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First synthesis of asperopterin A, an isoxanthopterin glycoside from *Aspergillus oryzae*

Abstract: The key precursor, N^2-(*N,N*-dimethylaminomethylene)-6-hydroxymethyl-8-methyl-3-[2-(4-nitrophenyl)ethyl]-7-xanthopterin (**16**) was efficiently prepared from 2,5-diamino-6-methylamino-3*H*-pyrimidin-4-one (**5**) and ethyl 3-(*tert*-butyldimethylsilyloxy)-2-oxopropionate (**12**), followed by the protection of the pteridine ring. Glycosylation of **16** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**18**) in the presence of tin(IV) chloride yielded the corresponding β-D-ribofuranoside. Successive removal of the protecting groups of the resulting D-ribofuranoside provided asperopterin A (**4b**).

Keywords: asperopterin; glycosylation; isoxanthopterin; protecting groups; pterin glycoside.

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Introduction

Certain pterins carrying various types of sugars attached to a hydroxyalkyl side chain at C-6 of the pteridine ring were found to be produced by some prokaryotes as exemplified by glycosides of biopterin (**1a**): 2′-*O*-(α-D-glucopyranosyl)biopterin (**1b**) isolated from various types of cyanobacteria [1–4] and limipterin (**1c**) isolated from a green sulfur photosynthetic bacterium [5] (Figure 1). With regard to glycosides of other pterins, tepidopterin (**2b**) and solfapterin (**3b**) were isolated from a green sulfur bacterium and a thermophilic archaebacterium, respectively [6, 7]. Most of the parent pteridine moieties of these glycosides consist of pterins such as biopterin (**1a**), ciliapterin (**2a**), neopterin (**3a**) and 6-hydroxymethylpterin [8]. By contrast, asperopterin A (**4b**) isolated from *Aspergillus oryzae* [9, 10] is a unique example of pteridine glycosides in an aspect of having an isoxanthopterin (7-xanthopterin) structure as a parent ring. Although its structure has been assigned to be the β-D-ribofuranoside of 6-hydroxymethyl-8-methyl-7-xanthopterin (asperopterin B) (**4a**) [11], the preparation of **4b** has remained unreported.

Methods

We have undertaken a synthetic study of various types of pterin glycosides owing to interest in their physiological functions and biological activities, as well as structural proof of these natural products [12–18]. Here, an efficient synthesis of asperopterin A (**4b**) as the first synthetic example of a natural isoxanthopterin glycoside is presented.

Results and discussion

With regard to preparation of asperopterin B (**4a**), the aglycone of asperopterin A (**4b**), two synthetic pathways starting with 2,5-diamino-6-methylamino-3*H*-pyrimidin-4-one (**5**) have been reported, as shown in Scheme 1. One is the condensation of **5** with ethyl glyoxalate and the subsequent hydoxymethylation of the resulting 9-methyl-7-xanthopterin (**6**) with methanol and ammonium peroxydisulfate [19, 20], and the second is the condensation of **5** with ethyl pyruvate and the subsequent bromination and hydroxylation of the resulting 6,7-dimethyl-7-xanthopterin (**7**) [11, 19]. Despite some modification of the reported procedures for preparation of **4a**, no improvements of its total yield were obtained.

We thus undertook a novel alternative way to prepare a 6-hydroxymethyl-7-xanthopterin derivative directly by condensation of pyrimidine derivative (**5**) with the 2-oxopropinonate derivative (**12**) (Scheme 2). Namely, oxidation of ethyl acrylate with potassium permanganate yielded ethyl 2,3-dihydoxypropionate (**10**). The selective protection of **10** with *tert*-butyldimethylsilyl (TBS) group gave **11**, which was then oxidized with Dess-Martin periodinane to provide ethyl 3-(*tert*-butyldimethylsilyloxy)-2-oxopropionate (**12**).

The pteridine ring formation of the pyrimidine derivative (**5**) with **12** afforded the 6-(*tert*-butyldimethylsilyloxymethyl)-7-xanthopterin derivative

