HETEROCYCLES, Vol. 84, No. 2, 2012, pp. 801 - 813. © 2012 The Japan Institute of Heterocyclic Chemistry
Received, 28th June, 2011, Accepted, 10th August, 2011, Published online, 18th August, 2011
DOI: 10.3987/COM-11-S(P)57

# FIRST SYNTHESIS OF A NATURAL ISOXANTHOPTERIN GLYCOSIDE, ASPEROPTERIN-A

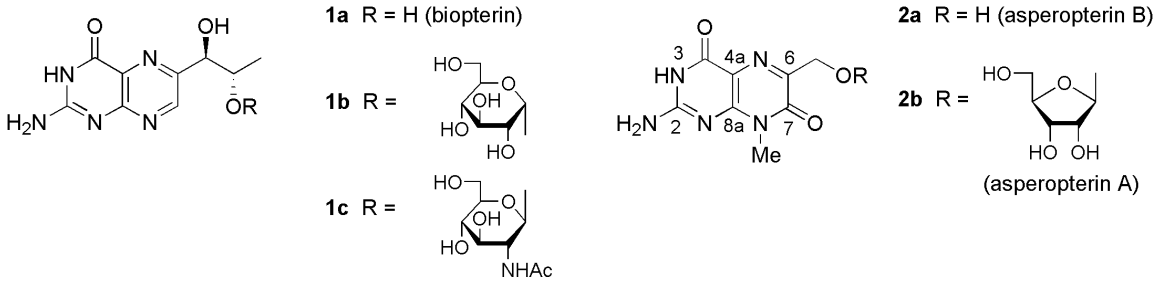
**Tadashi Hanaya,* Kazumasa Ejiri, and Hiroshi Yamamoto**[†]

Department of Chemistry, Faculty of Science, Okayama University, Tsushima-naka, Kita-ku, Okayama 700-8530, Japan. E-mail: hanaya@cc.okayama-u.ac.jp
[†] School of Pharmacy, Shujitsu University, Nishigawara, Naka-ku, Okayama 703-8516, Japan

**Abstract** – The key precursor, $N^2$-(*N*,*N*-dimethylaminomethylene)-6-hydroxymethyl-8-methyl-3-[2-(4-nitrophenyl)ethyl]-7-xanthopterin (**9**) was efficiently prepared from 2,5-diamino-6-methylamino-3*H*-pyrimidin-4-one (**3**) and ethyl 3-(*tert*-butyldimethylsilyloxy)-2-oxopropionate (**11**). The first synthesis of asperopterin-A (**2b**) was achieved by treatment of **9** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**15**) in the presence of tin(IV) chloride, followed by removal of the protecting groups.

## INTRODUCTION

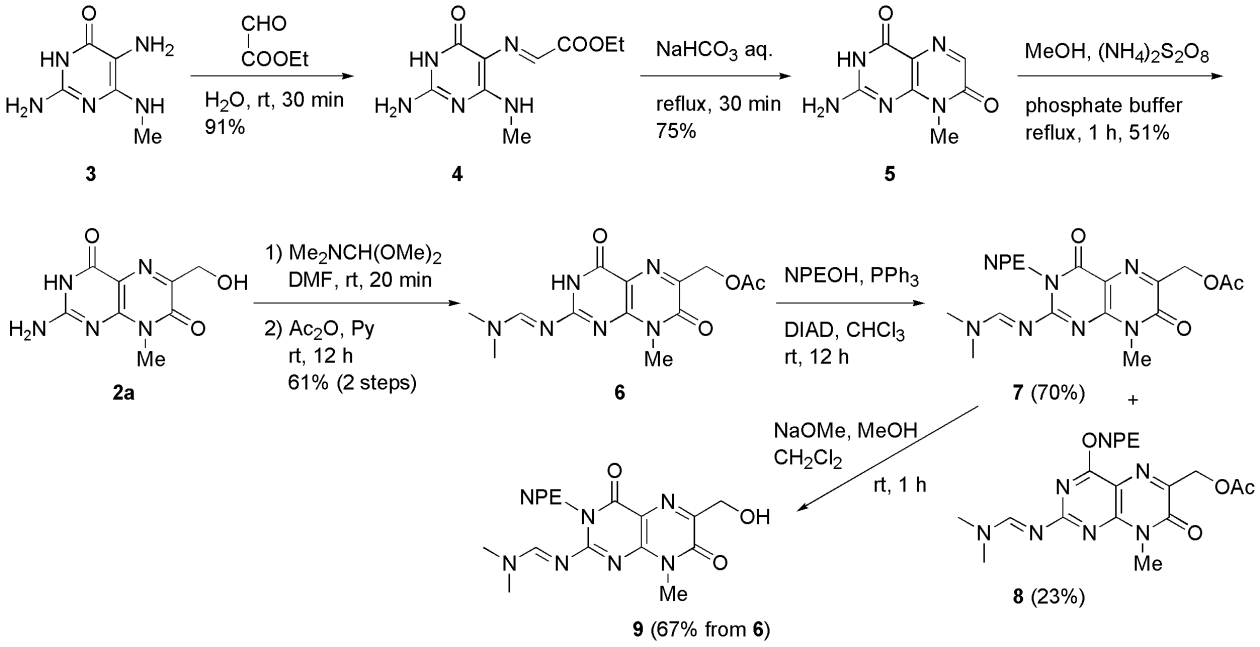
Some pteridines having various kind of sugars attached to the hydroxyalkyl side-chain at C-6 are known to occur in certain prokaryotes as exemplified by glycosides of biopterin (**1a**): *e.g.*, α-D-glucopyranosyl (**1b**)[1] and 2-acetamido-2-deoxy-β-D-glucopyranosyl (**1c**)[2] derivatives. So far, they have been found mostly in cyanobacteria[1,3,4] and also, to a lesser extent, in anaerobic photosynthetic bacteria[2,5] and chemoautotrophic archaebacteria.[6a] Most of the parent pteridine moieties of these glycosides consist of pterins such as biopterin (**1a**),[1,2] ciliapterin (L-*threo*-biopterin),[3,5] neopterin,[6] and 6-hydroxymethylpterin.[3] On the other hand, asperopterin-A (**2b**)[7,8] isolated from *Aspergillus oryzae* is a unique example of pteridine glycosides in an aspect of having an isoxanthopterin (7-xanthopterin)[9] structure as a parent ring. Its structure has been assigned to be the β-D-ribofuranoside of 6-hydroxymethyl-8-methyl-7-xanthopterin (asperopterin-B) (**2a**), the preparation of which, however, has remained unreported.




