Communications to the Editor

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EFFICIENT SYNTHESIS OF NOVEL MONOSACCHARIDE ANALOGS OF LIPIDS A[1)]

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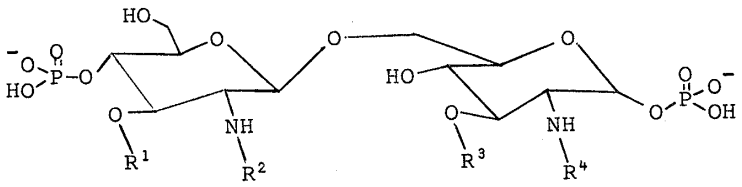
The efficient synthesis of the monosaccharide analogs of lipids A bearing two 3-acyloxytetradecanoyl and phosphoryl groups at the C-2,3 and C-4 positions of the glucosamine skeleton is described. Also a preliminary analysis of their biological activities is presented.

KEYWORDS —— lipid A analog; glucosamine derivative; 4-phosphorylated monosaccharide; endotoxic lethal activity; antitumor activity

Although many attempts have been made to synthesize lipids A and related analogs according to their wrongly assigned structures.[2)] Few synthetic and biological works based on the reversed structure of lipids A[3)] (1a-d) have been reported.[1,4)]

We describe here a new synthesis of the monosaccharide analogs (2a-d) of lipids A according to the corrected structures, and the preliminary results of their biological activities.

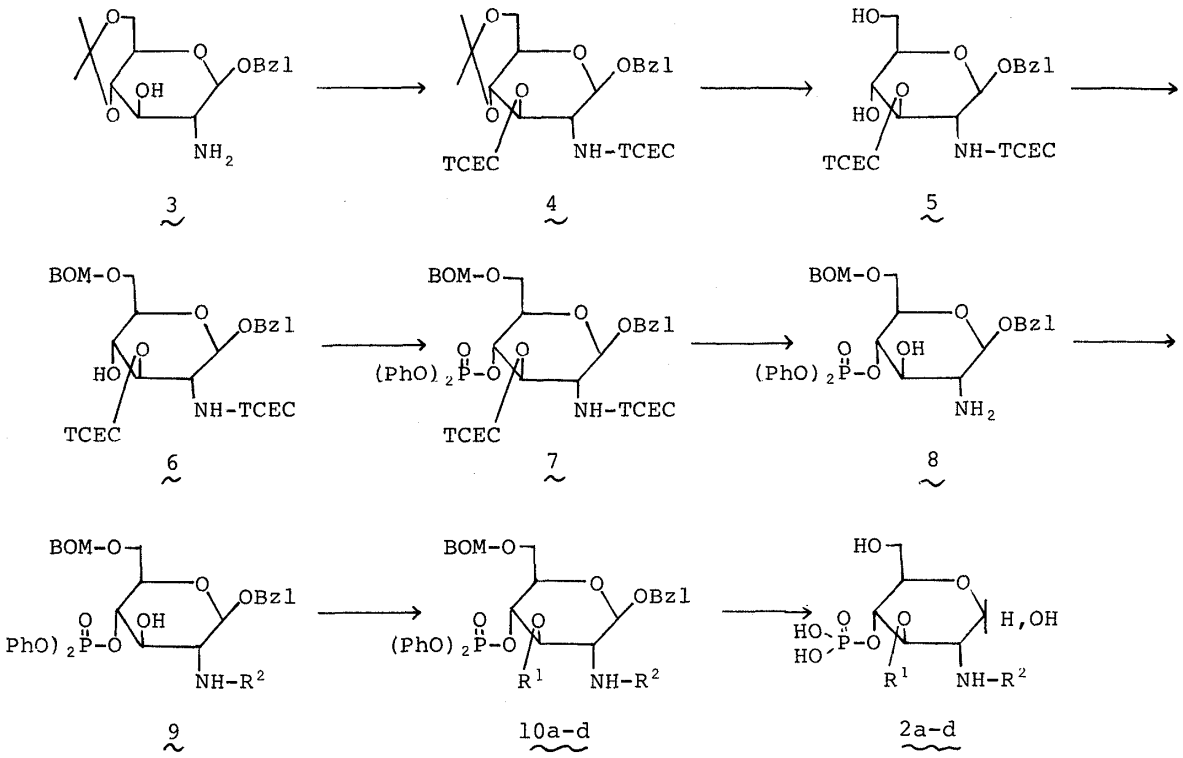


1a; R^1 = C_{14}-O-C_{14}, R^2 = C_{14}-O-C_{12}, R^3 = R^4 = C_{14}-OH
1b; R^1 = C_{14}-O-C_{14}, R^2 = C_{14}-O-C_{12}, R^3 = C_{14}-OH, R^4 = C_{14}-O-C_{16}
1c; R^1 = C_{14}-O-C_{14}, R^2 = C_{14}-O-C_{14}, R^3 = R^4 = C_{14}-OH
1d; R^1 = C_{14}-O-C_{14}, R^2 = C_{14}-O-C_{14}, R^3 = C_{14}-OH, R^4 = C_{14}-O-C_{16}

