Synthesis of D-Glucopyranosyl Cholestan-3β-yl Glutamate Derivatives

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Preferential formation of 1-(cholestan-3β-yl) *N*-CBZ-L-glutamate (3) or 5-(cholestan-3β-yl) *N*-CBZ-L-glutamate (4) was obtained when dicyclohexylamine or 4-(dimethylamino)pyridine was used as a basic catalyst for ester formation, respectively. Each glutamate was converted to an anomeric mixture of glucose derivatives: 1-(cholestan-3β-yl) 5-(2″,3″,4″,6″-tetra-*O*-benzyl-α- and -β-D-glucopyranosyl) *N*-CBZ-L-glutamates (7 and 8) or 5-(cholestan-3β-yl) 1-(2″,3″,4″,6″-tetra-*O*-benzyl-α- and -β-D-glucopyranosyl) *N*-CBZ-L-glutamates (9 and 10), using 2″,3″,4″,6″-tetra-*O*-benzyl-α-D-glucopyranose (11). After chromatographic separation of these isomers, their structures were determined by field desorption mass and nuclear magnetic resonance spectrometries.

Keywords glycoconjugate; ester; *N*-CBZ-L-glutamic acid anhydride; cholestanol

Recently the roles of various natural glycoconjugates *in vivo* have been clarified,[1] and consequently the biological activity of synthetic glycoconjugates has aroused much interest.[2] Synthetic sialosyl compounds have found to show biological activity, although they are simpler molecules than natural glycoconjugates.[3] From a practical point of view, the use of relatively low-molecular-weight substances that can be synthesized from common and inexpensive starting materials is desirable. Amphophilic substances derived from cholestanol, glutamic acid and glucose may be candidate esters fulfilling these requirements. As an initial trial, diester derivatives of *N*-carbobenzoxy L-glutamic acid (*N*-CBZ-L-glutamic acid (**7**, **8**, **9** and **10**) were synthesized and their chemical properties as well as nuclear magnetic resonance (NMR) spectra were examined.

Results and Discussion

Commercially available *N*-CBZ-L-glutamic acid was converted to an acid anhydride, *N*-CBZ-L-glutamic acid anhydride (**1**), by the 1,3-dicyclohexylcarbodiimide (DCC) method.[4] The compound (**1**) was reacted with cholestanol (**2**) in benzene, followed by chromatographic separation of 1-(cholestan-3β-yl) *N*-CBZ-L-glutamate (**3**),[5] its 5-isomer (**4**) and 1,5-(dicholestan-3β-yl) *N*-CBZ-L-glutamate (**5**). The **3**-to-**4** ratio was 1.3 : 1, and the yields of **3** plus **4** and that of **5** were 68% and 2%, respectively (Chart 1). All the carbons and protons of **3**, **4** and **5** were assigned by distortionless enhancement by polarization transfer (DEPT) and ^1H–^1H correlation spectroscopy (^1H–^1H COSY), respectively. Thus **3**, **4** and **5** were identified as esters from their NMR spectra[6] (Table Ia—b). This method, however, does not make clear whether the ester is the 1-ester or the 5-ester. The position of esterification was determined by considering the ^{13}C–^1H correlation in the NMR spectra taken in $CDCl_3$ solution. One of the esters shows the ester-bond and carboxylic group carbon signals at 171.3 and 177.5 ppm, respectively (Table Ib). Selective irradiation of the 2′ Proton (long-range selective proton decoupling)[7] of the glutamic acid moiety at 4.37 ppm (Table Ia) sharpens the ester-carbon peak at 171.3 ppm. This fact indicates that the compound has the ester bond at the C-1 position, leading to its identification as **3**. The other ester was identified as **4** through similar procedures.

