ELSEVIER

Carbohydrate Research 290 (1996) 225–232

CARBOHYDRATE RESEARCH

Note

Synthesis of glycolipids: dialkyl *N*-[*N*-(4-lactonamidobutyl)succinamoyl]-L-glutamates

Zhongzhi Zhang [a,*], Kimitoshi Fukunaga [a], Yoshiaki Sugimura [a], Katsumi Nakao [a], Toshimi Shimizu [b]

[a] *Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering, Yamaguchi University, Tokiwadai 2557, Ube 755, Japan*

[b] *National Institute of Materials and Chemical Research, Agency of Industrial Science and Technology, MITI, 1–1 Higashi, Tsukuba, Ibaraki 305, Japan*

Received 2 October 1995; accepted 30 April 1996

Keywords: Glycolipids; Galactolipids; Oligosaccharide; Peptide; Lipids for modified enzyme

Lipase-catalyzed transesterification in organic solvents has proved to be more effective, in many instances, than the conventional esterification procedure [1]. It can be used for the production of optically active materials and for the resolution of racemic compounds. In order to utilize enzymatic catalysis effectively in organic solvents, it is necessary to avoid the deactivation or denaturation of enzymes. Two approaches have been successfully developed to reach this goal. In the first method, a section of amino acids in an enzyme is modified with an amphiphilic synthetic polymer, poly(ethylene glycol) [2]; in the second one, the surface of the enzyme is coated with surfactant [3]. These modified enzymes are almost entirely soluble in organic solvents, and show higher reaction rates compared to the powder enzyme [4]. In the former, some of the enzymatic activities are decreased due to polymer modification, and their preparation is more difficult than that of the latter. Goto et al. [5] have prepared several surfactants for modifying enzymes and have investigated the effects of coating surfactants on the enzyme activity. The stability and reactivity of the enzymes used are strongly dependent on the type and structure of the detergents. Nonionic detergents were considered to be the most useful for modification of enzymes. Dialkyl glucosylglutamates containing a

* Corresponding author.

PII S0008-6215(96)00140-1

monosaccharide [6,7] were used as the glycolipids in the research field of lipase coated with glycolipid in enzymatic catalysis. To broaden the scope of this promising methodology, the development of chemically defined glycolipids is very important. Furthermore, glycolipids containing an oligosaccharide as a polar head group are considered to be very useful lipids for the modification of enzymes [6]. The present investigation has been aimed at developing an efficient synthetic method to prepare a series of glycolipids which have not only an oligosaccharide but also two long alkyl chains.

1. Results and discussion

The general synthetic procedure for dialkyl *N*-[*N*-(4-lactonamidobutyl)succinamoyl]-L-glutamates **7a–d** (**a**: R = dodecyl, **b**: R = tetradecyl, **c**: R = hexadecyl, **d**: R = dodecyl) is shown in Scheme 1. The synthetic route includes lactonization of lactonic acid (**1** → **2**), aminolysis of the lactonolactone with 1,4-butanediamine (**2** → **3**), esterification of L-glutamic acid with various higher alkyl alcohols (**4** → **5a–d**), condensation of the dialkyl L-glutamate and succinic anhydride (**5a–d** → **6a–d**), and coupling of compounds **3** and **6** (**3** + **6a–d** → **7a–d**). Each of the glycolipids is an amphiphile consisting of a hydrophilic oligosaccharide and two hydrophobic long alkyl groups. The properties of the synthetic compounds are listed in Table 1. In order to synthesize **7**, two kinds of reagents were used. In one, the aminobutyl-lactonamide **3** and a carboxylic acid **6** bearing two long alkyl chains condensed under the action of the coupling reagent diethyl phosphorocyanidate (DEPC). In the other, the carboxyl group of **6** was activated with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide (DCC) [8]; reaction with **3** then gave **7** (see Expt. no. **7a-1** of Table 2). The results for the coupling reagents and the effects of the three kinds of solvent on **7a** are shown in Table 2. The yield of **7a** in the case of reagent DEPC was higher than that with the DCC method. In the method using DEPC, because **3** was not completely soluble in THF, the yield of **7a** in THF was lower than that in Me_2SO or DMF. The best yield of **7a** was obtained in DMF. Since the boiling point of Me_2SO is higher than that of DMF, the solvent must be removed at a higher temperature, and the purification of the desired products was therefore difficult. As shown in Table 1, the coupling of **3** and **6a–d** was conveniently achieved with moderate yields, under the action of DEPC. The addition of triethylamine to generate a carboxylate anion from the carboxylic acid may be essential to promote the reaction [9]. However, the coupling reagent DCC was less effective than DEPC in this reaction system.

