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SYNTHETIC STUDIES ON NATURAL PTERIN GLYCOSIDES

Tadashi Hanaya* 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 – Some pterins having various kind of sugars attached to the hydroxyalkyl side-chain at C-6 are known to occur in certain prokaryotes as exemplified by 2'-*O*-(α-D-glucopyranosyl)biopterin isolated from various kinds of cyanobacteria. A synthetic exploration of various types of glycosides of biopterin and related pterins has been undertaken owing to a marked interest in their physiological functions and biological activities as well as the structural proof of those natural products. This review summarizes our synthetic studies on natural pterin glycosides by employing the appropriately protected N^2-(*N*,*N*-dimethylaminomethylene)-3-[2-(4-nitrophenyl)ethyl]pterin derivatives as glycosyl accepters.

CONTENTS

5. References

1. INTRODUCTION

Various pterin derivatives have been isolated from many living organisms: *e.g.*, microorganisms, algae, insects, fish, amphibian, and mammals.[1] Among them, some pterins having a hydroxyalkyl side-chain at C-6, such as biopterin (**1a**), ciliapterin (**2a**), and neopterin (**3a**), have been found as glycosidic forms in certain prokaryotes (Figure 1). As examples of glycosides of biopterin (**1a**), 2'-*O*-(α-D-gluco-pyranosyl)biopterin (**1b**)[2-5] and its β-D-ribofuranosyl analog (**1c**)[6] were isolated from cyanobacteria and limipterin [2'-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)biopterin] (**1d**)[7] was isolated from a green sulfur photosynthetic bacterium. Glycosides (**2b,c, 3b,c, 4b,c**) of other pterins such as ciliapterin (**2a**),[8,9] neopterin (**3a**),[10,11] and 6-hydroxymethylpterin (**4a**)[12,13] were also isolated from cyanobacteria, anaerobic photosynthetic bacteria, and chemoautotrophic archaebacteria. On the other hand, asperopterin-A (**5b**)[14] isolated from *Aspergillus oryzae* is a unique glycoside of **5a** having an isoxanthopterin (7-xanthopterin) structure as a parent ring. Various other glycosides consisting of different pterins and sugar moieties have

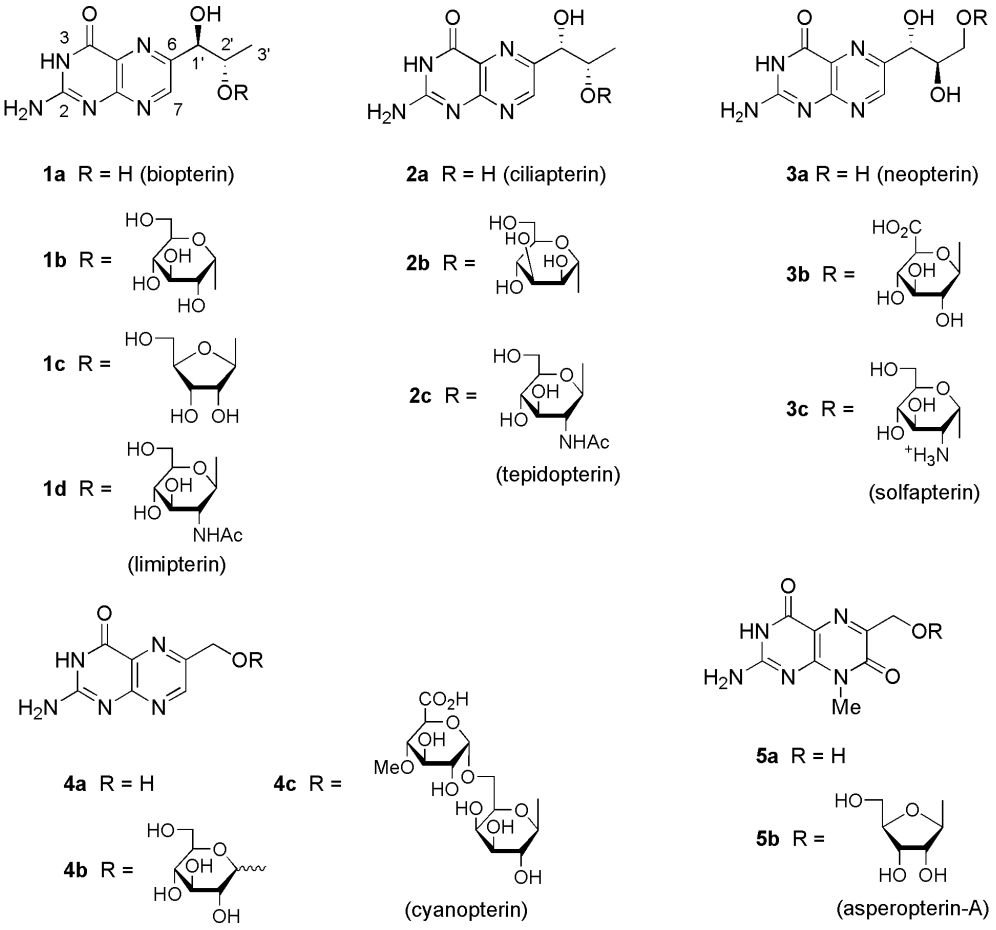


Figure 1. Naturally occurring pterin glycosides

also been found in nature, although some of them have remained unclear concerning the position and the anomeric structure of the glycosidic linkage.[8,12]

The physiological function of the parent pterins has been studied in detail: *e.g.*, **1a** exhibits enzyme cofactor activity in aromatic amino acid hydroxylation[15] and nitric oxide synthesis[16] as the form of its tetrahydro derivative, while neopterin (**3a**) has been shown to be a marker for the activation of cellular immunity or an inducer of apoptosis.[17] By contrast, the functional roles of pterin glycosides have remained obscure, although some inhibitory activities against tyrosinase[18] and photostabilization of photosynthetic pigments[19] were reported for **1b**. Despite a considerable interest from the viewpoint of their biological activities and functions as well as structural proof of hitherto reported natural products, there had been no report for preparation of pterin glycosides before our synthetic studies on them. For example, although biopterin α-D-glucoside (**1b**) is the most noteworthy among these pterin glycosides because of its abundant occurrence in various kinds of cyanobacteria, *Anacystis nidulans*,[2] *Oscillatoria* sp.,[3] *Synechococcus* sp.,[4] and *Spirulina platensis*,[5] no attempts at synthesis of **1b** had been made since its first discovery in 1958. In this review, we summarize our synthetic studies on natural pterin glycosides, taking up glycosides of biopterin in the first half and those of other pterins in the latter half.

