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A First Total Synthesis of a Hybrid-Type Ganglioside Associated with Amyotrophic Lateral Sclerosis-Like Disorder**

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Abstract: The hybrid ganglioside X1, which was identified in the bovine brain, was synthesized for the first time. Ganglioside X1 is believed to be involved in the development of amyotrophic lateral sclerosis-like disorders in patients with neurological disorders after treatment with bovine brain gangliosides. A convergent approach using two branched glycan units, the GM2-core trisaccharide and the lacto-ganglio tetrasaccharide, efficiently provided the highly branched heptasaccharide part of ganglioside X1, which was conjugated with the ceramide part to produce the protected ganglioside X1. Global deprotection delivered homogenous ganglioside X1, with which serum from the patient was reacted.

Keywords: gangliosides · glycosides · glycosylation · immunoassays · natural products · total synthesis

Introduction

Amyotrophic lateral sclerosis (ALS) is characterized by a progressive and selective loss of motor neurons in the brain and spinal cord, and is a devastating disorder of still unknown etiology and pathogenesis. Some patients who had been misdiagnosed as having ALS carried IgM autoantibodies against the GM1 ganglioside, which is a member of a group of sialic acid containing glycosphingolipids that is enriched in nervous tissues. The patients improved after plasmapheresis and immunosuppressant treatment, suggesting that the condition is autoimmune mediated.[1] In contrast, gangliosides extracted from bovine brain, which have been used as a therapeutic agent for many neurological disorders, might have caused the patient to display ALS-like disorder[2] The patient's IgM reacted strongly with GM2 and GalNAc-GD1a, which are minor gangliosides in bovine brain, but not with asialo-GM2, GM1, or GD1a, indicating that their terminal trisaccharides [GalNAcβ(1,4)(NeuAcα(2,3)Gal)] (GM2-core) are the epitope.[2,3] The patient who underwent plasmapheresis improved quickly, and the serum possessed killing activity to GM2-containing cells, suggesting that IgM antibodies with anti-GM2 reactivity function in the development of the ALS-like disorder.[2,4] By using the patient's IgM antibodies, further, novel GM2-core-containing gangliosides, X1 and X2 were identified in bovine brain. They were characterized as hybrid-type gangliosides, lacto-ganglio-series gangliosides, in which the core sequence of lacto-series (Galβ(1,3)GlcNAcβ(1,3)Gal) and ganglio-series (GalNAcβ(1,4)Gal) are hybridized.[3]

Their unusual structures may be immunogenic in humans to induce the pathogenic antibodies with anti-GM2 reactivity. Otherwise, X1 (**1**) and X2 (**2**) may be present in humans and may be target molecules for autoantibodies in some patients who are misjudged to have ALS. The novel GM2-core-containing gangliosides are required to identify such treatable patients with immunotherapy. Herein, we report a first total synthesis of **1** in detail.

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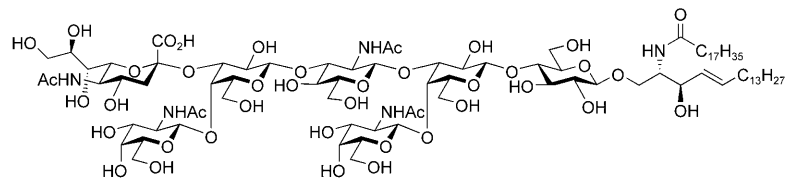
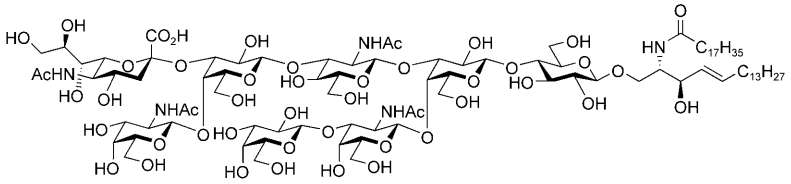
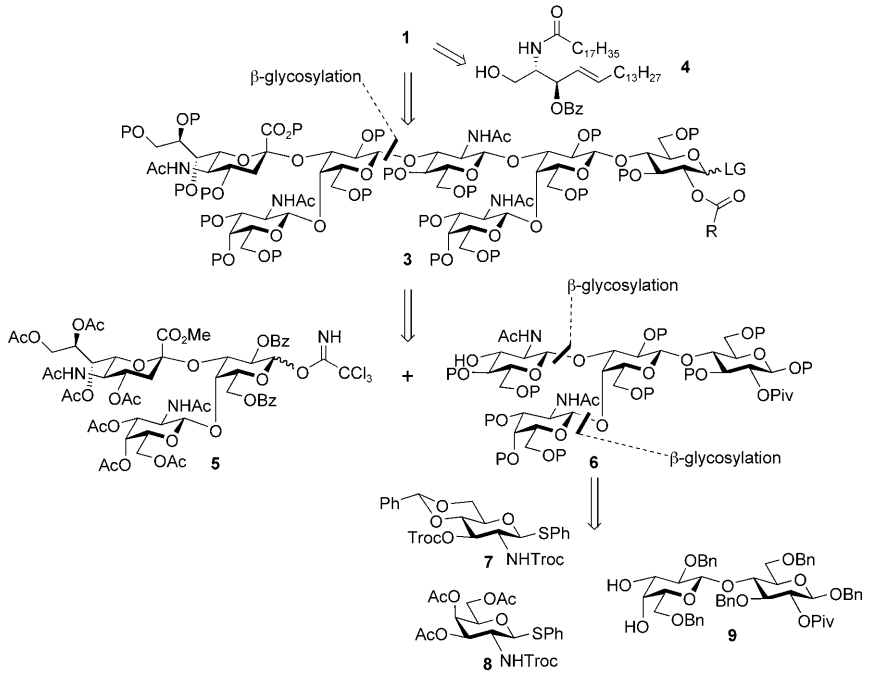


Ganglioside X1 (**1**)



Ganglioside X2 (**2**)

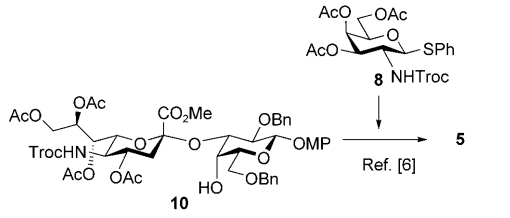
To build the entire ganglioside X1 structure, the glycan unit needed to be accessed through a highly efficient synthetic route and be designed to have a form suitable for straightforward introduction of the ceramide unit. The glycan structure of **1** has two galactosamine residues at the C4 hydroxyl groups of the external and internal galactose residues of sialyl lactotetraose. At first glance, direct and double galactosaminylation of the sialyl lactotetraose appeared to be a feasible approach to the assembly of the glycan part of target **1**. However, we also presumed that the glycan structure would defy this approach because the reactivity of the C4 hydroxyl group of galactose, flanked by the sialic acid residue at C-3, is markedly low. Similarly, this low reactivity could impede the selective protection–deprotection of the C4 hydroxyl group. Based on these conjectures, we devised a convergent approach to synthesize the target molecule (Scheme 1). First, the target (**1**) was divided into the glycan unit **3** and ceramide unit **4**. Then, the glycan unit was disassembled at the β(1,3)-linkage between Gal and GlcNAc, following a previous report on sialyl Lewis A,[5] thus providing two branched fragments, **5** and **6**. The left fragment, containing the GM2-core sequence, was designed as a trichloroacetimidate donor, which was developed by our research group.[6] The right fragment, containing the lacto- and ganglio-series glycan sequence, was further disassembled into glucosaminyl donor **7**, galactosaminyl donor **8**,[7] and lactose acceptor **9**.[8] For the final connection of the full-length glycan unit and ceramide, the lactose unit was designed with pivaloate at C2 to prevent orthoester formation.



Scheme 1. Retrosynthetic analysis of target compound **1**. P = protecting group, LG = leaving group, Bz = benzoyl, Piv = pivaloyl, Troc = 2,2,2-trichloroethoxycarbonyl, Bn = benzyl.

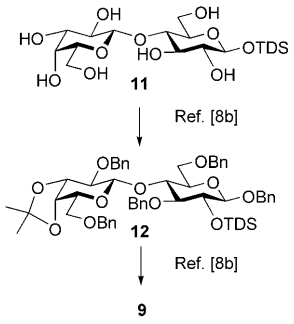
As shown in Scheme 2, the terminal GM2-core unit **5** could be readily synthesized according to a previously reported method from *N*-Troc galactosaminyl (GalN) donor **8** and *N*-Troc sialyl galactose **10**,[6,9] which can be rapidly produced on a large scale because of its high tendency to crystallize.

The synthesis of the tetrasaccharide counterpart commenced with the preparation of lactose acceptor **9**. The 2-*O*-pivaloyl derivative of lactoside was successfully prepared from 1-*O*-silylated lactose **11** through 2-*O*-silylated derivative **12** according to the procedure reported by Schmidt and coworkers (Scheme 3).[8b]



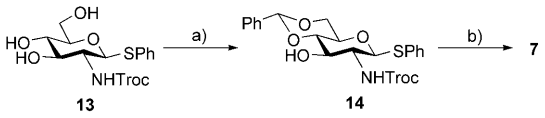
Scheme 2. Synthesis of terminal trisaccharide unit **5**. MP = *p*-methoxyphenyl.

The glucosaminyl (GlcN) donor was designed in a 4,6-benzylidenated form to further incorporate the GM2-core



Scheme 3. Synthesis of lactosyl acceptor **9** bearing a pivaloate at the C2 position. TDS = thexyldimethylsilyl.

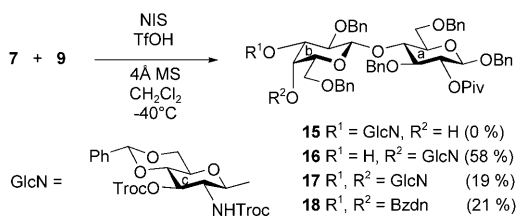
unit at the C3 hydroxyl group after assembly of the tetrasaccharide. The Troc group was chosen as the directing functionality for stereoselective β-glucosaminylation and also as a temporary protecting group for the C3 hydroxyl group, thus leading to structure **7**, which was synthesized from the reported compound **13**[10] through benzylidanation and introduction of the Troc group at the C3 hydroxyl group of **14** (Scheme 4).[11]



Scheme 4. Synthesis of glucosaminyl donor **7**. a) BDA, CSA/CH_3CN-THF, 61% (crystalline); b) TrocCl/Pyr, 94%. BDA = benzaldehyde dimethyl acetal, CSA = (±)-camphor-10-sulfonic acid, Pyr = pyridine.

