dried, to yield **2** (1 g, 78%), m.p. 165–167°, $[\alpha]_D^{25}$ +23° (*c* 1, methanol).

Anal. Calc. for $C_{54}H_{89}ClNO_{11}P$: C, 65.21; H, 9.02; N, 1.41. Found: C, 65.23; H, 8.96; N, 1.38.

*2-Acetamido-2-deoxy-1,3,4-tri-*O*-dodecanoyl-β-*D*-glucopyranose 6-(diphenyl phosphate)* (**3**). — A mixture of **2** (0.86 g, 0.83 mmol), acetic anhydride (0.3 ml), and pyridine (25 ml) was kept at 25° for 20 h, poured into ice–water (500 ml), and extracted with chloroform (2 × 50 ml). The extract was washed with dilute hydrochloric acid (2 × 50 ml), water (2 × 50 ml), saturated, aqueous sodium hydrogencarbonate (50 ml), and water (2 × 50 ml), dried, and evaporated. A final purification was carried out on a column of silica gel (chloroform–benzene–ethyl acetate, 4:5:1), to give **3** (0.75 g, 90%), m.p. 46.5–47.5° (from ethanol–water), $[\alpha]_D^{25}$ +6.5° (*c* 0.4, chloroform).

Anal. Calc. for $C_{56}H_{90}NO_{12}P$: C, 67.24; H, 9.07; N, 1.40. Found: C, 67.50; H, 9.05; N, 1.14.

*2-Deoxy-1,3,4-tri-*O*-dodecanoyl-2-dodecanoylamino-β-*D*-glucopyranose 6-(diphenyl phosphate)* (**4**). — Solutions of **2** (0.2 g, 0.2 mmol) in dry pyridine (2 ml) and dodecanoyl chloride (0.05 ml, 0.22 mmol) in dry chloroform (0.6 ml) were mixed at 25° and kept for 2 h. The mixture was then treated as described for **3**, to give **4** (0.114 g, 63%), m.p. 70.5–72° (from methanol), $[\alpha]_D^{25}$ +7° (*c* 1, chloroform).

Anal. Calc. for $C_{66}H_{110}NO_{12}P$: C, 69.51; H, 9.72; N, 1.23. Found: C, 69.43; H, 9.64; N, 0.95.

*2-Deoxy-1,3,4-tri-*O*-dodecanoyl-2-tetradecanoylamino-β-*D*-glucopyranose 6-(diphenyl phosphate)* (**5**). — A solution of dicyclohexylcarbodiimide (0.155 g, 0.75 mmol) in pyridine (3 ml) was slowly added to a solution of **2** (0.5 g, 0.5 mmol) and tetradecanoic acid (0.117 g, 0.5 mmol) in pyridine (5 ml). The mixture was stirred for 70 h at 25° and then treated as described for **3**. Recrystallisation of the product from benzene–hexane gave **5** (0.365 g, 62%), m.p. 66–67°, $[\alpha]_D^{26}$ +9° (*c* 1, chloroform).

Anal. Calc. for $C_{68}H_{114}NO_{12}P$: C, 68.69; H, 9.83; N, 1.20. Found: C, 68.83; H, 9.65; N, 0.97.

*2-Deoxy-1,3,4-tri-*O*-dodecanoyl-2-(*DL*-3-hydroxytetradecanoyl)amino-β-*D*-*

glucopyranose 6-(diphenyl phosphate) (**6**). — This compound (0.326 g, 55%), obtained from **2** (0.5 g, 0.5 mmol) as described for **5**, had m.p. 62.5–63° (from acetone–water), $[\alpha]_D^{25}$ +9° (*c* 1, chloroform).

Anal. Calc. for $C_{68}H_{114}NO_{13}P$: C, 68.95; H, 9.70; N, 1.18. Found: C, 68.93; H, 9.68; N, 1.06.

General method for preparing compounds **7–10**. — A solution of each of the diphenyl phosphoryl derivatives **3–6** (0.08 mmol) in glacial acetic acid (5 ml) was hydrogenolysed over Adams' catalyst (0.012 g) for 12 h under normal conditions. Each mixture was filtered, the solvent evaporated, and toluene evaporated from the residue to remove traces of acid. Each residue was subjected to column chromatography (chloroform–methanol–water, 65:25:4) and the product crystallised from chloroform–acetone. In this way, the following compounds were obtained.

2-Acetamido-2-deoxy-1,3,4-tri-*O*-dodecanoyl-β-D-glucopyranose 6-phosphate (**7**, 51%), m.p. 116–117°, $[\alpha]_D^{20}$ +7° (*c* 1, chloroform).

Anal. Calc. for $C_{44}H_{82}NO_{12}P \cdot 0.5H_2O$: C, 61.64; H, 9.76; N, 1.63; P, 3.61. Found: C, 61.76; H, 9.63; N, 1.44; P, 3.45.

2-Deoxy-1,3,4-tri-*O*-dodecanoyl-2-dodecanoylamino-β-D-glucopyranose 6-phosphate (**8**, 43.5%), m.p. 106–107°, $[\alpha]_D^{20}$ +6° (*c* 1, chloroform).

Anal. Calc. for $C_{54}H_{102}NO_{12}P \cdot H_2O$: C, 64.45; H, 10.16; N, 1.59; P, 3.15. Found: C, 64.47; H, 10.16; N, 1.39; P, 3.15.

2-Deoxy-1,3,4-tri-*O*-dodecanoyl-2-tetradecanoylamino-β-D-glucopyranose 6-phosphate (**9**, 37%), m.p. 103–104°, $[\alpha]_D^{20}$ +7.5° (*c* 1, chloroform).

Anal. Calc. for $C_{56}H_{106}NO_{12}P \cdot H_2O$: C, 65.02; H, 10.52; N, 1.36; P, 2.99. Found: C, 65.21; H, 10.43; N, 1.30; P, 3.00.

2-Deoxy-1,3,4-tri-*O*-dodecanoyl-2-(DL-3-hydroxytetradecanoyl)amino-β-D-glucopyranose 6-phosphate (**10**, 40%), m.p. 167–167.5°, $[\alpha]_D^{20}$ +12° (*c* 1, chloroform).

Anal. Calc. for $C_{56}H_{106}NO_{13}P \cdot H_2O$: C, 64.03; H, 10.36; N, 1.33; P, 2.95. Found: C, 64.13; H, 10.33; N, 1.35; P, 2.86.

REFERENCES

1 C. Lugowsky and E. Romanowska, *Eur. J. Biochem.*, 48 (1974) 81–87.
2 V. I. Gorbach, I. N. Krasikova, P. A. Luk'yanov, O. Yu. Razmakhnina, T. F. Solov'eva, and Yu. S. Ovodov, *Eur. J. Biochem.*, 98 (1979) 83–86.
3 F. Maley and H. A. Lardy, *J. Am. Chem. Soc.*, 78 (1956) 1393–1397.
4 M. Bergmann and L. Zervas, *Ber.*, 64 (1931) 976–980.
5 Y. Inouye, K. Onodera, S. Kitaoka, and S. Hirano, *J. Am. Chem. Soc.*, 78 (1956) 4722–4724.
6 A. S. Shashkov and O. S. Chizhov, *Bioorg. Khim.*, 2 (1976) 437–494.