C_{14}-OH: (R)-3-Hydroxytetradecanoyl, C_{14}-O-C_{12}: (R)-3-dodecanoyloxytetradecanoyl, C_{14}-O-C_{14}: (R)-3-tetradecanoyloxytetradecanoyl, C_{14}-O-C_{16}: (R)-3-hexadecanoyloxytetradecanoyl.

In the previously reported synthesis of the monosaccharide analogs of lipids A, the only method developed was the introduction of the desired fatty acid moiety at the C-2 and C-3 positions of the glucosamine skeleton in the early stage of the synthesis process.[5)]

Our present strategy includes the introduction of optically active fatty acid moieties at the desired positions in the last stage. Thus, the monosaccharide 4-phosphate (8) bearing one amino and one hydroxyl group at the C-2 and C-3 positions of the glucosamine skeleton was exploited as the key common intermediate. Efficient conversion of 8 into several 4-phosphorylated monosaccharides (2a-d) substituted with suitable fatty acid groups proceeded as shown below.



3 4 5 6 7 8 9 10a-d 2a-d

TCEC : CCl_3CH_2OCO, BOM : $C_6H_5CH_2OCH_2$, Bzl : $C_6H_5CH_2$,

10a, 2a ; $R^1 = C_{14}\text{-O-}C_{14}$, $R^2 = C_{14}\text{-O-}C_{12}$
10b, 2b ; $R^1 = C_{14}\text{-O-}C_{12}$, $R^2 = C_{14}\text{-O-}C_{12}$
10c, 2c ; $R^1 = C_{14}\text{-O-}C_{14}$, $R^2 = C_{14}\text{-O-}C_{14}$
10d, 2d ; $R^1 = C_{14}\text{-O-}C_{16}$, $R^2 = C_{14}\text{-O-}C_{16}$

The compound (4)[6] [96%, mp 65-68°C, $[\alpha]_D^{22}$ -32.5° (c=1.00, $CHCl_3$)] was readily prepared by treating the free amino and hydroxyl groups of benzyl 2-amino-2-deoxy-4,6-isopropylidene-β-D-glucopyranoside (3)[6] with 2,2,2-trichloroethoxycarbonyl chloride in the presence of a catalytic amount of 4-dimethylaminopyridine at room temperature for 2 h.[7] Subsequent removal of the isopropylidene group of 4 was accomplished by hydrolysis with aqueous 90% acetic acid at 90°C for 15 min to yield 5[6] [98%, mp 102-103°C, $[\alpha]_D^{18}$ -26.2° (c=1.20, $CHCl_3$)]. Treatment of the diol (5) with benzyloxymethyl chloride and tetramethylurea in CH_2Cl_2 at room temperature for 16 h followed by purification with silica gel column chromatography afforded the 6-benzyloxymethyl ether (6)[6] [85%, amorphous, $[\alpha]_D^{23}$ -24.2° (c=0.28, $CHCl_3$)]. Phosphorization of 6 was carried out with diphenyl phosphorochloridate,

