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FIRST SYNTHESIS OF BIOPTERIN α-D-GLUCOSIDE

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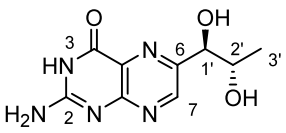
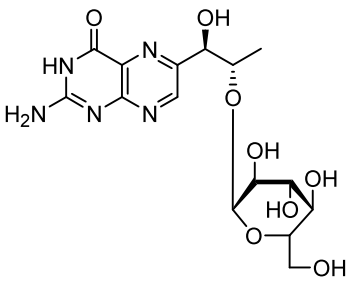
Abstract –A novel glycosyl donor, 4,6-di-*O*-acetyl-2,3-di-*O*-(4-methoxybenzyl)-α-D-glucopyranosy bromide (**15**) was efficiently prepared from D-glucose in 8 steps. The first synthesis of 2'-*O*-(α-D-glucopyranosyl)biopterin (**2**) was achieved by treatment of the key precursor, N^2-(*N*,*N*-dimethylaminomethylene)-1'-*O*-(4-methoxybenzyl)-3-[2-(4-nitrophenyl)ethyl]biopterin (**6**) with **15** in the presence of silver triflate and tetramethylurea, followed by removal of the protecting groups.

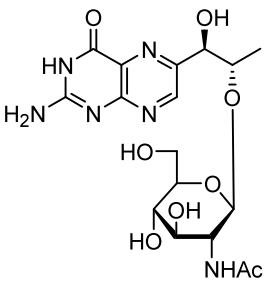
Some pterins having a hydroxyalkyl side-chain at C–6, a representative example being biopterin (**1**), have been found as glycosidic forms in certain prokaryotes; for example, 2'-*O*-(α-D-glucopyranosyl)biopterin (**2**)[1-4] isolated from cyanobacteria and limipterin [2'-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-biopterin] (**3**)[5] isolated from a green sulfur photosynthetic bacterium. Glycosides of other pterins such as ciliapterin (L-*threo*-biopterin),[6] neopterin,[7] and 6-hydroxymethylpterin[8] were also isolated from cyanobacteria, anaerobic photosynthetic bacteria, and chemoautotrophic archaebacteria. Although biopterin α-D-glucoside (**2**) is the most noteworthy among these pterin glycosides because of its abundant occurrence in various kinds of cyanobacteria, *Anacystis nidulans*,[1] *Oscillatoria* sp.,[2] *Synechococcus* sp.,[3] and *Spirulina platensis*,[4] there has been no report for synthesis of **2** since its first discovery in 1958.



1 (biopterin)



