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Experimental Details and Characterization Data

***N*-Benzyloxycarbonyl-L-serinoctylamide (3).** To a solution of *N*-benzyl-oxycarbonyl-L-serine (3.0 g, 12.5 mmole) and n-octylamine (2.28 mL, 13.8 mmole) in ethanol/benzene (1:1 v/v) (30 mL) was added EEDQ (3.4 g, 13.8 mmole). The mixture was stirred at room temperature for 24 h. The clear solution was concentrated *in vacuo* to give syrupy crude product. The crude material was then recryatalized from benzene to afford pure compound **3** (3.4 g, 78%): m.p. 95°C; $[\alpha]_D$= -14.1 ° (c 0.21, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 7.34 (m, 5 H, -Ph), 6.59 (br s, 1 H, NH_{Ser}), 5.87 (br d, 1 H, NH), 4.17 (ddd, 1 H, $H_{\alpha Ser}$), 4.09 (dd, 1 H, $H_{\beta Ser}$), 3.63 (dd, $H_{\beta Ser}$), 3.22 (dd, 2 H, $NHCH_2$), 1.48-1.26 (14 H, CH_2 x 7), 0.88 (t, 3 H, CH_3). Anal Calcd for $C_{19}H_{30}O_4N_2$: C, 65.11; H, 8.63; N, 7.99. Found: C, 65.09; H, 8.60; N, 7.98.

***N*-(Benzyloxycarbonyl)-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-galacto-pyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-L-serinoctyl-amide (4).** Powdered molecular sieves 4 A was added to a solution of compound **2** (3.1 g, 4.43 mmole) and **3** (1.03 g, 2.95 mmole) in dichloromethane (20 mL). Silver trifrate (AgOTf) (1.14 g, 4.43 mmole) was then added to the

mixture at -20 °C. The reaction mixture was stirred under nitrogen atmosphere at -20°C for 24 h. The mixture was filtered with celite, and the filtrate was washed with brine. The solution was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residual syrup was chromatographed on silica gel column and eluted with toluene/ethylacetate (2:1 v/v) to give glycoside **4** (1.49 g, 48 %): $[\alpha]^{23}_D$= + 6.8° (c 0.25, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 7.35 (m, 5 H, Ph), 6.33 (br s, 1 H, NH), 5.62 (br s, 1 H, NH), 5.35 (dd, 1 H, H-4'), 5.17-5.07 (m, 2 H, H-3 and H-2'), 4.96 (dd, 1 H, H-3'), 4.86 (dd, 1 H, H-2), 4.54-4.47 (4 H, H-1, H-1', $H\text{-}6_b$ and $H\text{-}6'_b$), 4.15-3.66 (8 H, $H\alpha_{Ser}$, $H\text{-}6_a$, $H\text{-}6'_a$, H-5', H-5, H-4 and $COCH_2$), 3.21 (br t, 4 H, $NHCH_2$ x 2), 2.78 (t, 2 H, $COCH_2$ x 2), 2.20-1.93 (s x 7, 21 H, OAc x 7), 1.62 (br s, 2 H, H_2O), 1.48 and 1.28 (each br s, 10 H, CH_2 x 5), 0.88 (t, 3 H, CH_3). Anal Calcd for $C_{45}H_{64}O_{21}N_2 \cdot H_2O$: C, 54.76; H, 6.74; N, 2.84. Found. C, 54.89; H, 6.63; N, 2.72.

***N*-((*N*-Acrylamido)-pentanoyl)-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-L-serinoctylamide (5).** 10% Palladium-carbon (300 mg) was added to a solution of compound **4** (1.5 g, 1.55 mmole) in methanol (40 mL) and the mixture was stirred under hydrogen atmosphere at room temperature for 2 h.

The mixture was filtrered with celite and the filtrate was evaporated *in vacuo* to give crude *O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-L-serinoctylamide. This intermediate was subsequently used for the next step without further purification.