2. SYNTHESIS OF BIOPTERIN GLYCOSIDES

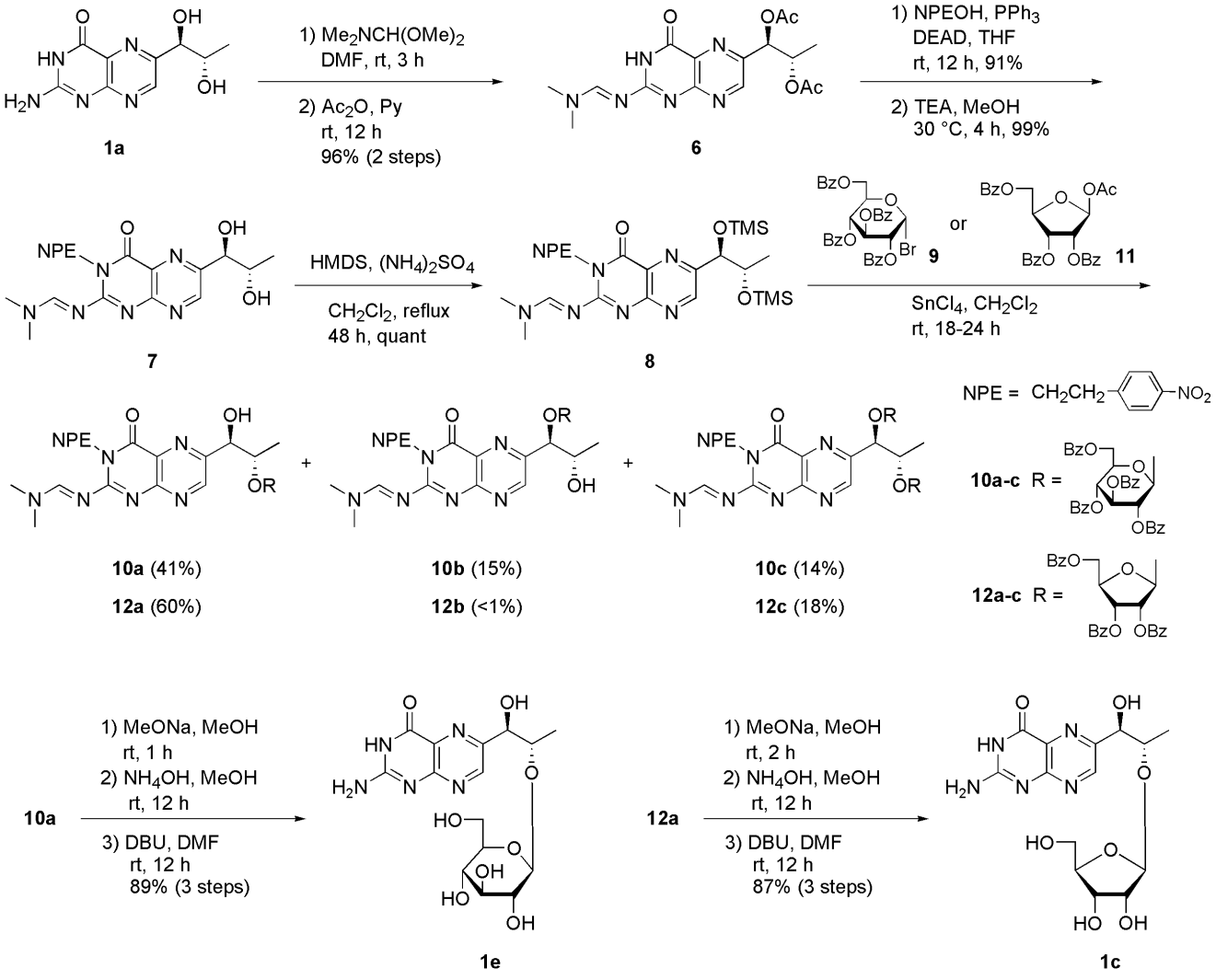
2-1. Synthesis of 2'-*O*-(β-D-ribofuranosyl)- and 2'-*O*-(β-D-glucopyranosyl)biopterin (1c and 1e)

In our initial studies on glycosylation of a side-chain hydroxy group, we had to devise suitable protecting and at the same time solubilizing groups for the pyrimidine ring and the side-chain hydroxy groups of the starting material biopterin (**1a**). Because of the effectively stabilized intramolecular hydrogen bondings in the solid state,[20] many pterin derivatives including **1a** are little soluble in nonpolar aprotic solvents in which glycosylation reactions smoothly proceed. To overcome this problem, we employed dimethylaminomethylene group for protection of 2-amino and 2-(4-nitrophenyl)ethyl (NPE) group for N(3) of the pteridine ring.[21] Thus, **1a** was converted in a four-step-procedure via the di-*O*-acetyl intermediate (**6**) into the sufficiently solubilized and versatile N^2-(*N*,*N*-dimethylaminomethylene)-3-NPE-biopterin (**7**) in 87% overall yield (Scheme 1)**.** Introduction of NPE group was achieved under Mitsunobu's conditions [NPE alcohol, triphenylphosphine, and diethyl azodicarboxylate (DEAD)].

For the purpose of further raising the solubility of compound **7** in dichloromethane, **7** was temporarily silylated with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) in the presence of ammonium sulfate, yielding the 1',2'-di-*O*-trimethylsilyl derivative (**8**) quantitatively. Glycosylation of **8** with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (**9**)[22] in the presence of tin(IV) chloride resulted in the formation of a mixture of 2'-*O*-(β-D-glucopyranosyl) **10a** (41%), 1'-*O*-glycosyl isomer **10b** (15%) and the 1',2'-di-*O*-

glycoside **10c** (14%). Removal of the protecting groups of the 2'-*O*-(β-D-glucopyranosyl) derivative (**10a**) was performed according to the following three-step-procedure: **10a** was treated with sodium methoxide in methanol to cleave benzoyl groups, and then treated with aqueous ammonia-methanol to remove the *N*,*N*-dimethylaminomethylene group. The NPE group was then removed by the action of DBU in DMF, thus affording 2'-*O*-(β-D-glucopyranosyl)biopterin (**1e**)[23] as the first synthetic example of pterin glycosides despite of the anomeric isomer of natural biopterin glycoside **1b**.

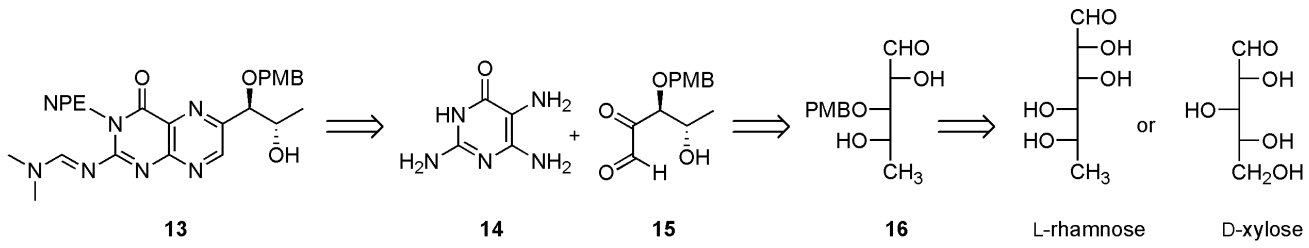
Meanwhile, similar treatment of **8** with 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzoyl-β-D-ribofuranose (**11**) afforded 2'-*O*-(β-D-ribofuranosyl) compound **12a** (60%) along with a minor portion of the 1',2'-di-*O*-glycoside **12c** (18%). Deprotection of **12a** by the same treatment described above provided natural product 2'-*O*-(β-D-ribofuranosyl)biopterin (**1c**).[6]



Scheme 1

2-2. Synthesis of limipterin (1d)

The results shown in Scheme 1 prompted us to pursue an effective preparation of a suitably 1'-*O*-protected biopterin derivative in order to achieve complete 2'-*O*-glycosylation. Taking into consideration the available combination of protecting groups employed for the synthetic pathways, we chose the biopterin derivative (**13**) whose 1'-hydroxy group was protected with *p*-methoxybenzyl (PMB) group, as a key glycosyl acceptor. As shown in a retrosynthetic analysis for **13** outlined in Scheme 2, the pteridine ring formation of **13** would be achieved by the condensation of 2,5,6-triaminopyrimid-4(3*H*)-one (**14**) with the 3-*O*-protected pentos-2-ulose (**15**), which would be obtainable from the 3-*O*-protected 5-deoxy-L-arabinose (**16**). Compound **16** would be derived by two different synthetic routes, starting with L-rhamnose involving C-1 cleavage, or starting with D-xylose involving C-4 inversion and C-5 deoxygenation. Thus the efficient total synthesis of limipterin [2'-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)biopterin] (**1d**) was attempted by selective glycosylation of the appropriately protected biopterin derivative (**13**) as follows.[24,25]



