Synthesis and Activity of Antitumor Agents, Methyl [*N'*-(2-Chloroethyl)-*N'*-nitrosoureido]deoxygentiobiosides

Yoshimasa FUKUDA and Tetsuo SUAMI*

Department of Applied Chemistry, Faculty of Engineering, Keio University, Hiyoshi, Kohoku-ku, Yokohama 223

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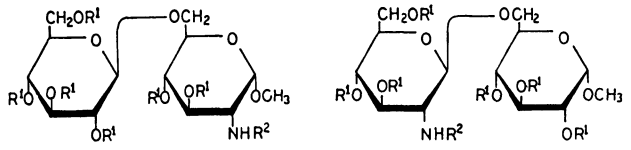
Synopsis. Chlorozotocin[1-4] and GANU[5-7] are nitrosoureido derivatives of D-glucose, exhibiting remarkable antitumor activities against leukemia L1210 in mice. We wish to report a synthesis of the derivatives of gentiobioside.

Tritylation of methyl 2-(benzyloxycarbonylamino)-2-deoxy-α-D-glucopyranoside,[8] followed by acetylation, afforded methyl 3,4-di-*O*-acetyl-2-(benzyloxycarbonylamino)-2-deoxy-6-*O*-trityl-α-D-glucopyranoside. Detritylation of the compound gave methyl 3,4-di-*O*-acetyl-2-(benzyloxycarbonylamino)-2-deoxy-α-D-glucopyranoside. Condensation of the compound with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[9] yielded methyl hexa-*O*-acetyl-2-(benzyloxycarbonylamino)-2-deoxy-α-gentiobioside, which was further converted into methyl 2-(benzyloxycarbonylamino)-2-deoxy-α-gentiobioside (**1**). Catalytic hydrogenolysis of **1**, followed by addition with 2-chloroethyl isocyanate, gave the 2-[*N'*-(2-chloroethyl)ureido] compound (**2**). Nitrosation of **2** with N_2O_3 gave the *N'*-nitroso compound (**3**).

Starting from methyl hexa-*O*-acetyl-2'-(benzyloxycarbonylamino)-2'-deoxy-α-gentiobioside (**4**), methyl 2'-[*N'*-(2-chloroethyl)-*N'*-nitrosoureido]-2'-deoxy-α-gentiobioside (**7**) was obtained by analogous reactions. The position of the nitroso group was demonstrated by ammonia-induced degradation[10] of **3** and **7**.



1, R^1= H, R^2= Cbz
2, R^1= H, R^2= $CONHCH_2CH_2Cl$
3, R^1= H, R^2= $CON(NO)CH_2CH_2Cl$
4, R^1= Ac, R^2= Cbz
5, R^1= H, R^2= Cbz
6, R^1= H, R^2= $CONHCH_2CH_2Cl$
7, R^1= H, R^2= $CON(NO)CH_2CH_2Cl$

Scheme 1.

TABLE 1. ANTITUMOR EFFECT OF **3** AND **7** ON LEUKEMIA L1210[a]

Dose mg/kg	Compound **3** ILS[b] (%)	Compound **3** 60-d survivors	Compound **7** ILS[b] (%)	Compound **7** 60-d survivors
56	25.7	0/5	28.2	0/5
48	42.9	0/5	30.8	0/5
40	226.3	1/5	202.6	1/5
32	118.4	0/5	466.7	3/5
16	84.2	0/5	241.7	1/5
8	39.5	0/5	94.4	0/5
4	28.9	0/5	61.1	0/5

a) Male BDF_1 hybrid mice were inoculated intraperitoneally with 10^6 cells of lymphoid leukemia L1210. Intraperitoneal injection of a compound was begun 24 h after the inoculation and performed once a day for 3 d. b) Percentage increase in life span of treated animals compared with control tumor bearers [100(T/C-1)].