A mixture of crude amino component (1.29 g, ~1.55 mmole), acrylamidocapric acid* (315 mg, 1.70 mmole), and EEDQ (421 mg, 1.70 mmole) in ethanol /benzene (1:1 v/v) (40 mL) was stirred at room temperature for 24 h. The clear solution was concentrated under reduced pressure. The residue was purified by silica gel chromatography with chroloform/methanol (50:1 v/v) as eluant to give compound **5** (1.21g, 78% from compound **4**): $[\alpha]^{23}_{D}$= +3.8° (c 0.37, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 6.43 (t, 1 H, NH), 6.39 (d, 1 H, NH_{Ser}), 6.28 (dd, 1 H, $CH{=}CH_2$), 6.10 (dd, 1 H, $CH{=}CH_{2trans}$), 5.62 (dd, 1 H, $CH{=}CH_{2cis}$), 5.35 (dd, 1 H, H-4'), 5.18 (t, 1 H, H-3), 5.11 (dd, 1 H, H-2'), 4.97 (dd, 1 H, H-3'), 4.86 (dd, 1 H, H-2), 4.60 (d, 2 H, H-1 and H-1'), 4.16-4.05 (3 H, $H\alpha_{Ser}$, $H\text{-}6_a$ and $H\text{-}6'_a$), 3.95 (dd, 1 H, H-5'), 3.88 (dt, 1 H, H-4), 3.81-3.66 (3 H, H-5 and $COCH_2$), 3.36-3.15 (4 H, $NHCH_2$ x 2), 2.23 (t, 2 H, $COCH_2$ x 2), 2.16-1.97 (7 x s, 21 H, OAc x 7), 1.73-1.29 (18 H, CH_2 x 9), 0.88 (t, 3 H, CH_3). Anal Calcd for $C_{46}H_{71}O_{21}N_3$; C, 55.14; H, 7.14; N, 4.17. Found. C, 54.99; H, 7.19; N, 4.15.

***N*-((*N*-Acrylamido)-pentanoyl)-*O*-(β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-L-serinoctylamide (6).** To a solution of compound **5** (394 mg, 0.39 mmole) in 1:1 (v/v)THF-methanol (10 mL) was added sodium methoxide (8.49 mg, 0.157 mmole). The mixture was stirred at room temperature for 1 h. The solution was neutralized with Dowex 50W X-8(H^+). The filtrate was evaporated *in vacuo* to afford pure glycomonomer **6** as amorphous powder (278 mg, >99%): $[\alpha]^{23}_D$= +8.2° (c 0.25, DMSO); ^{1}H-NMR (DMSO-d_6) δ 8.04 (t, 1 H, NH), 7.91 (d, 1 H, NH_{Ser}), 7.74 (t, 1 H, NH), 6.21 (dd, 1 H, $CH{=}CH_2$), 6.06 (dd, 1 H, $CH{=}CH_{2trans}$), 5.55 (dd, 1 H, $CH{=}CH_{2cis}$), 5.19 (br s, 2 H, CH_2), 5.10 (br s, 2 H, CH_2), 4.61 (t, 2 H, H-1 and H-1'), 4.52 (d, 1 H, H-4), 4.43 (ddd, 1 H, $H_{\alpha Ser}$), 4.18 (m, 2 H, CH_2), 3.91 (dd, 1 H, $H_{\beta Ser}$), 3.77 (m, 1 H, H-2), 3.62-3.29 (7 H, H-3, H-2', $H_{\beta Ser}$ and CH_2 x 2), 3.10 (dd, 2 H, $NHCH_2$), 3.04 (dd, 2 H, $NHCH_2$), 2.15 (t, 2 H, $COCH_2$), 1.53-1.24 (8 H, CH_2 x 4), 0.86 (t, 3 H, CH_3); ^{13}C-NMR (DMSO-d_6) δ 172.2, 169.3, and 164.4 (C=O), 131.9 ($CH{=}CH_2$), 124.7 ($CH{=}CH_2$), 103.9 (C-1), 103.2 (C-1'), 80.6 (C-4), 75.5 (C-4'), 74.8 (C-5'), 74.6 (C-5), 73.2 (C-3'), 73.0 (C-3), 70.5 (C-2'), 69.8 (C-2), 68.1 (C-6'), 67.4 (C-6), 60.4 ($C_{\beta Ser}$), 52.5 ($C_{\alpha Ser}$), 38.6, 38.4, 35.1, 31.2, 28.9, 28.8, 28.7, 28.6, 26.3, 26.1, 24.8, and 22.1 (CH_2 x 12), 13.9

(CH_3). Anal Calcd for $C_{32}H_{57}O_{14}N_3$: C, 54.30; H, 8.12; N, 5.94. Found. C, 54.18; H, 8.19; N, 5.86.

