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AN EFFICIENT SYNTHESIS OF 2'-*O*-(β-D-GLUCOPYRANOSYL)- AND 2'-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-L-BIOPTERINS

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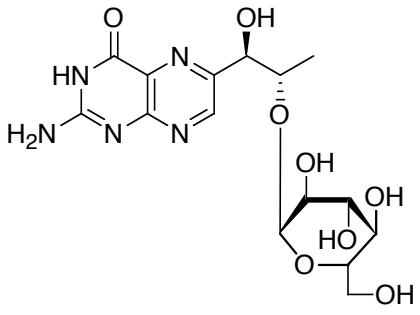
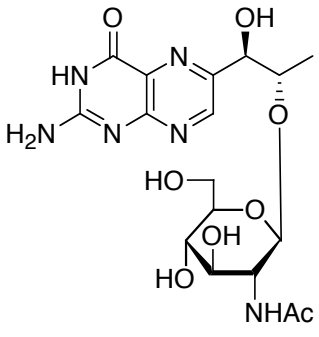
Abstract – N^2-(*N*,*N*-Dimethylaminomethylene)-3-[2-(4-nitrophenyl)ethyl]-1',2'-di-*O*-trimethylsilyl-L-biopterin (**6**) was prepared from L-biopterin in 5 steps. Glycosylation of **6** with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**9**) and 1,3,4,6-tetra-*O*-acetyl-2-phtalimido-β-D-glucopyranose (**10**) respectively afforded the corresponding 2'-*O*-(β-D-glucopyranosyl) derivatives (**12b**, **12c**) as major products. Removal of protecting groups of **12c** provided naturally occurring limipterin (**2**).

INTRODUCTION

Some pteridine glycosides having a glycosidated side-chain at C–6 of the pterin ring are known to occur in nature. For example, 2'-*O*-(α-D-glucopyranosyl)-L-biopterin (**1a**) was isolated from cyanobacterium, *Anacystis nidulans*,[1] *Synechococcus* sp. PCC 7942,[2] and *Spirulina (Arthrospira) platensis*,[3] whereas limipterin [2'-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-biopterin] (**2**) was isolated from a green sulfur photosynthetic bacterium *Chlorobium limicola f. thiosulfatophilum* NCIB 8327.[4] Although various types of glycosides of biopterin and related pteridins are considered to be of interest from the viewpoint of their biological activities and functions as well as the structural proof of hitherto reported



1a



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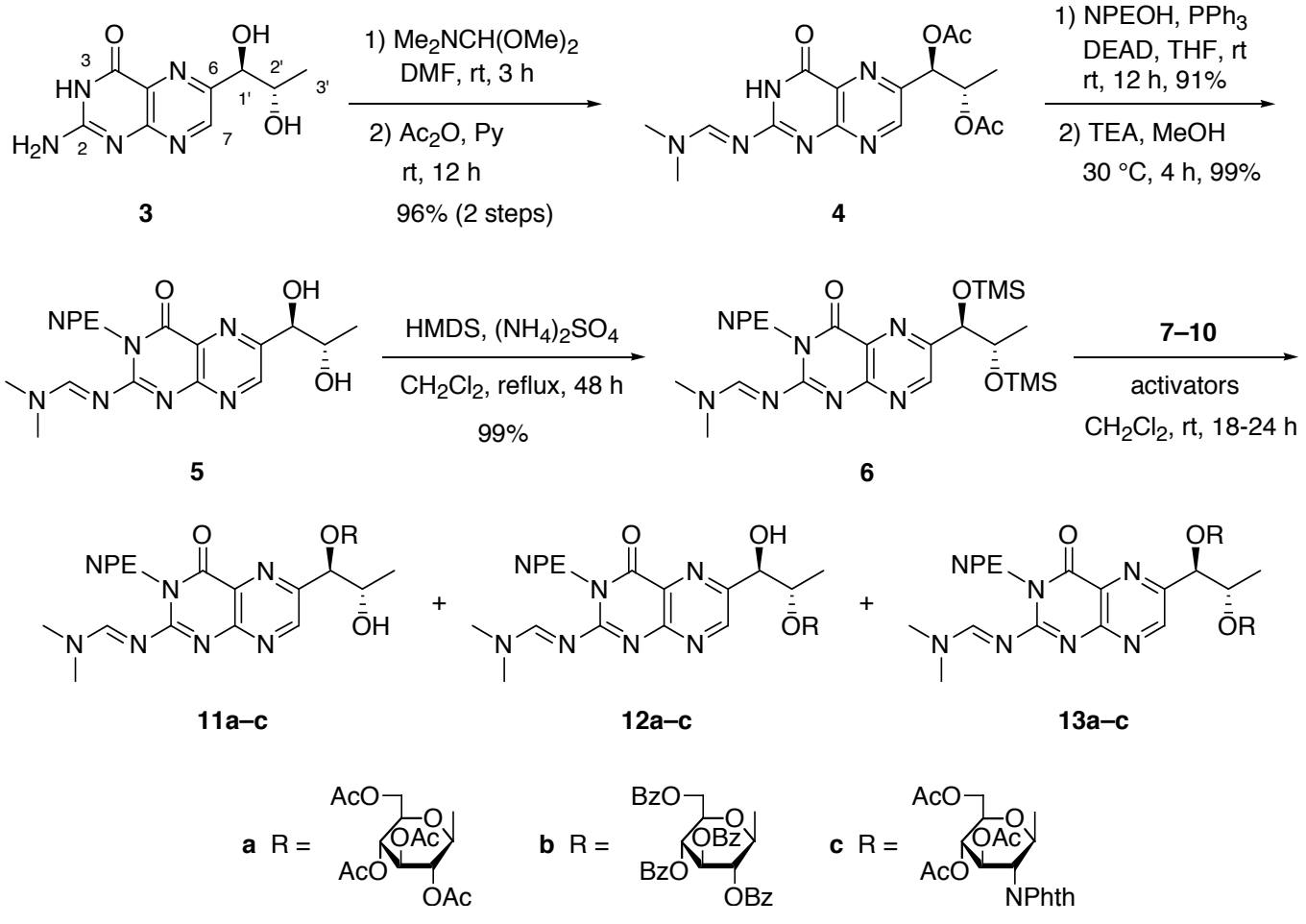
natural products, attempts for preparation of these compounds by glycosylation have not been reported so far. We describe herein the first efficient synthesis of 2'-*O*-(D-glucopyranosyl)-L-biopterins.[5]

