

Pteridines
Vol. 21, 2010, pp. 79 - 83

First Synthesis of a Natural Neopterin Glycoside: 3'-O-(β -D-Glucopyranosyluronic acid)neopterin

Tadashi Hanaya¹, Takafumi Hattori¹, Daisuke Takayama¹, Hiroshi Yamamoto²

¹Department of Chemistry, Faculty of Science, Okayama University, Tsushima-naka, Okayama 700-8530, Japan

²School of Pharmacy, Shujitsu University, Nishigawara, Okayama 703-8516, Japan

Date received: 2010/08/23

Abstract

1',2'-Di-O-acetyl-N²-(*N,N*-dimethylaminomethylene)-3-[2-(4-nitrophenyl)ethyl]neopterin (**1**) was prepared from neopterin in 5 steps. Glycosylation of **1** with methyl 2,3,4-tri-O-benzoyl- α -D-glucopyranosyluronate bromide in the presence of silver triflate and tetramethylurea afforded the corresponding 3'-O-(methyl β -D-glucopyranosyluronate)neopterin derivative (**2**) in 64% yield. The first synthesis of 3'-O-(β -D-glucopyranosyluronic acid)neopterin was achieved by successive removal (4 steps) of the protecting groups of **2**.

Key words: neopterin glycoside, D-glucuronic acid, glycosylation, protecting groups

Introduction

Some pterins having a hydroxyalkyl side-chain at C-6, a representative example being biopterin (**1**), have been found as glycosides in certain prokaryotes such as cyanobacteria and anaerobic photosynthetic bacteria: *e.g.*, 2'-O-(α -D-glucopyranosyl)biopterin (**2**) (1-4), its β -D-ribofuranosyl analog (**3**) (5), and limipterin (**4**) (6) (Figure 1). As for glycosides of neopterin (**5**), 3'-O-(β -D-glucopyranosyluronic acid)neopterin (**6**) was isolated from *Azotobacter agilis* (7) and *Bacillus subtilis* (8), whereas its 2-amino-2-deoxy- α -D-glucopyranosyl analog (solfapterin) (**7**) was isolated from thermophilic archaeobacterium *Sulfolobus solfataricus* (9). Various other glycosides consisting of different pterins such as ciliapterin and 6-hydroxymethylpterin have also been found in cyanobacteria and anaerobic photosynthetic bacteria (10-12).

The physiological function of parent pterins has been studied in detail: *e.g.*, biopterin (**1**) exhibits enzyme cofactor activity in aromatic amino acid hydroxylation (13-15) and nitric oxide synthesis (16-18) as the form of its tetrahydro derivative, while neopterin (**5**) has been shown to be a marker for the activation of cellular immunity or an inducer of apoptosis (19-22). By contrast, the functional roles of

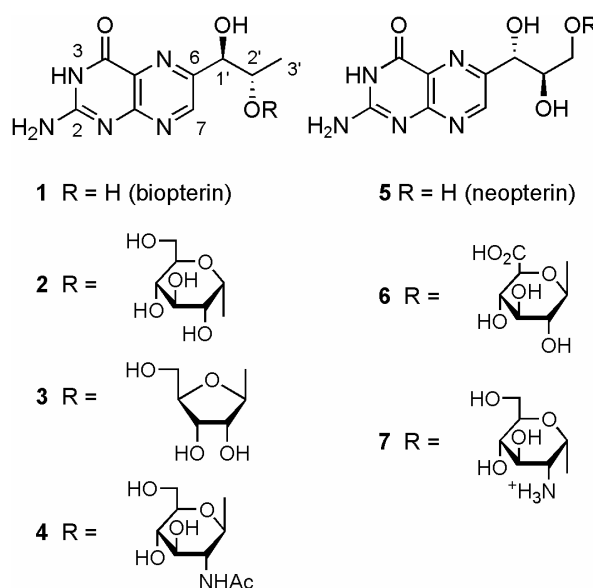


Figure 1: Structures of biopterin, neopterin, and their glycosides (**1-7**)

pterin glycosides have remained obscure, though some inhibitory activities against tyrosinase were reported for biopterin D-glucoside (**2**) (23). Despite a considerable interest from the viewpoint of their biological activities and functions as well as the structural proof

Correspondence to: Tadashi Hanaya, Department of Chemistry, Faculty of Science, Okayama University, Tsushima-naka, Okayama 700-8530, Japan, Fax: +81 86 251 7853; e-mail: hanaya@cc.okayama-u.ac.jp

of hitherto reported natural products, attempts at preparation of natural pterin glycosides have so far scarcely been made, except for our synthetic studies on biopterin and ciliapterin glycosides (24-31). We describe herein an efficient synthesis of 3'-*O*-(β -D-glucopyranosyluronic acid)neopterin (**6**) as the first synthetic example of a natural neopterin glycoside.

