SYNTHESIS OF BIOLOGICALLY ACTIVE DERIVATIVES OF D-GLUCOSAMINE-4-PHOSPHATE AND 1-THYMINYL-D-GLUCOSAMINE-4,6-DISULFATE

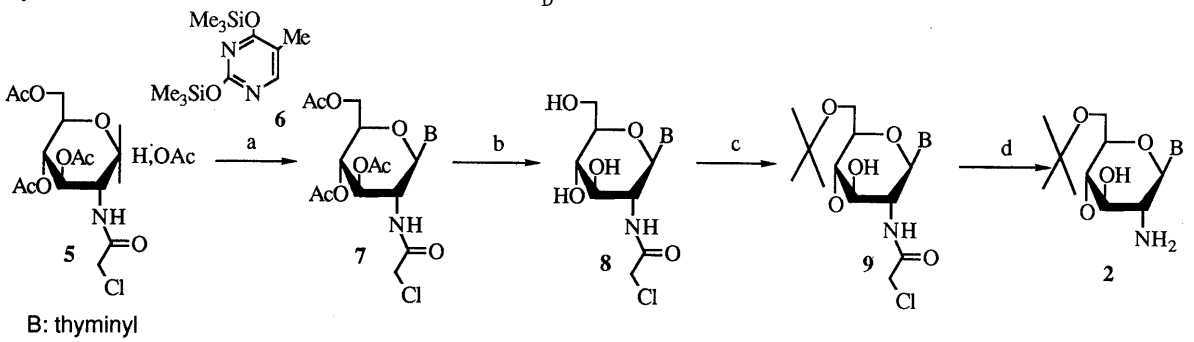
Keisuke IDEGAMI, Kiyoshi IKEDA, and Kazuo ACHIWA*
School of Pharmaceutical Sciences, University of Shizuoka, 395 Yada, Shizuoka 422, Japan

New 1-thyminyl-D-glucosamine-4-phosphate and 1-thyminyl-D-glucosamine-4,6-disulfate derivatives were synthesized. The 1-thyminyl-D-glucosamine-4,6-disulfate derivative showed antiviral activity against HIV.

KEYWORDS 1-thyminyl-D-glucosamine-4-phosphate; 1-thyminyl-D-glucosamine-4,6-disulfate; lipid A; antitumor activity; antiviral activity ; HIV

Lipid A is of considerable biological and pharmacological interest, because it is responsible for the expression of many biological activities of the lipopolysaccharide (LPS) of Gram-negative bacteria, e.g., endotoxicity, adjuvanticity, antitumor activity and so on.[1] Previously, we reported the synthesis of various derivatives of acyloxyacylglucosamine-4-phosphate as the nonreducing sugar moiety of lipid A.[2] These compounds exhibited mitogenic activity, antitumor activity, and lethal toxicity.[3] From our study concerning chemical modification of D-glucosamine-4-phosphate to yield more effective antitumor and immunopotent substances,[4] we report here the synthesis of the 1-thyminyl-D-glucosamine-4-phosphate (**1**) and 1-thyminyl-D-glucosamine-4,6-disulfate derivatives (**3**), (**4**). As our synthesis strategy, to prepare nucleosides containing glucosamine-4-phosphate, we designed a suitably functionalized key intermediate (**2**) carrying one amino and one hydroxyl group at the C-2 and C-3 positions of glucosamine skeleton, respectively.