In an attempt to obtain preferential synthesis of one

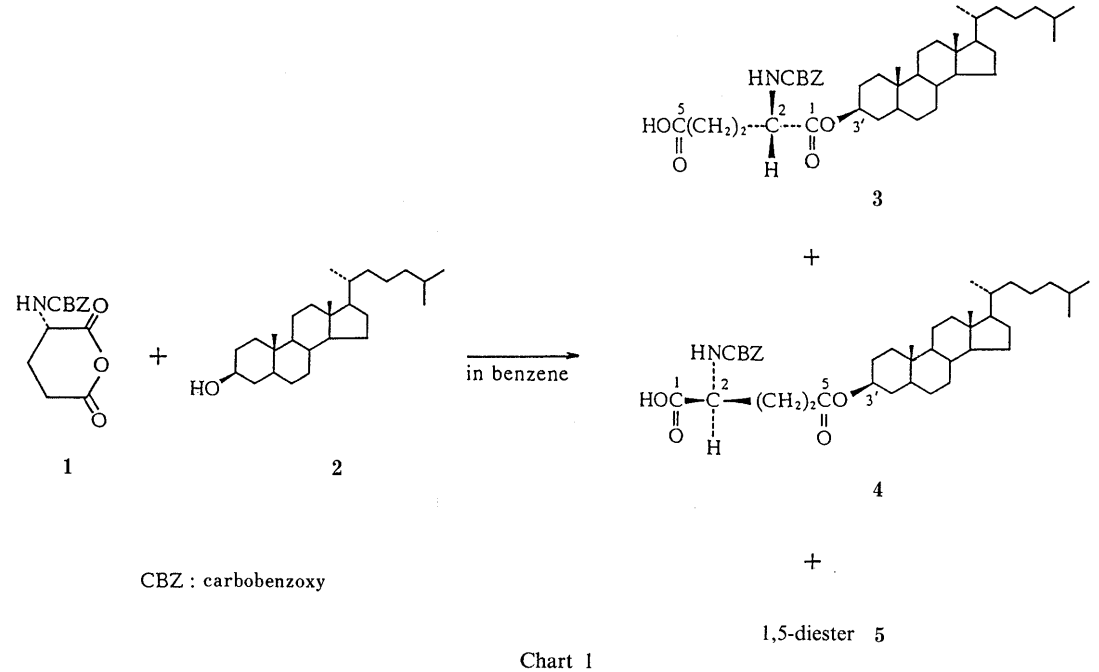

Chart 1

isomer in a good yield, several bases were examined for catalytic activity. The total yields of **3** and **4** and the ratios of **3** to **4** for each catalyst are shown in Table II. Triethylamine (TEA) did not increase the yield of **3**, while dicyclohexylamine (DCHA) did increase it. On the other hand, 4-(dimethylamino)pyridine (DMAP) gave more **4**.

Both isomers were separately converted to acid chlorides,[8] which were refluxed in the presence of pyridine with 2″,3″,4″,6″-tetra-*O*-benzyl-α-D-glucopyranose (**11**)[9] in dichloromethane (Chart 2). Each reaction mixture was loaded onto a silica gel column (Kieselgel 60, Merck) and eluted with a mixture of chloroform, *n*-hexane and 2-propanol (50 : 49 : 10). The first fraction containing these esters of **3** was rechromatographed under the conditions described above, using a mixture of benzene and 2-butanone (3 : 1) as an eluent. The ^{1}H-NMR spectrum of the separated material showed that it was a mixture (1 : 1.1) of **7** and **8** (Table IIIa). The yield of the mixture was about 30%. Preparative silica gel thin layer chromatography (p-TLC) was next applied to separate the α-glycoside derivative (**7**) from the β-glycoside derivative (**8**) using a mixture of dichloromethane and acetone (80 : 1) at 6 °C. Through the same procedure described above, a mixture (1 : 1.1) of α-glycoside derivative (**9**) and β-glycoside derivative (**10**) was obtained from **4** in 38% yield. These products were separated by the same p-TLC technique. The ^{1}H-NMR spectra of **9** and **10** indicate no epimerization at the C-2 position in the course of the synthesis (Table IIIb). Two spots other than those of **9** and **10** were found on the p-TLC plate but were not identified due to their small quantities. The synthesized compounds **7**, **8**, **9** and **10** were identified

