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First synthesis of tepidopterin [2′-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-*threo*-biopterin]

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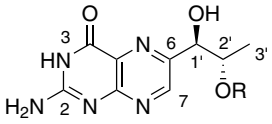
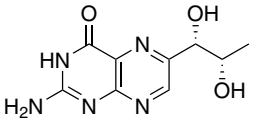
Abstract—N^2-(*N*,*N*-Dimethylaminomethylene)-1′-*O*-(4-methoxybenzyl)-3-[2-(4-nitrophenyl)ethyl]-L-*threo*-biopterin (**14**) was prepared from L-xylose in an 11-step-sequence. The first synthesis of tepidopterin (**3**) was achieved by treatment of **14** with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide in the presence of silver triflate and tetramethylurea, followed by removal of the protecting groups.

Keywords: Pterin glycoside; Pteridine; Tepidopterin; Glycosylation; L-*threo*-Biopterin

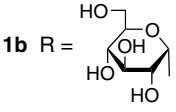
Some pterins having a hydroxyalkyl side chain at C-6, a representative example being L-biopterin (**1a**), have been found as glycosidic forms in certain prokaryotes such as cyanobacteria and anaerobic photosynthetic bacteria; for example, the 2′-*O*-(α-D-glucopyranosyl)-L-biopterin (**1b**) isolated from cyanobacterium, *Anacystis nidulans*,[1] *Synechococcus* sp.,[2] and *Spirulina platensis*,[3] and limipterin (**1c**) from a green sulfur photosynthetic bacterium, *Chlorobium limicola f. thiosulfatophilum*.[4] Various other glycosides consisting of different pterins and sugar moieties have also been found in nature, although the glycosidic linkages of some derivatives remain unclear.[5] The physiological function of the parent pterins has been studied in detail: for example, **1** exhibits enzyme cofactor activity in aromatic amino acid hydroxylation[6] and nitric oxide synthesis[7] as the form of its tetrahydro derivative. By contrast, the functional roles of pterin glycosides have remained obscure, although some inhibitory activities against tyrosinase[8] and photostabilization of photosynthetic pigments[9] were reported for L-biopterin D-glucoside (**1b**). Despite considerable interest from the viewpoint of their biological activities and functions, as well as structural proof, attempts at preparation of pterin glycosides have so far scarcely been made.[10]

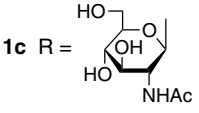


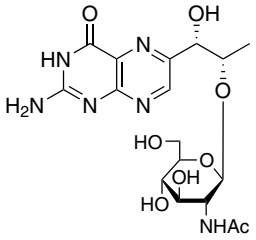
1a R = H (L-biopterin)



2 (L-*threo*-biopterin)







3 (tepidopterin)

Meanwhile, tepidopterin (**3**) was isolated from a green sulfur photosynthetic bacterium *Chlorobium tepidum* and was characterized as the 2′-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl) derivative of L-*threo*-biopterin (**2**).[11] The L-*threo* structure of **3** appears somewhat

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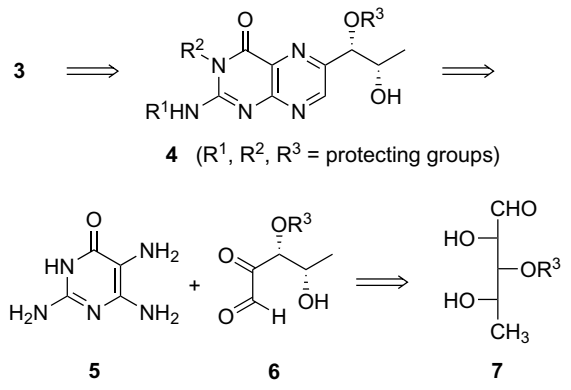
peculiar in view of the tendency that L-biopterin derivatives having an L-*erythro* form are common among natural pterin glycosides, and thus identifying the enzyme from *C. tepidum* has been attempted.[12] These facts prompted us to develop an efficient synthetic protocol for pterin glycosides as well as to prove the proposed structure of the reported natural product. We describe herein the first synthesis of tepidopterin (**3**) via an appropriately protected L-*threo*-biopterin.