Compounds **3** and **7** were subjected to determination of antitumor activity against leukemia L1210 in mice by the established protocol[11] (Table 1). Both compounds are highly active, **7** seeming to be more effective than **3**.

Experimental

Methyl 3,4-Di-O-acetyl-2-(benzyloxycarbonylamino)-2-deoxy-6-O-trityl-α-D-glucopyranoside. Trityl chloride (20.0 g) was added to a stirred solution of methyl 2-(benzyloxycarbonylamino)-2-deoxy-α-D-glucopyranoside[8] (10.0 g) in pyridine (120 ml), acetic anhydride (50 ml) being added after 24 h. After 16 h, the mixture was poured into ice cold water. The product precipitated was collected and recrystallized from ethanol, giving 8.7 g (44%) of the product, mp 90—91 °C, $[\alpha]_D^{20}$ +85° (*c* 1.2, chloroform).

Found: C, 69.67; H, 6.18; N, 2.30%. Calcd for $C_{38}H_{39}NO_9$: C, 69.82; H, 6.01; N, 2.14%.

Methyl 3,4-Di-O-acetyl-2-(benzyloxycarbonylamino)-2-deoxy-α-D-glucopyranoside. Acetic acid (0.3 ml) containing HBr was added under ice cooling with agitation to a solution of the trityl derivative (1.0 g) in glacial acetic acid (4 ml). After 1 min, the mixture was quenched in ice cold water and extracted with $CHCl_3$. The organic layer was washed with $NaHCO_3$ solution and water, and concentrated. Recrystallization of the residue from ether–petroleum ether gave 294 mg (37%) of the product, mp 112—113 °C, $[\alpha]_D^{20}$ +110° (*c* 0.8, chloroform). ^1H NMR (60 MHz, $CDCl_3$): δ 1.88 (s, 3, OAc), 2.03 (s, 3, OAc), 3.37 (s, 3, OCH_3), 4.76 (d, 1, J=4 Hz, H-1).

Found: C, 55.59; H, 6.14; N, 3.62%. Calcd for $C_{19}H_{25}NO_9$; C, 55.47; H, 6.13; N, 3.41%.

Methyl 2-(Benzyloxycarbonylamino)-2-deoxy-α-gentiobioside (1). A solution of the 3,4-di-*O*-acetyl derivative (995 mg) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[9] (2.0 g) in benzene (40 ml) was heated under reflux in the presence of $Hg(CN)_2$ (1.6 g) and Drierite (2.0 g) for 20 h. The mixture was diluted with $CHCl_3$ (60 ml) and filtered. The filtrate was washed with brine and water, and concentrated. The residue was purified on a silica gel column using 2 : 5 (v/v) acetone–benzene. Fractions (R_f 0.56 on TLC) were concentrated. The residue was deacetylated in methanolic ammonia and recrystallized from methanol to give 303 mg (42%) of **1**, mp 235—236 °C, $[\alpha]_D^{20}$ +50.9° (*c* 1.0, water).

Found: C, 51.78; H, 6.41; N, 2.75%. Calcd for $C_{21}H_{31}NO_{12}$: C, 51.53; H, 6.38; N, 2.86%.

Methyl 2-[N'-(2-Chloroethyl)ureido]-2-deoxy-α-gentiobioside (2). Compound **1** (850 mg) was hydrogenated in methanol (25 ml) with Pd black under H_2 (2.7 kg/cm²) for 2.5 h. The product was treated with 2-chloroethyl isocyanate (0.2 ml) in methanol (15 ml) overnight and the mixture was concetnrated. The residue was triturated in ethanol-ethyl acetate to give 620 mg (72%) of **2**, mp 151—152 °C (dec), $[\alpha]_D^{20}$ +54.2° (*c* 1.0, water).

Found: C, 41.74; H, 6.21; N, 5.88; Cl, 7.49%. Calcd for

$C_{16}H_{29}N_2ClO_{11}$: C, 41.70; H, 6.34; N, 6.08; Cl, 7.69%.