TABLE Ia. ^{1}H-NMR Chemical Shifts (δ ppm) of **3**, **4** and **5** in Chloroform-*d*

Compound	Chemical shifts ppm		
	3	**4**	**5**
CBZ-glutamic acid moiety			
2-H	4.37	4.40	4.34
3-Ha	2.20	2.23	2.15
3-Hb	1.97	2.03	1.95
4-Ha	2.42	2.39	2.35 (2H)
4-Hb	2.45	2.44	
CONH	5.47 (d)	5.56 (d)	5.39 (d)
$-CH_2-\langle\bigcirc\rangle$	5.11 (2H)	5.11 (2H)	5.10 (2H)
Cholestanol moiety			
3′-H	4.74	4.69	4.68, 4.72
18′-Me	0.65 (s)	0.64 (s)	0.65 (s)
19′-Me	0.82 (s)	0.82 (s)	0.81 (s)
21′-Me	0.90 (d)	0.90 (d)	0.90 (d)
26′-Me	0.86 (d)	0.86 (d)	0.86 (d)
27′-Me	0.86 (d)	0.86 (d)	0.86 (d)

TABLE Ib. ^{13}C-NMR Chemical Shifts (δ ppm) of **3**, **4** and **5** in Chloroform-*d*

Compound	Chemical shifts ppm		
	3	**4**	**5**
CBZ-glutamic acid moiety			
C-1	171.3	175.5	171.4
C-2	53.3	53.4	53.6
C-3	27.8	27.3	27.9
C-4	29.8	30.8	30.7
C-5	177.5	172.7	172.3
CONH	156.1	156.2	155.9
$-CH_2-\langle\bigcirc\rangle$	67.1	67.2	67.0
Cholestanol moiety			
C-3′	75.6	74.5	75.3, 74.2

TABLE II. Esterification of CBZ-L-glutamic Acid Anhydride with Cholestanol in Benzene

Catalyst	Temperature (°C)	Reaction time (h)	Total yield (%)	Ratio of **3**:**4**
None	Reflux	3.0	68	1.3 : 1
TEA	Reflux	3.0	60	1.5 : 1
TEA	19	3.0	63	1.9 : 1
TEA	6	3.0	45	1.6 : 1
DCHA	20	3.0	62	2.7 : 1
DCHA	6	3.0	50	2.5 : 1
DAP	19	3.0	54	1 : 5
DAP	6	2.5	45	1 : 5

DAP: 4-(dimethylamino)pyridine.

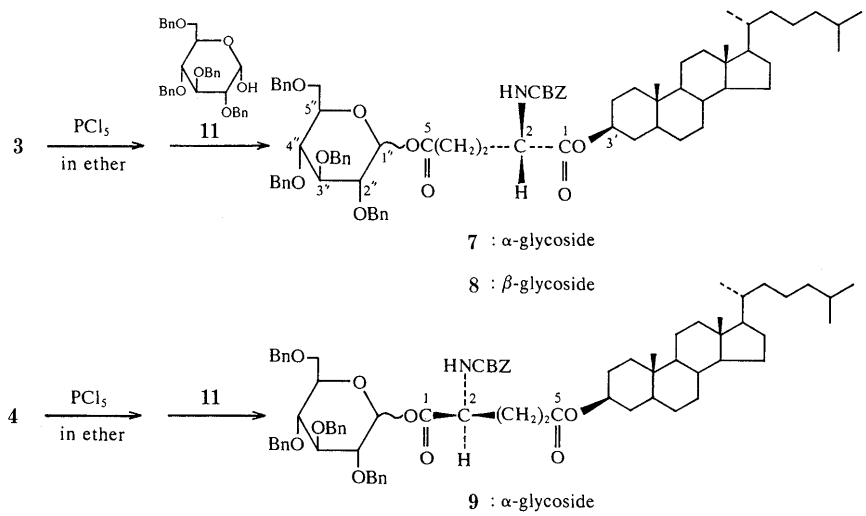

Chart 2

TABLE IIIa. Chemical Shifts (δ ppm) and Coupling Constants (Hz) of Anomeric Proton and Chemical Shifts (δ) of Glucose Carbons in **7**, **8**, **9** and **10**