Polyacrylamide Having LacCer Branches (Primer Support 7). To a solution of compound **6** (150 mg, 0.21 mmole) in DMSO (2.0 mL) was added a aqueous solution of acrylamide (60.25 mg, 0.84 mmole) in H_2O (2.0 mL). This clear solution was deaerated for a while using water pump, to which was added *N,N,N',N'*-tetramethylethylenediamine (TEMED) (12.7 μL, 84.0 μmole) and ammonium peroxodisulfate (APS) (7.67 mg, 33.6 μmole). The solution was stirred under nitrogen atmosphere at 50°C for 24h. The reaction mixture was directly subjected to gel filtration chromatography on Sephadex G-25 column (ϕ 30 mm x 400 mm) and eluted with deionized water. Polymer containing fractions were collected and concentrated to small volume, and the syrupy solution was lyophilized to afford polymer **7** as amorphous powder (193 mg, 92%): Molecular weight > 380,000 (GPC method). ^{1}H-NMR (D_2O) δ 4.34-4.32 (m, 4 H, H-1, H-1', H-4 and $H_{\alpha Ser}$), 4.02 (br d, 2 H, H-3 and H-3'), 3.84-3.78 (m, 4 H, H-2, H-2', H-6 and H-6'), 3.61 (m, 2 H, H-5 and H-5'), 3.50-3.39 (m, 2 H, H-4' and $H_{\beta Ser}$), 3.19 (d, 1 H, $H_{\beta Ser}$), 3.04 (m, 2 H, CH_2), 2.19 (4 H, $NHCH_2$ x 2), 2.06 (m, 2 H, $COCH_2$), 1.61-1.35 (13 H, CH_2 x 3 and CH x 7),

1.12 (s, 12 H, CH_2 x 6), 0.71 (s, 3 H, CH_3).

Polyacrylamide Having GM3 Trisaccharides (8). Primer polymer **7** (22 mg, ca. 20 μmole of Lactose residue), cytidine-5'-mono-phospho-*N* -acetylneu raminic acid (CMP-NeuAc) (15.0 mg, 24.4 μmol), α-2,3-sialyltransferase (0.3 unit), bovine serum albumin (BSA) (10.0 mg), and carf intestinal alkaline phosphatase (CIAP) (20 unit) were incubated in 50 mM sodium cacodylate buffer (pH 7.4, 5.0 mL) containing $MnCl_2$ (1.56 mg, 7.9 μmol) and Triton CF-54 (10 μL) at 37°C for 72 h. The reaction mixture was directly purified by chromatography on Sephadex G-25 column (ϕ 30 mm x 400 mm) eluted with deionized water. The polymer fractions were collected and lyophilized to give a glycopolymer having GM3 trisaccharide, **8** (22.0 mg, quantitative sialylation was estimated from integration data of ^{1}H-NMR spectrum): ^{1}H-NMR (D_2O) δ 4.16-3.57 (m, 11 H, H-1, H-1', H-2, H-3, H-3', H-4, H-4", H-5, H-5", H-6 and H-7"), 3.35 (d, 1 H, $H_{\beta Ser}$), 3.23-3.18 (m, 2 H, CH_2), 2.77 (dd, 1 H, H-3"$_{eq}$), 2.35-2.14 (m, $NHCH_2$ x 2 and $COCH_2$), 2.05 (s, 3 H, NHAc), 1.81 (t, 1 H, H-3"$_{ax}$), 1.66-1.13 (m, 34 H, CH_2 x 14 and CH x 6), 0.79 (br s, CH_3)#. ^{1}H- and ^{13}C-NMR spectra were listed in Figure 1 of the main text.

#Integration of this signal seemed to be larger than that of *N*-acetyl group of

sialic acid at 2.05 and this may indicate contamination of a faint amount of Triton CF-54.