To establish the hybrid branches stemming from the galactose residue, the GlcN donor **7** was first reacted with the 3′,4′-diol lactose acceptor **9**, aiming for regioselective glycosylation at the more reactive C3 hydroxyl group rather than at the C4 hydroxyl group.

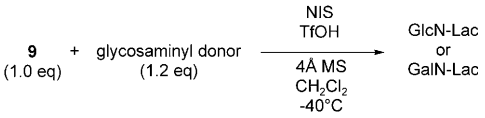
Although there are many examples of specific glycosylation of the C3 hydroxyl group of the 3,4-diol of galactose moiety by using glycosyl donors, especially sialic acid donors,[6,8,9,12] the GlcN donor **7** preferentially provided the β(1,4′)-linked product (Scheme 5). Thus, the reaction of **7** (1.2 equiv) and **9** (1.0 equiv), promoted by NIS-TfOH[13] in CH_2Cl_2 at −40°C, yielded trisaccharide **16** (58%) with double glycosylated **17** (19%) and 3′,4′-benzylidenated lac-

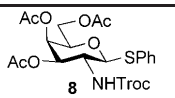
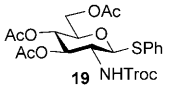
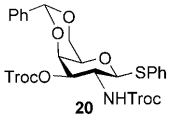


Scheme 5. Unexpected regioselectivity in the glycosylation of **9** with **7**. NIS = *N*-iodosuccinimide, TfOH = trifluoromethanesulfonic acid, MS = molecular sieves, Bzdn = benzylidene.

toside **18** (21%). The position of GlcN within **16** was confirmed by comparison of the chemical shifts in ^{1}H NMR spectra of **16** and its acetylated derivative. Upon acetylation (Ac_2O, Pyr), the H-3 of Gal shifted downfield from $\delta = 3.8$ to 4.9 ppm, indicating that the C3 hydroxyl group was free in compound **16**. The structures of compounds **17** and **18** were confirmed by ^{1}H NMR spectroscopic analysis and by mass spectrometry. In contrast, the desired product **15** was not obtained from this reaction. Disappointingly, replacing GlcN **7** with GalN donor **8** in this reaction resulted in unregioselective glycosylation, generating β(1,4′)-linked (35%), β(1,3′)-linked (42%), and double glycosylated (21%) products (Table 1).

Table 1. Glycosylation of **9** with glycosaminyl donors.

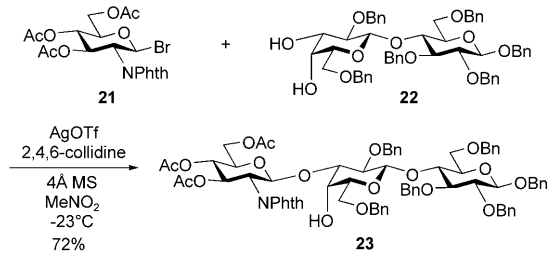


Entry	Donor	Yield of products [%] (1,3′)-linked	(1,4′)-linked	bis-linked
1	 **8**	42	35	21
2	 **19**	35	22	39
3	 **20**	63	15	20

Furthermore, triacetyl GlcN donor **19** and benzylidenated GalN donor **20**[14] were investigated. It was likely that the triacetyl GalN donor **8** and the triacetyl GlcN donor **19** were unselective (Table 1, entry 2). However, in the case of the benzylidenated GalN donor **20**, the C3 hydroxyl group was preferentially glycosylated to afford the (1,3′)-linked (63%), (1,4′)-linked (15%), and double glycosylated (20%) products as anomeric mixtures, showing a regioselectivity opposite to that observed with benzylidenated GlcN donor **7** (Table 1, entry 3). This unexpected regioselectivity also contrasts with the reaction of *N*-phthaloyl GlcN donor **21** and lactose acceptor **22** reported by Ogawa and co-workers,[15] in which the corresponding β(1,3′)glucosaminyl product **23** was obtained as the predominant product (Scheme 6).

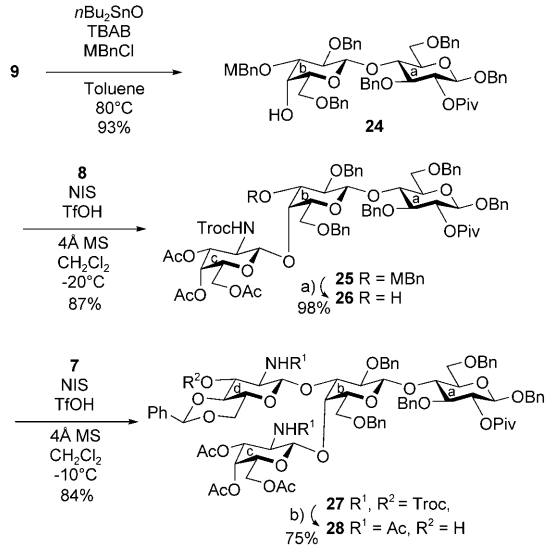
Although the unexpected results are interesting, the principle underlying these phenomena is still unclear. Because the regioselectivity is incongruous with the targeted structure, we redesigned the lactosyl acceptor as a monoalcohol derivative.

The C3 hydroxyl group of **9** was capped with the *p*-methoxybenzyl (MBn) group by stannylation and etherification (dibutyltin(IV) oxide (DBTO), MBnCl, and TBAB)[16] to



Scheme 6. The C3-selective glycosylation of 3′,4′-diol lactosyl acceptor **22** with glucosaminyl donor **21** developed by Ogawa et al.[15] Phth = phthaloyl.

provide **24** in 93% yield (Scheme 7). As expected, glycosylation of the lactose acceptor **24** (1.0 equiv) and GalN donor **8** (1.5 equiv) proceeded smoothly at −20°C to yield the gangliotriose (Gg_3) sequence **25** in 87% yield as a single



Scheme 7. Synthesis of unit **28** of the glycan part of the target compound. a) TFA/CH_2Cl_2, 98%; b) i) Zn, AcOH/MeOH; ii) Ac_2O/CH_2Cl_2-MeOH, 75%. TBAB = *n*-tetrabutylammonium bromide, MBn = *p*-methoxybenzyl, TFA = trifluoroacetic acid.

isomer. Upon treatment with trifluoroacetic acid (TFA) in CH_2Cl_2, the Gg_3 derivative was converted into C3-hydroxy acceptor **26** (98%). Subsequent glycosylation with GlcN donor **7**, mediated by NIS-TfOH in CH_2Cl_2, was also successful in converting Gg_3 into lacto-ganglio-tetraose **27** in 84% yield. Finally, successive unmasking of the three Troc groups by treatment with zinc in AcOH–MeOH and N-acetylation, delivered fragment **28** in 75% yield over two steps.

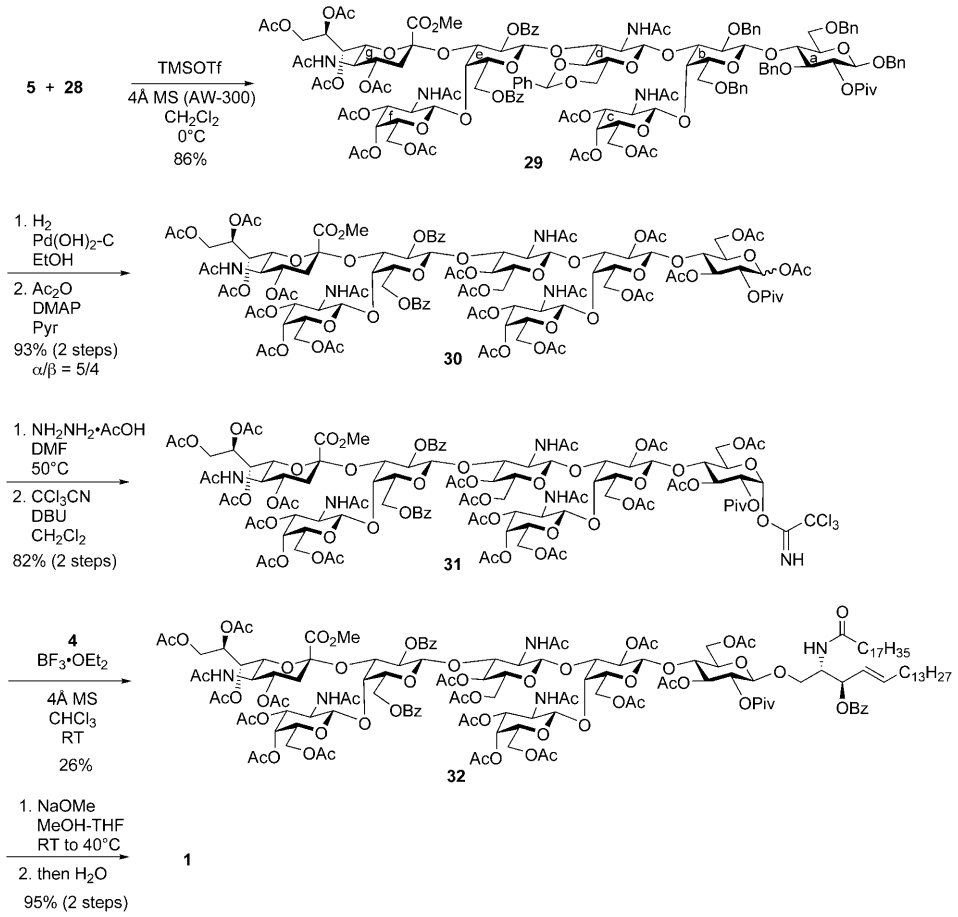
Fortunately, the convergent assembly of the heptasaccharide part proceeded in accordance with our initial expectation (Scheme 8). Thus, the left fragment, GM2-core donor **5**, was glycosidated with the right fragment **28** by using Schmidt's method[17] to provide the glycan framework of X1 **29** in 86% yield. The heptasaccharide structure of **29** was confirmed by mass spectrometry (ESI-TOF; *m*/*z* calcd for $C_{135}H_{160}N_4O_{52}$ $[1/2M+Na]^+$ 1357.4892; found 1357.4893 $[1/2M+Na]^+$). However, the stereochemistry of the new glycosidic bond between Gal and GlcN could not be defined as the β form because the signals from the H-1 and H-2 protons of Gal observed by ^1H NMR spectroscopic analysis overlapped with other signals such as the H-1 proton from the inner GalN, H-3 from the terminal GalN, H-4 from the terminal Neu5Ac, and the CH_2 protons from the benzyl group. The protecting groups of heptasaccharide **29** were then manipulated to generate the corresponding imidate donor. This process began with sequential hydrogenolysis of the benzyl groups catalyzed by $Pd(OH)_2/C$ and acetylation to provide 1-*O*-acetyl intermediate **30**, which was then advanced by hydrazinolysis of the anomer acetate and trichloroacetimidate formation by treatment with trichloroacetonitrile (CCl_3CN) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU),[18] to generate the imidate donor **31** in 76% over four steps. At this stage, the anomeric configuration of the terminal Gal was assigned as the β form from ^1H NMR spectroscopic analysis, whereby the H-1 proton was observed at $\delta = 4.88$ ppm as a doublet with a coupling constant of 7.5 Hz, and the H-2 proton was observed at $\delta = 5.29$ ppm as a double doublet ($J_{2,3} = 9.6$ Hz).