Compound	^{1}H-NMR		^{13}C-NMR					
	H-1″	$J_{1,2}$	C-1″	C-2″	C-3″	C-4″	C-5″	C-6″
7	6.36	3.5	90.4	78.8	81.7	76.9	73.0	68.1
8	5.60	7.9	94.3	81.1	84.7	77.2	75.5	68.2
9	6.41	3.4	91.6	78.9	81.6	76.7	73.4	67.9
10	5.63	7.6	94.9	80.4	84.7	77.2	75.8	68.1

TABLE IIIb. ^{1}H-NMR Chemical Shifts (δ ppm) of **7**, **8**, **9** and **10** in Chloroform-*d*

Compound	Chemical shifts ppm			
	7	**8**	**9**	**10**
Glucose moiety				
2″-H	3.67	3.57[a)]	3.69	3.60
3″-H	3.90	3.70[a)]	3.87	3.70
4″-H	3.73	3.75[a)]	3.75	3.73
5″-H	3.84	3.53[a)]	3.87	3.57
6″-H	3.64	3.71[a)]	3.64	3.70
Glutamic acid moiety				
2-H	4.36	4.33	4.45	4.39
3-Ha	1.95	1.95	2.00	1.96
3-Hb	2.22	2.22	2.15	2.19
4-H_2	2.48	2.48	2.36	2.36
CONH	5.39	5.34	5.44	5.36
$-CH_2-$⟨◯⟩	5.07, 5.11	5.11, 5.06	5.09 (2H)	5.08 (2H)
Cholestanol moiety				
3′-H	4.73 (dt)	4.73 (1H)	4.62 (1H)	4.65 (1H)
18′-Me	0.64	0.65	0.64	0.64
19′-Me	0.78	0.78	0.70	0.77
21′-Me	0.90 (d)	0.90 (d)	0.90 (d)	0.90 (d)
26′-Me	0.86 (d)	0.86 (d)	0.86 (d)	0.86 (d)
27′-Me	0.86 (d)	0.86 (d)	0.87 (d)	0.87 (d)

a) These data are uncertain due to the presence of a small amount of **7** as a contaminant.

by ^{1}H- and ^{13}C-NMR, DEPT, ^{1}H–^{1}H COSY, ^{1}H detected heteronuclear multiple quantum coherence (HMQC),[10] and ^{1}H detected heteronuclear multiple bond connectivity (HMBC)[10] spectroscopies. These results are listed in Tables IIIa and IIIb. The chemical shifts and J values of the proton in the anomeric position of the glucose moiety of **7** and **8** are 6.36 ppm ($J_{1,2}=3.5$ Hz) and 5.60 ppm ($J_{1,2}=7.9$ Hz), respectively. These values show that **7** and **8** have α- and β-glycoside bonds, respectively (Table IIIa). Similarly the derivatives of **4**, *i.e.* **9** and **10**, were confirmed to have α- and β-glycoside bonds. The ^{13}C- and ^{1}H-NMR signals are consistent with these assignments (Table IIIa).

Based on the foregoing spectroscopic data and analytical results described below, it is concluded that two pairs of compounds with cholestanol, glutamic acid and glucose moieties, 1-(cholestan-3β-yl) 5-(2″,3″,4″,6″-tetra-*O*-benzyl-α- and -β-D-glucopyranosyl)*N*-CBZ-L-glutamates (**7** and **8**), and 1-(2″,3″,4″,6″-tetra-*O*-benzyl-α- and -β-D-glucopyranosyl) 5-(chloestan-3β-yl)*N*-CBZ-L-glutamates (**9** and **10**), were synthesized.