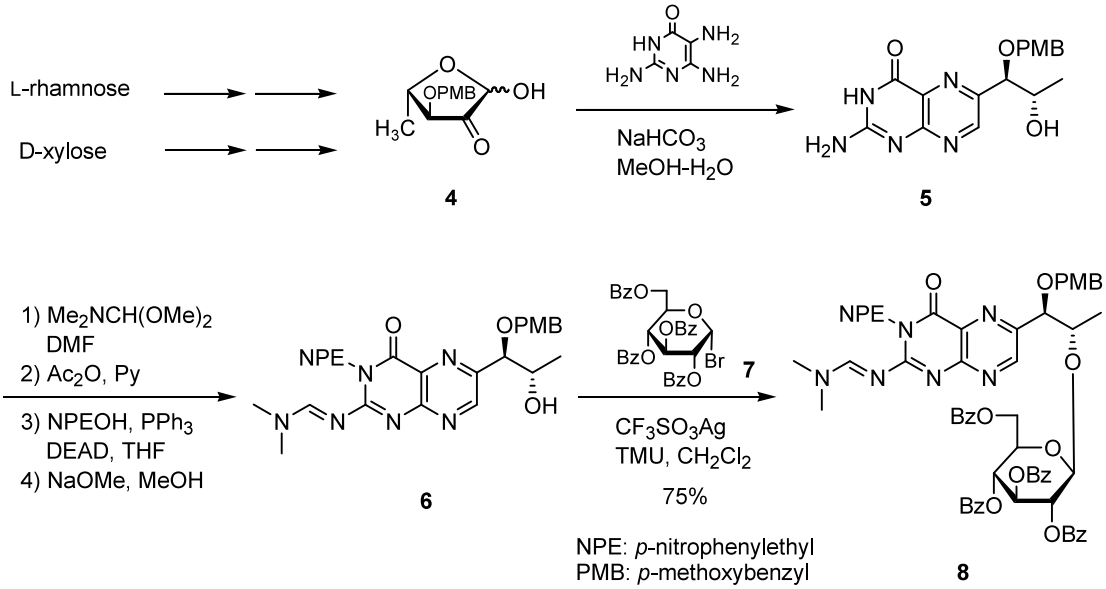
2



3

The physiological function of the parent pterins has been studied in detail: *e.g.*, **1** exhibits enzyme cofactor activity in aromatic amino acid hydroxylation[9] and nitric oxide synthesis[10] as the form of its

tetrahydro derivative. By contrast, the functional roles of pterin glycosides have remained obscure, although some inhibitory activities against tyrosinase[11] and photostabilization of photosynthetic pigments[12] were reported for **2**. Despite a considerable interest from the viewpoint of their biological activities and functions as well as structural proof of hitherto reported natural products, attempts at preparation of pterin glycosides have scarcely been made so far, except for our synthetic studies on limipterin (**3**) and ciliapterin glycosides.[13-15] In a previous paper,[14] we developed an efficient synthetic protocol for the pterin 2'-*O*-glycosides by way of the key intermediate, N^2-(*N*,*N*-dimethylamino-methylene)-1'-*O*-(4-methoxybenzyl)-3-[2-(4-nitrophenyl)ethyl]biopterin (**6**) derived from L-rhamnose (or D-xylose) via **4** and **5** (Scheme 1). Glycosylation of **6** with tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (**7**) in the presence of silver triflate afforded the 2'-*O*-(β-D-glucopyranosyl)biopterin derivative (**8**). In the present study, we therefore have undertaken to prepare an efficient glycosyl donor leading the preponderant production of pterin α-glycosides. We now describe the first synthesis of the representative, natural pterin glycoside, 2'-*O*-(α-D-glucopyranosyl)biopterin (**2**).

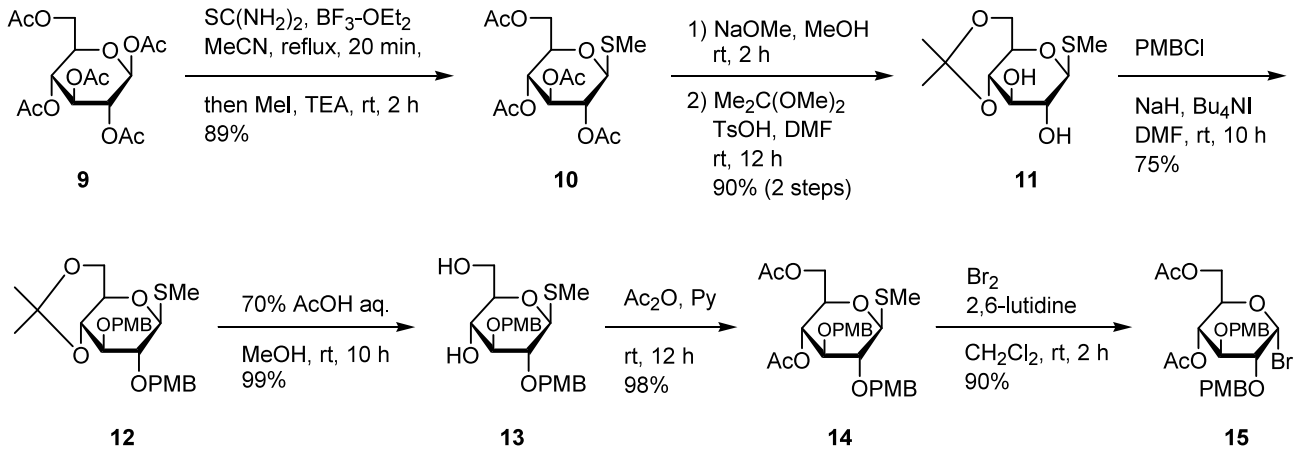


Scheme 1

The stereoselective formation of the β-glycoside (**8**) from **6** was mainly caused by participation of the 2-*O*-benzoyl group of the glycosyl donor (**7**) through the formation of an acyloxonium ion intermediate.[16] In order to avoid such a neighboring group participation, we sought to introduce an ether substituent for protection of 2-OH of a glycosyl donor. Taking into consideration the available combination of protecting groups employed for the synthetic pathway, *p*-methoxybenzyl (PMB) and acetyl groups were respectively chosen for protection of 2,3-OH and 4,6-OH of the glycosyl moiety.