Results and Discussion

The neopterin derivative (**10a**), whose pyrimidine moiety and 1',2'-hydroxy groups of the side chain are protected, can be perceived as the key precursor to achieve a selective 3'-*O*-glycosylation (Figure 2). As a starting material, neopterin (**5**) was prepared from D-arabinose according to the reported procedures (32). Treatment of **5** with *N,N*-dimethylformamide dimethyl acetal in DMF, followed by the selective 3'-*O*-protection with *tert*-butyldimethylsilyl (TBS) group and then 1',2'-di-*O*-acetylation, afforded the *N*-(*N,N*-dimethylaminomethylene)neopterin derivative (**8**) in a 76% total yield. The N-3 position of **8** was then protected with 2-(4-nitrophenyl)ethyl (NPE) group (33) by Mitsunobu reaction with 2-(4-nitrophenyl)ethanol in the presence of triphenylphosphine and diethyl azodicarboxylate (DEAD) to provide **9** in 84% yield.

Deprotection of 3'-*O*-TBS group of **9** with tetrabutylammonium fluoride (TBAF) resulted in the formation of the 1',3'-di-*O*- [**10b** (47%)], 2',3'-di-*O*- [**10c** (21%)], and 3'-*O*-acetate [**10d** (13%)] instead of the desired 1',2'-di-*O*-acetate (**10a**). Production of **10b-d** is likely to arise from the 1'-*O*- and 2'-*O*-acetyl group migration by the action of 3'-alkoxide derived from

desilylation. We thus attempted the cleavage of 3'-*O*-TBS group of **9** under acidic conditions. Treatment of **9** with trifluoroacetic acid (TFA) in dichloromethane was found to exclusively afford the 1',3'-di-*O*-acetate (**10b**), whereas hydrolysis of **9** in 60% acetic acid turned out to predominantly give the desired **10a** (84%), along with a minor amount of **10b** (10%).

Glycosylation of **10a** was examined by use of glycosyl donors, such as methyl 2,3,4-tri-*O*-acetyl- (**11a**) (34) and 2,3,4-tri-*O*-benzoyl- α -D-glucopyranosyluronate bromide (**11b**) (35), in the presence of an activator (Figure 3). Treatment of **10a** with 3.0 mol equiv. of **11a** in the presence of silver triflate (2.0 mol equiv.) and tetramethylurea (TMU) (1.0 mol equiv.) in dichloromethane at room temperature for 2.5 h resulted in the predominant formation of the 1',2',3'-tri-*O*-acetylneopterin derivative (**13**) in 53% yield; the desired 3'-*O*-(β -D-glucopyranosyluronate)neopterin derivative (**12**) was obtained in a minor portion (31% yield). Production of **13** can be perceived as the result of the subtraction of 2-*O*-acetyl group of **11a** by **10a** via the α -D-glucopyranosulunate-1,2-orthoacetate intermediate (31). Similar treatment of **10a** with 3.0 mol equiv. of the tri-*O*-benzoyl analog (**11b**), however, afforded the desired glycoside (**14**) in 64% yield, along with the recovery of **10a** (26%). Therefore **11b** seems to be a more suitable glycosyl donor for this work compared with **11a**. The β -anomeric configurations of thus synthesized neopterin glycosides (**12,14**) were assigned on the evidence of their $J_{1,2}$ values (7.3-7.6 Hz). Their stereoselective β -glycoside formation was mainly attained by participation of the neighboring groups (2-*O*-acyloxy groups of **11a,b**).

