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

Abstract: The key precursor, N^2 -(N,N -dimethylamino-methylene)-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 archaeobacterium, 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 hydroxymethylation 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-oxopropionate derivative (**12**) (Scheme 2). Namely, oxidation of ethyl acrylate with potassium permanganate yielded ethyl 2,3-dihydroxypropionate (**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

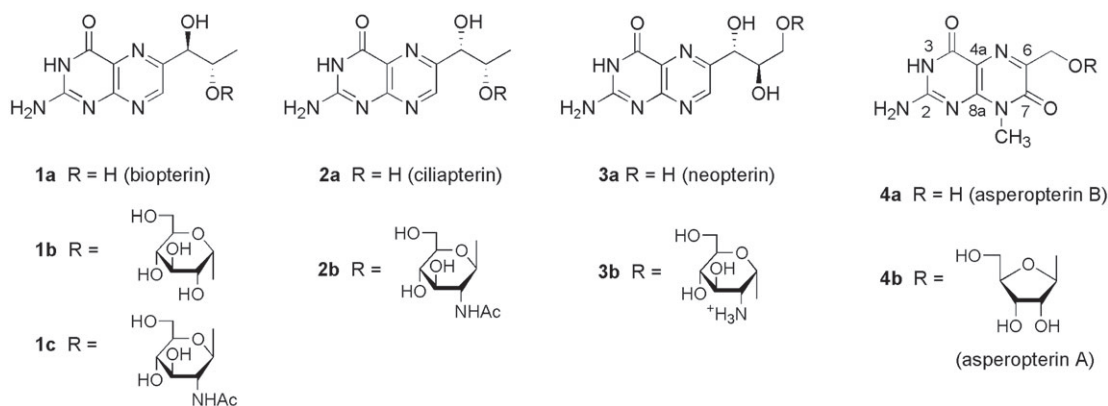


Figure 1 Naturally occurring pterin glycosides.

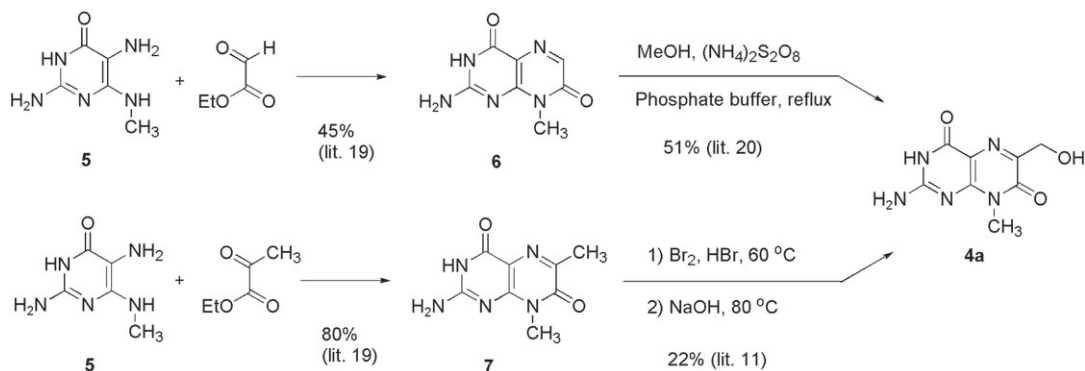
(13), which was treated with *N,N*-dimethylformamide dimethyl acetal in DMF to give the *N*²-(*N,N*-dimethylaminoethylene) derivative (14) in 48% overall yield. Mitsunobu reaction of 14 with *p*-nitrophenylethyl (NPE) alcohol in the presence of triphenylphosphine and diisopropyl azodicarboxylate (DIAD) 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].

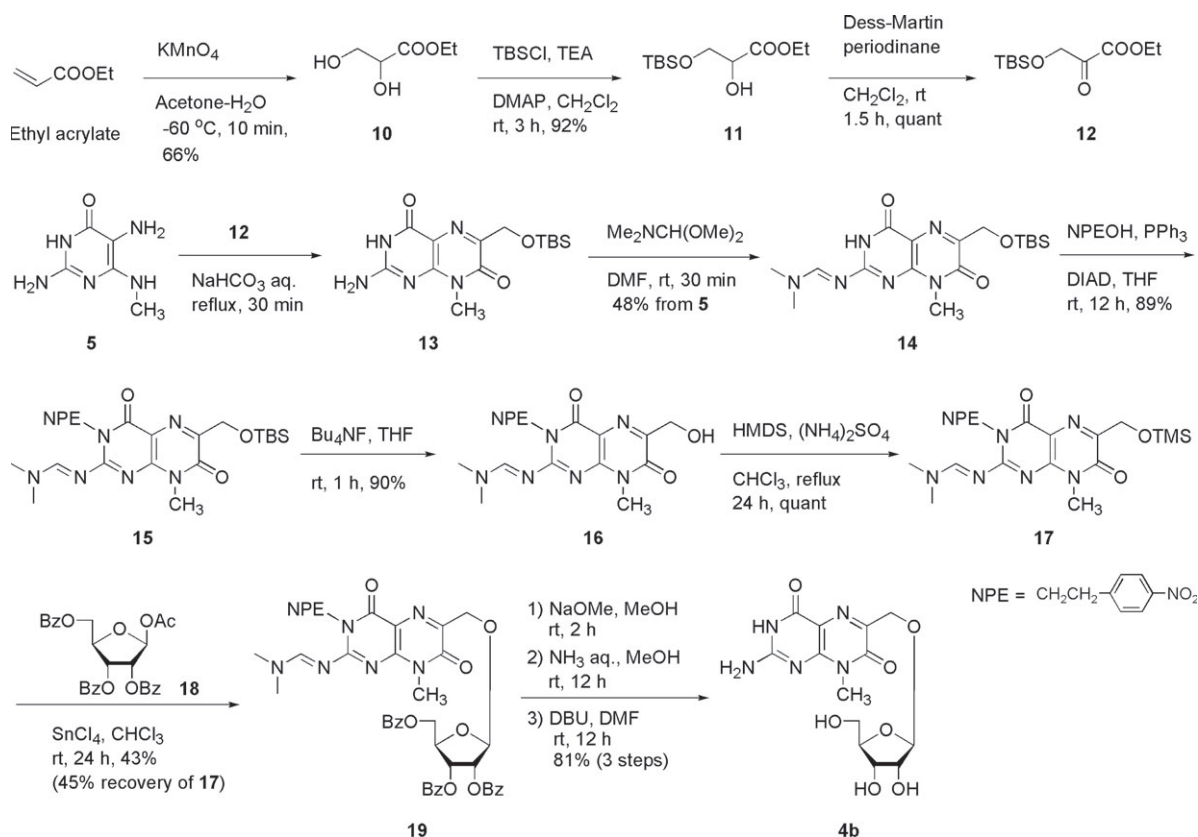
Glycosylation of 17 with glycosyl donor, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl- β -D-ribofuranose (18) was attempted

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 ^1H - 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.

Received October 31, 2012; accepted March 27, 2013

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