Finally, the obtained full-length glycan donor **31** was glycosidated with the known ceramide acceptor **4**.[19] The best result was obtained when the reaction was conducted in $CHCl_3$ at room temperature upon activation by $BF_3{\cdot}OEt_2$ (1.2 equiv for the donor), producing the protected ganglioside X1 (**32**) in 26% yield.[8a,20] In other approaches, use of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst diminished the coupling yield to around 5 to 10%. Under the optimized reaction conditions, 61% of the glycosyl donor was recovered as the hemiacetal, which could again be converted into the donor **31** to be used for the conjugation of ceramide. Formation of the orthoester was not observed. To approach ganglioside X1, the acyl protecting groups were removed by applying Zemplén's method, followed by saponification of the methyl ester on the sialic acid residue, to successfully yield 13.5 mg of target compound **1** in pure form.

The synthetic compound **1** was immunostained with IgM antibodies and compared to natural X1 on a thin-layer chromatographic plate. Figure 1 shows that synthetic **1** had similar mobility to natural X1 from bovine brain, and that serum IgM antibodies from the patient with ALS-like disorder[2] reacted with both natural and synthetic X1.

Conclusion

We have succeeded in the total synthesis of the lacto-ganglio series ganglioside X1. The convergent approach employing the GM2-core unit and the lacto-ganglio tetraosyl unit allowed access to the target structure with high efficiency, producing homogenous X1 in sufficient quantity for biological study. Furthermore, we confirmed that the patient's serum IgM bound to the synthesized **1** on a 96-well microtiter



Scheme 8. Assembly of glycan part **29** and final conjugation with ceramide to deliver target structure **1**. TMSOTf = trimethylsilyl trifluoromethanesulfonate, DMAP = 4-dimethylaminopyridine, DMF = *N*,*N*-dimethylformamide, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

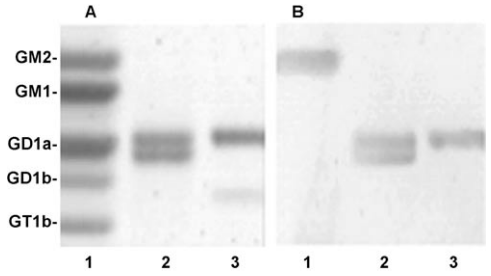


Figure 1. Reactivity of the synthesized X1 with serum that recognizes GM2 epitope [GalNAc β1-4 (NeuAc α2-3) Gal β]. A) TLC plate stained with the orcinol reagent for hexose. B) Immunostained chromatogram that had been overlaid first with serum from a patient with an ALS-like disorder[2] then with peroxidase-conjugated anti-human μ-chain specific antibodies. Lane 1: Authentic GM2, GM1, GD1a, GD1b, and GT1b. Lane 2: GM2-epitope containing gangliosides, X1 and X2, from bovine brain.[3] Lane 3: The synthesized X1 in the present study. Orcinol reagent stains GM2, GM1, GD1a, GD1b, GT1b, X1, and X2 from bovine brain and the synthesized X1. Serum IgM antibodies from the patient strongly bind to GM2, X1, and X2 from bovine brain, and the synthesized X1.

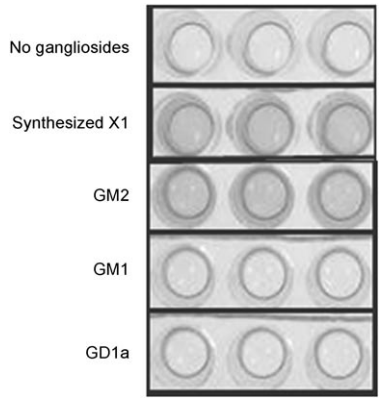


Figure 2. Reactivity of the synthesized X1 with serum that recognizes the GM2 epitope, developed on a microtiter plate. The patient's serum IgM antibodies react with X1 and GM2, but with neither GM1 nor GD1a. IgM antibodies against the X1, GM2, GM1, and GD1a (5 pmol/well) were measured in the patient's serum (1:500 dilution) according to reported procedure.[21] For structures of GM2, GM1, and GD1a, see the Supporting Information.

plate (Figure 2). Using the synthesized **1**, we will test serum samples from a number of patients who were misdiagnosed with ALS to identify those treatable with immunotherapy.

Experimental Section

General procedures: ^{1}H and ^{13}C NMR spectra were recorded with JEOL JNM-ECA600 spectrometers. ^{1}H NMR chemical shifts (δ) are expressed in ppm relative to the signal of Me_4Si as an internal standard, except when the samples were measured in [D_6]acetone, in which case the shift was referenced against the signal of acetone ($\delta = 2.09$ ppm). ^{13}C NMR chemical shifts (δ) are expressed in ppm relative to the signal of the solvent. High-resolution mass spectrometry (HRMS) was performed with a Bruker Daltonics micrOTOF (ESI-TOF) mass spectrometer. Specific rotations were measured with a Horiba SEPA-300 high-sensitivity polarimeter. Melting points were determined with an AS ONE ATM-01 apparatus. Molecular sieves were purchased from Wako Chemicals and dried at 300 °C for 2 h in a muffle furnace prior to use. Reactions were carried out under an atmosphere of argon unless otherwise specified. Solvents as reaction media were dried over molecular sieves and used without further purification. TLC analyses were performed on Merck TLC plates (silica gel 60F_{254} on glass), and compounds were visualized either by exposure to UV light (254 nm), by spraying with 10% H_2SO_4 solution in EtOH, or by treatment with ninhydrin reagent, followed by heating. Flash column chromatography on silica gel (Fuji Silysia Co., 80 mesh and 300 mesh) or Sephadex (Pharmacia LH-20) were performed with the solvent systems (v/v) specified. Evaporation and concentration were conducted in vacuo.

Compound 7: 2,2,2-Trichloroethyl chloroformate (200 μL, 1.45 mmol) was added to a solution of **14** (513 mg, 0.959 mmol) in pyridine (4.8 mL) at 0 °C, and the mixture was stirred for 30 min at RT. Upon completion of the reaction (confirmed by TLC analysis; EtOAc/hexane, 1:2), the reaction mixture was quenched by addition of MeOH at 0 °C and the residual solvent was removed by coevaporation with toluene. The residue was dissolved in EtOAc and the solution was washed with 2 M aqueous HCl, saturated aqueous $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:5) to give **7** (641 mg, 94 %), which was recrystallized from EtOAc/hexane. M.p. 158–160 °C; $[\alpha]_D = -26.0$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 7.51$–7.33 (m, 10H; 2 Ph), 5.52 (s, 1H; PhC*H*), 5.39 (t, $J_{2,3} = J_{3,4} = 9.7$ Hz, 1H; H-3), 5.29 (d, $J_{2,NH} = 8.3$ Hz, 1H; NH), 5.11 (d, $J_{1,2} = 10.3$ Hz, 1H; H-1), 4.79–4.71 (m, 4H; 2 CH_2), 4.41 (dd, $J_{5,6} = 5.2$ Hz, $J_{gem} = 10.7$ Hz, 1H; H-6), 3.82 (t, $J_{5,6'} = 10.3$ Hz, 1H; H-6′), 3.74 (t, $J_{4,5} = 9.7$ Hz, 1H; H-4), 3.68–3.60 ppm (m, 2H; H-2, H-5); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 153.9$, 153.8, 136.6, 133.1, 131.5, 129.2, 129.2, 128.6, 128.2, 126.1, 101.4, 95.2, 94.2, 86.8, 78.4, 76.9, 74.6, 70.4, 68.4, 55.5 ppm; HRMS: *m*/*z* calcd for $C_{25}H_{23}Cl_6NO_8SNa^+$: 729.9168 $[M+Na]^+$; found: 729.9168.

Compounds 16, 17, and 18: Molecular sieves (4 Å, 200 mg) were added to a solution of compounds **7** (98 mg, 137 μmol) and **9** (101 mg, 115 μmol) in CH_2Cl_2 (2.50 mL). The suspension was stirred for 30 min at −40 °C, whereupon NIS (51 mg, 227 μmol) and TfOH (2.0 μL, 22.6 μmol) were added. Stirring was continued for 40 min at −40 °C, at which point completion of the reaction was indicated by TLC (EtOAc/hexane 2:5, developed twice). The reaction mixture was filtered through Celite and the removed molecular sieves were washed with $CHCl_3$. The combined filtrate and washings were extracted with $CHCl_3$, and organic layer was washed with saturated aqueous $NaHCO_3$, saturated aqueous $Na_2S_2O_3$, and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexane 1:4→1:3→2:5) to give **16** (97 mg, 58 %), **17** (57 mg, 19 %), and **18** (30 mg, 21 %) as diastereoisomers (a/b = 13:17).