Although various types of pteridine glycosides are considered to be of interest from the viewpoint of their biological activities[10] and functions as well as structural proof of hitherto reported natural products, attempts at preparation of these compounds have so far scarcely been made, except for our synthetic studies on biopterin and ciliapterin glycosides.[11-14] We give herein an efficient synthesis of asperopterin-A (**2b**) as the first synthetic example of a natural isoxanthopterin glycoside.

## RESULTS AND DISCUSSION

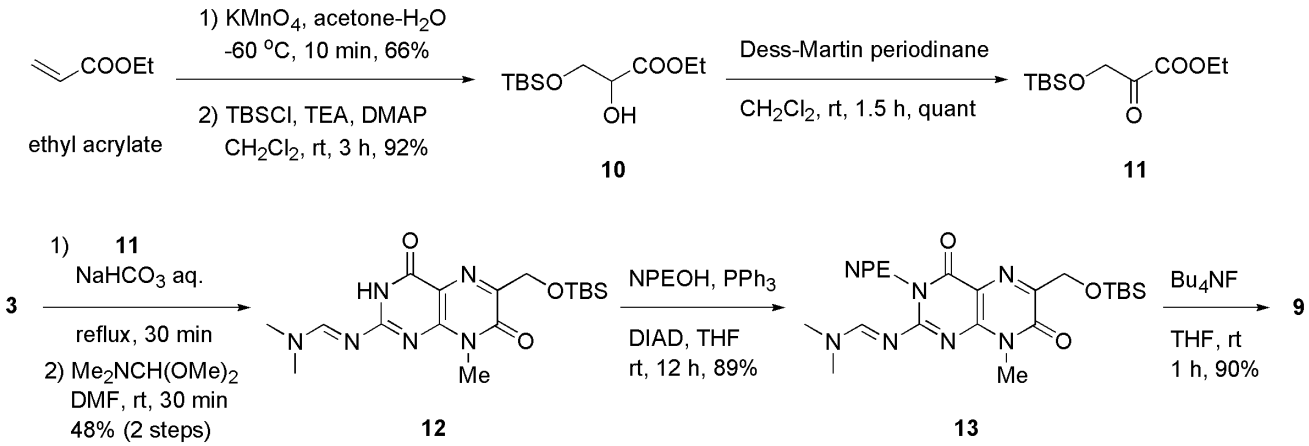
As the first step for the synthesis of asperopterin-A (**2b**), its aglycone asperopterin-B (**2a**) was prepared starting with 2,5-diamino-6-methylamino-3*H*-pyrimidin-4-one (**3**) (available from 2-amino-6-chloro-3*H*-pyrimidin-4-one) according to the reported procedures[15,16] with a slight modification (Scheme 1). Namely, condensation of **3** with ethyl glyoxalate afforded the imine (**4**), which was then cyclized under basic conditions to give 8-methyl-7-xanthopterin (**5**)[15] in an improved yield. Hydroxymethylation[16] of **5** with methanol and ammonium peroxydisulfate in a phosphate buffer provided **2a**; no improvement was achieved in the yield for this step despite various modifications of the reported procedures.




**Scheme 1**

For an effective glycosylation of the hydroxymethyl group, the pteridine moiety of **2a** was protected in a four-step procedure, affording the $N^2$-(*N*,*N*-dimethylaminomethylene)-3-[2-(4-nitrophenyl)ethyl] derivative (**9**). Thus, treatment of **2a** with *N*,*N*-dimethylformamide dimethyl acetal in DMF and the subsequent acetylation afforded the 6-acetoxymethyl-$N^2$-(*N*,*N*-dimethylaminomethylene) derivative (**6**). Mitsunobu reaction of **6** with *p*-nitrophenylethyl (NPE) alcohol in the presence of triphenylphosphine and diisopropyl azodicarboxylate (DIAD) in chloroform afforded a chromatographically inseparable mixture of the N(3)-NPE substituted product **7** (70% yield) and its $O^4$-NPE substituted isomer **8** (23%). As the Mitsunobu alkylation of $N^2$-(*N*,*N*-dimethylaminomethylene)pterins in THF (or dioxane) has been found to occur at N(3) position selectively,[17] the use of chloroform, instead of THF in which **6** is little soluble, may have caused the formation of byproduct $O^4$-alkylated derivative (**8**). The influence of solvents on the selectivity in this reaction remains to be clarified. Methanolysis of the mixture of **7** and **8** in the presence of sodium methoxide, followed by chromatographic separation, provided 6-hydromethyl derivative (**9**), a key precursor for glycosylation, in 67% overall yield from **6**.

We thus undertook a novel alternative way for preparation of the key intermediate **9** by condensation of pyrimidine derivative (**3**) with the 2-oxopropinonate derivative (**11**) (Scheme 2). Namely, oxidation of ethyl acrylate with potassium permanganate, followed by selective protection with *tert*-butyldimethylsilyl (TBS) group, afforded **10**, which was then oxidized with Dess-Martin periodinane to provide ethyl 3-(*tert*-butyldimethylsilyloxy)-2-oxopropionate (**11**).