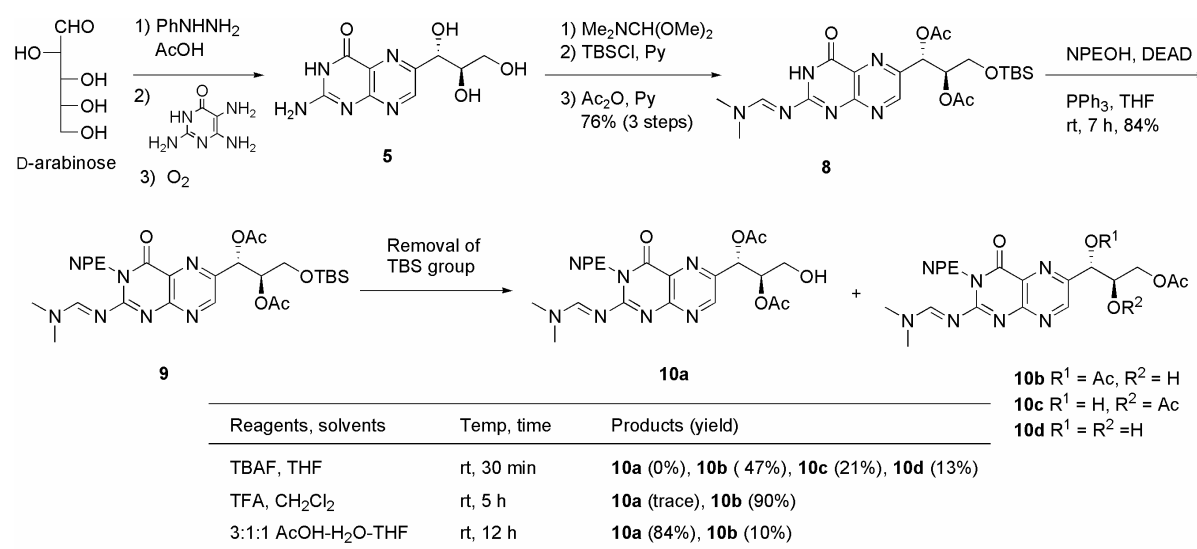


Figure 2: Synthesis of 1',2'-di-*O*-acetylneopterin derivative (**10a**)

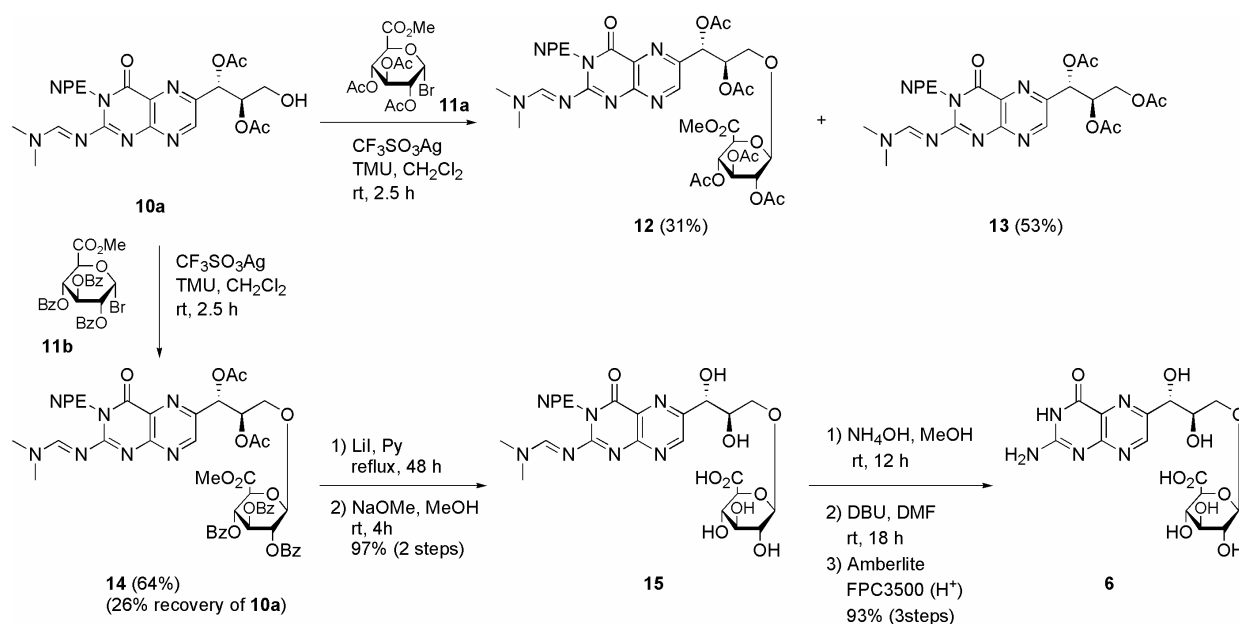


Figure 3: Synthesis of 3'-O-(β-D-glucopyranosyluronic acid)neopterin (**6**)

Removal of the protecting groups of the neopterin glycoside (**14**) was performed according to the following steps. First, attempted hydrolysis of all ester groups by use of aqueous sodium hydroxide resulted in the formation of an inseparable mixture of identified products. Selective cleavage of methyl ester of **14**, however, was achieved by use of lithium iodide in refluxing pyridine (**36**), followed by the action of sodium methoxide in methanol, affording the 3'-O-(β-D-glucopyranosyluronic acid)neopterin derivative (**15**).