Methyl 2-[N'-(2-Chloroethyl)-N'-nitrosoureid]-2-deoxy-α-gentiobioside (3). N_2O_3 was bubbled into a suspension of **2** (200 mg) in acetone (5 ml) at 0 °C for 3 min. The solution was concentrated and the residue was washed with hexane-diisopropyl ether to give 153 mg (73%) of **3**, mp 67—68 °C (dec), $[\alpha]_D^{22}$ +39.4° (*c* 1.0, water).

Found: C, 39.21; H, 5.99; N, 8.58; Cl, 7.24%. Calcd for $C_{16}H_{28}N_3ClO_{12}$: C, 38.97; H, 5.77; N, 8.92; Cl, 6.92%.

Degradation of **3** in methanolic ammonia gave methyl 2-deoxy-2-ureido-α-gentiobioside, which was identified by TLC and by comparison of its IR spectrum [(KBr) 1650, 1610, 1565 cm^{-1}] with that of an authentic sample prepared by a definite route.

Methyl 2,3,3',4,4',6'-Hexa-O-acetyl-2'-(benzyloxycarbonylamino)-2'-deoxy-α-gentiobioside (4). A mixture of methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranoside[12] (1.7 g) and 3,4,6-tri-*O*-acetyl-2-(benzyloxycarbonylamino)-2-deoxy-α-D-glucopyranosyl bromide[13] (2.9 g) in benzene (35 ml) was heated under reflux for 2 h in the presence of $Hg(CN)_2$ (2.7 g) and Drierite (3.5 g). The mixture was diluted with $CHCl_3$ and filtered. The filtrate was washed with brine and water, and concentrated. Recrystallization of the residue from ethanol gave 943 mg (26%) of **4**, mp 162—163 °C, $[\alpha]_D^{25}$ +68.7° (*c* 1.0, chloroform).

Found: C, 53.29; H, 5.96; N, 1.99%. Calcd for $C_{33}H_{43}NO_{18}$: C, 53.43; H, 5.84; N, 1.89%.

Methyl 2'-(Benzyloxycarbonylamino)-2'-deoxy-α-gentiobioside (5) A solution of **4** (195 mg) in methanolic ammonia was settled overnight and concentrated. Recrystallization of the residue from methanol afforded 91 mg (72%) of **5**, mp 212—214 °C, $[\alpha]_D^{20}$ +37.6° (*c* 1.0, water)

Found: C, 51.32; H, 6.30; N, 2.83%. Calcd for $C_{21}H_{31}NO_{12}$: C, 51.53; H, 6.38; N, 2.86%.

Methyl 2'-[N'-(2-Chloroethyl)ureido]-2'-deoxy-α-gentiobioside (6). Compound **5** (393 mg) was hydrogenated in a similar way to that described above, and the product was treated with 2-chloroethyl isocyanate to give 172 mg (47%) of **6**, mp 189—190 °C (dec), $[\alpha]_D^{20}$ +29.9° (*c* 0.6, water).

Found: C, 41.74; H, 6.21; N, 5.88, Cl, 7.49%. Calcd for $C_{16}H_{29}N_2ClO_{11}$: C, 41.70; H, 6.34; N, 6.08; Cl, 7.69%.

Methyl 2'-[N'-(2-Chloroethyl)-N'-nitrosoureido]-2'-deoxy-α-gentiobioside (7). Compound **6** (200 mg) was nitrosated in a similar way to that described above to give 198 mg (97%) of **7**, mp 116 °C (dec), $[\alpha]_D^{20}$ +27.1° (*c* 1.0, water).

Found: C, 39.53; H, 5.99; N, 8.24; Cl, 7.28%. Calcd for $C_{16}H_{28}N_3ClO_{12}$: C, 39.21; H, 5.76; N, 8.58; Cl, 7.24%.

Degradation of **7** in methanolic ammonia gave the 2'-ureido derivative as described above.

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