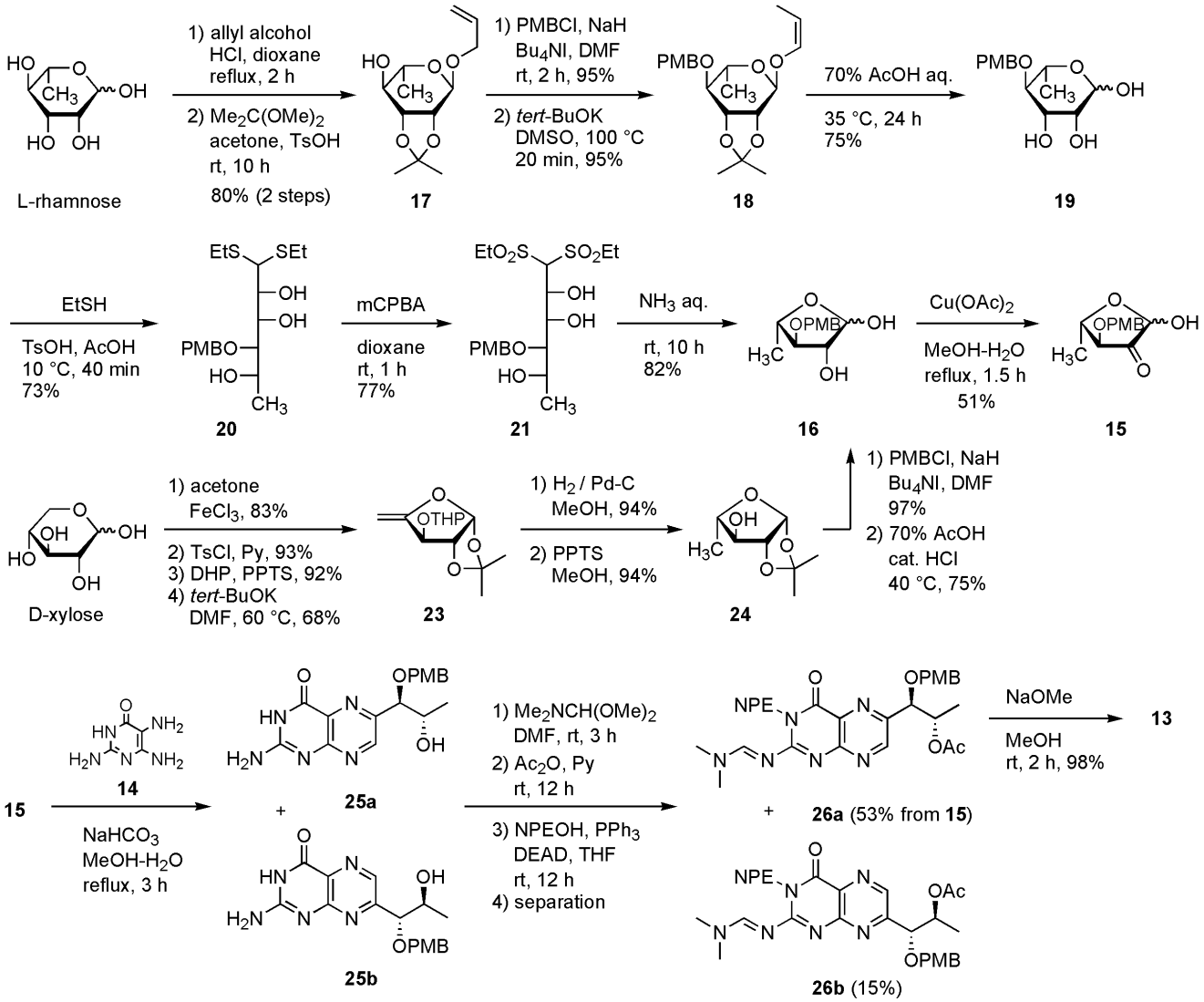
Scheme 2

A five-step synthetic conversion from L-rhamnose provided 4-*O*-PMB-6-deoxy-L-rhamnose (**19**) via the allyl (**17**)[26] and prop-1-enyl glycosides (**18**) as shown in Scheme 3. Treatment of **19** with ethanethiol in the presence of *p*-toluenesulfonic acid in acetic acid gave the dithioacetal (**20**), which was then oxidized with *m*-chloroperbenzoic acid (*m*CPBA) to the corresponding sulfone (**21**). Degradation of **21** with dilute aqueous ammonia[27] afforded 5-deoxy-3-*O*-PMB-L-arbinofuranose (**16**).[24]

Meanwhile, D-xylose also served as the starting material for preparation of **16**: the 5-deoxy-4-enofuranose derivative (**23**)[28] (prepared from D-xylose in four steps) was stereoselectively hydrogenated to afford the 5-deoxy-L-arabinose derivative (**24**), which was then converted into **16**. The selective oxidation of 2-hydroxy group of **16** with cupric acetate[29] provided the 3-*O*-PMB-L-*erythro*-pentos-2-ulose (**15**).[25]

Condensation of **15** with sulfate of **14** in an aqueous sodium bicarbonate solution afforded a 78:22 mixture of the biopterin derivative **25a** and its 7-substituted isomer **25b**. Successive treatment of the mixture with *N*,*N*-dimethylformamide dimethyl acetal in DMF, with acetic anhydride in pyridine, and

then with NPE alcohol (Mitsunobu reaction), gave the versatile biopterin derivative **26a** (53% overall yield from **15**) and the 7-substituted derivative **26b** (15%). Methanolysis of **26a** in the presence of sodium methoxide quantitatively provided the 1'-*O*-PMB derivative **13**, an ideal precursor for 2'-*O*-monoglycosylation.

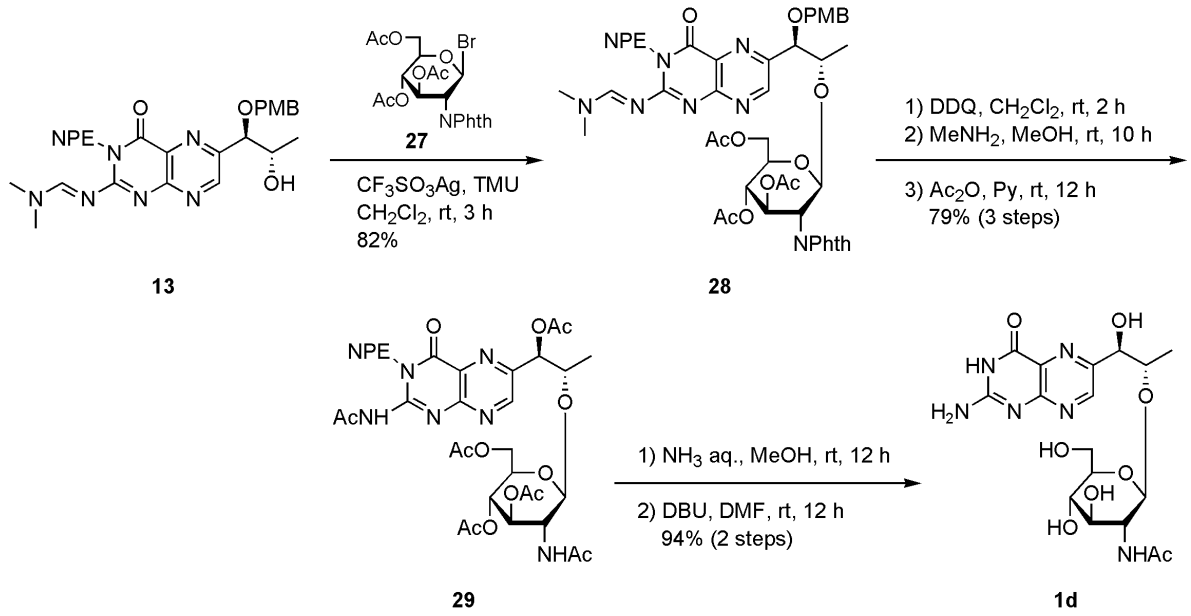


Scheme 3

The structural assignment of **26a** and **26b** was achieved primarily on the basis of their ^{1}H and ^{13}C-NMR spectral data. The signals of C-6 and C-7 of 6-alkylpteridines generally appear at a similar field, whereas C-7 signals of 7-alkyl derivatives shifts to a lower field (ca. 20 ppm) from those of C-6.[30] Therefore, the close values of **26a** (C-6: δ 150.71, C-7: δ 149.88) and the distant values of **26b** (C-6: δ 140.92, C-7: δ 159.98) indicate the 6-substituted pterin for the former and the 7-substituted pterin for the latter. These assignments are supported by the fact that H-7 signal (δ 8.96) of **26a** appears at a lower

field than that of H-6 of **26b** (δ 8.78) because of the lower electron density of C-7 than that of C-6 due to the conjugation with the 4-oxo group.[31]