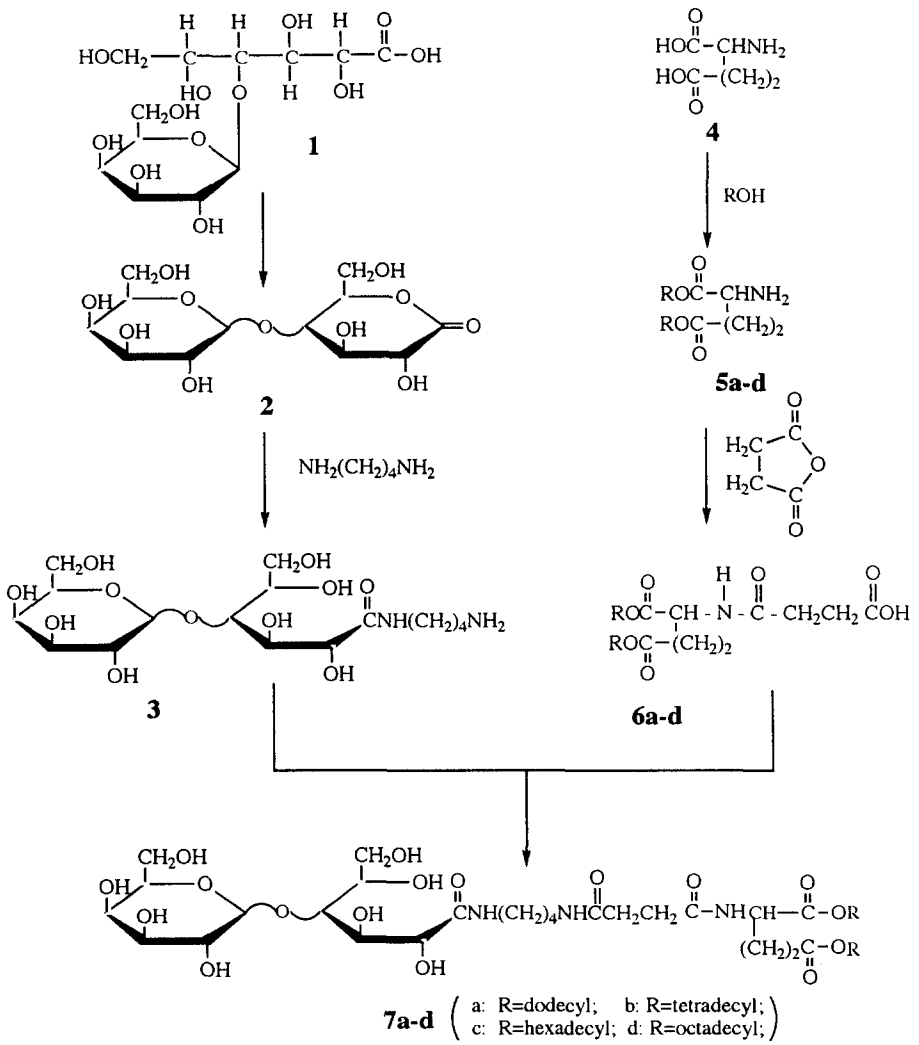
2. Conclusion

The glycolipids containing an oligosaccharide and two long alkyl chains have been prepared by a simple and efficient method. These glycolipids can be manufactured by this method on a large scale, when necessary. These glycolipids will be particularly useful for enzyme modification, to give organic-solvent-soluble reagents of lipase [10].

3. Experimental

General.—IR spectra were determined on a Hitachi IR-251 spectrophotometer. NMR spectra were recorded in $CDCl_3$ or Me_2SO-d_6 solution using a JEOL-EX-270 instrument with reference to tetramethylsilane as an internal standard. The optical rotations were measured on a HORIBA SEPA-200 high-sensitivity polarimeter. Melting points (Pyrex capillary) are uncorrected.

Materials.—Lactonic acid (lactobionic acid) and 1,4-butanediamine were purchased from Tokyo Kasei Co. Ltd., and succinic anhydride and diethyl phosphorocyanidate



Scheme 1.