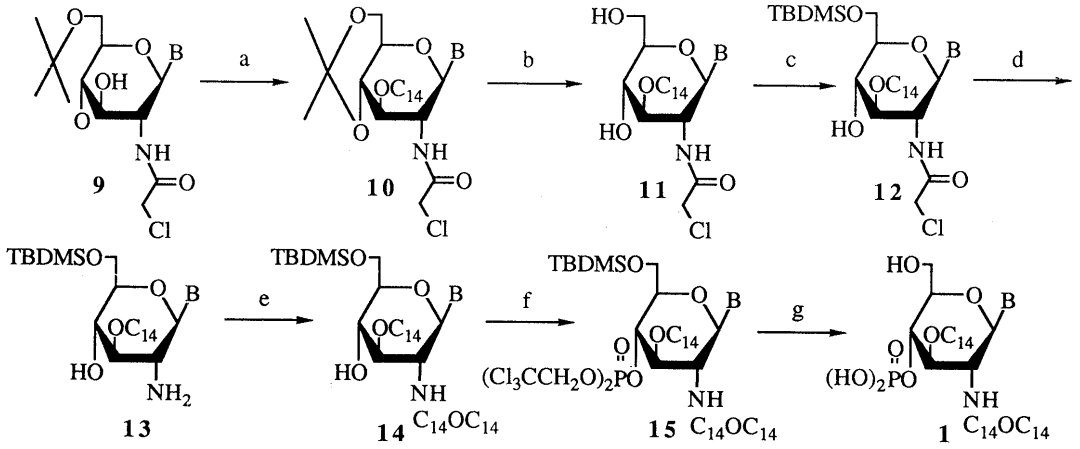
First, we describe a synthesis sequence for the 1-thyminyl-D-glucosamine-4-phosphate (**1**). The key intermediate (**2**) was prepared starting from 1,3,4,6-tetra-O-acetyl-2-chloroacetamido-2-deoxy-D-glucopyranose (**5**) in 4 steps. Condensation of **5** with the disilylated thymine (**6**) in the presence of $SnCl_4$ and Molecular Sieves 4A (MS4A) in $ClCH_2CH_2Cl$ afforded the N-glycoside (**7**) (64% mp 132-135°C, $[\alpha]_D$ -8.00°), as a single anomer, the glycosidic linkage of which was assigned the α-configuration from the anomeric proton (J=10Hz) at 6.04 ppm in the ^1H NMR spectrum of **7**. N-Glycoside (**7**) was O-deacetylated by NaOMe in MeOH to give the triol (**8**) (76%, mp 143-147°C, $[\alpha]_D$ +9.50°), and treated with 2,2-dimethoxypropane in DMF in the presence of a catalytic amount of p-toluenesulfonic acid to give 9 (78%, mp 219-221°C, $[\alpha]_D$ -16.6°). The chloroacetyl group of **9** was removed with thiourea and diisopropylethylamine in THF to give the key intermediate (**2**) (85%, mp 98-102°C, $[\alpha]_D$ +19.6°).



Reagents: a) **6** (1.2 eq), $SnCl_4$(1.5 eq), MS4A, $ClCH_2CH_2Cl$, 0°C, 1h then rt, 15 h; b) 0.1 M NaOMe, MeOH, 0°C, 1h then rt, 15h; c) 2,2-dimethoxypropane (5 eq), p-toluenesulfonic acid (0.2 eq), DMF, rt, 20 h; d) $(NH_2)_2C=S$ (2 eq), $(i\text{-}Pr)_2NEt$ (2 eq), MS4A, THF, reflux, 3h.

Chart 1

The key intermediate (**2**) thus obtained was used to synthesize of **1** as follows. We selected the tetradecanoyloxytetradecanoyl group at N-2 and the tetradecanoyl group at O-3 of the glucosamine skeleton of the GLA-27 type as the monosaccharide analogue of lipid A.[5] For further conversion of **2** into **1**, several attempts to condense the free amino group of **2** with the fatty acid residue in the presence of either dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) or DCC and N-hydroxysuccinimide (NSC) or the chloride as the activator of the carbonyl group led only to the recovery of **2**. The effective result was obtained as follows. The free hydroxyl group of **9** was acylated with tetradecanoyl chloride and pyridine in the presence of dimethylaminopyridine (DMAP) in CH_2Cl_2-DMF (10:1) to give **10** (82%, mp 164-167°C, $[\alpha]_D$ -28.3°), then the isopropylidene group of **10** was removed by hydrolysis with aqueous 90% AcOH to give the diol (**11**) (78%, mp 157-160°C, $[\alpha]_D$ -19.6°). The primary hydroxyl group of **11** was selectively protected with tert-butyldimethylsilyl chloride (TBDMS-Cl) and triethylamine in CH_2Cl_2 to give the 6'-O-TBDMS ether (**12**) (83%, mp 111-115°C, $[\alpha]_D$ -52.2°). The chloroacetyl group of **12** was removed with thiourea in THF to give **13** (78%, syrup, $[\alpha]_D$ +1.37°). Subsequently, the free amino group of **13** was acylated with optically pure (R)-3-tetradecanoyloxytetradecanoic acid, DCC, and DMAP in dry CH_2Cl_2 to afford the diacylate (**14**) (67%, syrup, $[\alpha]_D$ -13.1°). The free hydroxyl group of **14** was efficiently phosphorylated with bis(2,2,2-trichloroethyl)diisopropylaminophoshine[6] and 1H-tetrazole in CH_2Cl_2, then oxidized with t-BuO_2H[7] in CH_2Cl_2 to give the 4'-O-phosphate (**15**) (78%, syrup, $[\alpha]_D$ -5.88°). ^1H NMR spectrum of **15** showed two doublets (J=6.5Hz) (two protons each) at 4.60 and 4.58 ppm which could be assigned as the methylene protons of the trichloroethyl groups. Finaly, deprotection of the silyl group and the bis(2,2,2-trichloroethyl) group of **15** were simultaneously cleaved with Zn dust in aqueous 80% AcOH to yield the desired product (**1**) (85%, syrup, $[\alpha]_D$ -4.23°).