Penta-*O*-acetyl-β-D-glucopyranose (**9**),[17] derived from D-glucose, served as the starting material for preparation of methyl 4,6-di-*O*-acetyl-2,3-di-*O*-PMB-1-thio-β-D-glucopyranose (**14**) and its α-D-glucopyranosyl bromide derivative (**15**), the potential glycosyl donors for the pterin α-glycosides (Scheme 2). Treatment of **9** with thiourea and boron trifluoride etherate, followed by the action of

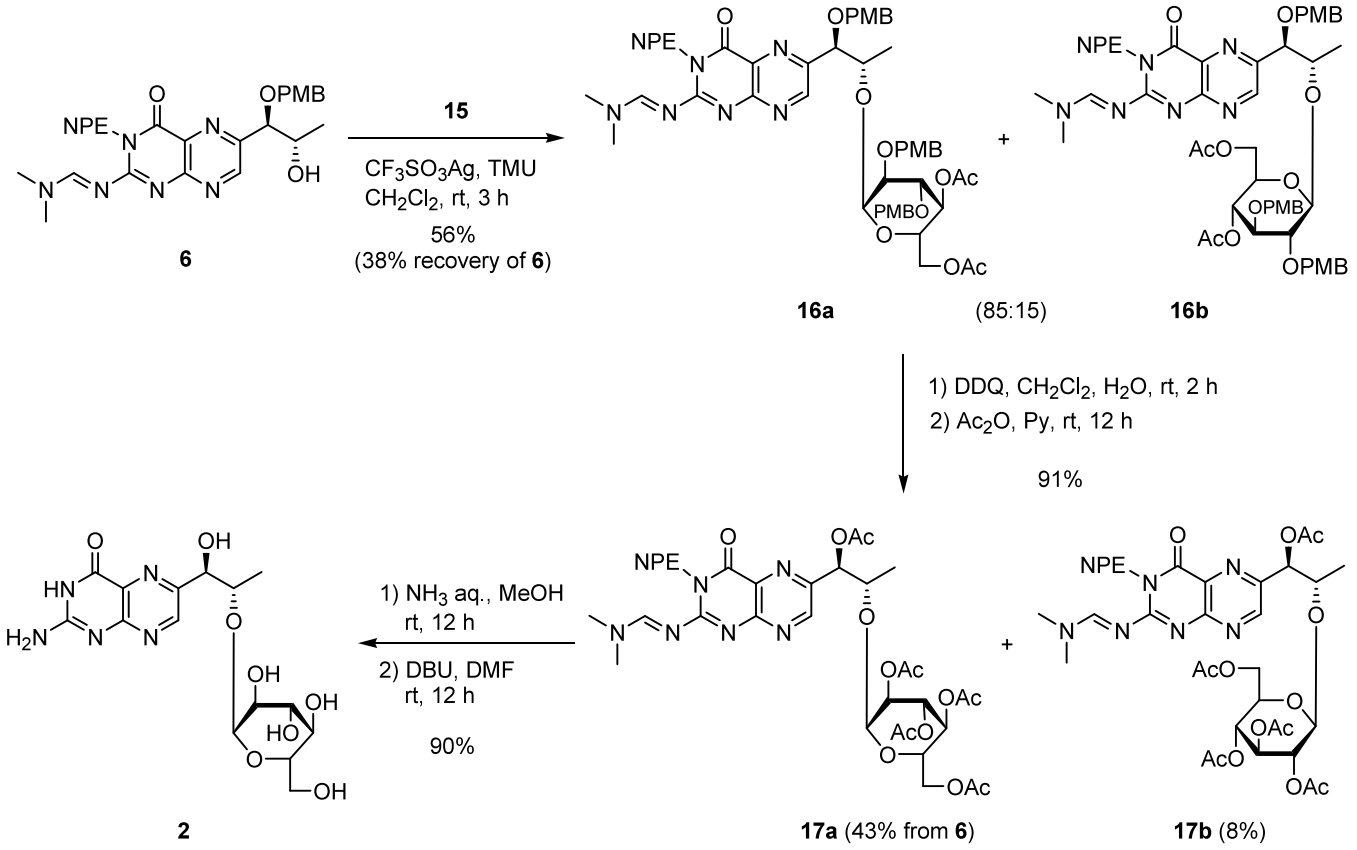
methyl iodide and triethylamine, afforded the methyl 1-thio-β-D-glucopyranose derivative (**10**).[18] Methanolysis of **10** in the presence of sodium methoxide and the subsequent acetalization with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid provided the 4,6-*O*-isopropylidene derivative (**11**).[19] Treatment of **11** with *p*-methoxybenzyl chloride and sodium hydride in DMF gave the 2,3-di-*O*-PMB derivative (**12**). Hydrolysis of **12** in 70% acetic acid afforded methyl 2,3-di-*O*-PMB-1-thio-β-D-glucopyranoside (**13**), which was then treated with acetic anhydride and pyridine to give the desired 4,6-di-*O*-acetyl derivative (**14**).[20] The transformation of the thioglycoside (**14**) to the D-glucopyranosyl bromide (**15**)[21] was achieved by reaction with bromine in dichloromethane in the presence of 2,6-lutidine.