RESULTS AND DISCUSSIN

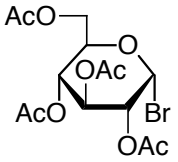
Because of the effectively stabilized intramolecular hydrogen bondings in the solid state,[6] many pterin derivatives including biopterin are little soluble in nonpolar aprotic solvents in which glycosidation reactions smoothly proceed. To overcome this probelem, L-biopterin (**3**) can be converted into a suitably protected and sufficiently solubilized derivative, N^2-(*N*,*N*-dimethylaminomethylene)-3-[2-(4-nitrophenyl)ethyl]-L-biopterin (**5**)[7] (Scheme 1). Namely, treatment of **3** with *N*,*N*-dimethylformamide dimethyl acetal in DMF and the following acetylation of hydroxy groups afforded the 1',2'-di-*O*-acetyl-N^2-(*N*,*N*-dimethylaminomethylene) derivative (**4**). The Mitsunobu reaction of **4** with *p*-nitrophenylethyl (NPE) alcohol in the presence of triphenylphosphine and diethyl azodicarboxylate (DEAD),[8] followed by methanolysis of acetyl groups provided a versatile intermediate (**5**). The reported procedures[7] for these steps were slightly modified to give **4** and **5** in higher yields.

For the purpose of raising the solubility of the N^2, *N*(3)-protected biopterin (**5**) in dichloromethane, **5** was temporarily silylated with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) in the presence of ammonium



Scheme 1

sulfate in dichloromethane under reflux for 12 h to give 1',2'-di-*O*-trimethylsilyl derivative (**6**) quantitatively. Glycosylation of **6** with various glucosyl donors (**7–10**) was extensively investigated under various conditions in the presence of activators. The yields of 1'-*O*- (**11**), 2'-*O*-glycosyl (**12**), and 1',2'-di-*O*-glycosyl compounds (**13**) depended upon the molar ratio of **6** to glycosyl donors, as summarized in Table 1.



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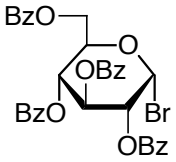

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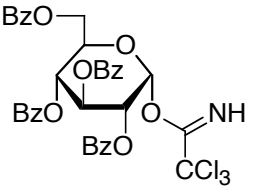

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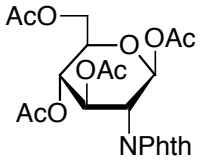

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Table 1. Glycosilation of L-biopterin derivative (**6**) [a]

Entry	Glycosyl donor (mol equiv.)	Activator (mol equiv.)	Products (yield) [b]		
1	**7** (1.5)	$SnCl_4$ (4.0)	**11a** (27%)	**12a** (32%)	**13a** (5%)
2	**7** (2.0)	$SnCl_4$ (4.0)	**11a** (31%)	**12a** (38%)	**13a** (8%)
3	**7** (2.5)	$SnCl_4$ (4.0)	**11a** (22%)	**12a** (26%)	**13a** (16%)
4	**7** (3.0)	$SnCl_4$ (4.0)	**11a** (16%)	**12a** (18%)	**13a** (27%)
5	**8** (2.0)	$SnCl_4$ (4.0)	**11b** (11%)	**12b** (28%)	**13b** (5%)
6	**8** (3.0)	$SnCl_4$ (4.0)	**11b** (15%)	**12b** (41%)	**13b** (14%)
7	**9** (2.0)	TMSOTf (0.4) [c]	**11b** (7%)	**12b** (47%)	**13b** (0%)
8	**9** (3.0)	TMSOTf (0.6) [c]	**11b** (5%)	**12b** (36%)	**13b** (9%)
9	**10** (2.5)	$SnCl_4$ (4.0)	**11c** (<1%)	**12c** (40%)	**13c** (5%)
10	**10** (2.5)	$SnCl_4$ (8.0)	**11c** (<1%)	**12c** (68%)	**13c** (8%)

