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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

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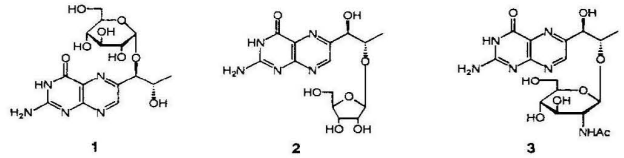
Introduction

Some pteridine compounds having a glycosidated side-chain are known to occur in nature; e. g., biopterin glucoside **1** isolated from a blue-green alga, *Anacystis nidulans* (1-3) and biopterin riboside **2** from a blue alga, *Spirulina platensis* (4, 5). Recently, limipterin was isolated from a green sulfur photosynthetic bacterium, *Chlorobium limicola f. thiosulfatophilum* NCIB 8327 and was characterized as 2'-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-biopterin (**3**) (6). Attempts at synthesizing some of these compounds by direct glycosidation have remained unsatisfactory. Various types of glycosides of biopterin and related pteridines are thus considered to be of interest in view of the structural proof of hitherto reported natural products as well as of their potential biological activities and functions. We report herein the first, efficient synthesis of limipterin via an N^2, N(3)-protected biopterin.

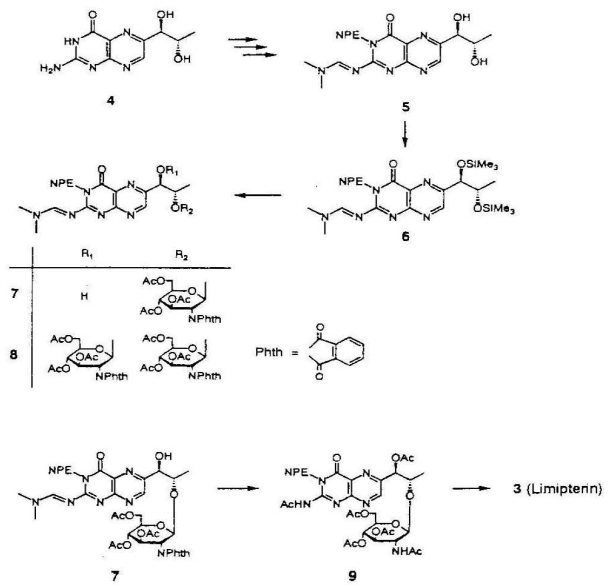


1 2 3

Results and Discussion

Pterin derivatives including biopterin are usually little soluble in nonpolar aprotic solvents (7) in which glycosidation reactions normally proceed smoothly. Thus, biopterin (4) was converted into the versatile N^2,N(3)-protected derivative, N^2-(N,N-dimethylamino)methylene-3-[2-(4-nitrophenyl)ethyl]-L-biopterin (5), in a 4-step sequence in ca. 55% overall yield (8). Then, this was quantitatively silylated with 1,1,1,3,3,3-hexamethyldisilazane in the presence of ammonium sulfate in dichloromethane under reflux for 12 h to give 1',2'-di-O-trimethylsilyl compound 6.

Direct glycosidations of **5** and glycosidations



Scheme 1. Synthesis of limipterin (**3**) via glycosidation of the N^2, N(3)-protected biopterin **5**: NPE=2-(4-Nitrophenyl) ethyl [p-$O_2NC_6H_4CH_2CH_2$-].

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Table 1. ^{1}H-NMR Parameters for Selected Biopterin Derivatives in $CDCl_3$*