Scheme 2

Glycosylation of the 1'-*O*-PMB-biopterin derivative (**6**) with glycosyl donors (**14**, **15**) was examined under various conditions in the presence of activators (Scheme 3). Treatment of **6** with the thioglycoside (**14**) in dichloromethane at room temperature in the presence of methyl triflate[22] or *N*-iodosuccinimide-silver triflate[23] as activators resulted in the formation of unidentified, decomposed compounds instead of the desired glycoside.[24] While glycosylation of **6** with 4.0 mol equiv. of the glycosyl bromide (**15**) in dichloromethane in the presence of tetrabutylammonium bromide and *N*-ethyldiisopropylamine[25] did not proceed, the same reaction in the presence of silver triflate (2.0 mol equiv.) and tetramethylurea (TMU)[26] (1.0 mol equiv.) afforded an inseparable anomeric mixture (85:15) of the 2'-*O*-(α-D-glucopyranosyl)biopterin derivative (**16a**) and its β-anomer (**16b**) in 56% yield, along with the recovery of **6** (38%). The α-anomeric structure of **16a** was derived from its $J_{1,2}$ value (3.5 Hz) of ^{1}H-NMR, while the larger $J_{1,2}$ value (8.0 Hz) confirmed the β-form of **16a**.

Separation of these isomers was achieved by removal of PMB groups and the subsequent acetylation. Thus, the mixture of **16a,b** was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dichloromethane, followed by acetylation with acetic anhydride in pyridine, afforded the 2'-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)biopterin derivative (**17a**) in 43% (total yield from **6**) and its β-anomer (**17b**) in 8%.[27]



Scheme 3

Removal of the protecting groups of **17a** was carried out according to the following steps: treatment with aqueous ammonia (to cleave the *N*,*N*-dimethylaminomethylene and acetyl groups) and then with DBU[28] (to cleave NPE group) furnished the desired 2'-*O*-(α-D-glucopyranosyl)biopterin (**2**) in 90% overall yield. The precise parameters obtained on ^{1}H- and ^{13}C-NMR spectra for **2** are listed in Table 1; the spectral data of the synthetic compound (**2**) were found to be essentially identical with those reported for natural product.[4,11]

The present work thus demonstrates the first synthesis of biopterin α-D-glucoside (**2**) by use of the key intermediate 1'-*O*-PMB-biopterin derivative (**6**) and the novel glycosyl donor (**15**) to preferentially provide an α-glycoside. Extension of this work including improvement of selectivity and yield for glycosylation as well as applications of these findings in synthesizing other natural pterin α-glycosides is in progress.