An efficient glycosylation was exemplified by the condensation of **13** with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**27**)[32] in the presence of silver triflate (2.2 mol equiv.) and tetramethylurea (TMU) (1.0 mol equiv.) in dichloromethane,[33] affording 2'-*O*-β-D-glucopyranosyl derivative (**28**) as a sole product in 82% yield (Scheme 4). Removal of the protecting groups of **28** was carried out by successive treatment of DDQ (to cleave PMB group) and then methylamine (to remove the phthaloyl, *N*,*N*-dimethylaminomethylene, and acetyl groups), followed by the action of and acetic anhydride, to give the fully-acetylated derivative **29**. Treatment of **29** with aqueous ammonia and then with DBU furnished limipterin (**1d**).[25]

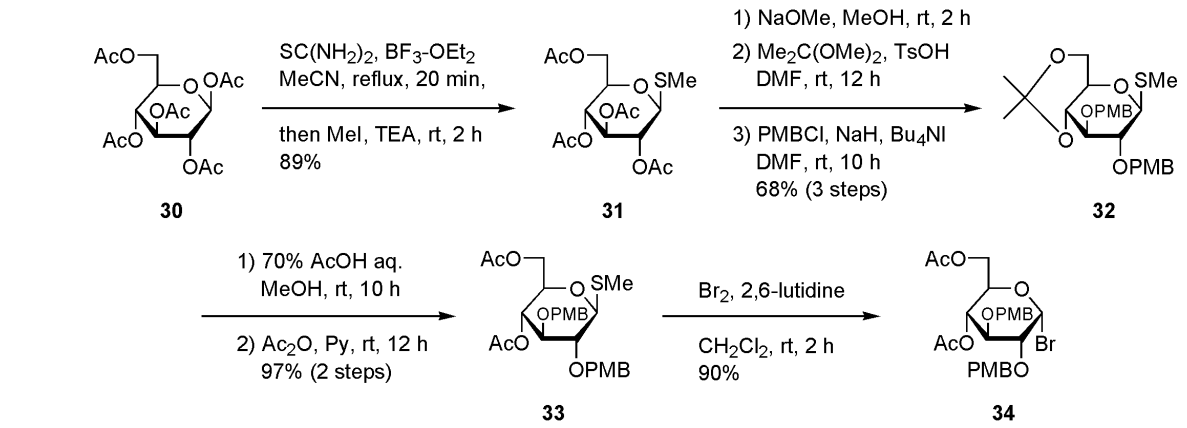


Scheme 4

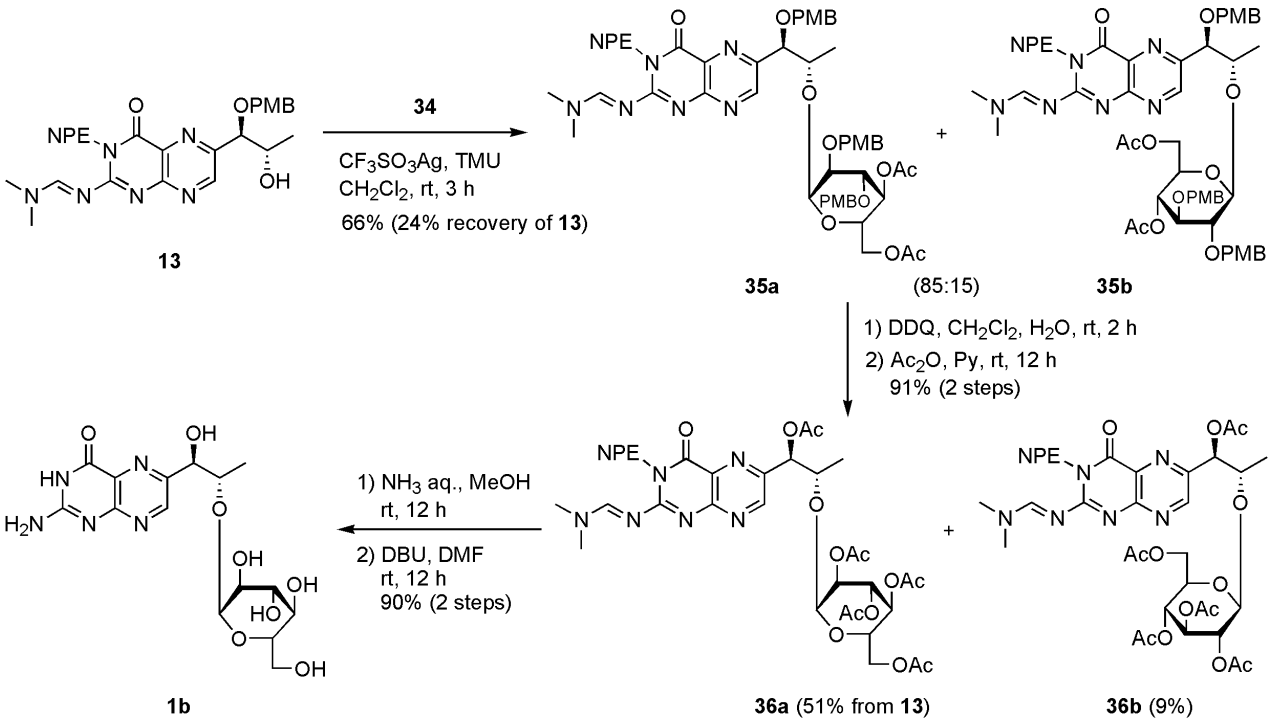
2-3. Synthesis of 2'-*O*-(α-D-glucopyranosyl)biopterin (1b)

The stereoselective formation of the β-glycosides (**10a–c**, **12a–c**, **28)** from **8** and **13** (in Scheme 1,4) was mainly caused by participation of the 2-*O*-benzoyl and 2-*N*-phthaloyl groups of the glycosyl donor (**9,11,27**) through the formation of an acyloxonium ion intermediate.[34] Accordingly, in order to avoid such a neighboring group participation in synthesis of biopterin α-D-glucoside (**1b**), we sought to introduce an ether substituent for protection of 2-OH of a glycosyl donor; thus PMB and acetyl groups were respectively chosen for protection of 2,3-OH and 4,6-OH of the glycosyl moiety. According to a synthetic route shown in Scheme 5, such an appropriately protected novel glycosyl donor (**34**) was prepared starting with penta-*O*-acetyl-β-D-glucopyranose (**30**) via 1-thio-β-D-glucopyranoside derivatives

(**31**–**33**).[35]



Scheme 5



Scheme 6

Then, glycosylation of **13** was found to give the best result when it was treated with 4.0 mol equiv. of the glycosyl bromide (**34**) in dichloromethane in the presence of silver triflate (2.0 mol equiv.) and TMU (1.0 mol equiv.), affording an inseparable anomeric mixture (85:15) of the 2'-*O*-(α-D-glucopyranosyl)-biopterin derivative (**35a**) and its β-anomer (**35b**) in 66% yield, along with the recovery of **13** (24%) (Scheme 6). Separation of these isomers was achieved by removal of PMB groups and the subsequent

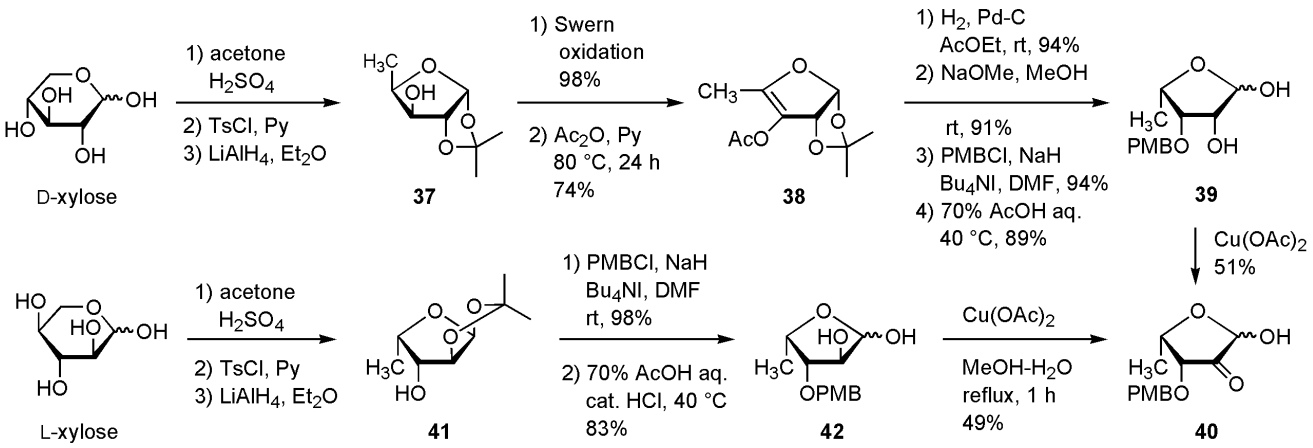
acetylation, affording the α-D-glucopyranoside (**36a**) in 51% (total yield from **13**) and its β-anomer (**36b**) in 9%. The α-anomeric structure of **36a** was derived from its $J_{1,2}$ value (3.9 Hz) of ^{1}H-NMR, while the larger $J_{1,2}$ value (8.1 Hz) confirmed the β-form of **36b**. Removal of the protecting groups of **36a** was accomplished as usual, furnishing the desired 2'-*O*-(α-D-glucopyranosyl)biopterin (**1b**).[35]

We thus achieved the first synthesis of biopterin α-D-glucoside (**1b**) by use of the key intermediate 1'-*O*-PMB-biopterin derivative (**13**) and the novel glycosyl donor (**34**) to preferentially provide an α-glycoside.