Table 1
Properties of synthetic compounds

Compound	Formula	Mp (°C)	Yield [a] (%)	$[\alpha]_D^{25}$ (deg)	Analytical data (%)		
					Found (Calcd)		
					C	H	N
6a	$C_{33}H_{61}NO_7$	56–58	64	–	67.50	10.69	2.53
					(67.88	10.53	2.40)
6b	$C_{37}H_{69}NO_7$	66–68	61	–	68.83	11.17	2.21
					(69.44	10.87	2.19)
6c	$C_{41}H_{77}NO_7$	76–77	84	–	70.87	11.62	1.97
					(70.75	11.15	2.01)
6d	$C_{45}H_{85}NO_7$	79–80	87	–	72.03	12.09	1.86
					(71.85	11.39	1.86)
7a	$C_{49}H_{91}N_3O_{17}$	154–156	44	+8.7 (*c* 1.0) [b]	59.81	9.12	4.16
					(59.91	9.23	4.23)
7b	$C_{53}H_{99}N_3O_{17}$	163–165	43	+7.2 (*c* 1.0) [b]	60.75	9.72	3.84
					(60.60	9.50	4.00)
7c	$C_{57}H_{107}N_3O_{17}$	150–152	40	+6.3 (*c* 0.8) [c]	61.56	9.72	3.76
					(61.87	9.75	3.80)
7d	$C_{61}H_{115}N_3O_{17}$	123–125	37	+5.6 (*c* 0.5) [d]	63.27	10.02	3.73
					(63.02	9.97	3.62)

[a] Described in Experimental.
[b] In Me_2SO.
[c] In 20:4:1 Me_2SO–$CHCl_3$–MeOH.
[d] In 5:4:1 Me_2SO–$CHCl_3$–MeOH.

(DEPC) from Wako Co. Ltd.. Tetrahydrofuran (THF) was dried over Na metal under reflux and distilled just before use. Dimethylformamide (DMF) was distilled under vacuum before use.

Table 2
The investigation of the coupling reagents and the effect of reaction solvents on glycolipid **7a**

Expt no.	**6a** (mmol)	HSI [a] (mmol)	DSS [b] (mmol)	Me_2SO (mL)	DMF (mL)	THF (mL)	DEPC [c] (mmol)	TEA [d] (mL)	**3** (mmol)	Me_2SO (mL)	Yield (%)
7a-1 [e]	1.72	2.57	2.57	4	–	–	–	–	1.72	3.5	15
7a-2 [f]	1.50	–	–	–	–	12	2.17	2.1	1.40	–	27
7a-3 [g]	1.50	–	–	–	12	–	2.17	2.1	1.40	–	44
7a-4 [h]	1.50	–	–	12	–	–	2.17	2.1	1.40	–	22

[a] HSI: *N*-Hydroxysuccinimide.
[b] DCC: dicyclohexylcarbodiimide.
[c] DEPC: diethyl phosphorocyanidate.
[d] TEA: triethylamine.
[e] r.t., activation, 1 day; r.t., amidation, 2 days.
[f] Reflux 19 h.
[g] r.t., 3 days.
[h] r.t., 3 days.

A typical example of the preparation of the **a** series ($R = C_{12}H_{25}$) of intermediates and glycolipids is given (the procedures for cases **b**: $R = C_{14}H_{29}$, **c**: $R = C_{16}H_{33}$, and **d**: $R = C_{18}H_{37}$ are omitted, but the corresponding compounds can be obtained according to the same method).

Lactonolactone (**2**) [11–14].—In a 500-mL flask, lactonic acid (**1**) (25 g, 69.77 mmol) was dissolved in 2-methoxyethanol (130 mL). The solution was heated and stirred, then diluted with toluene (65 mL) and concentrated. When the temperature of the distilled solution reached the boiling point of 2-methoxyethanol, addition of 2-methoxyethanol (130 mL) and toluene (65 mL) followed by concentration were repeated twice. Finally, after cooling of the solution crystalline lactonolactone was collected from the filter and washed with cooled 2-methoxyethanol. Yield: 17.17 g (72%); mp 196–197 °C, lit. [9] 195–196 °C; IR (KBr): ν 1740 cm^{-1} (lactone).

N-*(4-Aminobutyl)lactonamide* (**3**).—This compound was prepared from aminolysis of lactonolactone with 1,4-butanediamine according to ref. [15].