ACKNOWLEDGEMENTS

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Table 1. 600 MHz ^{1}H- and 151 MHz ^{13}C-NMR Spectral Parameters [chemical shifts (δ) and coupling constants (Hz)] for biopterin α-D-glucoside (**2**) in D_2O

	Pterin moiety				Glycosyl moiety					
Com-pound	H-7	H-1' ($J_{1',2'}$)	H-2' ($J_{2',3'}$)	H_3-3'	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6}$)	H_2-6
Synthetic [a]	8.79	4.83 (7.1)	4.07 (6.1)	1.30	5.00 (3.9)	3.41 (10.0)	3.32 (9.0)	3.22 (10.0)	2.40 (3.6)	3.43
Natural [b]	8.79	4.82 (7.3)	4.06 (5.9)	1.29	4.99 (3.3)	3.40 (9.2)	3.30 (9.2)	3.21 (9.2)	2.38 (3.4)	c

	Pterin moiety									Glycosyl moiety					
	C-2	C-4	C-4a	C-6	C-7	C-8a	C-1'	C-2'	C-3'	C-1	C-2	C-3	C-4	C-5	C-6
Synthetic [d]	155.7	165.4	128.3	152.2	149.6	155.2	75.6	75.5	14.9	95.8	71.6	73.4	69.5	72.5	60.5
Natural [e]	155.7	165.4	128.6	152.8	150.1	155.4	76.0	76.0	15.2	96.3	72.1	73.9	70.1	72.9	61.2

[a] The solvent peak (δ 4.79) was used as an internal standard.

[b] Ref. 4 (at 270 MHz). The internal or external standard is not shown.

[c] Not reported.

[d] 1,4-Dioxane (δ 67.2) was used as an internal standard.

[e] Ref. 11 (at 100 MHz). The internal or external standard is not shown.