3. SYNTHESIS OF CILIAPTERIN, NEOPTERIN, AND 6-HYDROXYPTERIN GLYCOSIDES

3-1. Synthesis of 2'-*O*-(α-D-mannopyranosyl)ciliapterin (2b) and tepidopterin (2c)

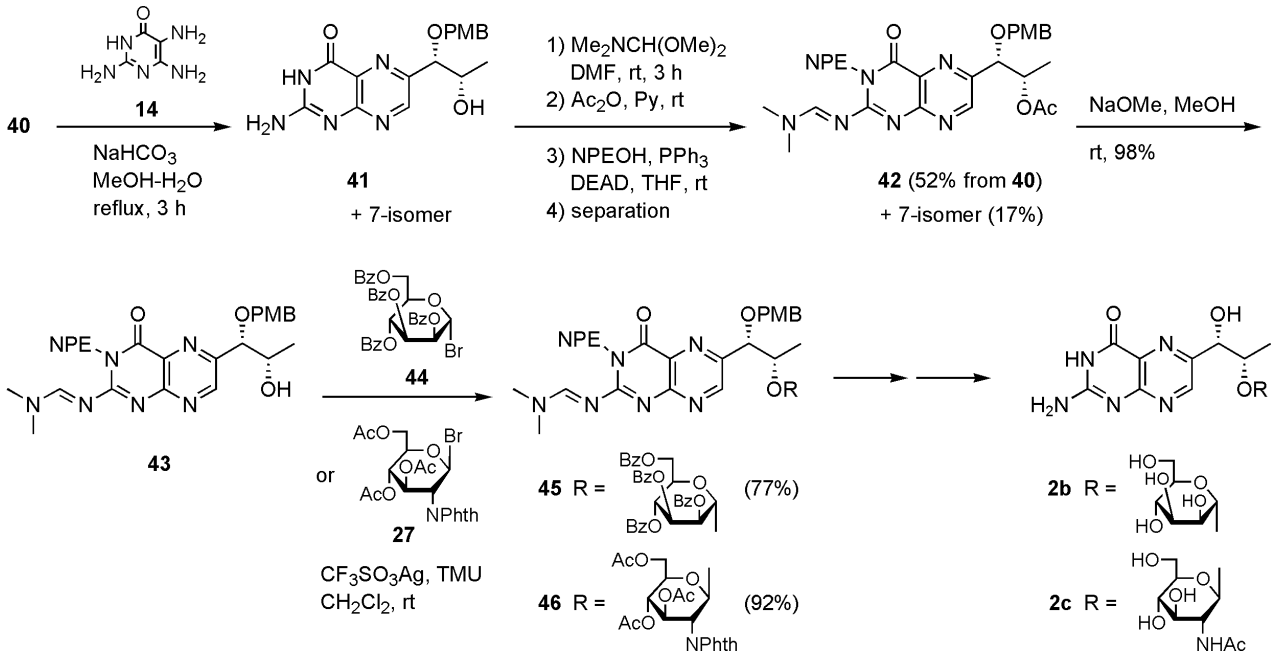
On the basis of the successful strategy for regioselective synthesis of biopterin 2'-*O*-glycosides (**1b,d**) by use of the 1'-*O*-protected precursor (**13**) in Schemes 4 and 6, preparation of ciliapterin 2'-*O*-glycosides (**2b,c**) was also attempted.[25,36] The 1'-*O*-protected ciliapterin derivative (**43**), corresponding to the 1'-epimer of **13**, was prepared by two synthetic routes starting with D-xylose or L-xylose (Schemes 7 and 8).



Scheme 7

5-Deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose (**37**)[37] derived from D-xylose was converted into the enol acetate (**38**) (Scheme 7). Catalytic hydrogenation of **38** afforded the 3-*O*-acetyl-L-lyxofuranose derivative, which was subjected to deacetylation, *p*-methoxybenzylation, and acetal cleavage, providing 5-deoxy-3-*O*-PMB-L-lyxose (**39**). The selective oxidation of **39** with cupric acetate afforded L-*threo*-pentose-2-ulose derivative (**40**). Meanwhile, 5-deoxy-1,2-*O*-isopropylidene-α-L-xylofuranose (**41**)[38] derived from L-xylose provided 5-deoxy-3-*O*-PMB-L-xylose (**42**), which was then oxidized to the 2-ulose **40**.

The condensation of **40** with sulfate of **14** in an aqueous sodium bicarbonate solution gave a 75:25 mixture of ciliapterin derivative (**41**) and its 7-substituted isomer (Scheme 8). These products were, as in the cases of **26a,b** from **25a,b** (Scheme 3), converted into the corresponding 2'-*O*-acetyl-N^2-(*N*,*N*-dimethylaminomethylene)-3-NPE-1'-*O*-PMB derivative (**42**) (52% overall yield from **40**) and the 7-substituted derivative (17%).



Scheme 8

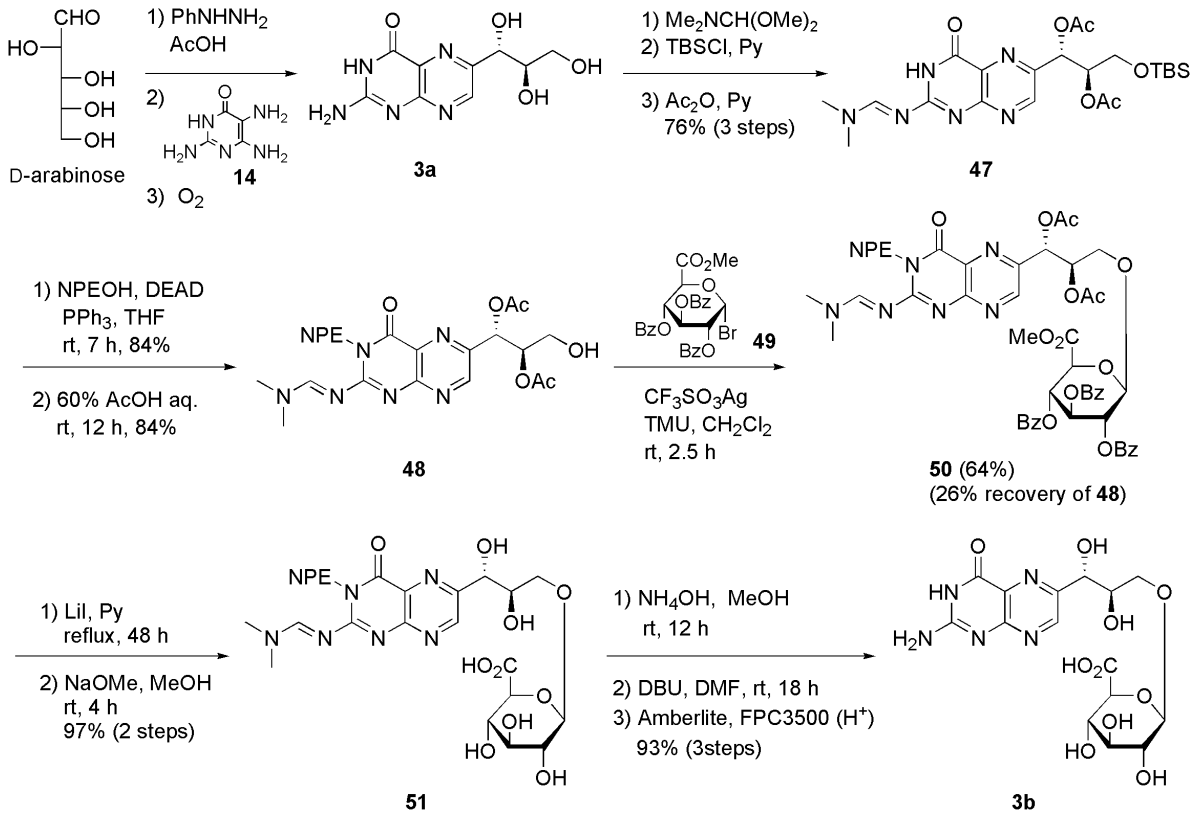
Methanolysis of **42** with sodium methoxide provided the 1'-*O*-PMB derivative (**43**), a versatile precursor for the 2'-*O*-monoglycosylation. Efficient glycosylation of **43** was attained by the D-mannopyranosyl bromide (**44**)[39] or the 2-deoxy-2-phthalimido-D-glucopyranosyl bromide (**27**) in the presence of silver triflate and TMU, affording the 2'-*O*-(α-D-mannopyranosyl)- (**45**) and 2'-*O*-(β-D-glucopyranosyl)-ciliapterin derivative (**46**), respectively. Deprotection of **45** and **46** by the similar treatment described in the preceding sections led to the first syntheses of ciliapterin α-D-mannopyranoside (**2b**) and tepidopterin (**2c**), respectively.