Dialkyl L-*glutamates* (**5**).—Compounds **5a–d** were synthesized by esterification of L-glutamic acid with higher alkyl alcohols (**a**: dodecanol, **b**: tetradecanol, **c**: hexadecanol, **d**: octadecanol), according to ref. [16].

Didodecyl N-*(3-carboxypropanoyl)*-L-*glutamate* (**6a**) [17,18].—Didodecyl L-glutamate hydrochloride (13 g, 24.99 mmol) was dissolved in dry THF (170 mL), Et_3N (5.4 mL, 38.81 mmol) was added, and the mixture was stirred for 20 min at 45–50 °C. The precipitate of triethylamine hydrochloride was removed by filtration to give didodecyl L-glutamate (**5a**) as the free amine. Succinic anhydride (2.5 g, 24.99 mmol) and dry THF (88 mL) were added and the mixture was heated under reflux for 6 h. Finally, the THF was removed in vacuo. The residue was extracted with $CHCl_3$ (130 mL), and washed with distilled water (150 mL) three times so that unreacted succinic anhydride was removed as succinic acid. Recrystallization of the residual solid from MeOH (30 mL) gave **6a** (9.29 g, 64%); mp 56–58 °C; IR (KBr): 3350 (N–H), 1720, 1680 cm^{-1} (C=O); ^{1}H NMR (270 MHz, $CDCl_3$, Me_4Si): δ 0.89 (t, 6 H, J 6.93 Hz, Me × 2), 1.28 (bs, 36 H, $Me(CH_2)_9 \times 2$), 1.62 (t, 4 H, J 6.76 Hz, $Me(CH_2)_9CH_2 \times 2$), 1.91–2.24 (m, 2 H, $NHCHCH_2$), 2.39 (m, 2 H, $NHCHCH_2CH_2$), 2.55, 2.68 (2 t, 4 H, J 6.85 Hz, $NHCO(CH_2)_2COOH$), 4.00–4.16 (m, 4 H, $CH_2OCO \times 2$), 4.60 (m, 1 H, $NHCH$), 6.78 (m, 1 H, NH), 9.88 (s, 1 H, COOH); ^{13}C NMR: δ 14.12 (Me × 2), 22.70–31.93 [$Me(CH_2)_{10} \times 2$, $NHCHCH_2CH_2$], 51.88 (NHCH), 65.08, 65.95 [$(CH_2)_2COOH$], 76.60, 77.56 [$Me(CH_2)_{10}CH_2OCO$], 172.09, 172.20 [$Me(CH_2)_{11}OCO$], 173.12 (NHCO), 176.82 (COOH).

Compounds **6b–6d** were prepared in a similar way.

6b: Yield 10.20 g (61%); mp 66–68 °C; IR (KBr): 3350 (N–H), 1720, 1680 cm^{-1} (C=O); ^{1}H NMR: δ 0.88 (t, 6 H, J 6.86 Hz, Me × 2), 1.28 (bs, 44 H, $Me(CH_2)_{11} \times 2$), 1.61 [t, 4 H, J 6.76 Hz, $Me(CH_2)_{11}CH_2 \times 2$], 1.91–2.24 (m, 2 H, $NHCHCH_2$), 2.37 (m, 2 H, $NHCHCH_2CH_2$), 2.55, 2.68 [2 t, 4 H, J 6.85 Hz, $NHCO(CH_2)_2COOH$], 4.00–4.16 (m, 4 H, $CH_2OCO \times 2$), 4.61 (m, 1 H, $NHCH$), 6.78 (m, 1 H, NH), 9.86 (s, 1 H, COOH); ^{13}C NMR: δ 14.12 (Me × 2), 22.70–32.69 [$Me(CH_2)_{12} \times 2$, $NHCHCH_2CH_2$], 51.81 (NHCH), 65.03, 65.88 [$(CH_2)_2COOH$], 76.60, 77.56 [$Me(CH_2)_{12}CH_2OCO$], 172.07, 172.18 [$Me(CH_2)_{13}OCO$], 173.06 (NHCO), 176.35 (COOH).