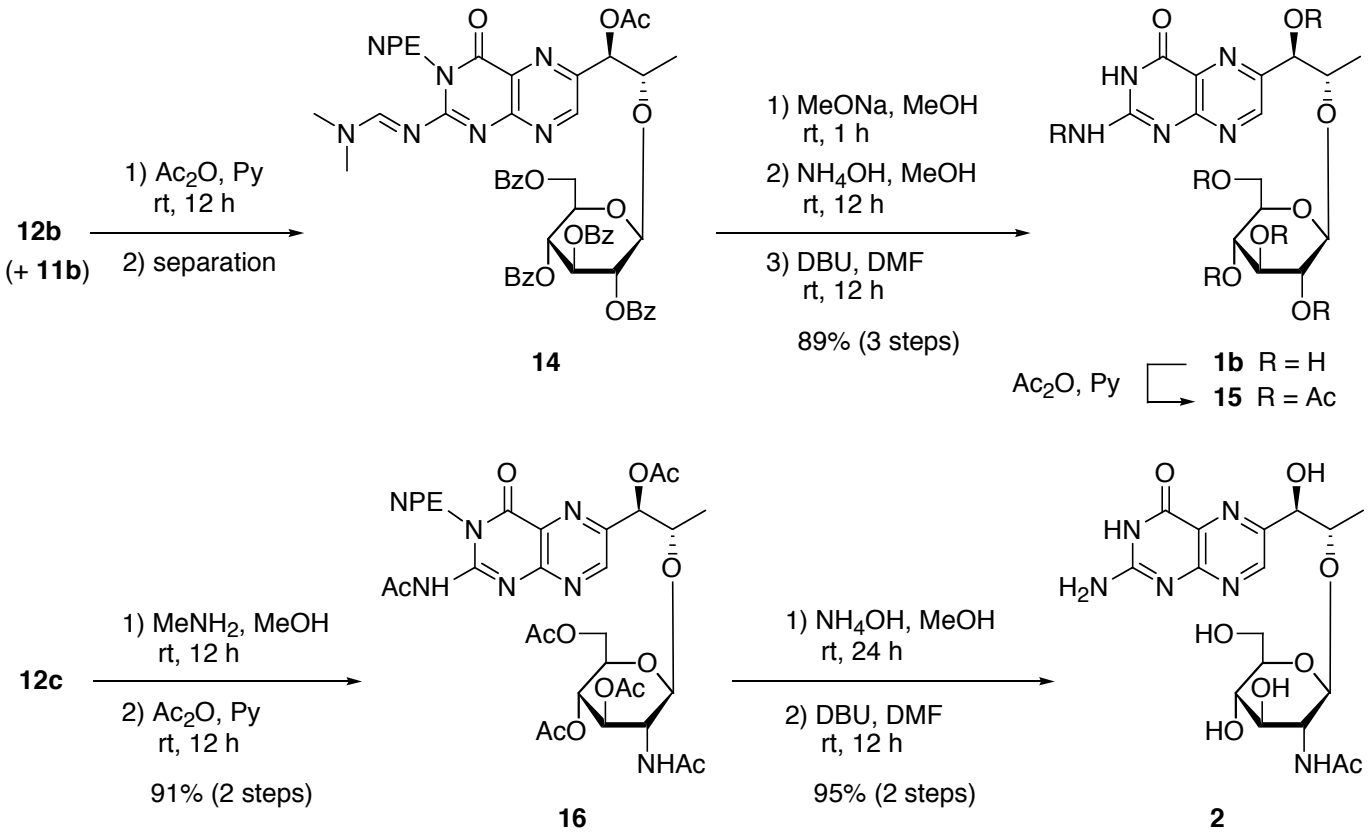
[a] All reactions were carried out in CH_2Cl_2 at rt (except for Entries 7, 8) for 18–24 h. [b] Yields of inseparable monoglycosides (**11**) and (**12**) were estimated by ^{1}H NMR spectra. [c] At –30 °C.

Glycosylation of **6** with 1.5–3.0 mol equiv. of tetera-*O*-acetyl-α-D-glucopyranosyl bromide (**7**)[9] in the presence of tin(IV) chloride (4.0 mol equiv.) in dichloromethane at room temperature for 24 h afforded 1'-*O*-(β-D-glucopyranosyl)-L-biopterin (**11a**), 2'-*O*-(β-D-glucopyranosyl) isomer (**12a**), and 1',2'-di-*O*-(β-D-glycopyranosyl) derivative (**13a**) (Entries 1–4).[10] Although the mono- and di-glycosyl compounds were chromatographically separable with a silica gel column, separation of the monoglucosides (**11a**) and (**12a**) has not been achieved. The 1'-*O*-substituted structure of **11a** was derived from a doublet of H-1' signal, whereas a triplet of H-1' for **12a** indicated the presence of 1'-OH group (Table 2). As for glucopyranosyl moieties of these compounds, all of their anomeric configurations were found to be β-form on the evidence of their $J_{1,2}$ values (7.7–7.9 Hz). Use of 2.0 mol equiv. of **7** (Entry 2) gave monoglycosides (**11a**, **12a**) in the highest yield among Entries 1–4, but 2'-*O*-preference of glycosylation

was little observed: 1'-*O*-glycoside: 2'-O-glycoside = 45:55. Meanwhile, in the case of same treatment of **6** with tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (**8**),[11] 3 mol equiv. of **8** rather than 2 mol afforded monoglycosides (**11b**, **12b**) in a higher yield and better 2'-*O*-selectivity (27:73) (Entries 5,6). An improvement in the 2'-*O*-selectivity is presumably ascribed to use of a larger protecting group of the glycosyl donor (**8**) than that of **7**. The 1'-*O*-monoglycoside (**11b**) and the 2'-*O*-glycoside (**12b**) were also obtained as an inseparable mixture and separation of them was achieved after having converted them into the corresponding acetates (see below).

Treatment of **6** with 2 mol equiv. of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**9**)[12] in the presence of trimethylsilyl triflate (TMSOTf)[13] at –30 °C revealed an improvement of 2'-*O*-selectivity (13:87) and absence of diglycoside (**13b**) (Entry 7). Use of 3 mol equiv. of **9** resulted in a lower yield of **12b** and formation of **13b** (Entry 8).

Glycosylation of **6** with 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**10**)[14] in the presence of tin chloride (4.0 equiv.) mainly afforded 2'-*O*-monoglycoside (**12c**) together with 1',2'-di-*O*-glycoside (**13c**) (Entry 9). Although a trace amount of byproduct speculated to be the 1'-*O*-glycoside (**11c**) in the fraction of **12**c was detectable from ^{1}H NMR spectrum, **11c** was removed by recrystallization of the fraction to give pure **12c**. Use of a larger amount (8.0 equiv.) of the activator brought about the highest yield (68%) of **12c** among this work. The stereoselective β-glycoside formation of all these products (**11–13a,b,c**) was mainly attained by participation of neighboring groups (acyloxy of **7–9** and phthalimido of **10**).