Transglycosidation Reaction by Ceramide Glycanase. To a mixture of glycopolymer **8** (22.0 mg, ca. 16.2 μmole of sialyllactose residue) and ceramide (C_{18}) (50 mg, 78.7 μmole) in 50 mM sodium citrate buffer (pH 6.0, 1 mL) was added Toriton CF-54 (1 drop), and the mixture was sonicated for 1 min in an ultrasonic water bath. The reaction was initiated by the addition of ceramide glycanase from leech (0.01 unit) and incubated at 37°C for 17 h. This mixture was directly chromatographed on Sephadex LH-20 column and eluted with chloroform / methanol / H_2O (60:30:4.6 (v/v)) to give GM3 (**1**) (11.6 mg, 61% calculated from compound **8**). A faint amount of hydrolytic product such as sialyllactose was obtained by the chromatographic purification: ^{1}H-NMR [DMSO-d_6 / D_2O (49:1)] δ 5.55 (dt, 1 H, *J* 6.6 Hz, H-5cer), 5.37 (dd, 1 H, *J* 6.8 Hz, H-4cer), 4.20(d, 1 H, *J* 7.8 Hz, H-1'), 4.14(d, 1 H, *J* 7.8 Hz, H-1), 2.75 (dd, 1 H, *J* 5.0 and 12.0 Hz, H-3"$_{eq}$), 1.89 (s, 3 H, NAc), 1.38 (t, 1 H, *J* 12.0 Hz, H-3"ax), 1.25 (br s, CH_2 of ceramide), and 0.85 (t, 6 H, *J* 6.8 Hz, CH_3 x 2). Anal Calcd for $C_{63}H_{116}O_{21}N_2$: C, 61.14; H, 9.45; N, 2.26. Found. C, 61.36; H, 9.49; N, 2.22.

Transfer of Ganglioside GM3 Oligosaccharide from a Water Soluble Polymer to Ceramide by Ceramideglycanase. A Novel Approach for the Chemical-Enzymatic Synthesis of Glycosphingolipids