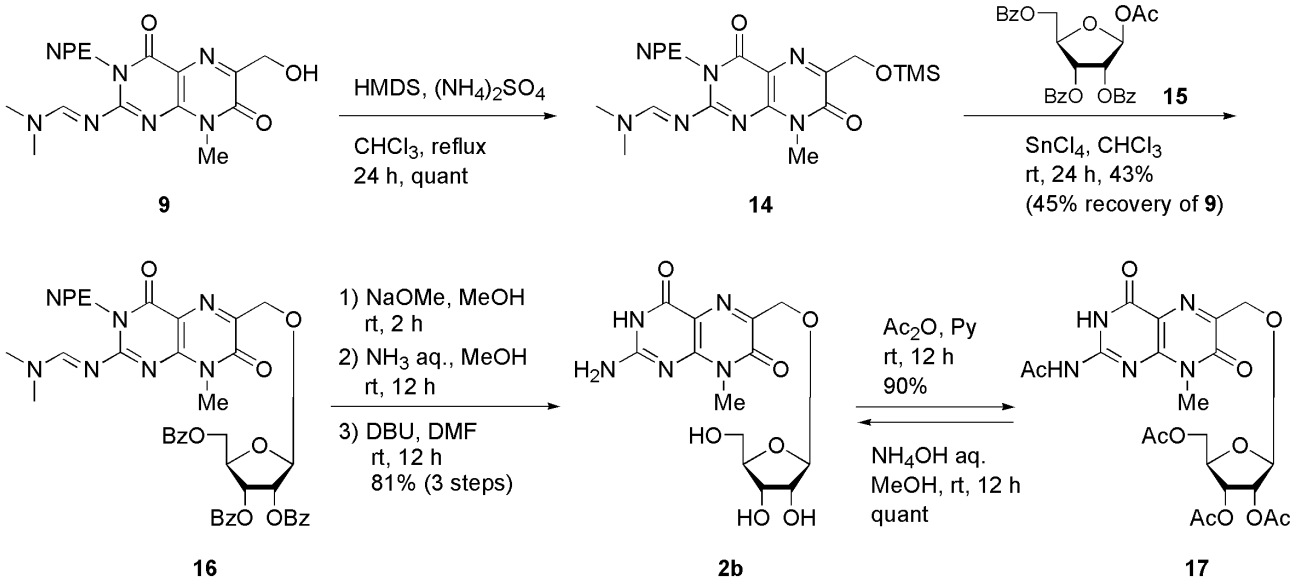

**Scheme 2**

The pteridine ring formation of the pyrimidine derivative (**3**) with **11** and the subsequent introduction of *N*,*N*-dimethylaminomethylene group afforded 6-(*tert*-butyldimethylsilyloxymethyl)-7-xanthopterin derivative (**12**) in 48% yield. Protection of **12** with NPE group under Mitsunobu conditions in THF yielded exclusively N(3)-NPE derivative (**13**), which was then treated with tetrabutylammonium fluoride

to provide 6-hydroxymethyl compound (**9**), the key precursor for glycosylation. Thus an improved preparation of **9** from **3** was achieved *via* a 3-step-shorter route in a ca. 3 times better overall yield, compared with the first route shown in Scheme 1.

Compound **9** was little soluble in chloroform and therefore was temporarily silylated with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) in the presence of ammonium sulfate in chloroform under reflux for 24 h, yielding the solubilized trimethylsilyl derivative (**14**) quantitatively.

Glycosylation of **14** with glycosyl donor, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**15**) was attempted under various conditions in the presence of activators (Scheme 3). Glycosylation of **14** with 2.0 mol equiv. of **15** in the presence of boron trifluoride etherate in chloroform at room temperature did not proceed due to precipitation of desilylated **9**. On the other hand, similar treatment of **14** with **15** in the presence of tin(IV) chloride (2.0 mol equiv.) afforded the D-ribofuranosyl derivative (**16**) in 43% yield, along with the recovery of **9** (45%).[18] Use of a larger amount of the glycosyl donor (3.0 equiv.) and the activator (3.0 equiv.) resulted in a lower yield of **16** (16%) and formation of a larger amount of **9** (68%). The β-anomeric configuration of the D-ribofuranoside (**16**) was assigned by its $J_{1,2}$ value (0 Hz). Its stereoselective β-glycoside formation was mainly attained by participation of the neighboring group (2-*O*-benzoyl group of **15**).




**Scheme 3**

Removal of the protecting groups of the isoxanthopterin glycoside (**16**) was performed according to the following three-step-procedures:[11,14] treatment of **16** with sodium methoxide in methanol to cleave benzoyl groups and then with aqueous ammonia-methanol to remove the *N*,*N*-dimethylaminomethylene

group, followed by the action of DBU in DMF to cleave the NPE group, furnished the target asperopterin-A (**2b**) in 81% overall yield from **16**. Structure of **2b** was unambiguously established as the corresponding pentaacetyl derivative (**17**) obtained by usual acetylation. Treatment of **17** with aqueous ammonia readily regenerated **2b** quantitatively. The precise structures of **2b** and **17** were established by $^{1}$H- and $^{13}$C-NMR spectra with the aid of 2D C-H COSY measurement (Table 1).

Thus the first synthesis of a natural isoxanthopterin glycoside, asperopterin-A (**2b**) was achieved utilizing an efficient synthesis of the key intermediate (**9**) from ethyl acrylate. Yields of ring formation, protection, and glycosylation of isoxanthopterin derivatives in this work seem to be relatively lower compared with those of pterin derivatives such as **1a–c**. Improvement of the yield of each step, as well as applications of these findings in synthesizing other pteridine glycosides having various types of sugar moieties, is in progress.

**Table 1**. 600 MHz $^{1}$H- and 151 MHz $^{13}$C-NMR Spectral parameters for asperopterin-A (**2b**) in DMSO-$d_6$ and its acetyl derivative (**17**) in $CDCl_3$

| Com-Pound | Chemical shifts/δ (coupling constants/Hz) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Pteridine moiety | | Glycosyl moiety | | | | | |
| | $CH_2$-6 ($^2J$) | $CH_3$-N(8) | H-1 ($J_{1,2}$) | H-2 ($J_{2,3}$) | H-3 ($J_{3,4}$) | H-4 ($J_{4,5a}$) | H$^a$-5 ($J_{5a,5b}$) | H$^b$-5 ($J_{4,5b}$) |
| **2b** [a] | 4.52, 4.36 (11.7) | 3.42 | 4.89 (0) | 3.72 (4.2) | 3.86 (6.8) | 3.76 (3.2) | 3.54 (11.6) | 3.40 (6.0) |
| **17** [b] | 4.84, 4.62 (11.9) | 3.51 | 5.25 (0) | 5.26 (4.8) | 5.31 (7.0) | 4.29 (3.8) | 4.34 (11.8) | 3.79 (6.3) |

| | Pteridine moiety | | | | | | | | Glycosyl moiety | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C-2 | C-4 | C-4a | C-6 | C-7 | C-8a | $CH_2O$ | $CH_3N$ | C-1 | C-2 | C-3 | C-4 | C-5 |
| **2b** | 156.53 | 160.39 | 111.20 | 144.22 | 155.40 | 151.92 | 66.31 | 28.09 | 107.23 | 74.55 | 71.24 | 83.94 | 63.34 |
| **17** [c] | 157.79 | 161.00 | 114.29 | 149.40 | 156.00 | 149.99 | 67.13 | 28.42 | 105.48 | 74.64 | 71.63 | 78.55 | 64.72 |

[a] The assignments were made by $D_2O$ exchange. [b] Additional signals: δ 2.03, 2.09, 2.095, 2.36 (4s, AcO-2,3,5, AcN), 9.43 [br s, H-N(3)], 12.08 (br s, AcN*H*). [c] Additional signals: δ 20.50, 20.58, 20.84, 24.49 ($CH_3CO$), 169.69, 169.75, 170 88, 172.63 ($CH_3CO$).