3-2. Synthesis of 3'-*O*-(α-D-glucopyranosyluronic acid)neopterin (3b)

As the first synthetic example of a natural neopterin glycoside, 3'-*O*-(α-D-glucopyranosyluronic acid)neopterin (**3b**) was prepared starting with neopterin (**3a**)[40] (available from D-arabinose), as illustrated in Scheme 9.[41] Namely, the key precursor (**48**), whose pyrimidine moiety and 1',2'-hydroxy groups of the side chain were protected, was prepared from **3a** via **47** in five steps. Treatment of **48** with

the methyl D-glucopyranosyluronate bromide (**49**)[42] in the presence of silver triflate and TMU afforded 3'-*O*-(β-D-glucopyranosyluronate)neopterin derivative (**50**) in 64% yield, along with the recovery of **48** (26%).

For removal of the protecting groups of **50**, attempted hydrolysis of all ester groups by use of aqueous sodium hydroxide resulted in the formation of an inseparable mixture of unidentified products. However, selective cleavage of methyl ester of **50** was achieved by use of lithium iodide in pyridine, followed by the action of sodium methoxide, providing the 3'-*O*-(β-D-glucopyranosyluronic acid)neopterin derivative (**51**). Treatment of **51** with aqueous ammonia and then with DBU, followed by acidification using an ion-exchange resin, furnished the target neopterin glycoside (**3b**).

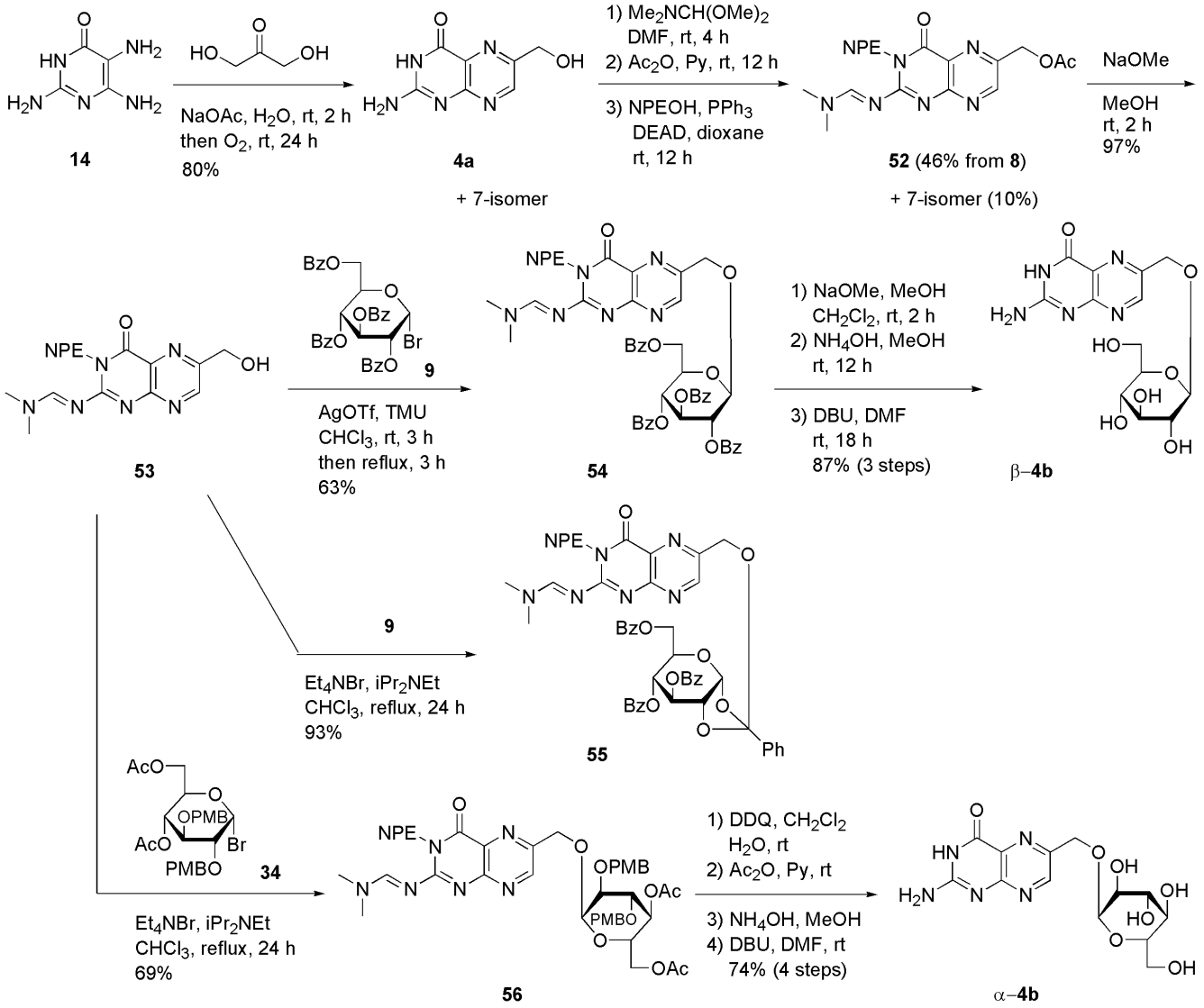


Scheme 9

3-3. Synthesis of 6-hydroxymethylpterin α- and β-D-glucopyranosides (4b)

As shown in Figure 1, anomeric structures of some pterin glycosides are α-type and those of others are β-type depending on the combination of the pterin and sugar moieties. Therefore we undertook the exploration of an efficient protocol for selective α- and β-glycosilation of pterin derivatives by employing 6-hydroxymethylpterin (**4a**) as a model pterin substrate, leading to the selective synthesis of each of 6-hydroxymethylpterin α- and β-D-glucopyranoside (**4b**).[43]

6-Hydoxymethylpterin (**4a**),[44] prepared from the pyrimidine derivative (**14**) and dihydroxyacetone, was converted into the N^2-(*N*,*N*-dimethylaminomethylene)-3-NPE derivative (**53**) via **52** in four steps. (Scheme 10). Glycosylation of **53** with two glycosyl donors (**9** and **34**) in chloroform was then extensively investigated under various conditions in the presence of activators. Remarkable results are shown in Scheme 10.