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18. F. M. Ibatullin, K. A. Shabalin, J. V. Jänis, and A. G. Shavva, *Tetrahedron Lett*., 2003, **44**, 7961; use of larger amounts of methyl iodide (10 mol equiv.) and TEA (12 mol equiv.) than those reported in this lit. led to a much improved yield (89%) of **10** from **9** (lit., 57% by use of 1.1 equiv. of methyl iodide and 3.1 equiv. of TEA).
19. All newly isolated compounds hereafter in this paper gave satisfactory analytical and ^{1}H-NMR (mostly at 600 MHz) data, which will be presented in a full paper in the near future.
20. ^{1}H-NMR for **14** (500 MHz, $CDCl_3$) δ = 1.94, 2.06 (3H each, 2s, AcO-4,6), 2.22 (3H, s, MeS-1), 3.48 (1H, dd, $J_{1,2}$ = 9.8, $J_{2,3}$ = 8.8 Hz, H-2), 3.54 (1H, ddd, $J_{4,5}$ = 10.1, $J_{5,6a}$ = 5.2, $J_{5,6b}$ = 2.4 Hz, H-5), 3.61 (1H, t, $J_{3,4}$ = 9.5 Hz, H-3), 3.80 (6H, s, MeO), 4.08 (1H, dd, $J_{6a,6b}$ = 12.2 Hz, H_b-6), 4.20 (1H, dd, H_a-6), 4.35 (1H, d, H-1), 4.59, 4.77 (2H each, 2d, ^2J = 11.0 Hz, CH_2O-2 or 3), 4.67, 4.81 (2H each, 2d, ^2J = 10.1 Hz, CH_2O-2 or 3), 5.02 (1H, t, H-4), 6.855, 6.86, 7.18, 7.30 (2H each, 4 d, $J_{o,m}$ = 8.8 Hz, C_6H_4).
21. ^{1}H-NMR for **15** (500 MHz, $CDCl_3$) δ = 1.95, 2.05 (3H each, 2s, AcO-4,6), 3.53 (1H, dd, $J_{2,3}$ = 9.2, $J_{1,2}$ = 3.9 Hz, H-2), 3.80, 3.81 (3H each, 2s, MeO), 3.92 (1H, t, $J_{3,4}$ = 9.5 Hz, H-3), 4.02 (1H, dd, $J_{6a,6b}$ = 12.5, $J_{5,6b}$ = 2.1 Hz, H_b-6), 4.14 (1H, ddd, $J_{4,5}$ = 10.4, $J_{5,6a}$ = 4.6, Hz, H-5), 4.26 (1H, dd, H_a-6), 4.58, 4.80 (2H each, 2d, ^2J = 11.3 Hz, CH_2O-2 or 3), 4.62, 4.67 (2H each, 2d, ^2J = 11.6 Hz, CH_2O-2 or 3), 5.05 (1H, dd, H-4), 6.26 (1H, d, H-1), 6.86, 6.88, 7.19, 7.29 (2H each, 4 d, $J_{o,m}$ = 8.6 Hz, C_6H_4).
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24. In model experiments using thioglycoside (**14**) with 2-propanol in CH_2Cl_2, the corresponding glycosides were obtained in good yields: 95% (α:β = 60:40) by use of MeOTf; 91% (56:44) by use of NIS-AgOTf.
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27. ^{1}H-NMR for **17a** (600 MHz, $CDCl_3$) δ 1.18 (3H, d, $J_{2',3'}$ = 6.4 Hz, H_3-3'), 1.98, 1.99, 1.995, 2.08, 2.19 (3H each, 5s, AcO-1', AcO-2,3,4,6*), 3.16 [2H, t, ^3J = 7.8 Hz, CH_2CH_2-N(3)], 3.19, 3.23 (3H each, 2s, Me_2N), 3.83 (1H, ddd, $J_{4,5}$ = 10.3, $J_{5,6a}$ = 4.2, $J_{5,6b}$ = 2.2 Hz, H-5*), 3.94 (1H, dd, $J_{6a,6b}$ = 12.5 Hz, H_b-6*), 4.19 (1H, dd, H_a-6*), 4.45 (1H, qd, $J_{1',2'}$ = 4.6 Hz, H-2'), 4.58, 4.60 [1H each, 2dt, ^2J = 12.0 Hz, CH_2-N(3)], 4.80 (1H, dd, $J_{2,3}$ = 10.3, $J_{1,2}$ = 3.9 Hz, H-2*), 5.00 (1H, dd, $J_{3,4}$ = 9.5 Hz, H-4*), 5.22 (1H, d, H-1*), 5.33 (1H, dd, H-3*), 6.06 (1H, d, H-1'), 7.42, 8.14 (2H each, 2d, $J_{o,m}$ = 8.8 Hz, C_6H_4), 8.86 (1H, s, CH=N-2), 8.90 (1H, s, H-7), *for glycosyl moiety. ^{1}H-NMR for **17b** (600 MHz, $CDCl_3$) δ 1.31 (3H, d, $J_{2',3'}$ = 6.4 Hz, H_3-3'), 1.88, 1.95, 2.01, 2.09, 2.13 (3H each, 5s, AcO-1', AcO-2,3,4,6*), 3.18 [2H, t, ^3J = 7.6 Hz, CH_2CH_2-N(3)], 3.19, 3.235 (3H each, 2s, Me_2N), 3.70 (1H, ddd, $J_{4,5}$ = 10.0, $J_{5,6a}$ = 5.1, $J_{5,6b}$ = 2.4 Hz, H-5*), 4.13 (1H, dd, $J_{6a,6b}$ = 12.2 Hz, H_b-6*), 4.23 (1H, dd, H_a-6*), 4.54 (1H, qd, $J_{1',2'}$ = 5.9 Hz, H-2'), 4.61, 4.63 [1H each, 2dt, ^2J = 12.4 Hz, CH_2-N(3)], 4.66 (1H, d, $J_{1,2}$ = 8.1 Hz, H-1*), 4.87 (1H, dd, $J_{2,3}$ = 9.5 Hz, H-2*), 5.01 (1H, dd, $J_{3,4}$ = 9.5 Hz, H-4*), 5.06 (1H, dd, H-3*), 5.94 (1H, d, H-1'), 7.43, 8.15 (2H each, 2d, $J_{o,m}$ = 8.5 Hz, C_6H_4), 8.75 (1H, s, H-7), 8.84 (1H, s, CH=N-2), *for glycosyl moiety.
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