Treatment of **15** with aqueous ammonia-methanol (to remove the *N,N*-dimethylaminomethylene group) and then with DBU in DMF (to cleave the NPE group), followed by acidification using an ion-exchange resin, furnished the target compound 3'-O-(β-D-glucopyranosyluronic acid)neopterin (**6**) in 92% (overall yield from **14**). The precise structure of **6** was established by ¹H- and ¹³C-NMR spectra with the aid of 2D C-H COSY measurement (Table 1).

Table 1: 600 MHz ¹H- and 151 MHz ¹³C-NMR Spectral parameters for 3'-O-(β-D-glucopyranosyluronic acid)neopterin (**6**) in D₂O^a

¹ H	Chemical shifts (δ)					Coupling constants (Hz)			
Neopterin moiety	H-7	H-1'	H-2'	H ^a -3'	H ^b -3'	<i>J</i> _{1',2'}	<i>J</i> _{2',3'a}	<i>J</i> _{2',3'b}	<i>J</i> _{3'a,3'b}
	8.87	5.01	4.22	4.09	3.79	6.4	3.2	6.1	11.0
Glycosyl moiety	H-1	H-2	H-3	H-4	H-5	<i>J</i> _{1,2}	<i>J</i> _{2,3}	<i>J</i> _{3,4}	<i>J</i> _{4,5}
	4.51	3.31	3.50	3.54	3.96	8.1	9.0	9.1	9.5
¹³ C	Chemical shifts (δ)								
Neopterin moiety	C-2	C-4	C-4a	C-6	C-7	C-8a	C-1'	C-2'	C-3'
	154.03	161.98	127.71	149.49	150.07	153.00	72.75	73.30	71.13
Glycosyl moiety	C-1	C-2	C-3	C-4	C-5	C-6			
	103.29	73.38	75.75	71.89	75.08	173.04			

^a Chemical shifts are reported as δ values relative to DOH (4.79 ppm) for ¹H and 1,4-dioxane (67.2 ppm) for ¹³C as an internal standard.

The present work thus demonstrates the first synthesis of a natural neopterin glycoside, 3'-*O*-(β -D-glucopyranosyluronic acid)neopterin (**6**) by use of the key intermediate (**10a**). Extension of this work including applications of these findings in synthesizing other neopterin glycosides having various types of sugar moieties is in progress.

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

- Forrest HS, van Baalen C, Myers J. Isolation and identification of a new pteridine from a blue-green alga. *Arch. Biochem. Biophys.* 1958; 78: 95-99.
- 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: 250-253.
- Noguchi Y, Ishii A, Matsushima A, Haishi D, Yasumuro K, Moriguchi T, Wada T, Koderia Y, Hiroto M, Nishimura H, Sekine M, Inada Y. Isolation of biopterin- α -glucoside from *Spirulina (Arthrospira) platensis* and its physiologic function. *Mar. Biotechnol.* 1999; 1: 207-210.
- 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: 121-125.
- Hanaya T, Torigoe K, Soranaka K, Fujita H, Yamamoto H, Pfeleiderer W. An efficient synthesis of 2'-*O*-(β -D-ribofuranosyl)biopterin. *Pteridines* 2008; 19: 72-78.
- Cha KW, Pfeleiderer W, Yim J. 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: 600-614.
- Suzuki R, Goto M. Neopterin-3'- β -D-glucuronide: isolation from *Azotobacter agilis*. *J. Biochem.* 1968; 63: 798-801.
- Kobayashi K, Forrest HS. Isolation and identification of a new pteridine neopteriny-3'- β -D-glucuronic acid from *Bacillus subtilis*. *Comp. Biochem. Physiol.* 1970; 33: 201-207.
- Lin XL, White RH. Structure of solfapterin (erythro-neopterin-3'-D-2-deoxy-2-aminogluco-pyranoside) isolated from the thermophilic archaebacterium *Sulfolobus solfataricus*. *J. Bacteriol.* 1988; 170: 1396-1398.
- 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.
- Ikawa M, Sasner JJ, Haney JF, Foxall TL. Pterins of the cyanobacterium *Aphanizomenon flos-aquae*. *Phytochem.* 1995; 38: 1229-1232.
- Lee HW, Oh CH, Geyer A, Pfeleiderer W, Park YS. Characterization of a novel unconjugated pteridine glycoside, cyanopterin, in *Synechocystis* sp. PCC 6803. *Biochim. Biophys. Acta* 1999; 1410: 61-70.
- Kaufman S, Fisher DB. In: Hayaishi O, ed. *Molecular Mechanisms of Oxygen Activation*. New York: Academic Press 1974; 285-369.
- Kaufman S, Kaufman EE. In: Blakley R, Benkovic SJ, eds. *Folates and Pterins*. New York: J. Wiley & Sons 1985; 2: 251-352.
- Fitzpatrick PF. Tetrahydropterin-dependent amino acid hydroxylases. *Annu. Rev. Biochem.* 1999; 68: 355-381.
- Crane BR, Arvai AS, Ghosh DK, Wu CQ, Getzoff ED, Stuehr DJ, Tainer JA. A structure of nitric oxide synthase oxygenase dimmer with pterin and substrate. *Science* 1998; 281: 2121-2126.
- Kwon NS, Nathan CF, Stuehr DJ. Reduced biopterin as a cofactor in the generation of nitrogen oxides by murine macrophages. *J. Biol. Chem.* 1989; 264: 20496-20501.
- Marletta MA. Nitric oxide synthase: aspects concerning structure and catalysis. *Cell* 1994; 78: 927-930.
- Wachter H, Werner ER, Reibnegger G, Fuchs D, Hausen A. Neopterin in clinical use. *Pteridines* 1989; 1: 3-10.
- Hausen A, Reibnegger G, Speck B, Wachter H. Neopterin as a new biochemical marker in the clinical monitoring of bone marrow transplant recipients. *Transplantation* 1984; 38: 497-500.
- Reibnegger G, Aulitzky W, Huber C, Margreiter R, Riccabona G, Wachter H. Neopterin in urine and serum of renal allograft recipients. *J. Clin. Chem. Biochem.* 1986; 24: 770-775.
- Hoffman G, Kenn S, Wirleitner B, Deetjen C, Frede S, Smolny M, Rieder J, Fuchs D, Baier-Bitterlich G, Schobersberger W. Neopterin induce nitric oxide-dependent apoptosis in rat vascular smooth muscle cells. *Immunobiology* 1998; 199: 63-73.
- Wachi Y, Yoshida S, Komatsu K, Matsunaga T. Biopterin glycoside, its manufacture with algae, and its use in cosmetic skin preparation. *Jpn. Patent*