Shin-Ichiro Nishimura and Kuriko Yamada

Fig.S-1

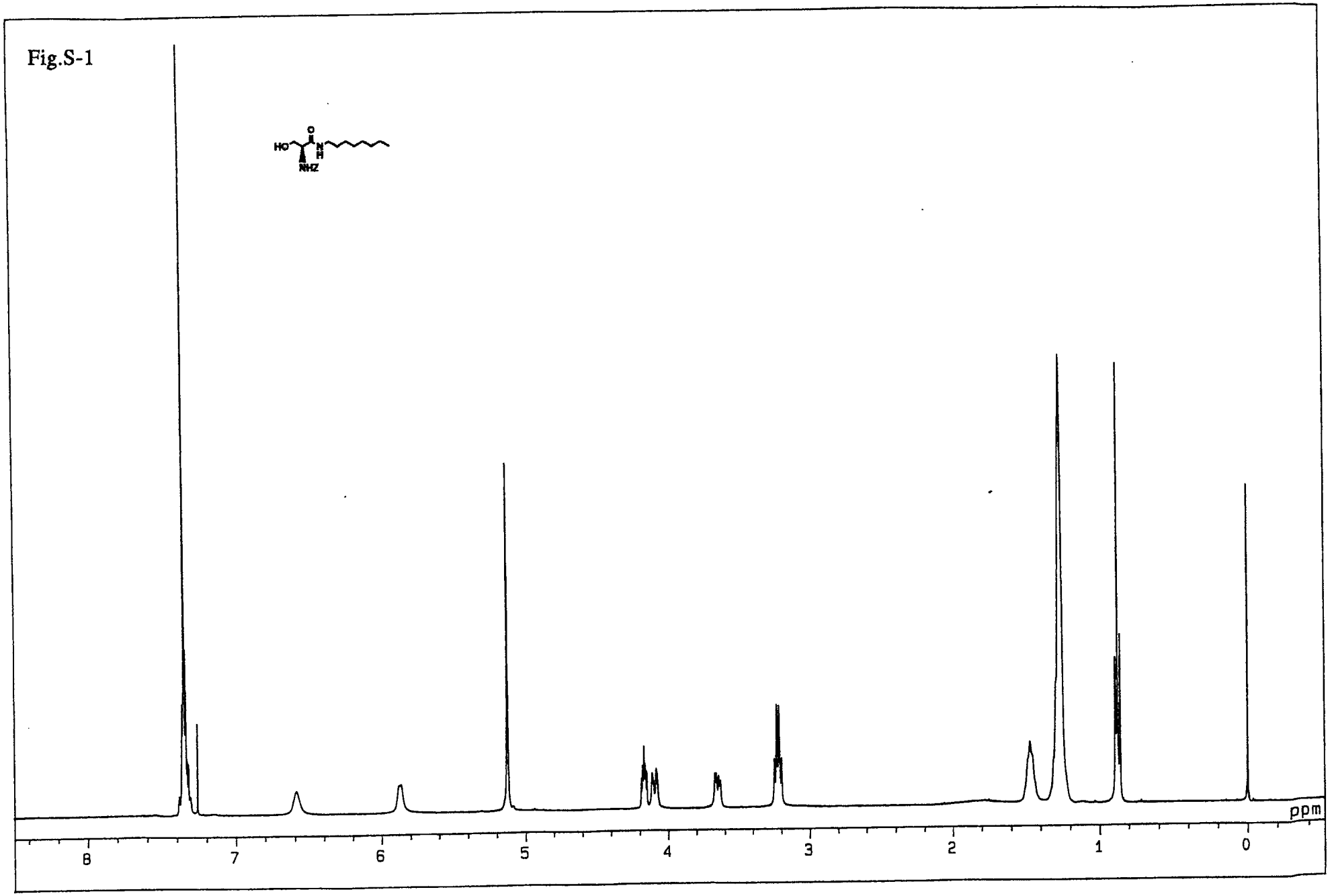

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Fig.S-2

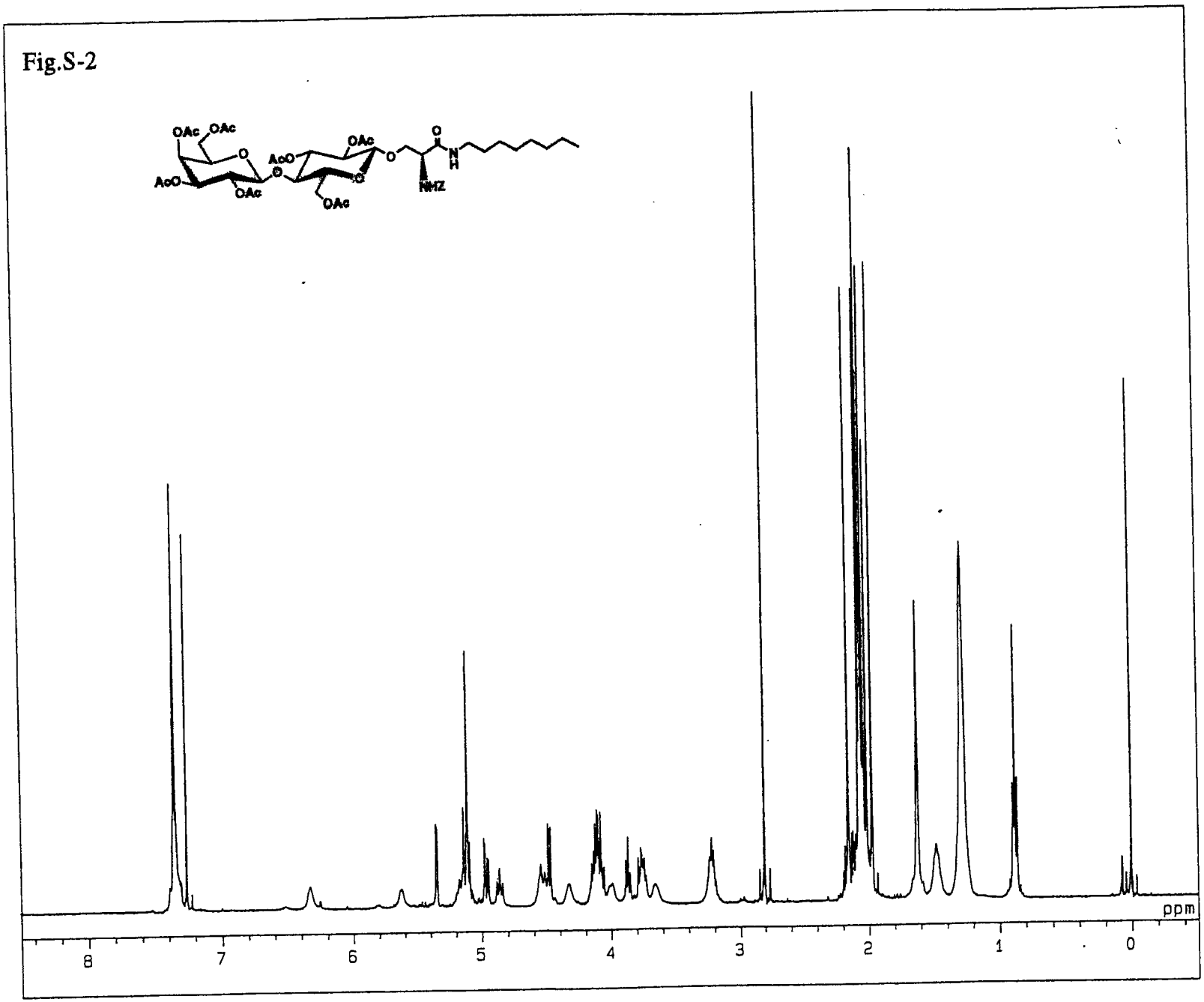

