Carbohydrate Research, 111 (1983) C9–C12
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Preliminary communication

Synthesis of *O*-{2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-β-D-glucopyranosyl 4-phosphate}-(1→6)-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-D-glucose The monosaccharide route

PATRICIA SZABÓ, S. ROBERT SARFATI, CHRISTIAN DIOLEZ, and LADISLAS SZABÓ

Équipe de Recherche No. 55 du Centre National de la Recherche Scientifique, Centre d'Études Pharmaceutiques, Université de Paris-Sud, F-92290 Châtenay-Malabry (France)

(Received August 3rd, 1982; accepted for publication, September 4th, 1982)

Endotoxins, lipopolysaccharides according to their composition, are major components of the outer membrane of Gram-negative bacteria. They exert numerous biological, in particular immunological, activities, and are widely used, in immunological studies, as B-cell mitogens, adjuvants, polyclonal activators, etc. Their general structure, the biosynthetic pathways of both of the hydrophilic regions, and detailed structures of a great number of repeating units present in the O-specific chains are known; data concerning the hydrophilic "core" region and the hydrophobic "Lipid A" region are, however, less abundant.

Analyses of numerous preparations of "Lipid A" by Hase and Rietschel[1] established the presence, in many of them, of the structure *O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-(1→6)-2-amino-2-deoxy-D-glucose. On the basis of previous studies, it was concluded that, in "Lipid A" preparations of enterobacterial origin, the amino groups of this disaccharide were substituted by d-(*R3*)-3-hydroxytetradecanoic acid residues and that the disaccharide carried two phosphate groups; one of these was glycosidically bound, but the chirality of the anomeric center carrying the phosphate group could not be established for any endotoxin preparation. According to Gmeiner *et al.*[2,3], in the "Lipid A" fragment of *Salmonella minnesota* Re mutant, the second phosphate group is located at C-4′. A similar assignment was made for the "Lipid A" fragment of the endotoxin of the *Escherichia coli* K 12, D31m4 strain by Rosner *et al.*[4].

In connection with work aimed at establishing the structure of the *Bordetella pertussis* endotoxin[5], and because of our current interest in the mechanism of the adjuvant effect exerted by this substance[6], compounds of known chemical structure were required. The synthesis of *O*-{2-deoxy-2-[(*3R*)-3-hydroxytetradecanamido]-β-D-glucopyranosyl 4-phosphate}-(1→6)-2-deoxy-[(*3R*)-3-hydroxytetradecanamido]-D-glucose (**11**) was, therefore, attempted. Syntheses of analogous structures have been reported recently by Kiso *et al.*[7], and Inage *et al.*[8].

2-Benzylideneamino-2-deoxy-D-glucopyranose[9] (**1**) was converted into the 4,6-*O*-benzylidene derivative **2** with benzaldehyde–zinc chloride[10], and then into the 1,3-diacetate

0008-6215/83/0000–0000/$03.00

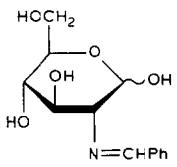
3 [^{1}H-n.m.r. (90 MHz, chloroform-*d*): δ 6, $J_{1,2}$ 8 Hz, H-1] with acetic anhydride–pyridine (0 to 20°, 16 h). Treatment of **3** in acetone with 5M hydrochloric acid at 4° gave the ammonium salt **4**, which was condensed with 3-hydroxytetradecanoic acid* in pyridine in the presence of 1 mol equiv. of triethylamine and dicyclohexylcarbodiimide. In preliminary experiments, the racemic acid was used, but in final experiments the pure D isomer[11]. Acetylation with acetic anhydride–sodium acetate (100°, 1 h) of the isolated, crude product gave the fully protected amide **5** [5:1 dichloromethane–ethyl acetate; yield 70% from **4**; $[\alpha]_D^{25}$ −23.6° (*c* 1, chloroform)]. Removal of the benzylidene group from **5** by acid hydrolysis failed, but could be accomplished by hydrogenolysis in ethanol in the presence of palladium on charcoal (50°, 1 bar) to yield the diol **6** (99:1 ethyl acetate–ethanol, yield 60%), m.p. 125–128°, $[\alpha]_D^{25}$ −6.8° (*c* 1, chloroform). A portion of **6** was selectively acetylated at O-6 by acetyl chloride in dichloromethane in the presence of 1 mol equiv. of pyridine from −50 to 20° for 16 h to give the tetraacetate **7** (ethyl acetate; yield 60%), m.p. 124–125°, $[\alpha]_D^{25}$ −20.8° (*c* 1, chloroform). Phosphorylation in benzene solution with diphenylphosphoryl chloride (2 mol. equiv.), in the presence of pyridine and of 4-dimethylaminopyridine (1.2 mol. equiv. each) for 16 h at 20°, of the remaining hydroxyl group led to the diphenyl phosphate **8** (3:1 dichloromethane–ethyl acetate; yield 80%), m.p. 79–81°, $[\alpha]_D^{25}$ −1.1° (*c* 1, chloroform). In the absence of 4-dimethylaminopyridine, phosphorylation was very slow, did not go to completion, and gave highly colored products; under the conditions just described, phosphorylation was rapid, the yield was high, and the product was colorless. Inage *et al.*[12] noted that introduction of a benzyl phosphate group at C-4′ of an otherwise completely protected 2-amino-6-*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranoside required "forced reaction conditions".

Whereas transformation of the phosphate **8** to the corresponding oxazoline derivative was easily accomplished, condensation of this with alcohols, including diol **6**, was always accompanied by simultaneous elimination of the acetoxy group of the amide-bound fatty acid.