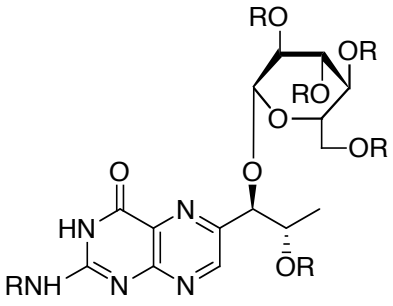


Scheme 2

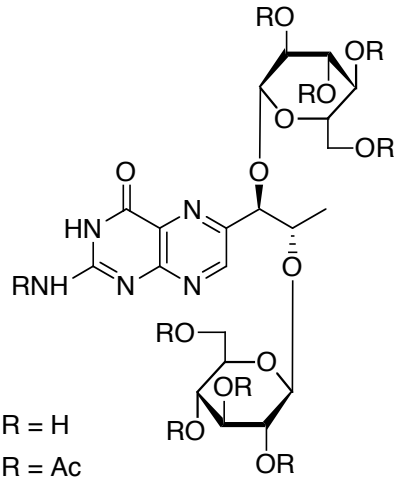
Removal of the protecting groups of the 2'-*O*-(tetra-*O*-benzoyl-β-D-glucopyranosyl)-L-biopterin derivative (**14**) isolated as the 1'-*O*-acetate from **12b** was carried out according to the following steps (Scheme 2). Namely, **14** was treated with sodium methoxide in methanol at room temperature for 2 h to cleave acetyl and benzoyl groups, and then treated with aqueous ammonia-methanol at room temperature for 12 h, to cleave the *N*,*N*-dimethylaminomethylene group. The NPE group was then removed by the treatment with DBU in DMF at room temperature for 12 h,[7,8] thus providing 2'-*O*-(β-D-glucopyranosyl)-L-biopterin (**1b**) in 89% (overall yield from **14**). Structure of **1b** was established as the corresponding per-*O*- and N^2-acetylated derivative (**15**) obtained by treatment of acetic anhydride in pyridine (Table 2).

For deprotection of 2'-*O*-(tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-L-biopterin derivative (**12c**), conversion of phthalimido group into acetamido group was carried out as an initial step. Namely, removal of the phthaloyl group of **12c** with methylamine in methanol, followed by acetylation with acetic anhydride in pyridine, afforded di-N^2:1'-*O*-acetyl-2'-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3-[2-(4-nitrophenyl)ethyl]-L-biopterin (**16**). Treatment of **16** with aqueous ammonium hydroxide, followed by the action of DBU, afforded limipterin (**2**). The spectral data of the synthetic compound (**2**) were in all respects with those of the natural product.[4]

By employing similar procedures described for those of **2** from **16**, deprotection of a mixture of 1'-*O*-glycoside (**11a**) and 2'-*O*-glycoside (**12a**) afforded a mixture of 1'-*O*-(β-D-glucopyranosyl)-L-biopterin (**17**) and **1b**. Although these compounds were converted into the corresponding acetylated derivatives for separation and structural assignment, isoration of di-N^2:2'-*O*-acetyl-1'-*O*-(2,3,4,6-tetra-*O*-β-D-glucopyranosyl)-L-biopterin (**18**) from **15** was not achieved. The similar treatment of 1',2'-di-*O*-(β-D-glucopyranosyl) derivatives (**13a**, **13b**) both afforded the same L-biopterin di-glycoside (**19**), which was converted into the acetylated compound (**20**).



17 R = H
18 R = Ac



19 R = H
20 R = Ac

The present work thus demonstrates an efficient way for preparation of 2'-*O*-(β-D-glucopyranosyl)-L-biopterin (**1b**) and natural product limipterin (**2**). Improvement of selectivity for 2'-*O*-monoglycosylation as well as preparation of naturally occurring 2'-*O*-(α-D-glucopyranosyl) derivative (**1a**), is in progress.

Table 2. 500 MHz ^{1}H-NMR Spectral Parameters for 1'- and 2'-*O*-(β-D-glucopyranosyl)-L-biopterin derivatives (**11–16**, **18, 20**) in $CDCl_3$

Com-pounds	Biopterin moiety				Chemical shifts / δ (coupling constants / Hz) Me_2NCH=N-2			$ArCH_2CH_2$-N(3)			Other signals
	H-7	H-1' ($J_{1',2'}$)	H-2' ($J_{2',3'}$)	H_3-3'	Me_2N	CH=N	Ar(*m*) ($J_{o,m}$)	Ar(*o*)	CH_2CH_2N ($^3J_{H,H}$)	
11a	8.80	5.07 (3.7)	4.30 (6.7)	1.07	3.24, 3.19	8.86	8.15 (8.6)	7.43	3.18, 4.61 (7.5)	2.68 (HO-2', $J_{2',OH}$ = 6.1)
12a	8.89	4.95 (5.0)	4.25 (6.4)	1.24	3.24, 3.19	8.86	8.14 (8.6)	7.42	3.18, 4.61 (7.5)	3.27 (HO-1', $J_{1',OH}$ = 5.2)
13a	8.80	5.12 (2.8)	4.57 (6.7)	1.12	3.24, 3.19	8.81	8.15 (8.6)	7.42	3.19, 4.62 (7.5)	
11b	8.72	5.14 (3.4)	4.32 (6.7)	1.03	3.24, 3.18	8.81	8.14 (8.5)	7.45	3.14, 4.58 (7.6)	2.82 (HO-2', $J_{2',OH}$ = 5.9)
12b	8.85	4.93 (5.2)	4.35 (6.4)	1.22	3.24, 3.19	8.84	8.14 (8.5)	7.41	3.14, 4.55 (7.6)	3.15 (HO-1', $J_{1',OH}$ = 4.3)
13b	8.75	4.93 (3.4)	4.50 (6.4)	1.23	3.24, 3.19	8.79	8.14 (8.6)	7.41	3.12, 4.50 (7.6)	
12c	8.67	4.31 (5.2)	4.26 (6.4)	1.22	3.25, 3.20	8.83	8.31 (8.7)	7.41	3.13, 4.55 (7.6)	4.79 (HO-1', $J_{1',OH}$ = 5.3)
13c	8.21	4.65 (6.0)	4.38 (6.4)	1.29	3.26, 3.21	8.89	8.14 (8.6)	7.42	3.14, 4.53 (7.8)	
14	8.65	5.90 (5.5)	4.58 (6.4)	1.34	3.25, 3.21	8.82	8.15 (8.6)	7.45	3.19, 4.59 (7.9)	1.74 (AcO-1')
15	8.86	5.94 (5.2)	4.47 (6.4)	1.29			10.08 [H-N(3)]			2.15 (AcO-1')[a] 2.44 (AcN-2), 12.57 (NH-2)
16	8.70	5.91 (5.2)	4.44 (6.4)	1.26			8.19 (8.8)	7.50	3.17, 4.55 (7.6)	2.16 (AcO-1')[a] 2.34 (AcN-2), 13.9 (NH-2)
18	8.94	5.13 (5.2)	5.32 (6.4)	1.24			9.35 [H-N(3)]			2.12 (AcO-2')[a] 2.47 (AcN-2), 12.39 (NH-2)
20	8.90	5.15 (2.7)	4.57 (6.4)	1.10			9.35 [H-N(3)]			2.36 (AcN-2), 12.40 (NH-2)