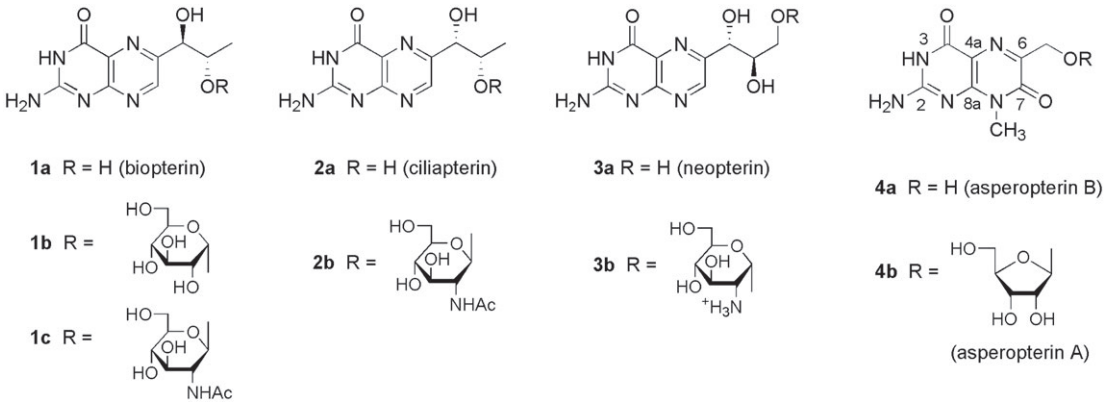


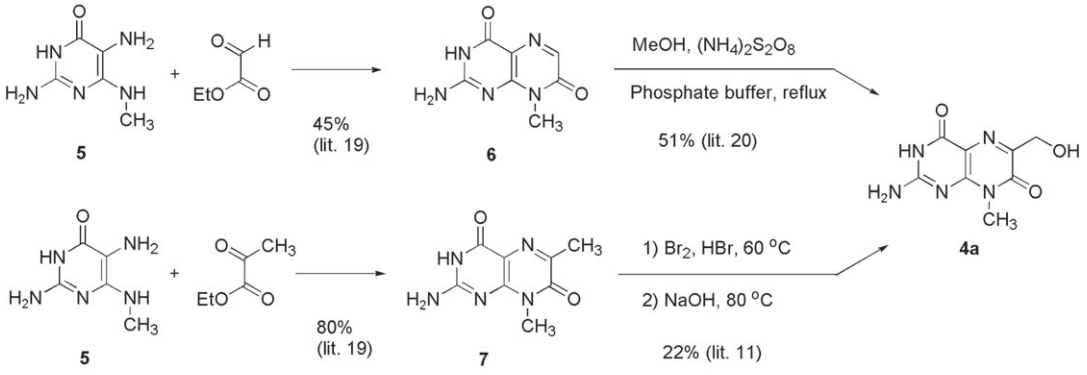
Figure 1 Naturally occurring pterin glycosides.

(**13**), which was treated with *N,N*-dimethylformamide dimethyl acetal in DMF to give the N^2-(*N,N*-dimethylaminomethylene) derivative (**14**) in 48% overall yield. Mitsunobu reaction of **14** with *p*-nitrophenylethyl (NPE) alcohol in the presence of triphenylphosphine and diisopropyl azodicarboxylate (DIAD) in THF yielded the N(3)-NPE derivative (**15**) [21], which was then treated with tetrabutylammonium fluoride to provide 6-hydroxymethyl compound (**16**), the key precursor for glycosylation. Thus, an improved synthesis of the 6-hydroxymethyl-7-xanthopterin derivative from **5** was accomplished in an appreciably better yield, compared with the reported routes shown in Scheme 1.