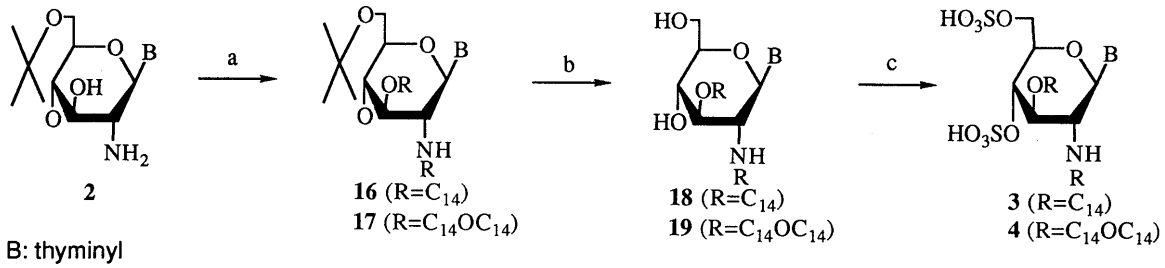


B: thymin-1-yl; C_{14}-: $CH_3(CH_2)_{12}C(O)$-; $C_{14}OC_{14}$-: $CH_3(CH_2)_{10}\overset{(R)}{C}HCH_2C(O)$-
$CH_3(CH_2)_{12}C(O)O$

Reagents: a) $C_{14}OCl$ (1.2 eq), pyridine (2 eq), DMAP (0.5eq), CH_2Cl_2-DMF (10 : 1), 0°C, 1h then rt, 15 h; b) AcOH-H_2O (9 : 1), 90-95°C, 1h; c) t-Butyl$(Me)_2SiCl$ (3 eq), NEt_3 (5 eq), DMAP (0.5eq), CH_2Cl_2, 0°C, 1h then rt, 15 h; d) $(NH_2)_2C$=S (5 eq), MS4A, THF, 50-60°C, 2.5 h; e) $C_{14}OC_{14}OH$ (1.5 eq), DCC (1.8 eq), CH_2Cl_2, 0°C, 1 h then rt, 20 h; f) 1) $(TCEO)_2PN(i\text{-}Pr)_2$ (4 eq), 1H-Tetrazole (6 eq), CH_2Cl_2, rt, 1 day; 2) t-BuO_2H (1.5 eq), CH_2Cl_2, - 40°C, 1h then 0°C, 20 h; g) Zn dust, AcOH-H_2O (8 : 2), 50-60°C, 5h.

Chart 2

Next, for the synthesis of 1-thyminyl-D-glucosamine-4,6-disulfate derivatives, the amino and hydroxyl groups of **2** were simultaneously acylated with either tetradecanoic acid or (R)-3-tetradecanoyloxy-tetradecanoic acid in the presence of DCC and DMAP in CH_2Cl_2-DMF (10:1) to give the corresponding diacylate (**16**), (**17**) in 36% and 8% yield, respectively. The isopropylidene group of **16, 17** was subsequently deprotected with 90% AcOH to give the diol (**18**), (**19**) in 54% and 39% yield, respectively. The 4,6-hydroxyl groups of **18, 19** were sulfated with Me_3NSO_3 complex in DMF, then treated with CF_3CO_2H in CH_2Cl_2 to afford the disulfate (**3**), (**4**) in 36% and 67% yield, respectively, after gelfiltration with Sephadex LH-20 ($CHCl_3$-MeOH=2:1).



2

16 (R=C_{14})
17 (R=$C_{14}OC_{14}$)

18 (R=C_{14})
19 (R=$C_{14}OC_{14}$)

3 (R=C_{14})
4 (R=$C_{14}OC_{14}$)

B: thyminyl

Reagents: a) $C_{14}OH$ or $C_{14}OC_{14}OH$ (1.5 eq), DCC (1.5 eq), DMAP (1.5 eq), CH_2Cl_2-DMF (5 : 2), 0°C, 1h then rt, 15 h; b) AcOH-H_2O (9:1), 90-95°C, 1h; c) 1) Me_3NSO_3 (3.5 eq), DMF, 50°C, 20 h; 2) CF_3CO_2H (1.5 eq), CH_2Cl_2, rt, 1h.

Chart 3

Preliminary examination of the biological activity of three synthetic compounds revealed that **1** showed weak antitumor activity and **4** exhibited significant antiviral activity against HIV (Humman Immunodeficiency Virus). In addition, **4** had little or no cytotoxicity.

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