## EXPERIMENTAL

All reactions were monitored by TLC (Merck Silica gel 60 $F_{254}$ or Merck Cellulose F) with an appropriate solvent system [(*A*) 4:1 AcOEt-hexane, (*B*) 1:19, (*C*) 1:9 MeOH-$CHCl_3$, (*D*) 5:3:1 2-PrOH-AcOEt-$H_2O$, (*E*) 4:1:1 BuOH-AcOH-$H_2O$] and the values of each TLC are described in terms of $R_f$(s) for silica gel and

$R_f$(c) for cellulose. Column chromatography was performed with Daiso Silica Gel IR-60/210w. Components were detected by exposing the plates to UV light and/or 20% $H_2SO_4$-EtOH, with subsequent heating. The NMR spectra were measured in $CDCl_3$ with Varian Unity Inova AS600 (600 MHz for $^1$H, 151 MHz for $^{13}$C) at 23 °C, unless otherwise stated. The solvent peak was used as an internal standard for chemical shifts: in $CDCl_3$, δ 7.26 for $^1$H, 77.00 for $^{13}$C; in DMSO-$d_6$, δ 2.50 for $^1$H, 39.70 for $^{13}$C. The assignments of $^{13}$C signals were made with the aid of 2D C-H COSY measurements.

**2-Amino-5-(ethoxycarbonylmethylenimino)-6-methylamino-3*H*-pyrimidin-4-one (4).**[15]

The following modification of the literature procedures[15] was made. To a solution of dihydrochloride of **3** (1.20 g, 5.26 mmol) in water (20 mL) was added a solution of 50% ethyl glyoxalate in toluene (2.00 mL, 10.1 mmol). After stirring at rt for 1 h, the pH value was adjusted to ca. 6 by adding 1M aqueous NaOH and then the mixture was cooled at 0 °C. The precipitate was collected by suction and washed with cold water to give **4** (1.15 g, 91%) as yellow crystals: mp 236–238 °C (lit.,[15] mp 240 °C, 90% yield); $R_f$(c) = 0.32 (*E*); $^1$H-NMR (DMSO-$d_6$) δ 1.24 (3H, t, $^3J$ = 7.1 Hz, $CH_2CH_3$), 2.90 (3H, d, $J_{NH,Me}$ = 4.9 Hz, $CH_3$NH-6), 4.17 (2H, q, $CH_2CH_3$), 6.20–7.40 (2H, br s, $NH_2$-2), 6.91 (1H, q, NH-6), 8.86 (1H, s, CH=N-5), 10.26 [1H, br s, H–N(3)]; $^{13}$C-NMR (DMSO-$d_6$) δ 14.50 ($CH_2CH_3$), 28.03 ($CH_3$NH), 59.81 ($CH_2CH_3$), 101.65 (C-5) 134.65 (*C*H=N–C-5), 155.09 (C-6),* 156.80 (C-2),* 162.88 ($CO_2$Et), 165.64 (C-4), * The assignment may have to be interchanged.

**8-Methyl-7-xanthopterin (5).**[15]

The following modification of the literature procedures[15] was made. A suspension of **4** (1.09 g, 4.56 mmol) in 1M aqueous $NaHCO_3$ (80 mL) was refluxed for 30 min. After adding activated carbon, the mixture was stirred at 70 °C for 30 min and the carbon was filtered off. The pH value of the filtrate was adjusted to ca. 4 by adding 2M aqueous acetic acid and then the mixture was cooled at 0 °C. The precipitate was collected by suction and washed with cold water to give **5** (664 mg, 75%) as a pale yellow solid: mp > 300 °C (lit.,[15] mp > 350 °C, 50% yield); $R_f$(c) = 0.15 (*E*); $^1$H-NMR (DMSO-$d_6$) δ 3.42 [3H, s, $CH_3$–N(8)], 6.50–7.80 (2H, br s, $NH_2$-2), 7.60 (1H, s, H-6), 11.25 [1H, br s, H–N(3)]; $^{13}$C-NMR (DMSO-$d_6$) δ 27.68 [$CH_3$–N(8)], 112.27 (C-4a), 138.53 (C-6), 151.73 (C-8a), 154.86 (C-7), 156.99 (C-2), 159.32 (C-4).

**6-Hydroxymethyl-8-methyl-7-xanthopterin (Asperopterin B) (2a).**[16]

The following modification of the literature procedures[16] was made. A suspension of **5** (300 mg, 1.55 mg) in methanol (30 mL) and 0.5 M ammonium phosphate (pH 5–6, 120 mL) was refluxed for 30 min. Solid ammonium peroxydisulfate (2.50 g, 11.0 mmol) was added and then the mixture was refluxed for

1.5 h with keeping the pH value at ca. 5–6 by adding 2M aqueous KOH. The mixture was evaporated in vacuo to about a quarter volume and then adjusted to pH 2 with 2M HCl. The mixture was passed through a column of activated carbon (3 g) and Celite (9 g). After washing the column with water till the eluate showed pH 5–6, the products were eluted with a 1:1:2 mixture (300 mL) of 5% aqueous ammonia, pyridine, and ethanol. The eluate was evaporated in vacuo and the residue was dissolved in 0.1M aqueous KOH. The solution was acidified by adding 1M formic acid and then cooled. The precipitate was collected by suction and washed with a small amount of water to give **2a** [177 mg, 51% (lit.,[16] 53%)] as pale yellow crystals: mp >300 °C (lit.,[8] mp >300 °C); $R_f$(c) = 0.22 (*E*); $^1$H-NMR (DMSO-$d_6$) δ 3.43 [3H, s, $CH_3$-N(8)], 4.42 (2H, d, $J_{CH2,OH}$ = 4.6 Hz, $CH_2$-6), 4.89 (1H, t, OH), 6.50–7.80 (2H, br s, $NH_2$-2), 11.14 [1H, br s, H–N(3)]; $^{13}$C-NMR (DMSO-$d_6$) δ 27.88 [$CH_3$–N(8)], 60.97 ($CH_2$OH), 110.67 (C-4a), 147.81 (C-6), 151.41 (C-8a), 154.51 (C-7), 156.03 (C-2), 159.26 (C-4).