Experimental

Melting points were recorded without correction on a Yanagimoto micromelting point apparatus, model MP-S3. Optical rotations were measured with a JASCO polarimeter, model DIP-140. Field desorption mass spectra (FD-MS), fast atom bombardment MS (FAB-MS), ultraviolet (UV) and infrared (IR) spectra were measured with JEOL JMS-DX300, Hitachi 330, and Perkin-Elmer 983 spectrometers, respectively. ^{1}H- and ^{13}C-NMR data were recorded on Varian XL-400 and JEOL JNM-GSX500 spectrometers. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) in $CDCl_3$ as an internal standard. TLC was performed using precoated Kieselgel 60F_{254} plates (Merck) and solvents of the following compositions: solvent I, $CHCl_3$–*n*-hexane-2-propanol (50 : 49 : 10); solvent II, benzene–2-butanone (3 : 1). Spots on the plates were detected under UV light or by spraying 5% sulfuric acid solution followed by heating the plates. Column chromatography was carried out on Kieselgel 60 (70—230 and 230—400 mesh, Merck) using the same solvents as for TLC.

1-(Cholestan-3β-yl)*N*-CBZ-L-glutamate (3) and 5-(Cholestan-3β-yl)*N*-CBZ-L-glutamate (4) (i) Synthesis without Catalyst: Compound **1** (3.3 g, 12.5 mmol) was added to a dried benzene solution (20 ml) of **2** (3.9 g, 10 mmol) and the reaction mixture was refluxed for 3 h under an argon atmosphere. The reaction mixture was evaporated *in vacuo* to a syrup, which was dissolved in $CHCl_3$, washed with slightly acidic cold water, dried over Na_2SO_4 and concentrated under reduced pressure to a syrup. This syrup was charged on a silica gel column and eluted with $CHCl_3$–*n*-hexane-2-propanol (50 : 49 : 10) (solvent I) to give two monoester fractions. The first fraction contained **3** (2.5 g, 38%), **3**: mp 104—106 °C. IR ν_{max}^{KBr} cm^{-1}: 3340, 2940, 1710, 1525, 1260—1050. $[\alpha]_D^{20}$ +11.22° ($c=2$, $CHCl_3$). The NMR ($CDCl_3$) data are summarized in Tables Ia and Ib. MS (FD) *m/z*: 652 ($M^+ + 1$). UV $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 244 (2.28), 251 (2.35), 256 (2.42), 261 (2.35). *Anal.* Calcd for $C_{40}H_{61}NO_6$: C, 73.68, H, 9.44, N, 2.15. Found: C, 73.50, H, 9.41, N, 2.24.

The second fraction gave **4** (1.9 g, 30%). **4**: mp 94—95 °C. IR ν_{max}^{KBr} cm^{-1} 3350, 2930, 1725, 1530, 1210—1050. $[\alpha]_D^{20}$ +11.72° ($c=2$, $CHCl_3$). The NMR ($CDCl_3$) data are summarized in Tables Ia and Ib. MS (FD) *m/z*: 652 ($M^+ + 1$). UV $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 251 (2.32), 256 (2.35), 264 (2.27). *Anal.* Calcd for $C_{40}H_{61}NO_6$: C, 73.68, H, 9.44, N, 2.15. Found: C, 73.68, H, 9.47, N, 2.01.

A fraction containing **5** (0.13 g, 2%), was also obtained. **5**: mp 138—139 °C; IR ν_{max}^{KBr} cm^{-1}, 3290, 2930, 1730, 1522, 1204, 1075, 1051, 1000. NMR ($CDCl_3$) data are summarized in Tables Ia and Ib. MS (FD) *m/z*: 1023 ($M^+ + 1$). *Anal.* Calcd for $C_{67}H_{107}NO_6$: C, 78.69, H, 10.54, N, 1.37. Found: C, 78.45, H, 10.46, N, 1.65.