pyridine and 4-dimethylaminopyridine in benzene.[4b] The reaction was complete in 2 h at room temperature to give 7[6] [87%, mp 119-120°C, $[\alpha]_D^{23}$ -8.57° (c=0.98, $CHCl_3$)]. Deprotection of the 2,2,2-trichloroethoxycarbonyl group by treatment with zinc powder in acetic acid at room temperature for 5 h[8] afforded 8[6] [96%, amorphous, $[\alpha]_D^{21}$ -9.50° (c=1.14, $CHCl_3$)]. Acylation of this common intermediate with the desired acyl groups proceeded smoothly. Thus the amino-hydroxy-compound (8) was first acylated at the amino group with (R)-3-dodecanoyloxytetradecanoic acid in the presence of dicyclohexylcarbodiimide in CH_2Cl_2 at 0-5°C to yield 9a[6] [68%, mp 97-99°C, $[\alpha]_D^{23}$ -13.1° (c=0.61, $CHCl_3$)] and then at the hydroxyl group with (R)-3-tetradecanoyloxytetradecanoic acid, dicyclohexylcarbodiimide, and 4-dimethylaminopyridine in the same solvent[9] to give 10a[6] [70%, mp 71-73°C, $[\alpha]_D^{25}$ -8.94° (c=0.94, $CHCl_3$)]. The protective benzyl and phenyl groups of 10a were removed stepwise by hydrogenolysis catalyzed by 10% Pd-on-carbon at 45°C for 5 h and PtO_2 at room temperature for 16 h in methanol[8] to yield 2a[6] [56%, mp 116-118°C, $[\alpha]_D^{25}$ +33.5° (c=0.40, $CHCl_3$)]. Similary, the diacylated compounds (10b-d)[6,9] were obtained by simultaneous acylation of the amino and hydroxyl groups of 8 with the corresponding fatty acids in the presence of dicyclohexylcarbodiimide and 4-dimethylaminopyridine in CH_2Cl_2 at room temperature for 16 h and the respective monosaccharide analogs of lipids A (2b-d)[6,10] were afforded by hydrogenolysis as described above for 2a.

Preliminary studies of the biological activity of 2a-d revealed that the order of potency of the endotoxic lethal activity was 2a>2c>2b>2d. The antitumor effect on the ascites form of Ehrlich carcinoma in mice was also observed.[11]

REFERENCES AND NOTES

1) Lipids A and Related Compounds. III., Part II; K. Ikeda, S. Nakamoto, T. Takahashi, and K. Achiwa, Chem. Pharm. Bull., submitted.
2) T. Shiba, and S. Kusumoto, " Chemistry of Endotoxin, " Vol. I, ed. by E. Th. Rietschel, Elsevier, New York, 1984, pp. 284-302.
3) a) K. Takayama, N. Qureshi, and P. Mascagni, J. Biol. Chem., 258, 12801 (1983); b) M. Imoto, S. Kusumoto, T. Shiba, H. Naoki, T. Iwashita, E. T. Rietschel, H. W. Wollenweber, C. Galanos, and O. Luderitz, Tetrahedron Lett., 24, 4017 (1983); c) U. Seydel, Lindner, H. W. Wollenweber, and E. T. Rietschel, Eur. J. Biochem., 145, 505 (1984).
4) a) M. Imoto, H. Yoshimura, N. Sakaguchi, S. Kusumoto, and T. Shiba, Tetrahedron Lett., 26, 1545 (1985), and the references therein; b) S. Kusumoto, M. Yamamoto, and T. Shiba, Tetrahedron Lett., 25, 3727 (1984); c) T. Takahashi, C. Shimizu, S. Nakamoto, K. Ikeda, and K. Achiwa, Chem. Pharm. Bull., 33, 1760 (1985).
5) a) M. Kiso, H. Ishida, and A. Hasegawa, Agric. Biol. Chem., 48, 251 (1984); b) D. Charon, R. Chaley, A. Malinvaud, M. Mondange, and L. Szabo, Biochem., 24 2736 (1985).
6) Satisfactory analytical and spectral data were obtained for this compound.
7) S. Kusumoto, H. Yoshimura, M. Imoto, T. Shimamoto, and T. Shiba, Tetrahedron Lett., 26, 909 (1985).

8) C. Dioleg, M. Mondange, S. R. Sarfati, L. Szabo, and P. Szabo, J. Chem. Soc., Perkin Trans. 1., 1984, 275.
9) 10b: mp 55-57°C, $[\alpha]_D^{21}$ -6.36° (c=0.88, $CHCl_3$).
10c: mp 62-64°C, $[\alpha]_D^{21}$ -9.75° (c=0.80, $CHCl_3$).
10d: mp 60-63°C, $[\alpha]_D^{20}$ -11.5° (c=1.00, $CHCl_3$).
10) 2b: mp 172-174°C, $[\alpha]_D^{25}$ +5.65° (c=0.46, $CHCl_3$).
2c: mp 155-157°C, $[\alpha]_D^{24}$ +10.5° (c=0.40, $CHCl_3$).
2d: mp 157-159°C, $[\alpha]_D^{24}$ +19.5° (c=1.24, $CHCl_3$).
11) T. Shimizu, S. Akiyama, T. Masuzawa, Y. Yanagihara, S. Nakamoto, T. Takahashi, K. Ikeda, and K. Achiwa, Chem. Pharm. Bull., submitted.

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