**6-Acetoxymethyl-$N^2$-(*N*,*N*-dimethylaminomethylene)-8-methyl-7-xanthopterin (6).**

To a supension of **5** (40.0 mg, 0.179 mmol) in dry DMF (2.5 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (0.030 mL, 0.226 mmol). The mixture was stirred at rt for 20 min and concentrated in vacuo. The residue was dissolved in dry pyridine (2.0 mL) and acetic anhydride (0.40 mL, 4.2 mmol) was added at 0 °C. The mixture was stirred at rt for 12 h and then concentrated in vacuo. The residue was purified by column chromatography with 1:19 MeOH-$CHCl_3$ to give **6** (29.4 mg, 51% yield from **5**) as pale yellow crystals: mp 170–172 °C (from AcOEt); $R_f$(s) = 0.25 (*B*), 0.47 (*C*); $^1$H-NMR ($CDCl_3$) δ 2.13 (3H, s, Ac), 3.20, 3.29 (3H each, 2s, $Me_2$N), 3.65 [3H, s, $CH_3$–N(8)], 5.24 (2H, s, $CH_2$-6), 4.89 (1H, t, OH), 8.73 (1H, br s, CH=N-2), 9.08 [1H, br s, H–N(3)]; $^{13}$C-NMR ($CDCl_3$) δ 20.85 ($CH_3$CO), 28.22 [$CH_3$–N(8)], 35.80, 42.00 ($Me_2$N), 63.51 ($CH_2$–C-6), 113.90 (C-4a), 145.93 (C-6), 150.58 (C-8a), 156.41 (C-7), 157.86 (C-2), 159.36 (CH=N), 160.46 (C-4), 170.67 ($CH_3CO$). Anal. calcd for $C_{13}H_{16}N_6O_4$: C, 48.75; H, 5.03. Found: C, 48.62; H, 5.19.

**6-Acetoxymethyl-$N^2$-(*N*,*N*-dimethylaminomethylene)-8-methyl-3-[2-(4-nitrophenyl)ethyl]-7-xanthopterin (7) and 6-Acetoxymethyl-$N^2$-(*N*,*N*-dimethylaminomethylene)-8-methyl-$O^4$-[2-(4-nitrophenyl)ethyl]-7-xanthopterin (8).**

To a solution of **6** (43.0 mg, 0.134 mmol), 2-(*p*-nitrophenyl)ethanol (45.0 mg, 0.267 mmol) and triphenylphosphine (72.0 mg, 0.275 mmol) in dry $CHCl_3$ (2.0 mL), was added a solution of 40% DIAD in toluene (0.140 mL, 0.266 mmol). The mixture was stirred at rt for 12 h and then concentrated in vacuo. The residue was purified by column chromatography with 1:2 AcOEt-hexane and then 2% MeOH-$CHCl_3$ to give a pale yellow foam (58.6 mg), which consisted of **7** (44.0 mg, 70% yield) and **8** (14.6 mg, 23%): $R_f$(s) = 0.44 (*B*).

$^1$H-NMR for **7** ($CDCl_3$) δ 2.15 (3H, s, Ac), 3.13 [2H, t, $^3J$ = 7.4 Hz, C$H_2$C$H_2$–N(3)], 3.17, 3.26 (3H each, 2s, $Me_2$N), 3.61 [3H, s, $CH_3$–N(8)], 4.54 [2H, t, $CH_2$–N(3)], 5.25 (2H, s, $CH_2$-6), 7.38, 8.13 (2H each, 2d, $J_{o,m}$ = 8.6 Hz, $C_6H_4$), 8.57 (1H, br s, CH=N-2).

$^1$H-NMR for **8** ($CDCl_3$) δ 2.17 (3H, s, Ac), 3.30 [2H, t, $^3J$ = 6.7 Hz, C$H_2$C$H_2$O-4], 3.22, 3.225 (3H each, 2s, $Me_2$N), 3.70 [3H, s, $CH_3$–N(8)], 4.79 [2H, t, $CH_2$O-4], 5.30 (2H, s, $CH_2$-6), 7.50, 8.16 (2H each, 2d, $J_{o,m}$ = 8.6 Hz, $C_6H_4$), 8.73 (1H, br s, CH=N-2).

### $N^2$-(*N*,*N*-dimethylaminomethylene)-6-hydroxymethyl-8-methyl-3-[2-(4-nitrophenyl)ethyl]-7-xanthopterin (9)

**A. From 7**. An inseparable mixture (75:25) of **7** and **8** (52.0 mg, 0.111 mmol) was dissolved in dry 1:1 $CH_2Cl_2$-MeOH (2.0 mL) and then sodium methoxide (28% in MeOH, 0.006 mL, 0.03 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 separated by column chromatography with 5% MeOH-$CHCl_3$ into two fractions.

The faster-eluted fraction [$R_f$(s) = 0.38–0.31 (B)] gave an inseparable mixture (15.0 mg) which consisted of NPE alcohol and unidentified products derived from **8**.

The slower-eluted fraction [$R_f$(s) = 0.27 (*B*), 0.51 (*C*)] afforded **9** (34.0 mg, 67% from **6**) as pale yellow crystals: mp 263–265 °C (from AcOEt); $^1$H-NMR ($CDCl_3$) δ 3.14 [2H, t, $^3J$ = 7.6 Hz, C$H_2$C$H_2$–N(3)], 3.19, 3.27 (3H each, 2s, $Me_2$N), 3.60–3.75 (1H, br s, OH), 3.63 [3H, s, $CH_3$–N(8)], 4.55 [2H, t, $CH_2$–N(3)], 4.79 (2H, s, $CH_2$-6), 7.39, 8.13 (2H each, 2d, $J_{o,m}$ = 8.7 Hz, $C_6H_4$), 8.57 (1H, br s, CH=N-2); $^{13}$C-NMR ($CDCl_3$) δ 27.67 [$CH_3$–N(8)], 34.15 (*C*$H_2$C$H_2$–N(3)], 35.66, 41.82 ($Me_2$N), 43.43 [$CH_2$–N(3)], 62.30 (*C*$H_2$–C-6), 112.78 (C-4a), 123.67 [C(*m*) of NPE], 129.82 [C(*o*) of NPE], 146.73 [C(*ipso*) of NPE], 146.74 (C-6), 147.79 [C(*p*) of NPE], 150.64 (C-8a), 156.22 (C-7), 157.53 (C-2), 158.69 (CH=N), 160.02 (C-4). Anal. calcd for $C_{19}H_{21}N_7O_5$: C, 53.39; H, 4.95. Found: C, 53.18; H, 5.07.