6c: Yield 15.72 g (84%); mp 76–77 °C; IR (KBr): 3350 (N–H), 1720, 1680 cm^{-1} (C=O); ^1H NMR: δ 0.88 (t, 6 H, J 6.60 Hz, Me × 2), 1.26 [bs, 52 H, $Me(CH_2)_{13} \times 2$], 1.61 [t, 4 H, J 6.76 Hz, $Me(CH_2)_{13}CH_2 \times 2$], 1.95–2.23 (m, 2 H, $NHCHCH_2$), 2.38 (m, 2 H, $NHCHCH_2CH_2$), 2.56, 2.69 [2 t, 4 H, J 6.85 Hz, $NHCO(CH_2)_2COOH$], 4.03–4.15 (m, 4 H, $CH_2OCO \times 2$), 4.60 (m, 1 H, NHCH), 6.78 (m, 1 H, NH), 9.84 (s, 1 H, COOH); ^{13}C NMR: δ 14.14 (Me × 2), 22.71–31.95 [$Me(CH_2)_{14} \times 2$, $NHCHCH_2CH_2$], 51.88 (NHCH), 65.08, 65.97 [$(CH_2)_2COOH$], 76.58, 77.54 [$Me(CH_2)_{14}CH_2OCO$], 171.86, 172.02 [$Me(CH_2)_{15}OCO$], 173.13 (NHCO), 176.73 (COOH).

6d: Yield 17.0 g (87%); mp 79–80 °C; IR (KBr): 3350 (N–H), 1720, 1680 cm^{-1} (C=O); ^1H NMR: δ 0.88 (t, 6 H, J 6.61 Hz, Me × 2), 1.26 [bs, 60 H, $Me(CH_2)_{15} \times 2$], 1.61 [t, 4 H, J 6.76 Hz, $Me(CH_2)_{15}CH_2 \times 2$], 1.91–2.35 (m, 2 H, $NHCHCH_2$), 2.40 (m, 2 H, $NHCHCH_2CH_2$), 2.57, 2.69 [2 t, 4 H, J 6.85 Hz, $NHCO(CH_2)_2COOH$], 4.00–4.16 (m, 4 H, $CH_2OCO \times 2$), 4.61 (m, 1 H, NHCH), 6.78 (m, 1 H, NH), 9.84 (s, 1 H, COOH); ^{13}C NMR: δ 14.12 (Me × 2), 22.71–31.95 [$Me(CH_2)_{16} \times 2$, $NHCHCH_2CH_2$], 51.88 [NHCH], 65.08, 65.97 [$(CH_2)_2COOH$], 76.58, 77.52 [$Me(CH_2)_{16}CH_2OCO$], 171.84, 172.02 [$Me(CH_2)_{17}OCO$], 173.13 (NHCO), 176.73 (COOH).

Didodecyl N-*[*N-*(4-lactonamidobutyl)succinamoyl]*-L-*glutamate* (**7a**).—Compound **3** (1.80 g, 4.20 mmol) was completely dissolved in DMF (30 mL) with heating, and triethylamine (0.87 mL, 6.3 mmol) and **6a** (2.64 g, 4.53 mmol) were added successively. The reaction system was then cooled to 15 °C, coupling reagent DEPC was slowly added, and the mixture was stirred for 3 days at 25–30 °C. Finally, solvent DMF was removed in vacuo (oil bath < 78 °C). The residue was recrystallized from acetone (60 mL), and chromatography on silica gel with 1:3 MeOH–$CHCl_3$ as the eluent then yielded **7a** (1.84 g, 44%); mp 154–156 °C; IR (KBr): 3375 (OH), 1740, 1680 cm^{-1} (C=O); ^1H NMR (270 MHz, Me_2SO-d_6, Me_4Si): 0.87 (t, 6 H, 6.77 Hz, Me × 2), 1.08–1.47 (m, 40 H, $Me(CH_2)_9 \times 2$, $NHCH_2(CH_2)_2CH_2NH$], 1.47–1.61 [m, 4 H, $Me(CH_2)_9CH_2 \times 2$], 1.74–2.04 [m, 4 H, $NHCH(CH_2)_2COO$], 2.20–2.45 [m, 4 H, $NHCO(CH_2)_2CONH$], 2.51–4.94 [m, 29 H, sugar-$CONHCH_2(CH_2)_2CH_2NH$, $CH_2OCO \times 2$, sugar hydrogens], 5.18 (m, 1 H, NHCH), 7.58 (m, 1 H, sugar-NH), 7.80 (m, 1 H, $NHCOCH_2CH_2CO$), 8.23 (m, 1 H, $NHCHCH_2CH_2$); ^{13}C NMR: δ 14.50 (Me × 2), 22.70–31.92 [$Me(CH_2)_{10} \times 2$, $NHCH(CH_2)_2$, $NH(CH_2)_4$], 52.62 (NHCH), 61.28, 62.93 (sugar-$CH_2OH \times 2$), 64.51, 64.96 ($NHCOCH_2CH_2CO$), 71.07, 71.76 (CH_2OCO), 83.58, 105.21 (sugar methine carbons), 172.35 ($CH_2OCO \times 2$), 172.62, 172.74 ($CH_2NHCOCH_2$, sugar-CONH, CHNHCO).