The retrosynthetic analysis for **3** is outlined in Scheme 1. The L-*threo*-biopterin derivative (**4**), whose pyrimidine ring moiety and 1′-hydroxy group of the side chain are protected, can be perceived as the key precursor to achieve the selective 2′-O-glycosylation. The pteridine ring formation of **4** would be achieved by condensation of 2,5,6-triamino-4-hydroxypyrimidine (**5**) with the pentos-2-ulose (**6**), which would be derived from the 3-O-protected 5-deoxy-L-xylose (**7**). Taking into consideration the available conditions to remove the protecting groups of the glycoside derived from **4**, we employed

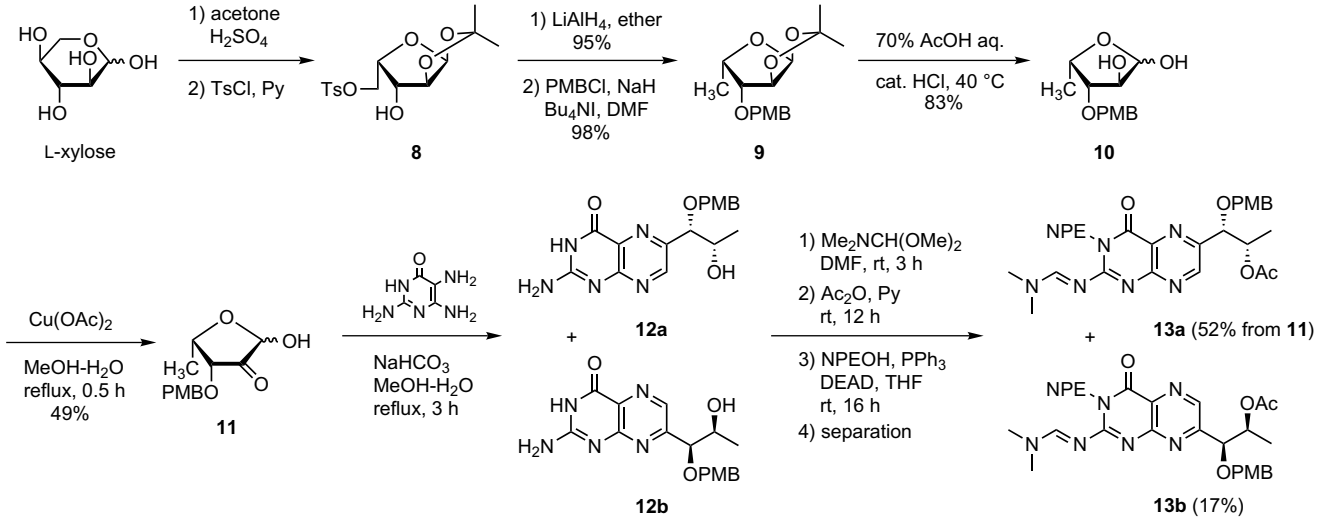


Scheme 1.