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Fig.S-3

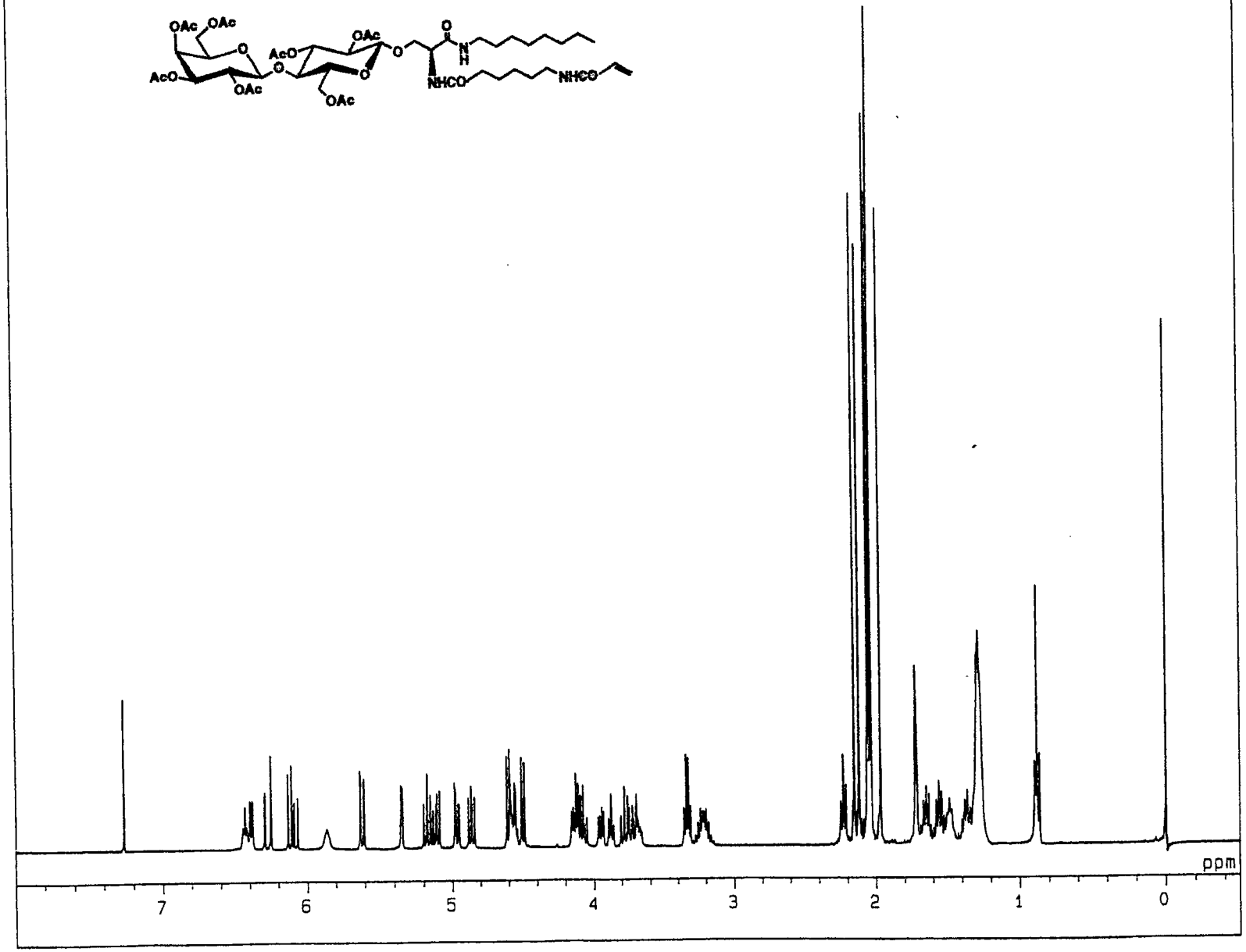

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Fig.S-4

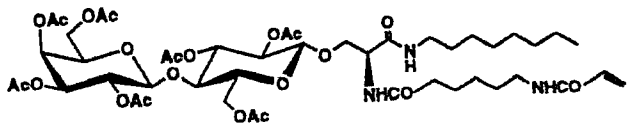

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Fig.S-5