Scheme 10

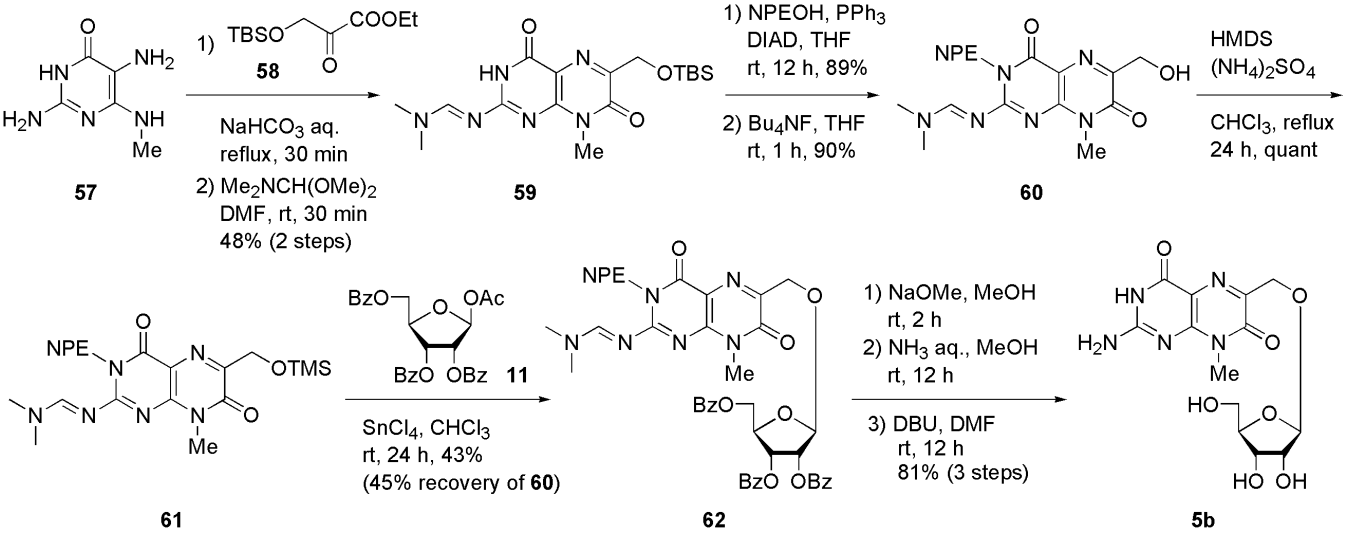
Glycosylation of **53** with **9** in refluxing chloroform in the presence of silver triflate (2.0 mol equiv.) and TMU (1.0 mol equiv.) provided 6-[(β-D-glucopyranosyloxy)methyl]pterin derivative (**54**) in 63% yield, whereas the same reaction in the presence of tetraethylammonium bromide (2.0 mol equiv.) and *N*-ethyldiisopropylamine (2.0 mol equiv.) afforded α-D-glucopyranose-1,2-orthobenzoate derivative (**55**) (in 93% yield) instead of the pterin glycoside.

Meanwhile glycosylation of **53** with **34** in refluxing chloroform in the presence of silver triflate and TMU resulted in the formation of decomposed compounds, but the same reaction in the presence of tetraethylammonium bromide and *N*-ethyldiisopropylamine resulted in the formation of 6-[(α-D-glucopyranosyloxy)methyl]pterin derivative (**56**) as a sole product (69% yield).

Removal of the protecting groups of β- (**54**) and α-D-glucoside (**56**) was performed as usual, furnishing 6-hydroxymethylpterin β- (β-**4b**) and α-D-glucopyranoside (α-**4b**), respectively.

3-4. Synthesis of asperopterin-A (5b)

Synthetic methodology for pterin glycosides described so far was applied to preparation of asperopterin-A (**5b**) known as the sole example of a natural isoxanthopterin glycoside, as illustrated in Scheme 11.[45]



Scheme 11

The 2-oxopropionate derivative (**58**) was prepared from ethyl acrylate in three steps. The pteridine ring formation of the pyrimidine derivative (**57**) with **58** and the subsequent introduction of *N*,*N*-dimethylaminomethylene group afforded the isoxanthopterin derivative (**59**) in 48% yield. Protection of **59** with NPE group and the following removal of TBS group provided 6-hydroxymethyl compound (**60**), which was then temporarily silylated to give the trimethylsilyl derivative (**61**).

Glycosylation of **61** with the glycosyl donor (**11**) was attempted in the presence of tin(IV) chloride (2.0 mol equiv.) to afford the D-ribofuranosyl derivative (**62**) in 43% yield, along with the recovery of **60** (45%). The β-anomeric configuration of the D-ribofuranoside (**62**) was assigned by its $J_{1,2}$ value (0 Hz). Removal of the protecting groups of **62** was performed by the successive three-step-procedure, furnishing the target asperopterin-A (**5b**) in 81% overall yield.

4. CONCLUSION

We have developed a novel, effective way for selective preparation of both pterin 2'-*O*-β- and 2'-*O*-α-glycosides. As the representative examples of this work, the first synthesis of biopterin α-D-glucoside (**1b**) was achieved by use of the key intermediate 1'-*O*-PMB-biopterin derivative (**13**) and the novel glycosyl donor (**34**), while the biopterin β-D-glucoside (limipterin) (**1d**) was selectively prepared from **13** and the glycosyl donor (**27**). This synthetic strategy using N^2-(*N*,*N*-dimethylaminomethylene)-3-NPE-protected pterin derivatives has proved a useful method applicable to preparation of various natural pterin glycosides having diverse pterin and sugar moieties.

The spectral data of the synthetic pterin glycosides were found to be essentially identical with those reported for the natural products and thus the validity of their proposed structures was confirmed. Although some ambiguous NMR parameters were included in the previous reports for natural compounds, their complete assignments have been established by this work.

5. REFERENCES

1. (a) W. Pfleiderer, *Angew. Chem., Int. Ed. Engl.*, 1964, **3**, 114; (b) H. Rembold and W. L. Gyure, *Angew. Chem., Int. Ed. Engl.*, 1972, **11**, 1061; (c) T. Hama and T. Obika, *Nature*, 1960, **187**, 326; (d) H. S. Forrest and C. Van Baalen, *Ann. Rev. Microbiol.*, 1970, **24**, 91; (e) I. Ziegler and R. Harmsen, *Advan. Insect. Physiol.*, 1969, **6**, 139.
2. H. S. Forrest, C. Van Baalen, and J. Myers, *Arch. Biochem. Biophys.*, 1958, **78**, 95.
3. T. Matsunaga, J. G. Burgess, N. Yamada, K. Komatsu, S. Yoshida, and Y. Wachi, *Appl. Microbiol. Biotechnol.*, 1993, **39**, 250.
4. Y. K. Choi, Y. K. Hwang, Y. H. Kang, and Y. S. Park, *Pteridines*, 2001, **12**, 121.
5. Y. Noguchi, A. Ishii, A. Matsushima, D. Haishi, K. Yasumuro, T. Moriguchi, T. Wada, Y. Kodera, M. Hiroto, H. Nishihara, M. Sekine, and Y. Inada, *Mar. Biotechnol.*, 1999, **1**, 207.
6. T. Hanaya, K. Torigoe, K. Soranaka, H. Fujita, H. Yamamoto, and W. Pfleiderer, *Pteridines*, 2008, **19**, 72.
7. K. W. Cha, W. Pfleiderer, and J. J. Yim, *Helv. Chim. Acta*, 1995, **78**, 600.
8. M. Ikawa, J. J. Sasner, J. F. Haney, and T. L. Foxall, *Phytochemistry*, 1995, **38**, 1229.
9. S.-H. Cho, J.-U. Na, H. Youn, C.-S. Hwang, C.-H. Lee, and S.-O. Kang, *Biochim. Biophys. Acta*, 1998, **1379**, 53.
10. (a) R. Suzuki and M. Goto, *J. Biochem.*, 1968, **63**, 798; (b) K. Kobayashi and H. S. Forrest, *Comp. Biochem. Physiol.*, 1970, **33**, 201.
11. X. Lin and R. H. White, *J. Bacteriol.*, 1988, **170**, 1396.
12. D. L. Hatfield, C. van Baalen, and H. S. Forrest, *Plant Physiol.*, 1961, **36**, 240.