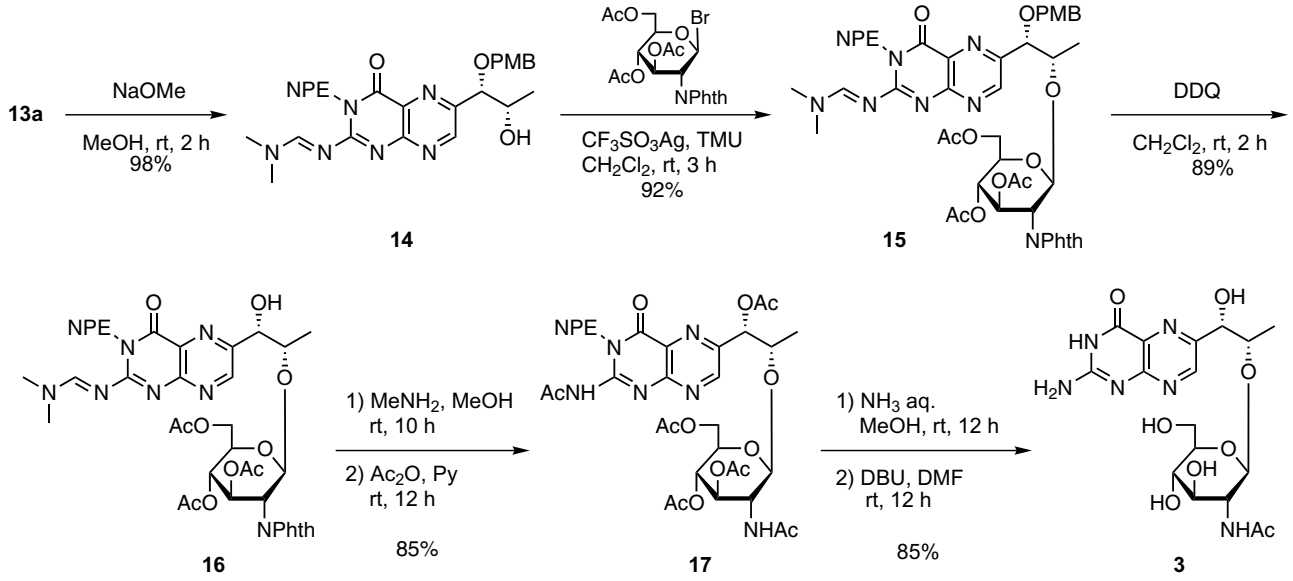
p-methoxybenzyl (PMB) group for protection of 1′-hydroxy, *N*,*N*-dimethylaminomethylene group for 2-amino, and 2-(4-nitrophenyl)ethyl (NPE) group for N-3 of the ring.[13]

The synthesis commenced from 1,2-*O*-isopropylidene-5-*O*-tosyl-α-L-xylofuranose (**8**), which was prepared from L-xylose according to the reported procedures[14] (Scheme 2). Reduction of **8** with lithium aluminum hydride in ether afforded the 5-deoxy derivative (in 95% yield), which was then treated with *p*-methoxybenzyl chloride and sodium hydride in the presence of tetrabutylammonium iodide to give the 3-*O*-PMB derivative (**9**) in 98% yield. Hydrolysis of **9** in 70% acetic acid containing catalytic hydrochloric acid afforded 5-deoxy-3-*O*-PMB-L-xylofuranose (**10**) in 83% yield.

The selective oxidation of 2-hydroxy group of **10** with cupric acetate[15] provided the L-*threo*-pentos-2-ulose derivative (**11**). The pteridine ring formation of **11** with sulfate of 2,5,6-triamino-4-hydroxypyrimidine (**5**) under neutral conditions afforded an inseparable mixture (35:65) of the 6-substituted pterin (**12a**) and its 7-substituted isomer (**12b**), while the same condensation in an aqueous sodium bicarbonate solution resulted in the preferential formation of the desired **12a** in a ratio of 75:25. These products were separated and characterized after having been converted into the fully protected derivatives (**13a**,**b**) by the following three steps: treatment of **12a**,**b** with *N*,*N*-dimethylformamide dimethyl acetal in DMF, followed by acetylation of a hydroxy group, afforded 2′-*O*-acetyl-N^2-(*N*,*N*-dimethylaminomethylene)-1′-*O*-PMB derivatives, whose N-3 position was then protected with NPE group by Mitsunobu reaction with 2-(4-nitrophenyl)ethanol in the presence of triphenylphoshine and diethyl azodicarboxylate (DEAD) to give **13a** and **13b**. These products were separated by column chromatography on silica gel to provide the



Scheme 2.



Scheme 3.