[a] The assignments of acetyl groups may have to be interchanged. [b] The assignments of H-1,2,3 signals may have to be interchanged. [c] Upper two lines for 1'-*O*-glucopyranosyl moiety, lower two lines for 2'-*O*-glucopyranosyl moiety. Correlations of protons were confirmed by 2D-COSY measurement. [d] The assignments of Bz(*o*) signals may have to be interchanged. [e] The assignments of phthaloyl groups may have to be interchanged.

	Glucopyranosyl moiety							
	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6a}$)	H^a-6 ($J_{6a,6b}$)	H^b-6 ($J_{5,6b}$)	Other signals
11a	4.52	5.11[b]	5.10[b]	5.09[b]	3.68	4.25	4.17	2.13, 2.03, 2.01, 1.99 (AcO-2,3,4,6)
	(7.7)	(9.5)	(9.5)	(9.8)	(5.5)	(12.2)	(2.4)	
12a	4.64	4.96	5.14	5.04	3.69	4.23	4.13	2.08, 2.04, 2.02, 1.98 (AcO-2,3,4,6)
	(7.9)	(9.5)	(9.5)	(9.8)	(5.5)	(12.2)	(2.1)	
13a[c]	4.33	5.12[b]	5.10[b]	5.08[b]	3.57	4.34	4.12	2.19, 2.09, 1.99, 1.98 (AcO-2,3,4,6)
	(7.9)	(9.5)	(9.5)	(9.8)	(4.6)	(12.2)	(2.1)	
	5.11	4.90	5.32	5.08	3.80	4.27	4.17	2.16, 2.14, 2.02, 1.98 (AcO-2,3,4,6)
	(7.9)	(9.5)	(9.5)	(9.8)	(4.7)	(12.2)	(2.4)	
11b	4.96	5.65	5.85	5.72	4.17	4.73	4.47	8.09, 7.98, 7.89, 7.80 [Bz(*o*)]
	(7.9)	(9.4)	(9.7)	(9.9)	(2.8)	(12.1)	(5.6)	7.54–7.26 [Bz(*m*,*p*)]
12b	5.08	5.50	5.89	5.65	4.19	4.64	4.49	8.03, 7.91, 7.84, 7.80 [Bz(*o*)]
	(7.9)	(9.8)	(9.6)	(9.8)	(3.1)	(12.2)	(5.8)	7.54–7.26 [Bz(*m*,*p*)]
13b[c]	4.85	5.19	5.72	5.46	4.17	4.56	4.51	8.10, 7.98, 7.92, 7.79 [Bz(*o*)][d]
	(7.9)	(9.5)	(9.9)	(9.8)	(3.0)	(12.2)	(5.8)	7.55–7.15 [Bz(*m*,*p*)]
	5.07	5.47	5.88	5.57	4.03	4.57	4.37	7.98, 7.92, 7.80, 7.57 [Bz(*o*)][d]
	(7.9)	(9.6)	(9.8)	(9.8)	(3.0)	(12.2)	(5.8)	7.55–7.15 [Bz(*m*,*p*)]
12c	5.53	4.29	5.70	5.13	3.91	4.28	4.17	2.11, 2.02,1.82 (AcO-3,4,6)
	(8.6)	(10.7)	(9.2)	(10.1)	(5.2)	(12.0)	(2.4)	7.76, 7.65 (Phth)
13c[c]	5.13	3.83	5.52	5.00	3.73	4.29	4.17	2.08, 1.98, 1.77 (AcO-3,4,6)[a]
	(8.5)	(10.7)	(9.2)	(10.0)	(5.5)	(12.2)	(2.4)	7.69, 7.56 (Phth)[e]
	5.24	4.16	5.65	5.03	3.81	4.25	4.11	2.15, 2.00, 1.79 (AcO-3,4,6)[a]
	(8.2)	(10.6)	(9.2)	(10.0)	(5.2)	(12.2)	(2.4)	7.78, 7.69 (Phth)[e]
14	5.16	5.44	5.90	5.61	4.22	4.64	4.48	8.02, 7.91, 7.77, 7.76 [Bz(*o*)]
	(7.9)	(9.8)	(9.5)	(10.1)	(3.1)	(11.6)	(5.5)	7.54–7.24 [Bz(*m*,*p*)]
15	4.62	4.85	5.10	5.00	3.68	4.21	4.11	2.08, 2.02, 1.96, 1.94 (AcO-2,3,4,6)[a]
	(7.9)	(9.5)	(9.5)	(9.8)	(5.2)	(12.2)	(2.4)	
16	4.82	4.20	5.27	5.00	3.77	4.22	4.13	2.02, 2.02, 1.86 (AcO-3,4,6)[a]
	(8.5)	(10.5)	(9.5)	(9.9)	(5.5)	(12.2)	(2.1)	2.08 (AcN-2),[a] 5.83 (NH-2)
18	4.46	5.06[b]	5.07[b]	5.08[b]	3.61	4.20	4.12	2.00, 1.99, 1.97, 1.94 (AcO-2,3,4,6)[a]
	(7.9)	(9.5)	(9.5)	(9.8)	(5.2)	(12.2)	(2.4)	.
20[c]	4.31	5.12[b]	5.10[b]	5.08[b]	3.58	4.33	4.12	2.14, 2.06, 2.03, 1.99 (AcO-2,3,4,6)[a]
	(7.9)	(9.5)	(9.5)	(9.8)	(4.6)	(12.2)	(2.1)	
	5.11	4.91	5.33	5.09	3.80	4.26	4.16	2.21, 2.09, 2.04, 2.00 (AcO-2,3,4,6)[a]
	(7.9)	(9.5)	(9.5)	(9.8)	(4.6)	(12.2)	(2.4)	