**B. From 13.** Tetrabutylammonium fluoride (1.0 M THF solution, 0.060 mL, 0.060 mmol) was added dropwise to a solution of **13** (27.0 mg, 0.0498 mmol) in dry THF (1.0 mL) at 0 °C. The mixture was stirred at rt for 1 h, diluted with water, and extracted with $CHCl_3$ three times. The combined organic layers were dried ($Na_2SO_4$) and evaporated in vacuo. The residue was purified by column chromatography to give **9** (19.2 mg, 90%).

### Ethyl 3-(*tert*-Butyldimethylsilyloxy)-2-hydroxypropionate (10).

To a solution of potassium permanganate (2.40 g, 15.2 mmol) dissolved in water (20 mL) and acetone (30 mL) was slowly added ethyl acrylate (1.50 mL, 13.8 mmol) at –60 °C. The mixture was stirred at –60 °C for 10 min and allowed to warm up to rt. The mixture was filtered and the precipitate was

washed with acetone. The combined filtrates were evaporated in vacuo (50 mmHg) at 20–30 °C. The residual aqueous solution was extracted with ethyl acetate three times. The combined organic layers were dried ($Na_2SO_4$), and evaporated in vacuo to give ethyl glycerate [1.22 g, 66% (lit.,[19] 56%)] as a colorless oil: $R_f$(s) = 0.24 (*C*).

This oil (1.22 g, 9.10 mmol) was dissolved in dry $CH_2Cl_2$ (10 mL) and then TEA (1.40 mL, 10.0 mmol) and DMAP (100 mg, 0.819 mmol) were added. To the mixture was added a solution of *tert*-butyldimethylsilyl chloride (1.51 g, 10.0 mmol) dissolved in dry $CH_2Cl_2$ (2 mL). The mixture was stirred at rt for 3 h and then diluted with $CHCl_3$ (20 mL). The mixture was washed with water, dried ($Na_2SO_4$), and evaporated in vacuo (50 mmHg). The residue was purified by column chromatography with 1:4 AcOEt-hexane as an eluant to give **10** (2.07 g, 92%) as a colorless oil: $R_f$(s) = 0.38 (*A*), 0.80 (*C*); $^1$H-NMR δ 0.04, 0.06 (3H each, 2s, $Me_2Si$), 0.87 (9H, s, $Me_3C$), 1.30 (3H, t, $^3J$ = 7.1 Hz, $CH_2CH_3$), 3.03 (1H, d, $J_{2,OH}$ = 8.1 Hz), 3.85, 3.93 (1H each, 2dd, $J_{3,3'}$ = 10.3, $J_{2,3}$ = 3.0 Hz, $H_2$-3), 4.19 (1H, dt, H-1), 4.24 (2H, q, $CH_2CH_3$); $^{13}$C-NMR δ –5.61, –5.46 (2s, $Me_2Si$), 14.21 ($CH_2CH_3$), 18.22 ($Me_3C$), 25.71 ($Me_3C$), 61.55 ($CH_2CH_3$), 65.06 (C-3), 71.93 (C-2), 172.75 (C-1). Anal. Calcd for $C_{11}H_{24}O_4Si$: C, 53.19; H, 9.74. Found: C, 53.02; H, 9.88.

**Ethyl 3-(*tert*-Butyldimethylsilyloxy)-2-oxopropionate (11).**

To a solution of **10** (129 mg, 0.519 mmol) in dry $CH_2Cl_2$ (2.0 mL) was added a solution of Dess-Martin periodinane (330 mg, 0.778 mmol) at 0 °C. The mixture was stirred at rt for 1.5 h and then diluted with $CHCl_3$ (20 mL). The mixture was washed with saturated sodium thiosulfate and then saturated $NaHCO_3$, dried ($Na_2SO_4$), and evaporated in vacuo (50 mmHg) to give **11** (127 mg, quant) as a colorless oil: $R_f$(s) = 0.30–0.24 (*A*). The product was spectroscopically pure and used for the next step without further purification. $^1$H-NMR δ 0.10 (6H s, $Me_2Si$), 0.91 (9H, s, $Me_3C$), 1.36 (3H, t, *J* = 7.2 Hz, $CH_2CH_3$), 4.32 (2H, q, $CH_2CH_3$), 4.74 (2H, s, $H_2$-3); $^{13}$C-NMR δ –5.47 ($Me_2Si$), 13.97 ($CH_2CH_3$), 18.37 ($Me_3C$), 25.68 ($Me_3C$), 62.39 ($CH_2CH_3$), 67.63 (C-3), 160.78 (C-1), 192.42 (C-2).

**6-(*tert*-Butyldimethylsilyloxymethyl)-$N^2$-(*N*,*N*-dimethylaminomethylene)-8-methyl-7-xanthopterin (12).**

To a solution of dihydrochloride of **3** (41.0 mg, 0.180 mmol) and $NaHCO_3$ (60.0 mg, 0.714 mmol) in water (1.0 mL) was added a solution of **11** (49.0 mg, 0.199 mmol) in ethanol (1.0 mL). The mixture was refluxed for 30 min and then concentrated in vacuo. The residue was dissolved in DMF and filtered, and then the filtrate was concentrated in vacuo. The residue was dissolved in dry DMF (1.5 mL) and *N*,*N*-dimethylformamide dimethyl acetal (0.020 mL, 0.151 mmol) was added. The mixture was stirred at rt for 30 min and concentrated in vacuo. The residue was purified by column chromatography with

1:19 MeOH-$CHCl_3$ to give **12** (34.0 mg, 48% yield from **3**) as pale yellow crystals: mp 252–254 °C (from AcOEt); $R_f$(s) = 0.24 (B), 0.44 (*C*); $^1$H-NMR ($CDCl_3$) δ 0.16 (6H s, $Me_2Si$), 0.93 (9H, s, $Me_3C$), 3.20, 3.27 (3H each, 2s, $Me_2N$), 3.63 [3H, s, $CH_3$–N(8)], 4.82 (2H, s, $CH_2$-6), 8.71 (1H, br s, CH=N-2), 9.84 [1H, br s, H–N(3)]; $^{13}$C-NMR ($CDCl_3$) δ –5.24 ($Me_2Si$), 18.72 ($Me_3C$), 26.00 ($Me_3C$), 28.09 [$CH_3$–N(8)], 35.73, 41.91 ($Me_2N$), 63.64 ($CH_2$–C-6), 113.62 (C-4a), 150.32 (C-6), 150.33 (C-8a), 156.45 (C-7), 157.53 (C-2), 159.22 (CH=N), 160.71 (C-4). Anal. calcd for $C_{17}H_{28}N_6O_3Si$: C, 52.02; H, 7.19. Found: C, 52.19; H, 7.30.