Table 1. ^{1}H and ^{13}C NMR spectral parameters for tepidopterin (**3**) in DMSO-d_6[a,b]

	Chemical shifts (δ)							Coupling constants (Hz)		
Pterin moiety	H-7	H-1′	H-2′	H_3-3′	H_2N-2	H–N(3)	HO-1′	$J_{1',2'}$	$J_{2',3'}$	$J_{1',OH}$
	8.65	4.59	4.01	1.12	6.99	11.55	5.31	4.4	6.4	5.3

Glycosyl moiety[c]	H-1	H-2	H-3	H-4	H-5	H[a]-6	H[b]-6	Ac	NH-2	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$	$J_{2,NH}$
	4.32	3.32	3.20	3.02	3.05	3.67	3.42	1.71	7.42	8.3	9.9	8.6	9.5	2.0	6.1	11.7	8.8

	Chemical shifts (δ)								
Pterin moiety	C-2	C-4	C-4a	C-6	C-7	C-8a	C-1′	C-2′	C-3′
	156.82	161.27	127.63	150.72	149.12	153.91	74.82	78.43	18.20

Glycosyl moiety	C-1	C-2	C-3	C-4	C-5	C-6	$COCH_3$	$CO\mathit{C}H_3$
	101.88	56.07	74.62	70.83	77.02	61.32	169.58	23.31

[a] The solvent peak was used as an internal standard (δ 2.50 for ^{1}H, 39.70 for ^{13}C).
[b] The assignments were made with the aid of D_2O exchange and 2D C–H COSY measurements.
[c] δ 4.95 (HO-3, $J_{3,OH} = 4.8$ Hz), 4.85 (HO-4, $J_{4,OH} = 5.1$ Hz), 4.47 (HO-6, $J_{6,OH} = 5.8$ Hz).

desired 6-substituted pterin derivative (**13a**) (52% overall yield from **11**) and the 7-substituted derivative (**13b**) (17%).

The structural assignment of **13a** and **13b** was made 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 shift to a lower field (ca. 20 ppm) from those of C-6.[16] Therefore, the close values of **13a** (C-6: δ 151.14, C-7: δ 149.35) and the distant values of **13b** (C-6: δ 140.24, C-7: δ 160.50) 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 **13a** appears at a lower field than H-6 signal (δ 8.78) of **13b** due to conjugation with the 4-oxo group.

Methanolysis of 2′-*O*-acetyl-1′-*O*-PMB-L-*threo*-biopterin derivative (**13a**) in the presence of sodium methoxide provided the 1′-*O*-PMB derivative (**14**), a versatile precursor for the 2′-O-monoglycosylation (Scheme 3). Efficient glycosylation of **14** was attained by condensation with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimindo-β-D-glucopyranosyl bromide[17] in the presence of silver triflate and tetramethylurea (TMU) in dichloromethane at room temperature for 3 h, affording the 2′-*O*-(β-D-glucopyranosyl)-L-*threo*-biopterin derivative (**15**) in 92% yield.

Removal of the protecting groups of **15** was carried out according to the following steps: cleavage of PMB

[†] All new compounds gave elemental analysis and/or MS (high-resolution) consistent with their structures. Synthetic procedures for compounds **13a**,**b** from **10** and the NMR spectral parameters of **13**–**17** are available as Supplementary data.

group of **15** was achieved by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), to afford **16** (89%), followed by removal of the phthaloyl and *N*,*N*-dimethylaminomethylene group of **16** with methylamine. Then the product was acetylated with acetic anhydride in pyridine to give the fully acetylated derivative (**17**) in 85% overall yield from **16**. Treatment of **17** with aqueous ammonia (to cleave acetyl groups except for 2-acetamido of the sugar) and then with DBU (to cleave the NPE group) furnished tepidopterin (**3**) in 85% overall yield. The precise parameters obtained on 600 MHz ^{1}H and 125 MHz ^{13}C NMR spectra for **3** are listed in Table 1;[‡] the spectral data of the synthetic compound (**3**) were found to be essentially identical with those reported for the natural product.[11]

The present work thus demonstrates the first synthesis of tepidopterin (**3**) from L-xylose via 1′-*O*-PMB-L-*threo*-biopterin derivative (**14**). This synthetic strategy has proved to provide a useful method that can be applied to a series of other natural pterin glycosides and their analogs.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2007.06.017.

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[‡] The complete assignments of **3** have been established by the present work; some ambiguous parameters were included in the previous report for the natural compound.