EXPERIMENTAL

All reactions were monitored by TLC (Merck silica gel 60F, 0.25 mm) with an appropriate solvent system [(*A*) AcOEt, (*B*) 1:9 MeOH–$CHCl_3$, and (*C*) 5:3:1 2-PrOH–AcOEt–H_2O]. Column chromatography was performed by Daiso Silica Gel IR-60/210w. Components were detected by exposing the plates to UV light and/or spraying them with 20% sulfuric acid–ethanol (with subsequent heating). The NMR spectra were measured in CDCl3 with Hitachi R-1900 (90 MHz) and Varian VXR-500 (500 MHz) spectrometers at 23 °C. Chemical shifts are reported as δ values relative to $CHCl_3$ (7.26 ppm) as an internal standard. The MS spectra were taken on a VG-70SE instrument and are given in terms of *m/z* (relative intensity) compared with the base peak.

N^2-(*N*,*N*-Dimethylaminomethylene)-3-[2-(4-nitrophenyl)ethyl]-L-biopterin (5).[7]

The following modification of the literature procedures[7] was made. To a suspension of **3** (100 mg, 0.421 mmol) in dry DMF (6.0 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (0.150 mL, 1.13 mmol). The mixture was stirred at rt for 3 h and evaporated in vacuo. The residue was dissolved in 5% MeOH-$CHCl_3$ and stirred at rt for 2 h. The mixture was evaporated in vacuo and the residue was dissolved in pyridine (5.0 mL) and acetic anhydride (2.5 mL) at 5 °C. The mixture was stirred at rt for 12 h and evaporated in vacuo. The residue was purified by column chromatography with 2% MeOH-$CHCl_3$ as an eluant to give 1',2'-di-*O*-acetyl-N^2-(*N*,*N*-dimethylaminomethylene)-L-biopterin (**4**) (152 mg, 96% from **3**) as pale yellow prisms: mp 192–193 °C (from AcOEt-hexane, lit.,[7] mp 194–195 °C, 77% yield); R_f = 0.43 (*B*).

To a solution of **4** (152 mg, 0.404 mmol), NPE alcohol (81. 0 mg, 0.485 mmol), and triphenylphosphine (159 mg, 0.608 mmol) in dry THF (4.0 mL) was added DEAD (0.100 mL, 0.654 mmol). The mixture was stirred at rt for 12 h and evaporated in vacuo. The residue was purified by column chromatography with 1:2 AcOEt-hexane and then 2% MeOH-$CHCl_3$ to give the 3-NPE derivative (193 mg, 91%) as pale yellow crystals: mp 204–205 °C (from AcOEt-hexane, lit.,[7] mp 205–206 °C, 80% yield); R_f = 0.60 (*B*).

To a solution of the above product (193 mg, 0.367 mmol) in MeOH (3.0 mL) was added TEA (1.0 mL, 7.2 mmol). The solution was stirred at 30 °C for 4 h and evaporated in vacuo. The residue was recrystallized from EtOH to give **5** (160 mg, 99%) as pale yellow crystals: mp 136–138 °C (lit.,[7] mp 138–140 °C, 97% yield); R_f = 0.42 (*B*).

N^2-(*N*,*N*-Dimethylaminomethylene)-3-[2-(4-nitrophenyl)ethyl]-1',2'-di-*O*-trimethylsilyl-L-biopterin (6).

A suspension of **5** (96.0 mg, 0.217 mmol), ammonium sulfate (66.2 mg, 0.502 mmol), and HMDS (0.680 mL, 3.28 mmol) in dry CH_2Cl_2 (5.0 mL) was refluxed for 48 h. The precipitate was filtered off and the filtrate was evaporated in vacuo. The residue was purified by short-path column chromatography with 1% MeOH-$CHCl_3$ to give **6** (126 mg, 99%) as a pale yellow foam: R_f = 0.65 (*B*); ^{1}H NMR (90 MHz) δ = 0.06, 0.09 (3H each, 2s, $SiMe_3$), 1.07 (3H, d, $J_{2',3'}$ = 6.4 Hz, H_3-3'), 3.09 [2H, m, CH_2–C–N(3)], 3.17, 3.22 (3H each, 2s, NMe_2), 4.15 (1H, dq, $J_{1',2'}$ = 4.0 Hz, H-2'), 4.60 [2H, m, CH_2–N(3)], 4.89 (1H, d, H-

1'), 7.41, 8.12 (2H each, 2d, $J_{o,m}$ = 8.7 Hz, $C_6H_4NO_2$), 8,85 (1H, s, CH=N^2), 8.93 (1H, s, H-7).

N^2-(*N*,*N*-Dimethylaminomethylene)-3-[2-(4-nitrophenyl)ethyl]-1'-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-biopterin (11a), its 2'-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) isomer (12a), and N^2-(*N*,*N*-dimethylaminomethylene)-3-[2-(4-nitrophenyl)ethyl]-1',2'-bis-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-biopterin (13a).