Compound 16: $[\alpha]_D = -45.5$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (600 MHz, [D$_6$]acetone): $\delta = 7.50$–7.21 (m, 30H; 6 Ph), 7.18 (d, $J_{2,NH} = 8.9$ Hz, 1H; NH), 5.72 (s, 1H; PhC*H*), 5.38 (t, $J_{2,3} = J_{3,4} = 9.7$ Hz, 1H; H-3c), 5.36 (d, $J_{1,2} = 8.2$ Hz, 1H; H-1c), 5.21 (d, $J_{gem} = 10.3$ Hz, 1H; CH_2), 5.01 (t, $J_{1,2} = J_{2,3} = 8.6$ Hz, 1H; H-2a), 4.99 (d, $J_{gem} = 11.0$ Hz, 1H; CH_2), 4.89 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.87–4.73 (m, 6H; OH, 4 CH_2), 4.66 (d, $J_{1,2} = 8.6$ Hz, 1H; H-1a), 4.62 (d, $J_{gem} = 10.3$ Hz, 1H; CH_2), 4.57 (d, $J_{1,2} = 7.6$ Hz, 1H; H-1b), 4.54 (d, $J_{gem} = 11.0$ Hz, 1H; CH_2), 4.52 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.42 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.39 (d, $J_{gem} = 12.3$ Hz, 1H; CH_2), 4.21–4.18 (m, 2H; H-6c, CH_2), 4.13 (d, $J_{3,4} = 1.4$ Hz, 1H; H-4b), 4.06 (t, $J_{3,4} = J_{4,5} = 9.3$ Hz, 1H; H-4a), 3.95 (t, $J_{4,5} = 9.7$ Hz, 1H; H-4c), 3.93–3.75 (m, 5H; H-6a, H-6′a, H-3b, H-2c, H-6′c), 3.72 (t, $J_{2,3} = J_{3,4} = 8.6$ Hz, 1H; H-3a), 3.68–3.57 (m, 4H; H-5a, H-2b, H-6b, H-5c), 3.55 (t, $J_{5,6} = J_{5,6'} = 5.5$ Hz, 1H; H-5b), 3.32 (dd, $J_{5,6'} = 5.5$ Hz, $J_{gem} = 9.6$ Hz, 1H; H-6′b), 1.16 ppm (s, 9H; *t*Bu); ^{13}C NMR (150 MHz, [D$_6$]acetone): $\delta = 176.9$, 155.4, 154.5, 140.3, 140.2, 139.5, 138.6, 138.5, 129.6, 129.0, 129.0, 128.9, 128.9, 128.8, 128.8, 128.6, 128.5, 128.3, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.1, 103.5, 102.7, 101.9, 100.8, 96.9, 95.7, 81.9, 81.8, 79.6, 78.3, 77.4, 77.3, 77.1, 76.0, 75.1, 74.9, 74.6, 74.5, 73.6, 73.5, 73.1, 70.9, 70.0, 69.0, 68.9, 66.7, 58.0, 39.2, 27.4 ppm; HRMS: *m*/*z* calcd for $C_{71}H_{77}Cl_6NO_{20}Na^+$: 1496.3062 $[M+Na]^+$; found: 1496.3063.

Compound 17: $[\alpha]_D = -37.0$ ($c = 0.5$, $CHCl_3$); ^{1}H NMR (600 MHz, [D$_6$]DMSO): $\delta = 8.24$ (d, $J_{2,NH} = 8.9$ Hz, 1H; NHd), 7.41–7.19 (m, 35H; 7 Ph), 5.77 and 5.75 (2 s, 2H; 2 PhC*H*), 5.37 (t, $J_{2,3} = J_{3,4} = 9.6$ Hz, 1H; H-3c), 5.30–5.27 (m, 2H; H-1c, H-3d), 5.04 (d, $J_{gem} = 10.3$ Hz, 1H; CH_2), 4.99 (d, $J_{1,2} = 8.2$ Hz, 1H; H-1d), 4.98–4.94 (m, 2H; 2 CH_2), 4.90 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.85–4.82 (m, 3H; 3 CH_2), 4.76–4.68 (m, 4H; H-1a, H-2a, 2 CH_2), 4.63 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.58 (d, $J_{gem} = 11.7$ Hz, 1H; CH_2), 4.53 (d, $J_{gem} = 11.7$ Hz, 1H; CH_2), 4.37–4.32 (m, 4H; H-1b, H-6d, 2 CH_2), 4.26 (d, $J_{gem} = 11.7$ Hz, 1H; CH_2), 4.23 (d, $J_{gem} = 12.3$ Hz, 1H; CH_2), 4.20 (t, $J_{3,4} = J_{4,5} = 9.6$ Hz, 1H; H-4d), 4.15 (brs, 1H; H-4b), 4.14–4.07 (m, 3H; H-2d, H-6c, CH_2), 4.05 (d, $J_{gem} = 10.3$ Hz, 1H; CH_2), 3.91 (t, $J_{3,4} = J_{4,5} = 9.6$ Hz, 1H; H-4c), 3.84–3.71 (m, 6H; H-4a, H-3b, H-2c, H-5c, H-6′c, H-6′d), 3.61 (dd, $J_{5,6} = 4.5$ Hz, $J_{gem} = 12.0$ Hz, 1H; H-6a), 3.58–3.42 (m, 5H; H-3a, H-5a, H-6′a, H-2b, H-5d), 3.41 (t, $J_{5,6} = J_{5,6'} = 5.5$ Hz, 1H; H-5b), 3.16 (m, 1H; H-6′b), 1.11 ppm (s, 9H; *t*Bu); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta = 176.2$, 154.6, 153.6, 153.4, 139.1, 139.1, 139.0, 138.4, 137.6, 137.4, 137.4, 129.1, 128.4, 128.2, 128.2, 128.2, 127.8, 127.8, 127.0, 127.6, 127.6, 127.5, 127.3, 127.3, 127.2, 126.4, 126.3, 102.6, 101.7, 100.5, 100.5, 100.3, 99.6, 96.4, 95.8, 95.0, 95.0, 81.9, 80.5, 78.8, 77.9, 77.0, 76.6, 76.5, 76.2, 76.1, 74.7, 74.5, 74.4, 74.2, 73.6, 73.6, 72.5, 72.4, 72.3, 72.1, 70,2, 68.9, 67.9, 67.5, 65.3, 64.7, 56.6, 55.8, 38.4, 27.0 ppm; HRMS: *m*/*z* calcd for $C_{90}H_{94}Cl_{12}N_2O_{28}Na^+$: 2093.2148 $[M+Na]^+$; found: 2093.2139.

Compound 18a: $[\alpha]_D = -8.4$ ($c = 1.6$, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 7.41$–7.16 (m, 30H; 6 Ph), 5.95 (s, 1H; PhC*H*), 5.15 (t, $J_{1,2} = J_{2,3} = 8.6$ Hz, 1H; H-2a), 4.92 (d, $J_{gem} = 11.0$ Hz, 1H; CH_2), 4.88 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.82 (d, $J_{gem} = 11.7$ Hz, 1H; CH_2), 4.74 (d, $J_{gem} = 11.0$ Hz, 1H; CH_2), 4.62–4.59 (m, 3H; 3 CH_2), 4.48 (d, $J_{1,2} = 8.6$ Hz, 1H; H-1a), 4.48 (d, $J_{1,2} = 8.3$ Hz, 1H; H-1b), 4.45 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.39 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.34 (t, $J_{2,3} = J_{3,4} = 6.2$ Hz, 1H; H-3b), 4.23 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.13 (d, $J_{4,5} = 1.4$, 9.6 Hz, 1H; H-4b), 4.09 (t, $J_{3,4} = J_{4,5} = 9.3$ Hz, 1H; H-4a), 3.90 (dd, $J_{5,6} = 4.1$ Hz, $J_{gem} = 11.0$ Hz, 1H; H-6a), 3.77 (dd, $J_{5,6'} = 1.4$ Hz, $J_{gem} = 11.0$ Hz, 1H; H-6′a), 3.67 (t, $J_{1,2} = J_{2,3} = 8.6$ Hz, 1H; H-3a), 3.65–3.61 (m, 2H; H-5b, H-6b), 3.49–3.44 (m, 3H; H-5a, H-2b, H-6′b), 1.13 ppm (s, 9H; *t*Bu); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 176.7$, 138.7, 138.2, 138.2, 137.2, 129.2, 128.4, 128.4, 128.3, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.2, 126.3, 103.3, 102.0, 99.8, 81.1, 80.3, 77.9, 76.5, 75.5, 73.9, 73.7, 73.5, 73.4, 73.3, 72.3, 72.2, 70.1, 69.0, 68.0, 38.7, 27.1 ppm; HRMS: *m*/*z* calcd for $C_{59}H_{64}O_{12}Na^+$: 987.4290 $[M+Na]^+$; found: 987.4290.

Compound 18b: $[\alpha]_D = -15.4$ ($c = 1.3$, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 7.43$–7.16 (m, 30H; 6 Ph), 5.91 (s, 1H; PhC*H*), 5.13 (t, $J_{1,2} = J_{2,3} = 8.6$ Hz, 1H; H-2a), 4.89 (d, $J_{gem} = 10.3$ Hz, 1H; CH_2), 4.87 (d, $J_{gem} = 11.0$ Hz, 1H; CH_2), 4.68 (d, $J_{gem} = 11.7$ Hz, 1H; CH_2), 4.60–4.55 (m, 4H; 4 CH_2), 4.46–4.41 (m, 3H; H-1a, H-1b, CH_2), 4.39 (d, $J_{gem} = 11.7$ Hz, 1H; CH_2), 4.25 (d, $J_{gem} = 11.0$ Hz, 1H; CH_2), 4.19–4.16 (m, 2H; H-3b, H-4b), 4.06 (t, $J_{3,4} = J_{4,5} = 9.3$ Hz, 1H; H-4a), 3.85 (dd, $J_{5,6} = 6.9$ Hz, $J_{gem} = 9.7$ Hz, 1H; H-6a), 3.76–3.72 (m, 2H; H-6′a, H-5b), 3.65 (dd, $J_{5,6} = 6.9$ Hz, $J_{gem} = 9.7$ Hz, 1H; H-6b), 3.61 (dd, $J_{2,3} = 8.6$ Hz, $J_{3,4} = 9.3$ Hz, 1H; H-3a), 3.45 (dd, $J_{5,6'} = 6.2$ Hz, $J_{gem} = 9.7$ Hz, 1H; H-6′b), 3.42 (m, 1H; H-5a), 3.37 (m, 1H; H-2b), 1.13 ppm (s, 9H; *t*Bu); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 176.7$, 138.7, 138.3, 138.2, 138.2, 137.8, 137.2, 129.2, 128.4, 128.3, 128.3, 128.3, 128.2, 128.0, 127.8, 127.8, 127.8, 127.7, 127.6, 127.5, 127.5, 127.1, 126.6, 104.3, 102.1, 99.8, 81.1, 80.9, 79.0, 76.3, 76.1, 75.4, 73.6, 73.5, 73.3, 73.3, 72.2, 71.8, 70.1, 68.8, 68.0, 38.7, 27.1 ppm; HRMS: *m*/*z* calcd for $C_{59}H_{64}O_{12}Na^+$: 987.4290 $[M+Na]^+$; found: 987.4290.