(ii) With Catalyst: Compound **1** (3.3 g, 12.5 mmol) was added to a dried benzene solution (20 ml) of **2** (3.9 g, 10 mmol) under an argon atmosphere with a catalyst (12.5 mmol) such as TEA, DCHA or DMAP. Each mixture was stirred for 3 h at various temperatures as shown in Table II and evaporated *in vacuo* to a syrup. The residue was dissolved in $CHCl_3$, poured into ice water and then extracted with $CHCl_3$. The extract was washed successively with 0.5 N HCl, ice water and 0.5 N aqueous $NaHCO_3$ solution, dried over Na_2SO_4, and concentrated to a syrup under reduced pressure. The procedure for purification of **3**, **4**, and **5** was the same as that described in (i) above.

1-(Cholestan-3β-yl) 5-(2″,3″,4″,6″-tetra-*O*-benzyl-α- and -β-D-glucopyranosyl)*N*-CBZ-L-glutamates (7) and (8) To a suspension of finely pulverized PCl_5 (170 mg, 0.82 mmol) in 10 ml of anhydrous ether was added **3** (500 mg, 0.77 mmol), and the mixture was stirred for 1 h at −8 °C under an argon atmosphere. The ether was evaporated under reduced pressure, then the residue was dissolved in 8 ml of CH_2Cl_2, and 0.07 ml (0.87 mmol) of pyridine was added. A previously prepared CH_2Cl_2 solution (8 ml) of 2″,3″,4″,6″-tetra-*O*-benzyl-α-D-glucopyranose (**11**) (330 mg, 0.62 mmol) was slowly added to the CH_2Cl_2–pyridine solution, then the mixture was refluxed for 2 h and allowed to stand overnight at room temperature. After being dried under reduced pressure and diluted with $CHCl_3$, the reaction mixture was poured into cold water. The $CHCl_3$ layer was washed successively with 0.1 N HCl, water, 0.1 N $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated to dryness. The product was roughtly purified by silica gel (Kieselgel 60, 70—230 mesh) column chromatography using solvent I descrived above, and further purified on a second silica gel column (Kieselgel 60, 230—400 mesh) using solvent II. The fraction containing **7** and **8** was concentrated to a syrup and then separated by *p*-TLC at 6 °C using CH_2Cl_2–acetone solution (80 : 1, $Rf=0.31$ for **7** and $Rf=0.23$ for **8**). Yields of **7** and **8** were 95 mg (14%) and 110 mg (16%), respectively. MS (FD) *m/z*: 1174 ($M^+ + 1$). **7**: IR ν_{max}^{KBr} cm^{-1}: 2931, 2865, 1739, 1726, 1496, 1454, 1210, 1155, 1072, 1027, 734, 696. UV $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 251 (2.89), 256 (2.95), 262 (2.88).

Anal. Calcd for $C_{74}H_{95}NO_{11}$: C, 75.66, H, 8.16, N, 1.19. Found: C,

75.59, H, 8.25, N, 1.12. **8**: IR ν_{max}^{KBr} cm^{-1}: 2933, 2868, 1726, 1496, 1467, 1210, 1153, 1074, 1027, 734, 698. UV $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 251 (2.82), 256 (2.98), 263 (2.84). *Anal.* Calcd for $C_{74}H_{95}NO_{11}$: C, 75.66, H, 8.16, N. 1.19. Found: C, 75.73, H, 8.27, N, 1.32.

1-(2″,3″,4″,6″-Tetra-*O*-benzyl-α- and -β-D-glucopyranosyl) 5-(cholestan-3β-yl) *N*-CBZ-L-glutamates (9) and (10) Except for the starting material **4**, the same preparative procedures as described above were applied to this compound. *Rf*=0.41—0.46 for **9** and *Rf*=0.36 for **10**. The yields of **9** and **10** were 130 mg (18%) and 140 mg (20%), respectively. Their IR and UV data were similar to those of **7** and **8**, **9**: *Anal.* Calcd for $C_{74}H_{95}NO_{11}$: C, 75.66, H. 8.16, N, 1.19. Found: C, 75.88, H, 8.35, N, 1.20. **10**: *Anal.* Calcd for $C_{74}H_{95}NO_{11}$: C, 75.66, H, 8.16, N, 1.19. Found: C, 75.75, H, 8.05, N, 1.28.

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