To a solution of **7**[9] (180 mg, 0.44 mmol) in CH_2Cl_2 (2.0 mL) was added $SnCl_4$ (0.10 mL, 0.88 mmol) at 0 °C. After stirring at 0 ° C for 0.5 h, a solution of **6** (130 mg, 0.222 mmol) in CH_2Cl_2 (1.5 mL) was added and then the mixture was stirred at rt for 24 h. After addition of saturated aqueous $NaHCO_3$, the mixture was extracted with $CHCl_3$. The organic layer was dried (Na_2SO_4) and evaporated in vacuo. The residue was separated by column chromatography with 1:2 AcOEt-hexane and then 2:3 AcOEt-$CHCl_3$ into two fractions.

The faster-eluting fraction [R_f = 0.63 (*B*)] gave **13a** (18 mg, 7.3%) as a pale yellow powder: mp 184–186 °C (from AcOEt-hexane): ^{1}H NMR, see Table 2; FAB MS *m/z* 1102 (M+1; 72), 772 (14), 727 (12), 424 (26), 247 (16), 154 (100). Found: *m/z* 1102.3760. Calcd for $C_{48}H_{60}N_7O_{23}$: M+1, 1102.3742.

The slower-eluting fraction [R_f = 0.47 (*B*)] gave a pale yellow powder (117 mg), which consisted of **11a** (53 mg, 31%) and **12a** (64 mg, 38%), the ratio being estimated by ^{1}H NMR spectrum: ^{1}H NMR for **11a** and **12a**, see Table 2; FAB MS *m/z* 772 (M+1, 15), 424 (12), 307 (20), 289 (14), 154 (100). Found: *m/z* 772.2780. Calcd for $C_{34}H_{42}N_7O_{14}$: M+1, 772.2792.

N^2-(*N*,*N*-Dimethylaminomethylene)-3-[2-(4-nitrophenyl)ethyl]-1'-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-L-biopterin (11b), its 2'-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl) isomer (12b), and N^2-(*N*,*N*-dimethylaminomethylene)-3-[2-(4-nitrophenyl)ethyl]-1',2'-bis-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-L-biopterin (13b).

(A) From **8**. By use of the same procedures described above, compound (**6**) (54.0 mg, 0.0922 mmol) was treated with **8**[11] (183 mg, 0.278 mmol) and $SnCl_4$ (0.044 ml, 0.38 mmol) at rt for 20 h. The products were separated by column chromatography with 1:2 AcOEt-hexane and then 2% MeOH-$CHCl_3$ into two fractions.

The faster-eluting fraction [R_f = 0.75 (*B*)] gave **13b** (20.8 mg, 14%) as a pale yellow powder: ^{1}H NMR, see Table 2. *Anal*. Calcd for $C_{88}H_{75}N_7O_{23}$: C, 66.12; H, 4.73. Found: C, 66.38; H, 4.82.

The slower-eluting fraction [R_f = 0.53 (*B*)] gave a pale yellow powder (52.8 mg), which consisted of **11b** (14.0 mg, 15%) and **12b** (38.8 mg, 41%), the ratio being estimated by ^{1}H NMR: ^{1}H NMR for **11b** and **12b**, see Table 2.

(B) From **9**. To a solution of **9**[12] (121 mg, 0.164 mmol) in CH_2Cl_2 (2.0 mL) was added TMSOTf (0.006 mL, 0.033 mmol) at –30 °C. After stirring at same temperature for 0.5 h, a solution of **6** (47.8 mg, 0.0816 mmol) in CH_2Cl_2 (1.0 mL) was added and then the mixture was stirred at –30 °C for 18 h. After addition of saturated $NaHCO_3$, the mixture was extracted with $CHCl_3$. The organic layer was dried (Na_2SO_4) and evaporated in vacuo. The residue was separated by column chromatography to give an inseparable mixture (45.2 mg) of **11b** (6.0 mg, 7%) and **12b** (39.2 mg, 47%).

N^2-(*N*,*N*-Dimethylaminomethylene)-3-[2-(4-nitrophenyl)ethyl]-2'-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-L-biopterin (12c) and N^2-(*N*,*N*-dimethylaminomethylene)-3-[2-(4-nitrophenyl)ethyl]-1',2'-di-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-L-biopterin (13c).

By use of the same procedures as those for preparation of **11–13a**, compound (**6**) (315 mg, 0.538 mmol) was treated with **10**[14] (644 mg, 1.35 mmol) and $SnCl_4$ (0.51 mL, 4.3 mmol) at rt for 24 h. The products were separated by column chromatography with 1:2 AcOEt-hexane and then 2% MeOH-$CHCl_3$ into two fractions.

The faster-eluting fraction [R_f = 0.58 (*B*)] gave **13c** (56 mg, 8.2%) as pale yellow crystals: mp 134–135 °C (from AcOEt-hexane); ^{1}H NMR, see Table 2. *Anal*. Calcd for $C_{60}H_{61}N_9O_{23}$: C, 56.469; H, 4.82. Found: C, 56.528; H, 4.90.

The slower-eluting fraction [R_f = 0.48 (*B*)] gave pale yellow solid (324 mg) of **12c** contaminated with a trace amount of **11c**, which was recrystallized from AcOEt-hexane to give **12c** (315 mg, 68%) as pale yellow crystals: mp 166–167 °C; ^{1}H NMR, see Table 2. *Anal*. Calcd for $C_{40}H_{42}N_8O_{14}$: C, 55.94; H, 4.93. Found: C, 56.02; H, 5.01.

1'-*O*-Acetyl-N^2-(*N*,*N*-dimethylaminomethylene)-3-[2-(4-nitrophenyl)ethyl]-2'-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-L-biopterin (14).