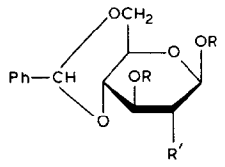
The diphenyl phosphate **8** was, therefore, treated with hydrogen bromide–acetic acid in dry chloroform (~5 mol. equiv., 20°, 2 h) to give bromide **9** which, because of its instability, was not purified. After removal of the solvents (*in vacuo*, codistillation with dry toluene) it was immediately condensed with diol **6** in 1:1 nitromethane–toluene in the presence of mercury dicyanide and molecular sieve 4A, for 18 h at 20° to give disaccharide **10** [(3:1 ethyl acetate–dichloromethane), $[\alpha]_D^{20}$ −7.1° (*c* 1, chloroform); ^{1}H-n.m.r. (400 MHz, chloroform-*d*): δ 4.70 and 5.46 ($J_{1,2} = J_{1',2'}$ 9 Hz, H-1 and -1′)], in 20% yield when **6** and **8** contained the DL acid, but only 7% with derivatives of the D acid; the reasons for the differences in yields is not clear, but it could be due to the lesser solubility, in nitromethane–toluene, of the diol **6** derived from the D acid.

Finally, deprotection of disaccharide **10** by hydrogenolysis in ethanol in the pre-

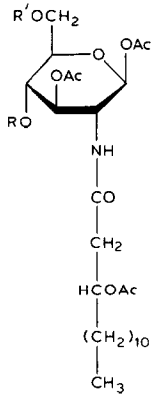
*All compounds containing 3-hydroxytetradecanoic acid were purified by column chromatography on silica gel with the mixtures of eluent (v/v) indicated; yields and optical rotations are given for the purified products containing the D acid.



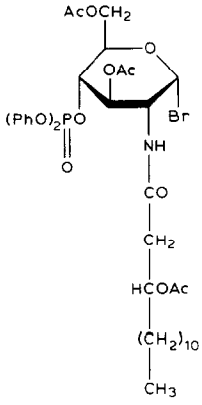
1



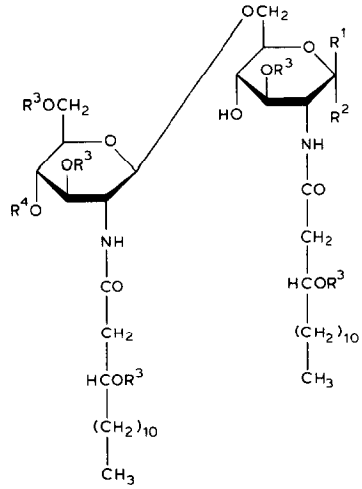
2 R = H, R′ = N=CHPh
3 R = Ac, R′ = N=CHPh
4 R = Ac, R′ = NH_3^+ Cl^-
5 R = Ac, R′ = $NHCOCH_2CHOAc(CH_2)_{10}CH_3$



6 R = R′ = H
7 R = H, R′ = Ac
8 R = $OP(OPh)_2$, R′ = Ac



9



10 R^1 = OAc, R^2 = H, R^3 = Ac, R^4 = $OP(OPh)_2$
11 R^1, R^2 = H, OH; R^3 = H; R^4 = OPO_2^{2-}

sence of platinum, followed by *O*-deacetylation with ammonia in methanol gave **11**, which was recovered by precipitation from its methanolic solution with acetone and isolated as the monoammonium salt in 55% yield, $[\alpha]_D^{25}$ +21° (*c* 0.255, 1:1 pyridine–methanol). The disaccharide phosphate **11** has the structure assigned to "Compound II" isolated, by Rosner *et al.*[4], from the endotoxin of the heptoseless *E. coli* K12, D31m4 strain[13], and had similar R_F value[4] (t.l.c. on DEAE-cellulose: 66:1:33, v/v, isobutyric acid–conc. ammonia–water). ^{1}H-n.m.r. spectra and elementary analyses of all compounds agreed with the proposed structures. Starting from 2-amino-2-deoxy-D-glucose hydrochloride, **4** was obtained with an overall yield of about 55% by use of the crude products.

REFERENCES

1 S. Hase and E. T. Rietschel, *Eur. J. Biochem.*, 63 (1976) 101–107.
2 J. Gmeiner, O. Lüderitz, and O. Westphal, *Eur. J. Biochem.*, 7 (1969) 370–379.
3 J. Gmeiner, M. Simon, and O. Lüderitz, *Eur. J. Biochem.*, 21 (1971) 355–356.
4 M. R. Rosner, J.-Y. Tang, I. Barzilay, and H. G. Khorana, *J. Biol. Chem.*, 254 (1979) 5906–5917.
5 A. Le Dur, R. Chaby, and L. Szabó, *J. Bacteriol.*, 143 (1980) 78–88.
6 N. Haeffner-Cavaillon, R. Chaby, J.-M. Cavaillon, and L. Szabó, *J. Immunol.*, 128 (1982) 1950–1954.
7 M. Kiso, H. Nishiguchi, K. Nishihori, and A. Hasegawa, *Carbohydr. Res.*, 88 (1981) C10–C13.
8 M. Inage, H. Chaki, S. Kusumoto, and T. Shiba, *Chem. Lett.*, (1980) 1373–1376.
9 W. O. Cutler, W. N. Haworth, and S. Peat, *J. Chem. Soc.*, (1937) 1979–1983.
10 D. M. Hall, *Carbohydr. Res.*, 86 (1980) 158–160.
11 M. Demary, G. Puzo, and J. Asselineau, *Nouv. J. Chim.*, 2 (1978) 373–378.
12 M. Inage, H. Chaki, S. Kusumoto, and T. Shiba, *Tetrahedron Lett.*, (1981) 2281–2284.
13 D. A. Monner, S. Jonsson, and H. G. Boman, *J. Gen. Virol.*, 24 (1974) 67–75.