Compound 24: Dibutyltin(IV) oxide (37 mg, 148 μmol), 4-methoxybenzyl chloride (18.6 μL, 137 μmol), and tetrabutylammonium bromide (45 mg, 138 μmol) were added to a solution of **9** (101 mg, 115 μmol) in toluene (1.1 mL). The mixture was stirred for 9 h at 80 °C (completion of the reaction was confirmed by TLC analysis; EtOAc/hexane, 1:1), then triethylamine was added and the mixture was concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexane 1:3) to give **24** (106 mg, 93 %). $[\alpha]_D = -7.0$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 7.34$–7.17 (m, 27H; 6 ArH), 6.84 (d, $J = 8.2$ Hz, 2H; ArH), 5.13 (t, $J_{1,2} = J_{2,3} = 8.3$ Hz, 1H; H-2a), 4.97 (d, $J_{gem} = 11.0$ Hz, 1H; CH_2), 4.87 (d, $J_{gem} = 11.7$ Hz, 1H; CH_2), 4.76 (s, 2H; CH_2), 4.63 (d, $J_{gem} = 11.0$ Hz, 1H; CH_2), 4.62–4.57 (m, 3H; 3 CH_2), 4.56 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.46 (d, $J_{1,2} = 8.3$ Hz, 1H; H-1a), 4.42–4.40 (m, 2H; H-1b, CH_2), 4.33 (d, $J_{gem} = 12.0$ Hz, 1H; CH_2), 4.28 (d, $J_{gem} = 11.0$ Hz, 1H; CH_2), 4.05 (t, $J_{3,4} = J_{4,5} = 9.3$ Hz, 1H; H-4a), 3.96 (brs, 1H; H-4b), 3.82 (dd, $J_{5,6} = 4.5$ Hz, $J_{gem} = 10.3$ Hz, 1H; H-6a), 3.79 (s, 3H; OCH_3), 3.73 (d, $J_{gem} = 10.3$ Hz, 1H; H-6′a), 3.63 (dd, $J_{2,3} = 8.3$ Hz, $J_{3,4} = 9.3$ Hz, 1H; H-3a), 3.57 (t, $J_{1,2} = J_{2,3} = 8.6$ Hz, 1H; H-2b), 3.52 (dd, $J_{5,6} = 7.2$ Hz, $J_{gem} = 8.6$ Hz, 1H; H-6b), 3.43 (dd, $J_{4,5} = 9.3$ Hz, $J_{5,6} = 4.5$ Hz, 1H; H-5a), 3.35 (dd, $J_{2,3} = 8.6$ Hz, $J_{3,4} = 4.1$ Hz, 1H; H-3b), 3.35 (dd, $J_{5,6'} = 4.1$ Hz, $J_{gem} = 8.6$ Hz, 1H; H-6′b), 3.31 (dd, $J_{5,6} = 7.2$ Hz, $J_{5,6'} = 4.1$ Hz, 1H; H-5b), 2.39 (s, 1H; OH), 1.13 ppm (s, 9H; *t*Bu); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 176.7$, 159.3, 138.8, 138.6, 138.2, 138.0, 137.2, 129.9, 129.4, 128.3, 128.2, 128.2, 128.0, 127.8, 127.7, 127.6, 127.6, 127.6, 127.5, 127.4, 127.0, 113.8, 102.7, 99.7, 81.0, 80.7, 79.4, 76.4, 75.5, 75.3, 73.8, 73.4, 73.1, 72.8, 72.2, 71.7, 70.0, 68.5,

68.1, 66.2, 55.2, 38.7, 27.1 ppm; HRMS: *m/z* calcd for $C_{60}H_{68}O_{13}Na^+$: 1019.4552 $[M+Na]^+$; found: 1019.4552.

Compound 25: Molecular sieves (4 Å, 415 mg) were added to a solution of **8** (125 mg, 218 μmol) and **24** (145 mg, 145 μmol) in CH_2Cl_2 (3.65 mL). The suspension was stirred for 30 min at −20 °C, whereupon NIS (74 mg, 327 μmol) and TfOH (2.9 μL, 32.7 μmol) were added. Stirring was continued for 15 min at −20 °C, when completion of the reaction was indicated by TLC (EtOAc/hexane 1:2, developed twice). The reaction mixture was filtered through Celite and the removed molecular sieves were washed with $CHCl_3$. The combined filtrate and washings were extracted with $CHCl_3$, and organic layer was washed with saturated aqueous $NaHCO_3$, saturated aqueous $Na_2S_2O_3$, and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:2→1:1→3:2, then EtOAc/Toluene, 1:5) to give **25** (184 mg, 87 %). $[\alpha]_D = -12.0$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (600 MHz, [D$_6$]DMSO): $\delta = 7.57$ (d, $J_{2,NH} = 9.0$ Hz, 1H; NH), 7.43–7.16 (m, 27H; 6 ArH), 6.84 (d, $J = 8.2$ Hz, 2H; ArH), 5.30 (d, $J_{3,4} = 2.7$ Hz, 1H; H-4c), 5.18 (dd, $J_{2,3} = 11.4$ Hz, $J_{3,4} = 2.7$ Hz, 1H; H-3c), 5.03 (d, $J_{gem} = 10.3$ Hz, 1H; CH_2), 4.84 (d, $J_{1,2} = 8.3$ Hz, 1H; H-1c), 4.78 (d, $J_{gem} = 11.7$ Hz, 1H; CH_2), 4.76–4.68 (m, 5H; H-1a, H-2a, 3 CH_2), 4.66 (d, $J_{gem} = 10.3$ Hz, 1H; CH_2), 4.55 (d, $J_{gem} = 11.7$ Hz, 1H; CH_2), 4.54 (d, $J_{gem} = 12.3$ Hz, 1H; CH_2), 4.44–4.41 (m, 3H; 3 CH_2), 4.38 (d, $J_{1,2} = 7.5$ Hz, 1H; H-1b), 4.31 (d, $J_{gem} = 11.7$ Hz, 1H; CH_2), 4.24 (d, $J_{gem} = 12.3$ Hz, 1H; CH_2), 4.11 (dd, $J_{5,6} = 6.2$ Hz, $J_{gem} = 11.0$ Hz, 1H; H-6c), 4.05 (dd, $J_{5,6'} = 6.2$ Hz, $J_{gem} = 11.0$ Hz, 1H; H-6′c), 4.02 (s, 1H; H-4b), 3.99 (t, $J_{5,6} = J_{5,6'} = 6.2$ Hz, 1H; H-5c), 3.96 (d, $J_{gem} = 12.3$ Hz, 1H; CH_2), 3.81 (t, $J_{3,4} = J_{4,5} = 9.3$ Hz, 1H; H-4a), 3.77–3.65 (m, 8H; H-3a, H-5a, H-6a, H-6′a, H-2c, OCH_3), 3.56 (dd, $J_{1,2} = 7.5$ Hz, $J_{2,3} = 8.6$ Hz, 1H; H-2b), 3.53 (dd, $J_{5,6} = 4.2$ Hz, $J_{gem} = 11.0$ Hz, 1H; H-6b), 3.47 (d, $J_{2,3} = 8.6$ Hz, 1H; H-3b), 3.40 (dd, $J_{5,6} = 4.2$ Hz, $J_{5,6'} = 5.2$ Hz, 1H; H-5b), 3.28 (dd, $J_{5,6'} = 5.2$ Hz, $J_{gem} = 11.0$ Hz, 1H; H-6′b), 2.12, 1.97 and 1.87 (3×s, 9H; 3 Ac), 1.14 ppm (s, 9H; *t*Bu); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta = 176.2$, 170.2, 170.1, 169.6, 158.9, 154.3, 139.0, 138.9, 138.8, 138.3, 137.5, 130.8, 129.6, 128.8, 128.4, 128.3, 128.3, 128.2, 127.8, 127.8, 127.7, 127.5, 127.5, 127.4, 127.2, 113.7, 102.1, 101.5, 99.6, 96.4, 80.8, 80.3, 79.5, 79.3, 76.4, 74.7, 74.4, 74.3, 73.5, 73.5, 73.4, 72.5, 72.3, 72.3, 71.0, 70.3, 70.2, 69.8, 69.7, 67.8, 66.7, 61.6, 55.1, 52.5, 40.2, 38.4, 27.0, 20.6, 20.6, 20.5 ppm; HRMS: *m/z* calcd for $C_{75}H_{86}Cl_3NO_{22}Na^+$: 1480.4599 $[M+Na]^+$; found: 1480.4599.