A 13:87 mixture (34.1 mg) of **11b** and **12b** was dissolved in pyridine (1.0 mL) and then acetic anhydride (0.40 mL, 4.2 mmol) was added at 0 °C. The mixture was stirred at rt for 12 h and evaporated in vacuo. The residue was chromatographed on a silica gel with AcOEt as an eluant to give **14** (25.1 mg, 81% from **12b**) as a yellow syrup: R_f = 0.24 (*A*); ^{1}H NMR, see Table 2. *Anal*. Calcd for $C_{56}H_{51}N_7O_{15}$: C, 63.33; H, 4.84. Found: C, 63.24; H, 4.99.

Besides pure **14**, a mixture (8.2 mg) which consisted of **14** and the 2'-*O*-acetyl derivative of **11b** was obtained: R_f = 0.24–0.20 (*A*).

Di-N^2:1'-*O*-acetyl-2'-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-biopterin (15).

Compound (**14**) (56.3 mg, 0.0530 mmol) was dissolved in MeOH (2.0 mL) and a 28% methanolic NaOMe (0.03 mL, 0.15 mmol) was added at 0 °C. The mixture was stirred at rt for 1 h and neutralized with Amberlite IR-120(H^+). The resin was filtered off and the filtrate was evaporated in vacuo. The residue was dissolved in MeOH (4.0 mL) and 28% aqueous ammonia solution (4.0 mL) was added. The mixture was stirred at rt for 12 h and evaporated in vacuo. The residue was dissolved in DMF (1.0 mL) and DBU (0.050 mL, 0.32 mmol) was added. The mixture was stirred at rt for 12 h and evaporated in vacuo. The residue was washed with $CHCl_3$ and dried under reduced pressure to give 2'-*O*-(β-D-glucopyranosyl)-L-biopterin (**1b**) (18.8 mg) as a yellow powder: R_f = 0.23 (*C*).

Compound (**1b**) was dissolved in pyridine (2.0 mL) and then acetic anhydride (1.0 mL) was added at 0 °C. The mixture was stirred at rt for 12 h and evaporated in vacuo. The residue was purified by column chromatography with AcOEt to give **15** (29.7 mg, 86% from **14**) as a pale yellow syrup: R_f = 0.52 (*B*); ^{1}H NMR, see Table 2. *Anal*. Calcd for $C_{27}H_{33}N_5O_{14}$: C, 49.77; H, 5.10. Found: C, 49.92; H, 5.02.

Di-N^2:1'-*O*-acetyl-3-[2-(4-nitrophenyl)ethyl]-2'-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-biopterin (16).

Compound (**12c**) (100 mg, 0.116 mmol) was dissolved in MeOH (5.0 mL) and 40% methanolic methylamine (2.0 mL) was added. The mixture was stirred at rt for 12 h and evaporated in vacuo. The residue was dissolved in pyridine (2.0 mL) and then acetic anhydride (1.0 mL, 11 mmol) was added at 0 °C. The mixture was stirred at rt for 12 h and evaporated in vacuo. The residue was purified by column chromatography with 1:2 AcOEt-hexane (to remove impurities) and then 2% MeOH-$CHCl_3$ as an eluant to give **16** (84.7 mg, 91%) as pale yellow crystals: mp 142–144 °C (from AcOEt-hexane): R_f = 0.42 (*B*); ^{1}H NMR, see Table 2. *Anal*. Calcd for $C_{35}H_{41}N_7O_{15}$: C, 52.56; H, 5.17. Found: C, 52.39; H, 5.29.

2'-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-biopterin (2).

Compound (**16**) (84.0 mg, 0.105 mmol) was dissolved in MeOH (8.0 mL) and 28% aqueous ammonia solution (3.0 mL) was added. The mixture was stirred at at for 24 h and evaporated in vacuo. The residue was dissolved in DMF (2.0 mL) and DBU (0.10 mL, 0.64 mmol) was added. The mixture was stirred at rt for 12 h and evaporated in vacuo. The residue was washed with $CHCl_3$ and dried under reduced pressure to give **2** (43.8 mg, 95%) as pale yellow crystals: mp 192–195 °C (from H_2O–2-PrOH): R_f = 0.38 (*C*). *Anal*. Calcd for $C_{17}H_{24}N_6O_8$: C, 46.36; H, 5.49. Found: C, 46.19; H, 5.58.

Di-N^2:2'-*O*-acetyl-1'-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-biopterin (18).

By use of the same procedures for **2** from **16**, a 45:55 mixture (130 mg) of **11a** and **11b** was converted into a mixture of 1'-*O*-(β-D-glucopyranosyl)-L-biopterin (**17**) and **1b**. The mixture was acetylated with acetic anhydride (1.0 mL, 11 mmol) in pyridine (2.0 mL) to give an inseparable mixture (53 mg) of **18** and **15** as a pale yellow syrup: overall yield 81 % from **11a** and **11b**; R_f = 0.50 (*B*); ^{1}H NMR for **18**, see Table 2.

N^2-Acetyl-1',2'-bis-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-biopterin (20).

By use of the same procedures for **2** from **16**, compound (**13a**) (43.2 mg) was converted into 1',2'-bis-*O*-(β-D-glucopyranosyl)-L-biopterin (**19**) (20.3 mg, 92%) as a yellow powder: R_f = 0.19 (*C*). By use of the same procedures for **14** from **1b**, compound (**13b**) (20.8 mg) was also converted into **19** (6.4 mg, 88%). Compound (**19**) (25.5 mg) was acetylated with acetic anhydride (0.40 mL, 4.2 mmol) in pyridine (1.0 mL) to give **20** (38.7 mg, 91%) as a pale yellow syrup: R_f = 0.47 (*B*); ^{1}H NMR, see Table 2. *Anal*. Calcd for $C_{39}H_{49}N_5O_{22}$: C, 49.84; H, 5.26. Found: C, 50.02; H, 5.29.

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