- 05,286,989; 1993 (Chem. Abstr. 1994; 120: 161782t).
- 24 Yamamoto H, Hanaya T, Harada K, Kawamoto H, Pfeleiderer W. An efficient synthesis of limipterin [2'-*O*-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-L-biopterin] via *N*²-(*N,N*-dimethylamino)methylene-3-[2-(4-nitrophenyl)ethyl]-L-biopterin. *Pteridines* 1996; 7: 110-112.
- 25 Hanaya T, Soranaka K, Harada K, Yamaguchi H, Suzuki R, Endo Y, Yamamoto H, Pfeleiderer W. 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.
- 26 Hanaya T, Baba H, Toyota H, Yamamoto H. Efficient total syntheses of natural pterin glycosides: limipterin and tepidopterin. *Tetrahedron* 2008; 64: 2090-2100.
- 27 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; 2075-2078.
- 28 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: 635-644.
- 29 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: 7989-7997.
- 30 Hanaya T, Yamamoto H, Pfeleiderer W. First synthesis of a representative, natural pterin glycoside: 2'-*O*-(α -D-glucopyranosyl)biopterin. *Pteridines* 2009; 20, Special issue: 36-41.
- 31 Hanaya T, Baba H, Ejiri K, Yamamoto H. Synthesis of 6-hydroxymethylpterin α - and β -D-glucosides. *Heterocycles* 2010; 80: 1013-1025.
- 32 Soyka R, Pfeleiderer W. Synthesis and characteristics of 5,6-dihydro-6-(1,2,3-trihydroxypropyl)pteridines: covalent intramolecular adducts. *Helv. Chim. Acta* 1990; 73: 808-826.
- 33 Hanaya T, Torigoe K, Soranaka K, Yamamoto H, Yao Q, Pfeleiderer 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.
- 34 Bollenback GN, Long JW, Benjamin DG, Lindquist JA. The synthesis of aryl-D-glucopyranosiduronic acids. *J. Am. Chem. Soc.* 1955; 77: 3310-3315.
- 35 Zorbach WW, Valiaveedan GD. Methyl (cortison-21-yl 2,3,4-tri-*O*-acetyl- β -D-glucosid)uronate. *J. Org. Chem.* 1964; 29: 2462-2463.
- 36 Elsinger F, Schreiber J, Eschenmoser A. Notiz über die Selektivität der Spaltung von Carbonsäuremethylestern mit Lithiumjodid. *Helv. Chim. Acta* 1960; 43: 113-118.