Compound 26: TFA (41.0 μL, 552 μmol) was added to a solution of **25** (101 mg, 69.0 μmol) in CH_2Cl_2 (1.4 mL) at 0 °C, and the mixture was stirred for 7.5 h at RT (completion of the reaction was confirmed by TLC; EtOAc/hexane, 1:1), then the reaction mixture was quenched by the addition of triethylamine at 0 °C. The residue was extracted with $CHCl_3$ and the solution was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexane 2:3) to give **26** (90 mg, 98 %). $[\alpha]_D = -23.5$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (600 MHz, [D$_6$]DMSO): $\delta = 7.65$ (d, $J_{2,NH} = 8.9$ Hz, 1H; NH), 7.44–7.18 (m, 25H; 5 Ph), 5.30 (d, $J_{3,OH} = 5.5$ Hz, 1H; OH), 5.29 (d, $J_{3,4} = 3.5$ Hz, 1H; H-4c), 5.12 (dd, $J_{2,3} = 11.0$ Hz, $J_{3,4} = 3.5$ Hz, 1H; H-3c), 5.03 (d, $J_{gem} = 10.3$ Hz, 1H; CH_2), 4.99 (d, $J_{1,2} = 8.3$ Hz, 1H; H-1c), 4.85 (d, $J_{gem} = 11.7$ Hz, 1H; CH_2), 4.83 (d, $J_{gem} = 10.3$ Hz, 1H; CH_2), 4.78–4.73 (m, 3H; H-2a, 2 CH_2), 4.70 (d, $J_{1,2} = 8.3$ Hz, 1H; H-1a), 4.60 (d, $J_{gem} = 11.7$ Hz, 1H; CH_2), 4.55 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.44–4.40 (m, 3H; H-1b, 2 CH_2), 4.35 (d, $J_{gem} = 11.7$ Hz, 1H; CH_2), 4.28 (d, $J_{gem} = 12.3$ Hz, 1H; CH_2), 4.10 (dd, $J_{5,6} = 5.5$ Hz, $J_{gem} = 11.0$ Hz, 1H; H-6c), 4.05 (dd, $J_{5,6'} = 6.2$ Hz, $J_{gem} = 11.0$ Hz, 1H; H-6′c), 4.00 (brs, 1H; H-5c), 3.96 (d, $J_{gem} = 12.3$ 1H; CH_2), 3.90 (s, 1H; H-4b), 3.84 (m, 1H; H-2c), 3.83 (t, $J_{3,4} = J_{4,5} = 9.6$ Hz, 1H; H-4a), 3.73 (dd, $J_{5,6} = 4.2$ Hz, $J_{gem} = 11.0$ Hz, 1H; H-6a), 3.70–3.61 (m, 4H; H-3a, H-5a, H-6′a, H-3b), 3.53 (dd, $J_{5,6} = 4.2$ Hz, $J_{gem} = 10.5$ Hz, 1H; H-6b), 3.50–3.46 (m, 2H; H-2b, H-5b), 3.25 (dd, $J_{5,6'} = 6.6$ Hz, $J_{gem} = 10.5$ Hz, 1H; H-6′b), 2.16, 1.97 and 1.87 (3×s, 9H; 3 Ac), 1.14 ppm (s, 9H; *t*Bu); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta = 176.2$, 170.1, 170.1, 169.6, 153.9, 139.3, 139.0, 138.8, 138.4, 137.6, 128.8, 128.4, 128.2, 128.2, 127.8, 127.7, 127.5, 127.5, 127.4, 127.3, 127.2, 102.3, 101.5, 99.7, 96.5, 80.7, 80.3, 76.4, 76.3, 74.8, 74.4, 73.6, 73.5, 73.0, 72.5, 72.3, 70.9, 70.2, 69.9, 69.6, 67.9, 66.9, 61.6, 52.6, 38.4, 27.0, 20.6, 20.6 ppm; HRMS: *m/z* calcd for $C_{67}H_{78}Cl_3NO_{21}Na^+$: 1360.4024 $[M+Na]^+$; found: 1360.4026.

Compound 27: Molecular sieves (4 Å, 450 mg) were added to a solution of **7** (130 mg, 183 μmol) and **26** (163 mg, 122 μmol) in CH_2Cl_2 (3.0 mL). The suspension was stirred for 30 min at −10 °C, whereupon NIS (83 mg, 366 μmol) and TfOH (3.2 μL, 36.6 μmol) were added. Stirring was continued for 40 min at −10 °C (completion of the reaction was indicated by TLC, EtOAc/hexane 1:1). The reaction mixture was filtered through Celite and the removed molecular sieves were washed with $CHCl_3$. The combined filtrate and washings were extracted with $CHCl_3$, and organic layer was washed with saturated aqueous $NaHCO_3$, saturated aqueous $Na_2S_2O_3$, and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexane 1:2→1:1) to give **27** (198 mg, 84 %). $[\alpha]_D = -35.0$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (600 MHz, [D$_6$] DMSO): $\delta = 8.23$ (d, $J_{2,NH} = 8.9$ Hz, 1H; NHd), 7.42–7.17 (m, 30H; 6 Ph), 7.08 (d, $J_{2,NH} = 9.6$ Hz, 1H; NHc), 5.68 (s, 1H; PhC*H*), 5.32 (d, $J_{3,4} = 3.4$ Hz, 1H; H-4c), 5.29 (t, $J_{2,3} = J_{3,4} = 10.0$ Hz, 1H; H-3d), 5.25 (dd, $J_{2,3} = 11.7$ Hz, $J_{3,4} = 3.4$ Hz, 1H; H-3c), 5.16 (d, $J_{1,2} = 8.2$ Hz, 1H; H-1c), 5.00 (d, $J_{1,2} = 8.9$ Hz, 1H; H-1d), 5.00 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.98 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.87 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.85 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.78 (d, $J_{gem} = 11.7$ Hz, 1H; CH_2), 4.74–4.69 (m, 4H; H-1a, H-2a, 2 CH_2), 4.58 (d, $J_{gem} = 11.7$ Hz, 1H; CH_2), 4.58 (d, $J_{gem} = 11.0$ Hz, 1H; CH_2), 4.53 (d, $J_{gem} = 11.7$ Hz, 1H; CH_2), 4.36 (d, $J_{1,2} = 7.6$ Hz, 1H; H-1b), 4.36 (d, $J_{gem} = 10.3$ Hz, 1H; CH_2), 4.32 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.30–4.21 (m, 5H; H-6c, H-4d, H-6d, 2 CH_2), 4.15 (d, $J_{3,4} = 2.1$ Hz, 1H; H-4b), 4.13–4.02 (m, 4H; H-5c, H-6′c, H-2d, CH_2), 3.95 (m, 1H; H-2c), 3.93 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 3.83 (t, $J_{5,6'} = J_{gem} = 10.0$ Hz, 1H; H-6′d), 3.77–3.73 (m, 2H; H-4a, H-3b), 3.62 (dd, $J_{5,6} = 3.9$ Hz, $J_{gem} = 10.7$ Hz, 1H; H-6a), 3.60–3.43 (m, 7H; H-3a, H-5a, H-6′a, H-2b, H-5b, H-6b, H-5d), 3.18 (dd, $J_{5,6'} = 7.9$ Hz, $J_{gem} = 12.3$ Hz, 1H; H-6′b), 2.19, 1.98 and 1.91 (3×s, 9H; 3×Ac), 1.13 ppm (s, 9H; *t*Bu); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta = 176.2$, 170.2, 170.0, 169.8, 154.6, 154.5, 153.4, 139.1, 139.0, 138.8, 138.4, 137.6, 137.3, 129.1, 128.7, 128.3, 128.2, 128.1, 127.7, 127.7, 127.7, 127.6, 127.5, 127.3, 127.2, 126.3, 102.5, 101.6, 100.6, 100.2, 99.6, 96.6, 95.7, 95.0, 82.3, 80.4, 78.7, 77.9, 76.5, 76.3, 76.1, 74.7, 74.6, 74.2, 73.7, 73.5, 73.0, 72.5, 72.3, 72.1, 71.0, 70.2, 69.8, 69.5, 67.9, 67.5, 67.2, 65.2, 61.7, 55.9, 52.5, 38.4, 27.0, 20.7, 20.6 ppm; HRMS: *m/z* calcd for $C_{86}H_{95}Cl_9N_2O_{29}Na^+$: 1957.3109 $[M+Na]^+$; found: 1957.3103.

Compound 28: Zinc powder (3.00 g) was added to a solution of **27** (204 mg, 105 μmol) in a mixture of MeOH (5.2 mL) and AcOH (5.2 mL), and the mixture was stirred for 30 min at RT (completion of the reaction was confirmed by TLC analysis; $CHCl_3$/MeOH, 20:1). The reaction mixture was filtered through Celite and the removed zinc powder was washed with $CHCl_3$. The combined filtrate and washings were concentrated and the residue was extracted with $CHCl_3$. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), concentrated, and exposed to high vacuum for 4 h. The residue was treated with a solution of Ac_2O (24.0 μL, 254 μmol) in MeOH (600 μL) and CH_2Cl_2 (600 μL) for 40 min (completion of the reaction was confirmed by TLC analysis; $CHCl_3$/MeOH, 15:1). The reaction mixture was extracted with EtOAc and this solution was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 50:1→40:1→30:1) to give **28** (118 mg, 75 %). $[\alpha]_D = -41.5$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (600 MHz, CD_3CN): $\delta = 7.52$–7.20 (m, 30H; 6 Ph), 7.16 (d, $J_{2,NH} = 10.3$ Hz, 1H; NHc), 6.75 (d, $J_{2,NH} = 9.7$ Hz, 1H; NHd), 5.63 (s, 1H; PhC*H*), 5.32 (d, $J_{3,4} = 3.4$ Hz, 1H; H-4c), 5.10 (d, $J_{1,2} = 8.9$ Hz, 1H; H-1c), 5.04 (dd, $J_{2,3} = 11.7$ Hz, $J_{3,4} = 3.4$ Hz, 1H; H-3c), 5.01 (d, $J_{gem} = 10.3$ Hz, 1H; CH_2), 4.78 (t, $J_{1,2} = J_{2,3} = 8.9$ Hz, 1H; H-2a), 4.77 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.69 (d, $J_{gem} = 11.0$ Hz, 1H; CH_2), 4.65 (d, $J_{1,2} = 8.9$ Hz, 1H; H-1d), 4.61 (d, $J_{gem} = 12.4$ Hz, 1H; CH_2), 4.56 (d, $J_{gem} = 11.0$ Hz, 1H; CH_2), 4.53 (d, $J_{1,2} = 8.9$ Hz, 1H; H-1a), 4.47 (d, $J_{gem} = 10.3$ Hz, 1H; CH_2), 4.42 (d, $J_{gem} = 11.7$ Hz, 1H; CH_2), 4.37 (m, 1H; H-2c), 4.35 (d, $J_{1,2} = 8.2$ Hz, 1H; H-1b), 4.30–4.26 (m, 3H; H-6d, 2 CH_2), 4.17 (dd, $J_{5,6} = 5.5$ Hz, $J_{gem} = 9.6$ Hz, 1H; H-6c), 4.08 (d, $J_{3,4} = 2.8$ Hz, 1H; H-4b), 4.07–3.98 (m, 4H; H-5c, H-6′c, H-2d, CH_2), 3.86 (t, $J_{3,4} = J_{4,5} = 9.3$ Hz, 1H; H-4a), 3.81 (t, $J_{5,6'} = J_{gem} = 10.0$ Hz, 1H; H-6′d), 3.77 (t, $J_{2,3} = J_{3,4} = 9.7$ Hz, 1H; H-3d), 3.72–3.66 (m, 3H; H-6a, H-4d, OH), 3.62–3.59 (m, 2H; H-3a, H-3b), 3.55 (d, $J_{gem} = 9.6$ Hz, 1H; H-6′a), 3.51–3.43 (m, 3H; H-5a, H-6b, H-5d), 3.40–3.37 (m, 2H; H-2b, H-5b), 3.18 (dd, $J_{5,6'} = 6.2$ Hz, $J_{gem} = 10.3$ Hz, 1H; H-6′b), 2.18, 1.96, 1.95, 1.92 and 1.75 (5×s, 15H; 5×Ac), 1.16 ppm (s, 9H; *t*Bu);

^{13}C NMR (150 MHz, CD_3CN): δ = 176.2, 170.3, 170.1, 169.9, 169.1, 139.0, 139.0, 138.8, 138.4, 137.9, 137.6, 129.1, 128.8, 128.3, 128.2, 128.1, 127.8, 127.6, 127.5, 127.5, 127.2, 127.2, 127.0, 126.6, 103.5, 101.5, 101.0, 101.0, 99.7, 81.5, 81.2, 80.2, 79.3, 76.3, 74.9, 74.8, 74.4, 74.2, 72.9, 72.4, 72.4, 72.2, 71.6, 70.2, 70.1, 69.7, 69.4, 68.1, 67.6, 67.1, 66.0, 61.9, 56.4, 49.1, 38.4, 27.0, 23.1, 23.1, 20.7, 20.6 ppm; HRMS: *m/z* calcd for $C_{81}H_{96}N_2O_{25}Na^+$: 1519.6194 $[M+Na]^+$; found: 1519.6194.