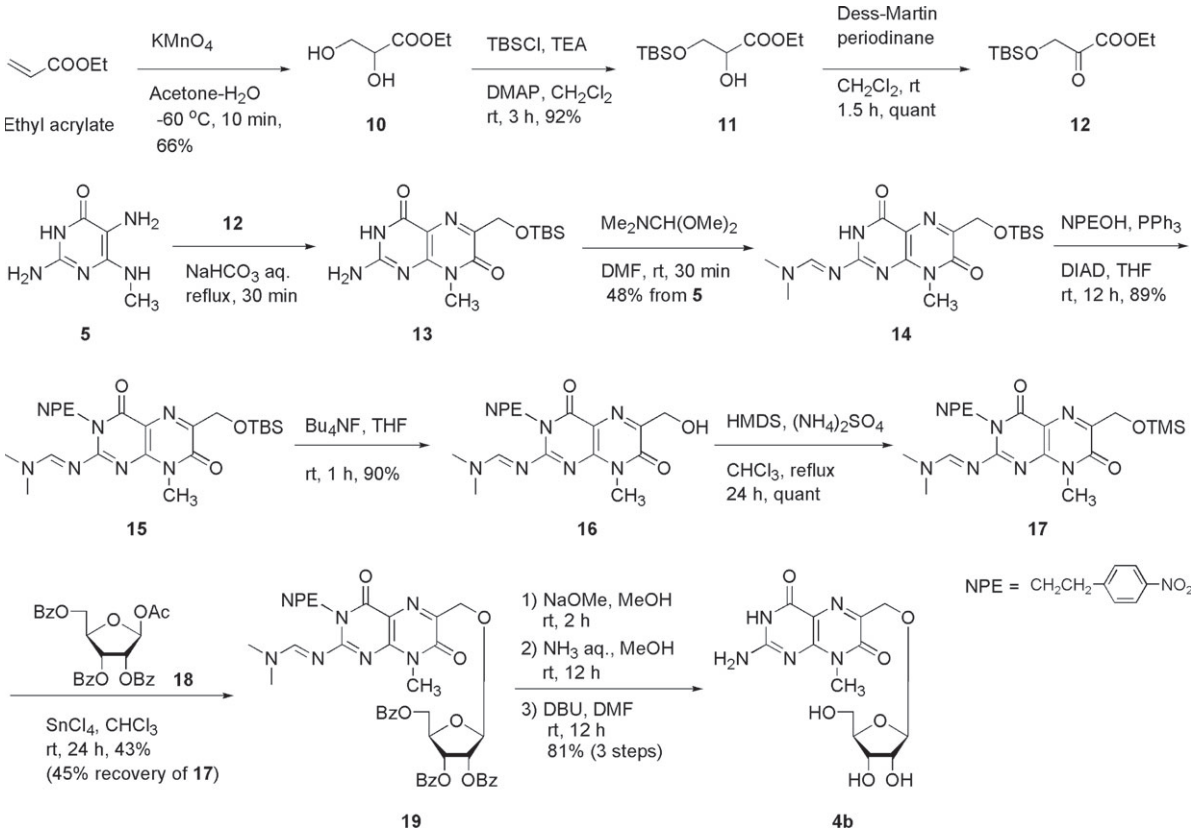
Owing to its low solubility in chloroform, compound **16** was temporarily silylated with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) in the presence of ammonium sulfate in chloroform under reflux for 24 h, yielding the solubilized trimethylsilyl derivative (**17**) quantitatively [12, 16].

Glycosylation of **17** with glycosyl donor, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**18**) was attempted under various conditions in the presence of activators (Scheme 2). Glycosylation of **17** with 2.0 mol equiv. of **18** in the presence of boron trifluoride etherate in chloroform at room temperature did not proceed due to precipitation of desilylated **16**. By contrast, similar treatment of **17** with **18** in the presence of tin(IV) chloride (2.0 mol equiv.) yielded the D-ribofuranosyl derivative (**19**) in 43% yield, along with the recovery of **16** (45%). Use of a larger amount of the glycosyl donor (3.0 equiv.) and the activator (3.0 equiv.) resulted in a lower yield of **19** (16%) and formation of a larger amount of **16** (68%). The β-anomeric configuration of the D-ribofuranoside (**19**) was assigned by its $J_{1,2}$ value (0 Hz). Its stereoselective β-glycoside formation was mainly caused by participation of the 2-*O*-benzoyl group of the glycosyl donor **18**.

Removal of the protecting groups of isoxanthopterin glycoside (**19**) was performed according to well-established procedures [12, 16]: treatment of **19** with sodium methoxide in methanol to cleave benzoyl groups and then with aqueous ammonia-methanol to remove the



Scheme 1



Scheme 2

N,N-dimethylaminomethylene group, followed by the action of DBU in DMF to cleave the NPE group, furnished the target asperopterin A (**4b**) in 81% overall yield from **19**. The precise structures of **4b** were established by ^{1}H- and ^{13}C-NMR spectra with the aid of HSQC measurement.

The first synthesis of a natural isoxanthopterin glycoside, asperopterin A (**4b**) was thus achieved utilizing an efficient preparation of the key intermediate (**16**) from ethyl acrylate. Yields of the ring formation, protection and glycosylation of isoxanthopterin derivatives in the present study remain relatively low compared with those of other pterin derivatives such as **1b,c** and **2b**. Improvement of the yields of some steps, as well as applications of these findings in synthesizing other pteridine glycosides having various types of sugar moieties, is in progress.