13. H. W. Lee, C. H. Oh, A. Geyer, W. Pfleiderer, and Y. S. Park, *Biochim. Biophys. Acta*, 1999, **1410**, 61.
14. (a) Y. Kaneko and M. Sanada, *J. Ferment. Technol.*, 1969, **47**, 8; (b) S. Matsuura, M. Yamamoto, and Y. Kaneko, *Bull. Chem. Soc. Jpn.*, 1972, **45**, 492.
15. (a) S. Kaufman and E. E. Kaufman, '*Folates and Pterins,*' ed. by R. Blakley and S. J. Benkovic, J. Wiley & Sons, New York, 1985, Vol. 2, pp. 251–352; (b) S. Kaufman and D. B. Fisher, '*Molecular Mechanisms of Oxygen Activation*,' ed. by O. Hayaishi, Academic Press, New York, 1974, pp. 285–369; (c) P. F. Fitzpatrick, *Annu. Rev. Biochem.*, 1999, **68**, 355.
16. (a) N. S. Kwon, C. F. Nathan, and D. J. Stuehr, *J. Biol. Chem.*, 1989, **264**, 20496; (b) M. A. Marletta, *Cell*, 1994, **78**, 927; (c) B. R. Crane, A. S. Arvai, D. K. Ghosh, C. Q. Wu, E. D. Getzoff, D. J. Stuehr, and J. A. Tainer, *Science*, 1998, **5359**, 2121.
17. (a) H. Wachter, E. R. Werner, G. Reibnegger, D. Fuchs, and A. Hausen, *Pteridines*, 1989, **1**, 3; (b) A. Hausen, G. Reibnegger, B. Speck, and H. Wachter, *Transplantation*, 1984, **38**, 497; (c) G. Hoffman, S. Kenn, B. Wirleitner, C. Deetjen, S. Frede, M. Smolny, J. Rieder, D. Fuchs, G. Baier-Bitterlich, and W. Schobersberger, *Immunobiology*, 1998, **199**, 63.
18. Y. Wachi, S. Yoshida, K. Komatsu, and T. Matsunaga, *Jpn. Patent*, 05,286,989, 1993 (*Chem. Abstr.*, 1994, **120**, 161782t).
19. T. Saito, H. Ishikawa, Y. Hada, K. Fukui, Y. Kodera, A. Matsushima, and Y. Inada, *Dyes Pigments*, 2003, **56**, 203.
20. W. Pfleiderer, '*Physical Methods in Heterocyclic Chemistry*', Vol. 1, ed. by A. R. Katritzky, Academic Press, New York, 1963, pp. 177–188.
21. T. Hanaya, K. Torigoe, K. Soranaka, H. Yamamoto, Q. Yao, and W. Pfleiderer, *Pteridines*, 1995, **6**, 1.
22. R. K. Ness, H. G. Fletcher, Jr., and C. S. Hudson, *J. Am. Chem. Soc.*, 1950, **72**, 2200.
23. T. Hanaya, K. Soranaka, K. Harada, H. Yamaguchi, R. Suzuki, Y. Endo, H. Yamamoto, and W. Pfleiderer, *Heterocycles*, 2006, **67**, 299.
24. T. Hanaya, H. Toyota, and H. Yamamoto, *Synlett*, 2006, 2075.
25. T. Hanaya, H. Baba, H. Toyota, and H. Yamamoto, *Tetrahedron*, 2008, **64**, 2090.
26. R. Gigg, S. Payne, and R. Conant, *J. Carbohydr. Chem.*, 1983, **2**, 207.
27. L. Hough and T. J. Taylor, *J. Chem. Soc.*, 1955, 3544.
28. J. Kiss, R. D'Souza, and P. Taschner, *Helv. Chim. Acta*, 1975, **58**, 311.
29. (a) J. Weinstock, *U.S. Patent*, 3,505,329, 1970 (*Chem. Abstr.*, 1970, **72**, 132787h); (b) E. C. Taylor and P. A. Jacobi, *J. Am. Chem. Soc.*, 1976, **98**, 2301.
30. (a) J. P. Geerts, A. Nagel, and H. C. Van der Plas, *Org. Magn. Reson.*, 1976, **8**, 607; (b) T. Hanaya,

D. Takayama, and H. Yamamoto, *Heterocycles*, 2006, **70**, 355.

31. (a) U. Ewers, H. Günther, and L. Jaenicke, *Chem. Ber.*, 1974, **107**, 3275; (b) S. Tobias, H. Günther, and W. Pfleiderer, *Chem. Ber.*, 1985, **118**, 354.
32. J. Farkas, M. Ledvina, J. Brokes, J. Jezek, J. Zajicek, and M. Zaoral, *Carbohydr. Res.*, 1987, **163**, 63.
33. (a) S. Hanessian and J. Banoub, *Carbohydr. Res.*, 1977, **53**, C13; (b) S. Hanessian and J. Banoub, *Methods Carbohydr. Chem.*, 1980, **8**, 247.
34. G. Wulff and G. Röhle, *Angew. Chem., Int. Ed. Engl.*, 1974, **13**, 157, and references cited therein.
35. T. Hanaya, H. Baba, H. Toyota, and H. Yamamoto, *Tetrahedron*, 2009, **65**, 7989.
36. T. Hanaya, H. Baba, M. Kanemoto, and H. Yamamoto, *Heterocycles*, 2008, **76**, 635.
37. B. Hildebrandt, Y. Nakamura, and S. Ogawa, *Carbohydr. Res.*, 1991, **214**, 87.
38. N. A. Jones, S. A. Nepogodiev, C. J. MacDonald, D. L. Hughes, and R. A. Field, *J. Org. Chem.*, 2005, **70**, 8556.
39. (a) R. K. Ness, H. G. Fletcher, and C. S. Hudson, *J. Am. Chem. Soc.*, 1950, **72**, 2200; (b) M. Mach, U. Schlueter, F. Mathew, B. Fraser-Reid, and K. C. Hazen, *Tetrahedron*, 2002, **58**, 7345.
40. R. Soyka and W. Pfleiderer, *Helv. Chim. Acta*, 1990, **73**, 808.
41. T. Hanaya, T. Hattori, D. Takayama, and H. Yamamoto, *Pteridines*, 2010, **21**, 79.
42. W. W. Zorbach and G. D. Valiaveedan, *J. Org. Chem.*, 1964, **29**, 2462.
43. T. Hanaya, H. Baba, K. Ejiri, and H. Yamamoto, *Heterocycles*, 2010, **80**, 1013.
44. C. M. Baugh and E. Shaw, *J. Org. Chem.*, 1964, **29**, 3610.
45. T. Hanaya, K. Ejiri, and H. Yamamoto, *Heterocycles*, 2012, **84**, 801.

Tadashi Hanaya is an associate professor at Graduate School of Natural Science and Technology, Okayama University. He received his M.Sc. degree in 1984 from Graduate School of Science, Okayama University. After having worked as a research fellow at Ogawa & Co., Ltd., he became a research associate at Okayama University in 1986 and was promoted to an associate professor in 1997. He received his Ph.D. degree from Okayama University in 1990 under the supervision of Professor Hiroshi Yamamoto. He stayed at Professor Wolfgang Pfleiderer's laboratory, University of Konstanz, Germany in 1995 as a Humboldt research fellow. His research interests include synthetic studies on natural pteridine derivatives and carbohydrate analogs having phosphorus.

Hiroshi Yamamoto is a professor of organic chemistry at School of Pharmacy, Shujitsu University. He received his B.Sc. (Okayama Univ. 1963), M.Sc. (Tohoku Univ. 1965) and Ph.D. (in Medical Chemistry, Prof. A. Albert, Australian Nat'l Univ. 1968). He became a research fellow (Okayama Univ. 1968-'72), a Humboldt research fellow (Prof. W. Pfleiderer, Univ. Konstanz, Germany 1970-'72), a post-doctoral fellow (Prof. S. Masamune, Univ. Alberta, Canada 1972-'74), an assistant professor (Okayama Univ. 1974-'85) and a professor (Fac. Sc., Okayama Univ. 1985-2006). He has then moved to Shujitsu Univ., Okayama (2006-). His research interests include synthetic studies on various *N*-heterocycles and *P*-in-the-ring carbohydrate analogs.