Compounds **7b–7d** were prepared in a similar way.

7b: Yield 1.89 g (43%); mp 163–165 °C; IR (KBr): 3375 (OH), 1740, 1680 cm^{-1} (C=O); ^1H NMR: δ 0.85 (t, 6 H, J 6.75 Hz, Me × 2), 1.08–1.47 [m, 48 H, $Me(CH_2)_{11} \times 2$, $NHCH_2(CH_2)_2CH_2NH$], 1.47–1.61 [m, 4 H, $Me(CH_2)_{11}CH_2 \times 2$), 1.74–2.04 (m, 4 H, $NHCH(CH_2)_2COO$], 2.20–2.45 [m, 4 H, $NHCO(CH_2)_2CONH$], 2.51–4.94 [m, 29 H, sugar-$CONHCH_2(CH_2)_2CH_2NH$, $CH_2OCO \times 2$, sugar hydrogens], 5.18 (m, 1 H, NHCH), 7.58 (m, 1 H, sugar-NH), 7.80 [m, 1 H, $NHCOCH_2CH_2CO$), 8.23 (m, 1 H, $NHCHCH_2CH_2$); ^{13}C NMR: δ 14.52 (Me × 2), 22.68–31.90 [$Me(CH_2)_{12} \times 2$, $NHCH(CH_2)_2$, $NH(CH_2)_4$], 52.62 (NHCH), 61.28,

62.93 (sugar-$CH_2OH \times 2$), 64.53, 64.95 ($NHCOCH_2CH_2CO$), 71.05, 71.78 (CH_2OCO), 83.56, 105.24 (sugar methine carbons), 172.34 (CH_2OCO), 172.61, 172.75 ($CH_2NHCOCH_2$, sugar-CONH, CHNHCO).

7c: Yield 1.86 g (40%); mp 150–152 °C; IR (KBr): 3375 (OH), 1740, 1680 cm^{-1} (C=O); ^1H NMR: δ 0.86 (t, 6 H, J 6.69 Hz, Me × 2), 1.08–1.47 [m, 56 H, $Me(CH_2)_{13} \times 2$, $NHCH_2(CH_2)_2CH_2NH$], 1.47–1.61 [m, 4 H, $Me(CH_2)_{13}CH_2 \times 2$], 1.74–2.04 [m, 4 H, $NHCH(CH_2)_2COO$], 2.20–2.47 [m, 4 H, $NHCO(CH_2)_2CONH$], 2.48–4.93 [m, 29 H, sugar-$CONHCH_2(CH_2)_2CH_2NH$, $CH_2OCO \times 2$, sugar hydrogens], 5.18 (m, 1 H, NHCH), 7.53 (m, 1 H, sugar-NH), 7.82 (m, 1 H, $NHCOCH_2CH_2CH_2CO$), 8.26 (m, 1 H, $NHCHCH_2CH_2$); ^{13}C NMR: δ 14.51 (Me × 2), 22.71–31.89 [$Me(CH_2)_{14} \times 2$, $NHCH(CH_2)_2$, $NH(CH_2)_4$], 52.59 (NHCH), 61.31, 62.94 (sugar $CH_2OH \times 2$), 64.51, 64.94 ($NHCOCH_2CH_2CO$), 71.06, 71.77 (CH_2OCO), 83.61, 105.18 (sugar methine carbons), 172.31 ($CH_2OCO \times 2$), 172.58, 172.71 ($CH_2NHCOCH_2$, sugar-CONH, CHNHCO).