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References

1. Forrest HS, van Baalen C, Myers J. Isolation and identification of a new pteridine from a blue-green alga. Arch. Biochem. Biophys. 1958;78:95–9.
2. Matsunaga T, Burgess JG, Yamada N, Komatsu K, Yoshida S, Wachi Y. An ultraviolet (UV-A) absorbing biopterin glucoside from the marine planktonic cyanobacterium Oscillatoria sp. Appl. Microbiol. Biotechnol. 1993;39:250–3.
3. Noguchi Y, Ishii A, Matsushima A, Haishi D, Yasumuro K, Moriguchi T, et al. Isolation of biopterin-α-glucoside from Spirulina (Arthrospira) platensis and its physiologic function. Mar. Biotechnol. 1999;1:207–10.
4. Choi YK, Hwang YK, Kang YH, Park YS. Chemical structure of 1-O-(L-erythro-biopterin-2′-yl)-α-glucose isolated from a cyanobacterium Synechococcus sp. PCC 7942. Pteridines 2001;12:121–5.
5. Cha KW, Pfleiderer W, Yim JJ. Isolation and characterization of limipterin (1-O-(L-erythro-biopterin-2′-yl)-β-N-acetylglucosamine) and its 5,6,7,8-tetrahydro derivative from green sulfur bacterium Chlorobium limicola f. thiosulfatophilum NCIB 8327. Helv. Chim. Acta 1995;78:600–14.
6. Cho SH, Na JU, Youn H, Hwang CS, Lee CH, Kang SO. Tepidopterin, 1-O-(L-threo-biopterin-2′-yl)-β-N-acetylglucosamine

from Chlorobium tepidum. Biochim. Biophys. Acta 1998;1379: 53–60.
7. Lin XL, White RH. Structure of solfapterin (erythro-neopterin-3′-D-2-deoxy-2-aminoglucopyranoside) isolated from the thermophilic archaebacterium Sulfolobus solfataricus. J. Bacteriol. 1988;170:1396–8.
8. Lee HW, Oh CH, Geyer A, Pfleiderer W, Park YS. Characterization of a novel unconjugated pteridine glycoside, cyanopterin, in Synechocystis sp. PCC 6803. Biochim. Biophys. Acta 1999;1410:61–70.
9. Kaneko Y. Studies on the fluorescent substances produced by Aspergillus fungi (VI). Purification and isolation of bluish purple-fluorescent substance as crystal. Nippon Nogei Kagaku Kaishi 1966;40:227–35.
10. Kaneko Y, Sanada M. Studies on the fluorescent substances produced by Aspergillus fungi (VIII). Purification and isolation of Asperopterin B and chemical properties of asperopterin B and A. J. Ferment. Technol. 1969;47:8–19.
11. Matsuura S, Yamamoto M, Kaneko Y. The structure of the pteridine glycoside from Aspergillus oryzae. Bull. Chem. Soc. Jpn. 1972;45:492–5.
12. Hanaya T, Soranaka K, Harada K, Yamaguchi H, Suzuki R, Endo Y, et al. An efficient synthesis of 2′-O-(β-D-glucopyranosyl)- and 2′-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-biopterins. Heterocycles 2006;67:299–310.
13. Hanaya T, Toyota H, Yamamoto H. Novel preparation of a 2′-O-acetyl-1′-O-(4-methoxybenzyl)-L-biopterin derivative, a versatile precursor for a selective synthesis of L-biopterin glycosides. Synlett 2006;13:2075–8.
14. Hanaya T, Baba H, Toyota H, Yamamoto H. Efficient total syntheses of natural pterin glycosides: limipterin and tepidopterin. Tetrahedron 2008;64:2090–100.
15. Hanaya T, Baba H, Kanemoto M, Yamamoto H. An efficient synthetic route for a versatile ciliapterin derivative and the first ciliapterin D-mannoside synthesis. Heterocycles 2008;76:635–44.
16. Hanaya T, Torigoe K, Soranaka K, Fujita H, Yamamoto H, Pfleiderer W. An efficient synthesis of 2′-O-(β-D-ribofuranosyl) biopterin. Pteridines 2008;19:72–8.
17. Hanaya T, Baba H, Toyota H, Yamamoto H. Synthetic studies on pterin glycosides: the first synthesis of 2′-O-(α-D-glucopyranosyl)biopterin. Tetrahedron 2009;65:7989–97.
18. Hanaya T, Hattori T, Takayama D, Yamamoto H. First synthesis of a natural neopterin glycosides: 3′-O-(β-D-glucopyranosyluronic acid)neopterin. Pteridines 2010;21:79–83.
19. Pfleiderer W, Rukwied M. Zur Struktur des Isoxanthopterins. Chem. Ber. 1961;94:1–12.
20. Sugimoto T, Murata S, Matsuura S, Pfleiderer W. Synthesis of asperopterin-B and some analogues. Tetrahedron Lett. 1986;27:4179–80.
21. Hanaya T, Torigoe K, Soranaka K, Yamamoto H, Yao Q, Pfleiderer W. Selective N(3)- and O^4-alkylation of L-biopterin: a convenient synthesis of 3- and O^4-methyl-L-biopterin and the versatile N^2-(N,N-dimethylaminomethylene)-N(3)-p-nitrophenethyl-protected L-biopterin. Pteridines 1995;6:1–7.