**6-(*tert*-Butyldimethylsilyloxymethyl)-$N^2$-(*N,N*-dimethylaminomethylene)-8-methyl-3-[2-(4-nitrophenyl)ethyl]-7-xanthopterin (13).**

To a solution of **12** (26.4 mg, 0.0673 mmol), 2-(4-nitrophenyl)ethanol (22.5 mg, 0.134 mmol) and triphenylphosphine (36.1 mg, 0.134 mmol) in dry THF (1.0 mL), was added a solution of 40% DIAD in toluene (0.070 mL, 0.133 mmol). The mixture was stirred at rt for 12 h and then concentrated in vacuo. The residue was purified by column chromatography with 1:2 AcOEt-hexane and then 2% MeOH-$CHCl_3$ to give **13** (32.5 mg, 89% yield) as pale yellow crystals: mp 169–171 °C (from AcOEt); $R_f$(s) = 0.50 (B), 0.72 (*C*); $^1$H-NMR ($CDCl_3$) δ 0.18 (6H s, $Me_2Si$), 0.94 (9H, s, $Me_3C$), 3.13 [2H, t, $^3J$ = 7.6 Hz, $CH_2CH_2$–N(3)], 3.16, 3.24 (3H each, 2s, $Me_2N$), 3.61 [3H, s, $CH_3$–N(8)], 4.55 [2H, t, $CH_2$–N(3)], 4.85 (2H, s, $CH_2$-6), 7.39, 8.13 (2H each, 2d, $J_{o,m}$ = 8.7 Hz, $C_6H_4$), 8.54 (1H, br s, CH=N-2). Anal. calcd for $C_{25}H_{35}N_7O_5Si$: C, 55.43; H, 6.51. Found: C, 55.30; H, 6.72.

**$N^2$-(*N,N*-Dimethylaminomethylene)-8-methyl-3-[2-(4-nitrophenyl)ethyl]-6-[(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)oxymethyl]-7-xanthopterin (16).**

A suspension of **9** (45.7 mg, 0.107 mmol), ammonium sulfate (33.0 mg, 0.250 mmol), and HMDS (0.300 mL, 1.45 mmol) in dry $CHCl_3$ (5.0 mL) was refluxed for 24 h. The precipitate was filtered off and the filtrate was evaporated in vacuo to give the trimethylsilylated compound (**14**) (53.5 mg) as a pale yellow foam: $R_f$(s) = 0.56 (*B*).

To a solution of the D-ribofuranose derivative (**15**) (110 mg, 0.218 mmol) in dry $CHCl_3$ (1.5 mL) was added a solution of $SnCl_4$ (1M in $CH_2Cl_2$, 0.210 mL, 0.210 mmol) at 0 °C. After stirring at this temp for 30 min, a solution of **14** (53.5 mg, 0.107 mmol) in dry $CHCl_3$ (3.0 mL) was added in small portions at 0 °C 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 2% MeOH-$CHCl_3$ into two fractions.

The faster-eluting fraction [$R_f$(s) = 0.46 (*B*)] gave **16** (40.2 mg, 43%) as a pale yellow foam: $^1$H NMR ($CDCl_3$) δ 3.13 [2H, t, $^3J$ = 7.6 Hz, $CH_2CH_2$-N(3)], 3.17, 3.25 (3H each, 2s, $Me_2N$), 3.58 [3H, s,

$CH_3$-N(8)], 4.53 [2H, t, $CH_2$-N(3)], 4.60 (1H, dd, $J_{5a,5b}$ = 11.5, $J_{4,5b}$ = 6.5 Hz, $H_b$-5*), 4.69 (1H, dd, $J_{4,5a}$ = 4.4 Hz, $H_a$-5*), 4.71 (1H, td, $J_{3,4}$ = 6.8 Hz, H-4*), 4.78, 4.98 (1H each, 2d, $^2J$ = 11.7 Hz, $CH_2$-6), 5.58 (1H, s, H-1*), 5.75 (1H, d, $J_{2,3}$ = 4.9 Hz, H-2*), 5.88 (1H, dd, H-3*), 7.26–7.39 [6H, m, Bz(*m*)], 7.39, 8.13 (2H each, 2d, $J_{o,m}$ = 8.8 Hz, $C_6H_4$), 7.40–7.55 [3H, m, Bz(*p*)], 7.82, 7.995, 8.00 [2H each, 3dd, $J_{o,m}$ = 8.5, $J_{o,p}$ = 1.3 Hz, Bz(*o*)], 8.51 (1H, s, CH=N-2), *for glycosyl moiety. Anal. Calcd for $C_{45}H_{41}N_7O_{12}$: C, 61.99; H, 4.74. Found: C, 61.81; H, 4.90.

The slower-eluting fraction [$R_f$(s) = 0.35 (*B*)] gave **9** (20.6 mg, 45% recovery).

**8-Methyl-6-[(β-D-ribofuranosyl)oxymethyl]-7-xanthopterin (Asperopterin A) (2b).**

**A. From 16.** Compound **16** (37.0 mg, 0.0424 mmol) was dissolved in dry 1:2 $CH_2Cl_2$-MeOH (2.1 mL) and then sodium methoxide (28% in MeOH, 0.020 mL, 0.098 mmol) was added at 0 °C. The mixture was stirred at rt for 2 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 (3.0 mL) and 28% aqueous ammonia solution (3.0 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 then DBU (0.040 mL, 0.27 mmol) was added. The mixture was stirred at rt for 12 h, diluted with water (2.0 mL), and neutralized with Amberlite FPC3500($H^+$). The resin was filtered off and the filtrate was evaporated in vacuo. The residue was washed with $CHCl_3$ and dried under reduced pressure to give **2b** (12.2 mg, 81%) as a pale yellow solid: $R_f$(s) = 0.27 (*D*), $R_f$(c) = 0.18 (*E*); $^1$H and $^{13}$C NMR (DMSO-$d_6$), see Table 1. An analytical sample was crystallized from water: mp 191–192 °C (lit.,[7] mp 193 °C). Anal. Calcd for $C_{13}H_{17}N_5O_7 \cdot H_2O$: C, 41.82; H, 5.13. Found: C, 41.99; H, 5.20.