Compound 29: AW-300 molecular sieves (4 Å, 500 mg) were added to a solution of **5** (153 mg, 115 μmol) and **28** (113 mg, 75.5 μmol) in CH_2Cl_2 (1.90 mL). The suspension was stirred for 30 min at 0 °C, whereupon TMSOTf (1.0 μL, 5.54 μmol) was added. Stirring was continued for 30 min at 0 °C (completion of the reaction was indicated by TLC analysis; $CHCl_3$/MeOH, 12:1; developed twice). The reaction mixture was filtered through Celite and the removed molecular sieves were washed with $CHCl_3$. The combined filtrate and washings were extracted with $CHCl_3$, and organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 40:1→35:1→30:1→25:1) to give **29** (174 mg, 86 %). $[\alpha]_D = -16.5$ (c = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, CD_3CN): δ = 8.30 (d, J = 7.5 Hz, 2H; Ph), 7.83 (d, J = 7.6 Hz, 2H; Ph), 7.65 (t, J = 7.3 Hz, 1H; Ph), 7.58 (t, J = 7.2 Hz, 1H; Ph), 7.50 (t, J = 7.3 Hz, 2H; Ph), 7.46–7.11 (m, 32H; 7 Ph), 7.03 (d, $J_{2,NH}$ = 9.6 Hz, 1H; NHc), 6.41 (d, $J_{2,NH}$ = 9.7 Hz, 1H; NHf), 6.30 (d, $J_{2,NH}$ = 9.6 Hz, 1H; NHd), 6.12 (d, $J_{5,NH}$ = 10.3 Hz, 1H; NHg), 5.62 (s, 1H; PhC*H*), 5.34 (d, $J_{3,4}$ = 3.5 Hz, 1H; H-4c), 5.22 (d, $J_{3,4}$ = 2.8 Hz, 1H; H-4f), 5.18–5.12 (m, 3H; H-3c, H-7g, H-8g), 5.08–4.99 (m, 5H; H-1c, H-1e, H-2e, H-3f, H-4g), 4.98 (d, J_{gem} = 10.3 Hz, 1H; CH_2), 4.86 (d, $J_{1,2}$ = 8.3 Hz, 1H; H-1f), 4.76 (t, $J_{1,2} = J_{2,3}$ = 9.0 Hz, 1H; H-2a), 4.75 (d, J_{gem} = 12.4 Hz, 1H; CH_2), 4.62–4.48 (m, 7H; H-1a, H-1d, H-3e, H-9g, 3 CH_2), 4.46 (d, J_{gem} = 10.3 Hz, 1H; CH_2), 4.40 (d, J_{gem} = 12.4 Hz, 1H; CH_2), 4.37 (q, $J_{1,2} = J_{2,3} = J_{2,NH}$ = 9.6 Hz, 1H; H-2c), 4.29 (d, $J_{1,2}$ = 8.2 Hz, 1H; H-1b), 4.26–4.22 (m, 3H; H-6d, 2 CH_2), 4.18–4.02 (m, 12H; H-4b, H-5c, H-6c, H-6′c, H-2d, H-3d, H-6e, H-2f, H-5f, H-5g, H-9g, CH_2), 3.99 (d, $J_{3,4}$ = 2.1 Hz, 1H; H-4e), 3.95–3.92 (m, 3H; H-5e, H-6′e, H-6f), 3.84–3.81 (m, 3H; H-4a, H-6′d, H-6g), 3.75 (s, 3H; $COOCH_3$), 3.71 (dd, $J_{5,6}$ = 6.2 Hz, J_{gem} = 11.0 Hz, 1H; H-6′f), 3.63–3.56 (m, 2H; H-6a, H-4d), 3.54 (dd, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 2.7 Hz, 1H; H-3b), 3.50 (d, J_{gem} = 10.3 Hz, 1H; H-6′a), 3.45–3.40 (m, 3H; H-5a, H-6b, H-5d), 3.36 (t, $J_{5,6} = J_{5,6'}$ = 6.2 Hz, 1H; H-5b), 3.33 (dd, $J_{1,2}$ = 8.2 Hz, $J_{2,3}$ = 9.6 Hz, 1H; H-2b), 3.16 (dd, $J_{5,6'}$ = 6.2 Hz, J_{gem} = 11.0 Hz, 1H; H-6′b), 2.22–2.15 (m, 7H; H-3g_{eq}, 2 Ac), 2.11, 2.05 and 1.98 (3×s, 9H; 3 Ac), 1.96–1.89 (m, 19H; H-3g_{ax}, 6 Ac), 1.82, 1.78 and 1.75 (3×s, 9H; 3 Ac), 1.16 ppm (s, 9H; *t*Bu); ^{13}C NMR (150 MHz, CD_3CN): δ = 177.6, 171.5, 171.3, 171.2, 171.1, 171.0, 171.0, 170.8, 170.6, 170.5, 170.4, 169.2, 166.4, 165.3, 140.0, 139.9, 139.7, 139.3, 138.4, 138.4, 134.3, 134.1, 130.9, 130.7, 130.7, 130.1, 129.8, 129.6, 129.4, 129.4, 129.2, 129.2, 129.1, 129.0, 128.9, 128.9, 128.7, 128.6, 128.4, 128.3, 128.2, 128.1, 127.9, 127.0, 103.9, 103.1, 102.9, 102.2, 101.9, 100.9, 100.8, 100.2, 82.2, 81.4, 81.3, 80.3, 79.0, 78.7, 77.0, 75.9, 75.8, 75.5, 75.3, 74.3, 74.0, 73.7, 73.5, 73.2, 72.6, 72.4, 72.2, 72.1, 71.7, 71.3, 71.2, 70.8, 70.6, 70.3, 69.1, 68.6, 68.5, 68.3, 67.8, 67.6, 67.0, 63.8, 63.0, 62.4, 62.2, 55.4, 53.9, 50.8, 50.4, 48.7, 39.3, 35.7, 27.4, 23.4, 23.2, 23.1, 23.0, 21.4, 21.0, 21.0, 20.8, 20.8, 20.7, 20.5 ppm; HRMS: *m/z* calcd for 1/2 $(C_{135}H_{160}N_4O_{52})+Na^+$: 1357.4892 $[1/2M+Na]^+$; found: 1357.4893.

Compound 30: $Pd(OH)_2$/C (20 %, 234 mg) was added to a solution of **29** (153 mg, 115 μmol) in EtOH (1.90 mL) and the suspension was stirred under a hydrogen stream for 62 h at RT (completion of the reaction was confirmed by TLC analysis; $CHCl_3$/MeOH/H_2O, 14:3:0.1). The reaction mixture was filtered through Celite and washed thoroughly with $CHCl_3$. The combined filtrate and washings were concentrated and exposed to high vacuum for 4 h. The residue was then treated with a solution of Ac_2O (500 μL, 5.29 mmol) and DMAP (1 mg, 8.15 μmol) in pyridine (500 μL) for 21 h (completion of the reaction was confirmed by TLC analysis; $CHCl_3$/MeOH, 18:1; developed twice), then the reaction mixture was coevaporated with toluene. The residue was extracted with EtOAc and the solution was washed with 2 M aqueous HCl, saturated aqueous $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 30:1→25:1) to give **30** (97 mg, 93 %, α/β = 5/4). ^{1}H NMR (600 MHz, CD_3CN): δ = 6.16 (d, $J_{1,2}$ = 3.4 Hz, 1H; H-1aα), 5.75 ppm (d, $J_{1,2}$ = 8.3 Hz, 0.8H; H-1aβ); HRMS: *m/z* calcd for 1/2 $(C_{107}H_{140}N_4O_{59})+Na^+$: 1235.3931 $[1/2M+Na]^+$; found: 1235.3931.

