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

Tadashi Hanaya[1], Takafumi Hattori[1], Daisuke Takayama[1], Hiroshi Yamamoto[2]

[1]Department of Chemistry, Faculty of Science, Okayama University, Tsushima-naka, Okayama 700-8530, Japan
[2]School of Pharmacy, Shujitsu University, Nishigawara, Okayama 703-8516, Japan

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Abstract

1',2'-Di-*O*-acetyl-N^2-(*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-glucronic 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 *Azotobactor agilis* (7) and *Bacillus subtilis* (8), whereas its 2-amino-2-deoxy-α-D-glucopyranosyl analog (solfapterin) **(7)** was isolated from thermophilic archaebacterium *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).

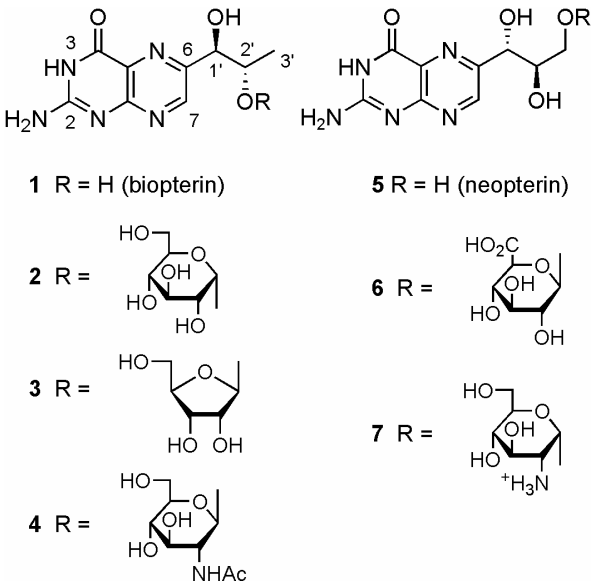


Figure 1: Structures of biopterin, neopterin, and their glycosides **(1-7)**

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 pterin glycosides have remaind 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

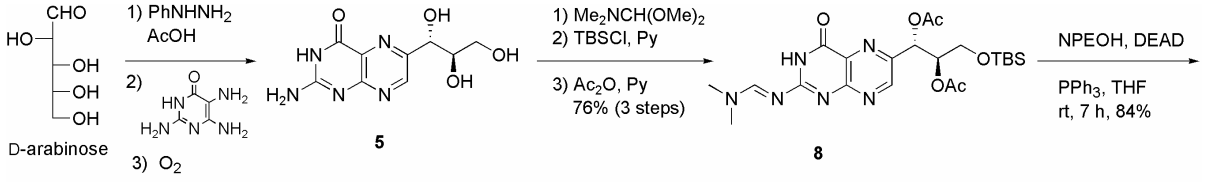
of hitherto reported natural products, attempts at preparation of natural pterin glycosides have so far scarcely been made, except for our synthetic studies on biopterin and ciliapterin glycosides (24-31). We describe herein an efficient synthesis of 3'-*O*-(β-D-glucopyranosyluronic acid)neopterin **(6)** 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^2-(*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-glucopyranosulonate-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 donar for this work compared with **11a**. The β-anomeric configurations of thus synthesized neoperin 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**).



Reagents, solvents	Temp, time	Products (yield)
TBAF, THF	rt, 30 min	**10a** (0%), **10b** (47%), **10c** (21%), **10d** (13%)
TFA, CH_2Cl_2	rt, 5 h	**10a** (trace), **10b** (90%)
3:1:1 AcOH-H_2O-THF	rt, 12 h	**10a** (84%), **10b** (10%)

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

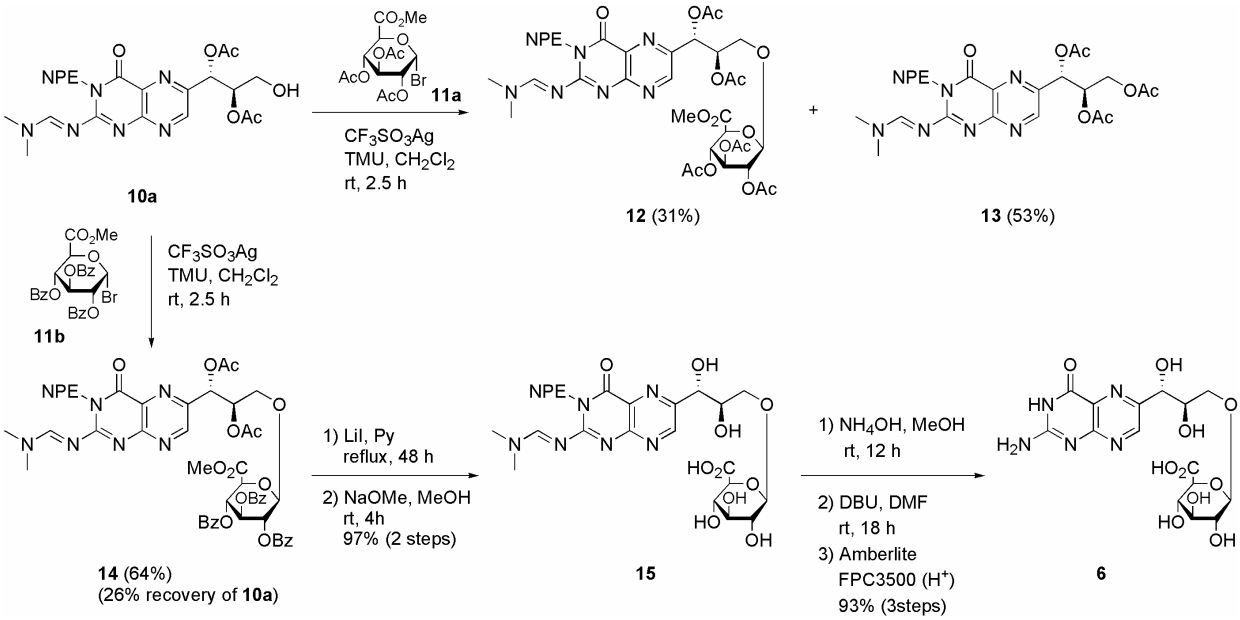


Figure 3: Synthesis of 3'-*O*-(β-D-glucopyranosyluronic acid)neopterin **(6)**

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 ^{1}H- and ^{13}C-NMR spectra with the aid of 2D C-H COSY measurement (Table 1).

Table 1: 600 MHz ^{1}H- and 151 MHz ^{13}C-NMR Spectral parameters for 3'-*O*-(β-D-glucopyranosyluronic acid)neopterin **(6)** in D_2O[a]

^{1}H	Chemical shifts (δ)					Coupling constants (Hz)			
Neopterin moiety	H-7	H-1'	H-2'	H^{a}-3'	H^{b}-3'	$J_{1',2'}$	$J_{2',3'a}$	$J_{2',3'b}$	$J_{3'a,3'b}$
	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	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
	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 ^{1}H and 1,4-dioxane (67.2 ppm) for ^{13}C 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

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