Compounds	Chemical shifts (δ)/Coupling constants (Hz)									
	Biopterin moiety									
	RN-2	R-N(3) ($J_{1',2'}$)	R-N(3) ($J_{o,m}$)		H-7	H-1' ($J_{1',2'}$)	RO-1'	H-2' ($J_{2'-3'}$)	RO-2'	H_3-3'
7	3.20s, 3.25s[a] 8.83s[f]	3.13brt, 4.55brt[b] (≈7.5)	7.4brd, 8.31brd[c] (≈8.8)		8.67s	4.30brd (5.2)	4.79brs[d]	4.26qd (6.4)	*e*	1.22d
9	2.34s[g] 13.9brs[d]	3.17brt[b, h] (≈7.5)[h]	7.5brd, 8.19brd[c] (≈8.8)		8.70s	5.91d (5.2)	2.16s[g]	4.44qd (6.4)	*e*	1.26d
	Glucopyranosyl moiety									
	H-1" ($J_{1",2"}$)	H-2" ($J_{2",3"}$)	RN-2" ($J_{2",NH}$)	H-3" ($J_{3",4"}$)	H-4" ($J_{4",5"}$)	H-5" ($J_{5",6a"}$)	H-6a" ($J_{6a",6b"}$)	H-6b" ($J_{5",6b"}$)	AcO-3, 4, 6	
7	5.53d (8.6)	4.29dd (10.7)	7.65dd[f], 7.76 m[f] (5.5, 3.1)[f]	5.70dd (9.2)	5.13dd (10.2)	3.91ddd (2.4)	4.17dd (12.0)	4.28dd (5.2)	2.11, 2.02, 1.82[k]	
9	4.82d (8.5)	4.20ddd (10.5)	5.83brd[d], 2.08[g, k] (7.3)	5.27dd (9.5)	5.00dd (9.9)	3.77ddd (2.1)	4.13dd (12.2)	4.22dd (5.5)	2.02, 2.02, 1.86[k]	

*Parameters were obtained at 500 MHz. br=broad. [a]R=Me_2NC. [b]R=$ArCH_2CH_2$. [c]R=*o,m*-C_6H_4CC. [d]R=H. [e]R=Glucopyranosyl (see below). [f]R=HC. [g]R=Ac. [h]R=$ArCCH_a$ at δ 4.54brt, R=$ArCCH_b$ at δ 4.55brt, $J_{1'a,1'b}$=12.0 Hz. $J_{1'b,2'}$=7.6 Hz. [i]H-4,7 of phthaloyl. [j]H-5, 6 of phthaloyl. [k]The assignments of the acetyl groups may have to be interchanged.

of the activated di-O-silyl compound **6** were extensively studied using various amino-protected sugar donors and activators. As a result, glycosidation with 1,3,4,6-tri-O-acetyl-2-phthalimido-β-D-glucopyranose (9) (3.0 equimolar amount) in the presence of tin(IV) chloride in dichloromethane at room temperature for 24 h turned out to be most satisfactory. Silica gel column chromatography of the crude products [first with ethyl acetate-hexane (1:2 v/v) and then with 1% methanol-chloroform as an eluant] gave 2'-O-(1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-N^2-(N,N-dimethylamino)methylene-3-[2-(4-nitrophenyl)-ethyl]-L-biopterin (7) [a pale yellow crystalline powder, m.p. 166-167°C (from AcOEt-hexane), 71% overall yield from **5**; for ^{1}H-NMR data, see Table 1] and 1',2'-di-O-glucopyranosyl compound **8** [a pale yellow crystalline powder, m.p. 134-135°C (from AcOEt-hexane), 8% overall yield].

Removal of the phthaloyl group of the above mono-glucopyranosyl biopterin 7 with 40% methylamine-methanol (1:5 v/v) at room temperature for 16 h, followed by acetylation with acetic anhydride in pyridine at room temperature for 24 h, afforded 1'-O-acetyl-N^2-acetyl-2'-(2-acetamido-1,3,-4,6-tetra-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-[2-(4-nitrophenyl)ethyl]-L-biopterin (9) [pale yellow microcrystals, m.p. 84-85°C (from AcOEt-hexane), 85% overall yield from 7; for ^{1}H-NMR data, see Table 1]. This compound was treated with 25% aqueous ammonium hydroxide-methanol (2:5 v/v) at room temperature for 24 h, to cleave all O-acetyl and N^2-acetyl groups, and then the 3-NPE group was removed by the treatment with DBU-DMF (1:10 v/v) at room temperature for 24 h (10), thus providing limipterin (3) [a pale orange crystalline power, m.p. 190-195°C (decomp., from water-2-propanol), 97% overall yield from 9). The spectral data of the synthetic compound **3** were identical in all respects with those of the natural product (6).

Acknowledgments

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