**B. From 17.** Compound **17** (12.1 mg, 0.0231 mmol) was dissolved in MeOH (1.0 mL) and 28% aqueous ammonia solution (1.0 mL) was added. The mixture was stirred at rt for 12 h and evaporated in vacuo to give **2b** (8.1 mg, 99%).

***N*$^2$-Acetyl-8-methyl-6-[(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)oxymethyl]-7-xanthopterin (17).**

Compound **2b** (12.8 mg, 0.0360 mmol) was dissolved in pyridine (1.5 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 purified by column chromatography with 2% MeOH-$CHCl_3$ as an eluant to give **17** (17.0 mg, 90%) as a pale yellow foam: $R_f$(s) = 0.45 (*C*); $^1$H and $^{13}$C NMR ($CDCl_3$), see Table 1. Anal. calcd for $C_{21}H_{25}N_5O_{11}$: C, 48.19; H, 4.81. Found: C, 48.31; H, 5.01.

**ACKNOWLEDGEMENTS**

We are grateful to the SC-NMR Laboratory of Okayama University for the NMR measurements and to

WESCO Scientific Promotion Foundation (to T. H.) which partially supported this work.

## REFERENCES AND NOTES


1. (a) H. S. Forrest, C. Van Baalen, and J. Myers, *Arch. Biochem. Biophys.*, 1958, **78**, 95; (b) T. Matsunaga, J. G. Burgess, N. Yamada, K. Komatsu, S. Yoshida, and Y. Wachi, *Appl. Microbiol. Biotechnol*., 1993, **39**, 250; (c) Y. Noguchi, A. Ishii, A. Matsushima, D. Haishi, K. Yasumuro, T. Moriguchi, T. Wada, Y. Kodera, M. Hiroto, H. Nishimura, M. Sekine, and Y. Inada, *Mar. Biotechnol*., 1999, **1**, 207; (d) Y. K. Choi, Y. K. Hwang, Y. H. Kang, and Y. S. Park, *Pteridines*, 2001, **12**,121.
2. K. W. Cha, W. Pfleiderer, and J. J. Yim, *Helv. Chim. Acta*, 1995, **78**, 600.
3. M. Ikawa, J. J. Sasner, J. F. Haney, and T. L. Foxall, *Phytochemistry*, 1995, **38**, 1229.
4. H. W. Lee, C. H. Oh, A. Geyer, W. Pfleiderer, and Y. S. Park, *Biochim. Biophys. Acta*, 1999, **1410**, 61.
5. S.-H. Cho, J.-U. Na, H. Youn, C.-S. Hwang, C.-H. Lee, and S.-O. Kang, *Biochim. Biophys. Acta*, 1998, **1379**, 53.
6. (a) X. Lin and R. H. White, *J. Bacteriol.*, 1988, **170**, 1396; (b) A. Suzuki and M. Goto, *J. Biochem*., 1968, **63**, 798.
7. (a) Y. Kaneko, *Nippon Nogei Kagaku Kaishi*, 1966, **40**, 227; (b) Y. Kaneko and M. Sanada, *J. Ferment. Technol.*, 1969, **47**, 8.
8. S. Matsuura, M. Yamamoto, and Y. Kaneko, *Bull. Chem. Soc. Jpn.*, 1972, **45**, 492.
9. The trivial name recommended by the International Society of Pteridinology: J. Ferre, K. B. Jocobson, and W. Pfleiderer, *Pteridines*, 1990, **2**, 129.
10. Some inhibitory activities against tyrosinase were reported for biopterin D-glucoside (**1b**): Y. Wachi, S. Yoshida, K. Komatsu, and T. Matsunaga, Jpn. Patent, 05,286,989, 1993 (*Chem. Abstr*., 1994, **120**, 161782t).
11. (a) T. Hanaya, H. Baba, H. Toyota, and H. Yamamoto, *Tetrahedron*, 2009, **65**, 7989; (b) T. Hanaya, K. Torigoe, K. Soranaka, H. Fujita, H. Yamamoto, and W. Pfleiderer, *Pteridines*, 2008, **19**, 72.
12. (a) T. Hanaya, H. Baba, H. Toyota, and H. Yamamoto, *Tetrahedron*, 2008, **64**, 2090; (b) T. Hanaya, K. Soranaka, K. Harada, H. Yamaguchi, R. Suzuki, Y. Endo, H. Yamamoto, and W. Pfleiderer, *Heterocycles*, 2006, **67**, 299.
13. (a) T. Hanaya, H. Baba, M. Kanemoto, and H. Yamamoto, *Heterocycles*, 2008, **76**, 635; (b) T. Hanaya, H. Baba, and H. Yamamoto, *Carbohydr. Res*., 2007, **342**, 2159.
14. T. Hanaya, H. Baba, K. Ejiri, and H. Yamamoto, *Heterocycles*, 2010, **80**, 1013.
15. W. Pfleiderer and M. Rukwied, *Chem. Ber*., 1961, **94**, 1.

16. T. Sugimoto, S. Murata, S. Matsuura, and W. Pfleiderer, *Tetrahedron Lett*., 1986, **27**, 4179.
17. (a) T. Hanaya, K. Torigoe, K. Soranaka, H. Yamamoto, Q. Yao, and W. Pfleiderer, *Pteridines*, 1995, **6**, 1; (b) Q. Yao and W. Pfleiderer, *Helv. Chim. Acta*, 2003, **86**, 1.
18. When **9** was, without the trimethylsilylation, treated in a larger amount of chloroform with **15** in the presence of $SnCl_4$, no glycosylation was found to proceed apparently due to deposition of a complex of **9** with the activator; the same reaction under reflux conditions resulted in the formation of unidentified, decomposed products.
19. D. Choi, J. P. Stables, and H. Kohn, *Bioorg. Med. Chem.*, 1996, **4**, 2105.