7d: Yield 1.79 g (37%); mp 123–125 °C; IR (KBr): 3375 (OH), 1740, 1680 cm^{-1} (C=O); ^1H NMR: δ 0.87 (t, 6 H, J 6.71 Hz, Me × 2), 1.08–1.47 [m, 64 H, $Me(CH_2)_{15} \times 2$, $NHCH_2(CH_2)_2CH_2NH$], 1.47–1.61 [m, 4 H, $Me(CH_2)_{15}CH_2 \times 2$], 1.74–2.04 [m, 4 H, $NHCH(CH_2)_2COO$], 2.20–2.47 [m, 4 H, $NHCO(CH_2)_2CONH$], 2.48–4.95 [m, 29 H, sugar-$CONHCH_2(CH_2)_2CH_2NH$, $CH_2OCO \times 2$, sugar hydrogens], 5.16 (m, 1 H, NHCH), 7.59 (m, 1 H, sugar-NH), 7.81 (m, 1 H, $NHCOCH_2CH_2CO$), 8.25 (m, 1 H, $NHCHCH_2CH_2$); ^{13}C NMR: δ 14.49 (Me × 2), 22.71–31.92 [$Me(CH_2)_{16} \times 2$, $NHCH(CH_2)_2$, $NH(CH_2)_4$], 52.59 (NHCH), 61.29, 62.91 (sugar $CH_2OH \times 2$), 64.50, 64.97 ($NHCOCH_2CO$), 71.07, 71.75 (CH_2OCO), 83.57, 105.20 (sugar methine carbons), 172.34 (CH_2OCO), 172.60, 172.71 ($CH_2NHCOCH_2$, sugar-CONH, CHNHCO).

Acknowledgements

The authors are grateful to Professor Kiyoshi Matsumoto of Kyoto University for his helpful suggestions on the synthesis of glycolipids.

References

[1] E. Santanniello, P. Ferraboschi, and P. Grisenti, *Enzyme Microb. Technol.*, 15 (1993) 367–381.
[2] K. Takahashi, H. Nishimura, T. Yoshimoto, Y. Sato, and Y. Inada, *Biochem. Biophys. Res. Commun.*, 121 (1984) 261–265.
[3] Y. Okahata, Y. Fujimoto, and K. Ijiro, *Tetrahedron Lett.*, 29 (1988) 5133–5134.
[4] Y. Okahata and K. Ijiro, *J. Chem., Soc., Chem. Commun.*, (1988) 1392–1394.
[5] M. Goto, H. Kameyama, M. Goto, M. Miyata, and F. Nakashio, *J. Chem. Eng. Jpn.*, 26 (1993) 109–111.
[6] W. Tsuzuki, T. Saki, and T. Suzuki, *J. Chem. Soc., Perkin Trans. 1*, (1991) 1851–1854.
[7] M. Goto, N. Kamiya, M. Miyata, and F. Nakashio, *Biotechnol. Prog.*, 10 (1994) 263–268.
[8] G.W. Anderson, J.E. Zimmerman, and F.M. Callahan, *J. Am. Chem., Soc.*, 86 (1964) 1839–1842.
[9] T. Shioiri, Y.Y. Koyama, Y. Kasai, and S. Yamada, *Tetrahedron*, 32 (1976) 2211–2217.
[10] W. Tsuzuki, H. Kasumomoto, and S. Kobayashi, *Analyst*, 118 (1993) 131–135.
[11] H.S. Isbell and H.L. Frush, *Methods Carbohydr. Chem.*, 2 (1963) 16–19.

[12] K. Hashimoto, S. Imanishi, M. Okada, and H. Sumitomo, *J. Polym. Sci., Part A: Polym. Chem.*, 29 (1991) 1271–1279.
[13] K. Kobayashi, H. Sumitomo, and Y. Ina, *Polym. J.*, 17 (1985) 567–575.
[14] T.J. Williams, N.R. Plessas, and I.J. Goldstein, *Arch. Biochem. Biophys.*, 195 (1979) 145–151.
[15] K. Kobayashi, H. Sumitomo, and Y. Ina, *Polym. J.*, 48 (1991) 253–256.
[16] Y. Ishikawa and T. Kunitake, *J. Am. Chem. Soc.*, 113 (1991) 621–630.
[17] D. Kippenberger, K. Rosenquist, L. Odberg, P. Tundo, and J.H. Fendler, *J. Am. Chem. Soc.*, 105 (1983) 1129–1135.
[18] H. Hachisako, Y. Motozato, R. Murakami, and K. Yamada, *Chem. Lett.*, (1992) 219–222.