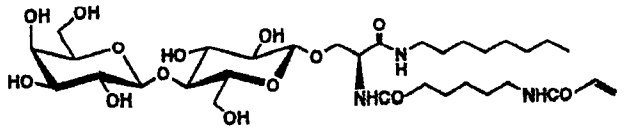

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Fig.S-6

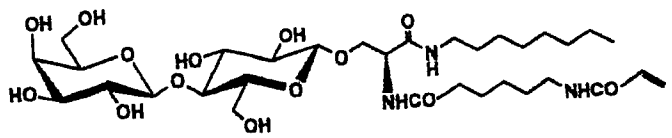

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Fig.S-7

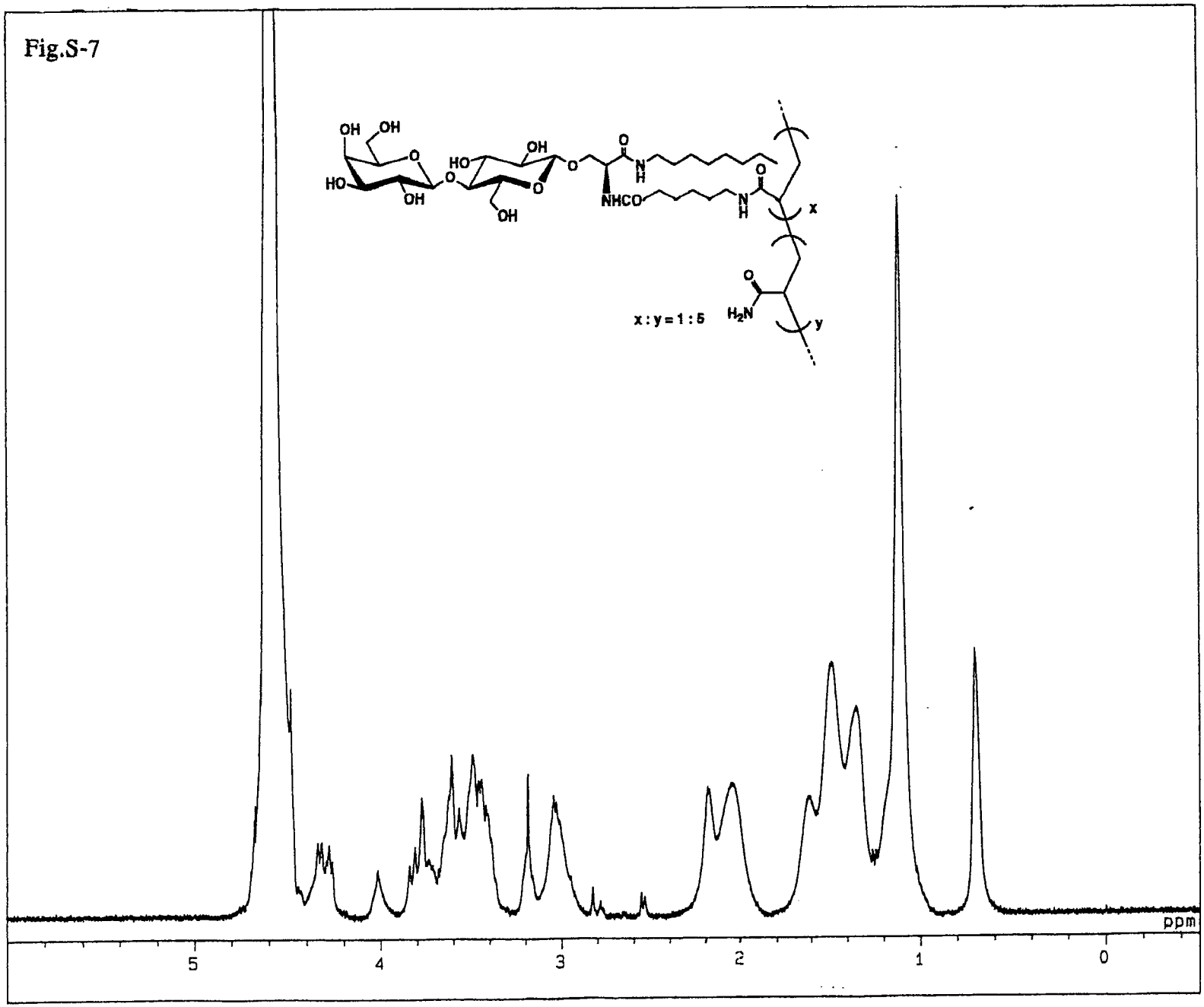

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Fig.S-8

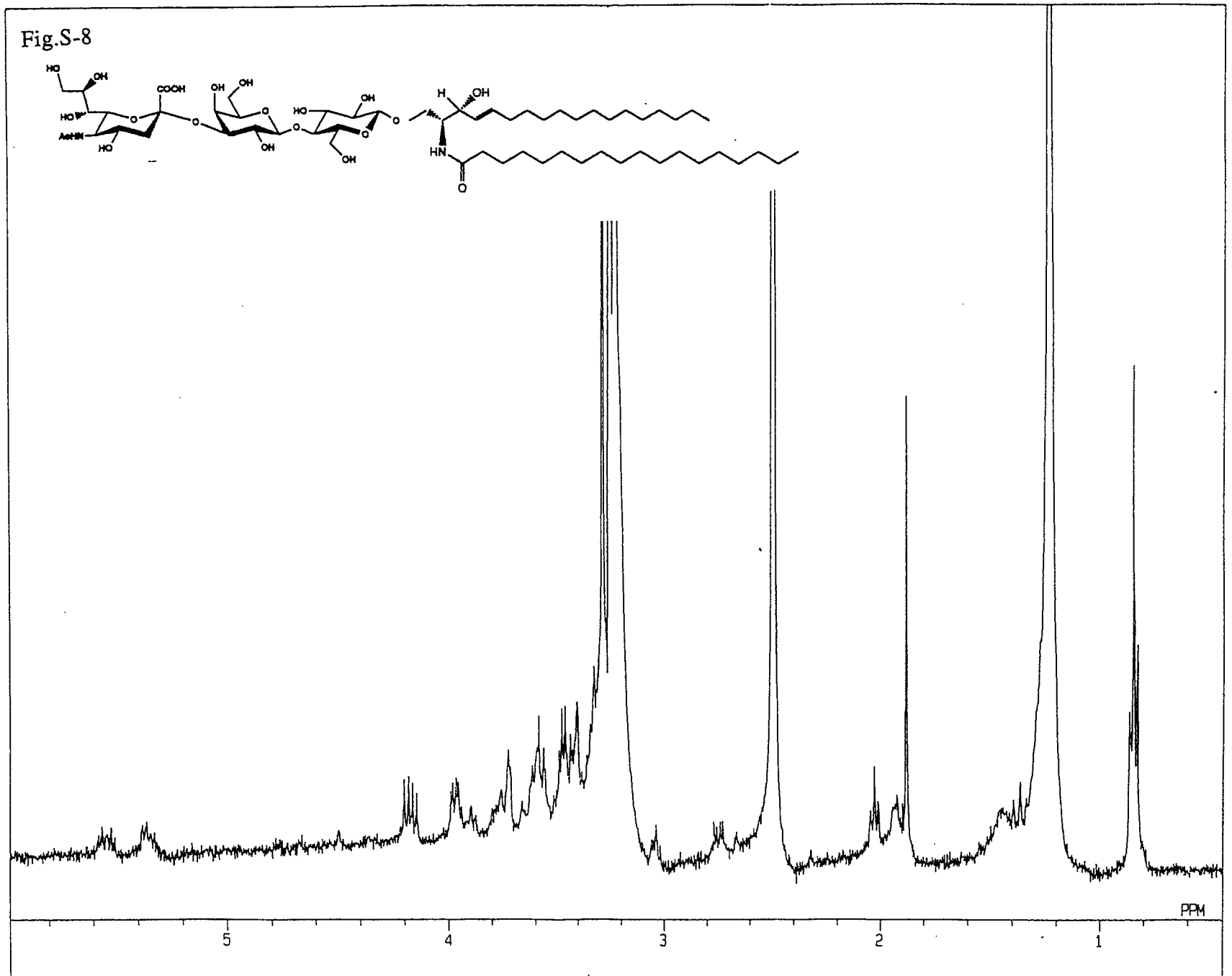

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Fig.S-8(2)