Compound 31: Hydrazine acetate (10 mg, 111 μmol) was added to a solution of **30** (97 mg, 40.0 μmol) in DMF (800 μL) and the mixture was stirred for 10 min at 50 °C (completion of the reaction was confirmed by TLC analysis; $CHCl_3$/MeOH, 15:1; developed twice). The reaction mixture was diluted with EtOAc and the solution was washed with water and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 20:1→15:1) to give the 1-OH derivative, which was exposed to high vacuum for 7 h. The residue was then treated with a solution of trichloroacetonitrile (80.0 μL, 79.8 μmol) and DBU (7.5 μL, 50.1 μmol) in CH_2Cl_2 for 50 min at RT (completion of the reaction was confirmed by TLC analysis; $CHCl_3$/MeOH, 10:1). The mixture was concentrated and the residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 25:1) to give **31** (83 mg, 82 %). $[\alpha]_D = -2.0$ (c = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.65 (s, 1H; NH), 8.14 (d, J = 6.9 Hz, 2H; Ph), 8.05 (d, J = 7.6 Hz, 2H; Ph), 7.59 (t, J = 7.2 Hz, 1H; Ph), 7.55 (t, J = 7.2 Hz, 1H; Ph), 7.48–7.43 (m, 4H; Ph), 6.49 (d, $J_{1,2}$ = 3.5 Hz, 1H; H-1a), 6.43–6.35 (m, 2H; NHc, NHf), 5.85 (d, $J_{2,NH}$ = 11.0 Hz, 1H; NHd), 5.53 (m, 1H; H-8g), 5.52 (t, $J_{2,3} = J_{3,4}$ = 10.0 Hz, 1H; H-3a), 5.37 (d, $J_{3,4}$ = 2.7 Hz, 1H; H-4c), 5.36 (d, $J_{3,4}$ = 3.4 Hz, 1H; H-4f), 5.29 (dd, $J_{1,2}$ = 7.5 Hz, $J_{2,3}$ = 9.6 Hz, 1H; H-2e), 5.23–5.20 (m, 3H; H-3c, H-3f, H-7g), 5.04 (dd,$J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.0 Hz, 1H; H-2a), 5.02–4.98 (m, 3H; H-1c, H-1f, NHg), 4.95 (dd, $J_{1,2}$ = 8.3 Hz, $J_{2,3}$ = 10.3 Hz, 1H; H-2b), 4.88 (d, $J_{1,2}$ = 7.5 Hz, 1H; H-1e), 4.83 (td, $J_{3ax,4} = J_{4,5}$ = 11.2 Hz, $J_{3eq,4}$ = 4.2 Hz, 1H; H-4g), 4.76 (brs, 1H; H-4d), 4.62 (dd, $J_{5,6}$ = 7.2 Hz, J_{gem} = 11.0 Hz, 1H; H-6e), 4.40–4.38 (m, 2H; H-6c, H-3e), 4.32 (dd, $J_{5,6'}$ = 4.8 Hz, J_{gem} = 11.0 Hz, 1H; H-6′e), 4.26 (d, $J_{1,2}$ = 8.3 Hz, 1H; H-1b), 4.26–4.23 (m, 3H; H-6b, H-1d, H-6f), 4.20 (dd, $J_{8,9}$ = 1.4 Hz, J_{gem} = 12.3 Hz, 1H; H-9g), 4.16–4.11 (m, 2H; H-6′b, H-6′c), 4.08–4.05 (m, 3H; H-5a, H-6a, H-6d), 4.03 (d, $J_{3,4}$ = 2.0 Hz, 1H; H-4b), 3.99 (dd, $J_{8,9'}$ = 5.5 Hz, J_{gem} = 12.3 Hz, 1H; H-9g), 3.96–3.78 (m, 10H; H-4a, H-6′a, H-3d, H-5d, H-6′d, H-4e, H-5e, H-5f, H-6′f, H-5g), 3.76 (dd, $J_{5,6}$ = 11.0 Hz, $J_{6,7}$ = 2.0 Hz, 1H; H-6g), 3.73 (s, 3H; $COOCH_3$), 3.62–3.57 (m, 5H; H-3b, H-5b, H-2c, H-2d, H-2f), 2.67 (dd, $J_{3eq,4}$ = 4.2 Hz, J_{gem} = 12.3 Hz, 1H; H-3g_{eq}), 2.15, 2.14, 2.14, 2.09, 2.08, 2.06, 2.05, 2.05, 2.04, 2.02, 2.01, 2.01 and 1.96 (14×s, 42H; 14 Ac), 1.84–1.79 (m, 10H; H-3g_{ax}, 3 Ac), 1.77 (s, 3H; Ac), 1.13 ppm (s, 9H; *t*Bu); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 178.0, 171.5, 171.3, 171.3, 171.2, 171.1, 171.0, 171.0, 170.7, 170.4, 170.3, 170.0, 169.1, 166.9, 165.3, 160.7, 134.4, 134.3, 131.0, 130.6, 130.6, 130.3, 129.6, 129.4, 103.6, 103.0, 102.0, 101.6, 100.8, 100.3, 93.4, 91.3, 80.0, 79.0, 78.7, 76.6, 75.9, 75.2, 74.4, 72.9, 72.8, 72.6, 72.3, 72.2, 72.1, 71.6, 71.3, 70.9, 70.5, 70.4, 70.3, 70.0, 68.5, 68.0, 67.9, 67.4, 64.4, 64.1, 63.2, 62.9, 62.5, 62.4, 62.1, 53.9, 50.9, 50.0, 48.7, 39.3, 35.8, 27.0, 23.4, 23.2, 23.0, 22.9, 21.4, 21.2, 21.0, 21.0, 20.9, 20.8, 20.8, 20.7, 20.7 ppm; HRMS: *m/z* calcd for $C_{107}H_{138}Cl_3N_5O_{58}Na^+$: 2548.6961 $[M+Na]^+$; found: 2548.6960.

Compound 32: Molecular sieves (4 Å, 250 mg) were added to a solution of **31** (88 mg, 34.8 μmol) and **4** (35 mg, 52.2 μmol) in $CHCl_3$ (880 μL). The suspension was stirred for 30 min at RT, whereupon $BF_3{\cdot}OEt_2$ (5.2 μL, 41.0 μmol) was added. Stirring was continued for 6.5 h at RT (completion of the reaction was indicated by TLC analysis; $CHCl_3$/MeOH, 20:1; developed twice). The reaction mixture was filtered through Celite and the removed molecular sieves were washed with $CHCl_3$. The combined filtrate and washings were extracted with $CHCl_3$, and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 40:1→30:1→20:1, then acetone/hexane, 3:2) to give **32** (27 mg, 26 %). $[\alpha]_D = -14.6$ (c = 2.3, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.12 (d, J = 6.9 Hz, 2H; Ph), 8.05 (d, J = 6.8 Hz, 2H; Ph), 8.00 (d, J = 6.9 Hz, 2H; Ph), 7.60–7.53 (m, 3H; 3 Ph), 6.44–6.31 (m, 2H; NHc, NHf), 5.86 (dt, $J_{4,5}$ = 15.2 Hz, $J_{5,6} = J_{5,6'}$ = 7.2 Hz, 1H, H-5^{Cer}), 5.82 (d, $J_{2,NH}$ = 10.3 Hz, 1H; NHd), 5.72 (d, $J_{2,NH}$ = 9.6 Hz, 1H; NH^{Cer}), 5.54–5.51 (m, 2H; H-8g, H-3^{Cer}), 5.45 (dd, $J_{3,4}$ = 7.9 Hz, $J_{4,5}$ = 15.2 Hz,1H, H-4^{Cer}), 5.37 (d, $J_{3,4}$ = 2.7 Hz, 1H; H-4c), 5.36 (d, $J_{3,4}$ = 3.4 Hz, 1H; H-4f), 5.28 (t, $J_{1,2} = J_{2,3}$ = 9.0 Hz, 1H; H-2e), 5.22–5.20 (m, 3H; H-3c, H-3f, H-7g), 5.15 (t, $J_{2,3} = J_{3,4}$ = 8.6 Hz, 1H; H-3a), 5.02–4.97 (m, 2H; H-1c, NHg), 4.91–4.87 (m, 4H; H-2a, H-2b, H-1e, H-1f), 4.83 (td, $J_{3ax,4} = J_{4,5}$ = 11.0 Hz, $J_{3eq,4}$ = 4.4 Hz, 1H; H-4g), 4.72 (brs, 1H; H-4d), 4.62 (dd, $J_{5,6}$ = 7.6 Hz, J_{gem} = 10.3 Hz, 1H; H-6e), 4.46 (m, 1H;

H-2^{Cer}), 4.38 (d, $J_{1,2}=7.6$ Hz, 1H; H-1a), 4.36 (m, 1H; H-3e), 4.32 (dd, $J_{5,6'}=4.5$ Hz, $J_{gem}=10.3$ Hz, 1H; H-6′e), 4.25–4.17 (m, 6H; H-1b, H-6c, H-1d, H-6d, H-6f, H-9g), 4.12 (dd, $J_{5,6'}=7.2$ Hz, $J_{gem}=12.0$ Hz, 1H; H-6′c), 4.08–3.79 (m, 14H; H-6a, H-6′a, H-4b, H-6b, H-6′b, H-3d, H-5d, H-6′d, H-4e, H-5e, H-6′f, H-5g, H-9g, H-1^{Cer}), 3.75 (dd, $J_{5,6}=11.0$ Hz, $J_{6,7}=2.1$ Hz, 1H; H-6 g), 3.73 (s, 3H; $COOCH_3$), 3.69 (dd, $J_{3,4}=8.6$ Hz, $J_{4,5}=9.6$ Hz, 1H; H-4a), 3.66–3.55 (m, 8H; H-3b, H-5b, H-2c, H-5c, H-2d, H-2f, H-5f, H-1′^{Cer}), 3.49 (m, 1H; H-5a), 2.64 (dd, $J_{3eq,4}=4.4$ Hz, $J_{gem}=10.3$ Hz, 1H; H3 g_{eq}), 2.15, 2.14, 2.13, and 2.10 (4×s, 12H; 4 Ac), 2.06–2.00 (m, 30H; H-3g_{ax}, H-6^{Cer}, H-6′^{Cer}, 9 Ac), 1.96, 1.92, 1.88, 1.83, 1.83, 1.81, and 1.75 (7×s, 21H; 7 Ac), 1.33–1.22 (m, 54H; 27 CH_2), 1.14 (s, 9H; *t*Bu), 0.88 ppm (2×t, $J=6.6$ Hz, 6H; 2 CH_2CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta=177.0$, 172.6, 171.8, 171.1, 170.8, 170.8, 170.7, 170.7, 170.5, 170.3, 170.3, 170.2, 170.1, 170.1, 170.0, 169.9, 169.9, 169.6, 168.9, 168.3, 165.7, 165.0, 164.8, 164.7, 137.7, 133.3, 133.2, 132.9, 130.3, 130.3, 129.8, 129.7, 129.6, 128.5, 128.5, 128.3, 124.7, 100.4, 100.2, 100.1, 99.6, 99.6, 97.9, 74.7, 73.8, 73.6, 73.5, 73.4, 73.0, 72.7, 72.1, 71.9, 71.9, 71.4, 71.2, 70.7, 70.5, 70.1, 69.9, 68.7, 68.6, 67.4, 67.1, 66.9, 66.9, 66.5, 63.2, 62.6, 62.4, 62.3, 62.1, 61.4, 61.2, 52.9, 52.8, 50.5, 49.2, 38.8, 36.9, 36.5, 32.3, 31.9, 30.0, 29.9, 29.8, 29.8, 29.7, 29.6, 29.5, 29.5, 29.4, 29.3, 29.2, 28.9, 27.1, 26.9, 25.7, 23.6, 23.3, 23.2, 23.1, 22.7, 21.2, 20.9, 20.8, 20.7, 20.7, 20.6, 20.6, 20.5, 20.4, 20.3, 14.1 ppm; HRMS: *m/z* calcd for 1/2 ($C_{148}H_{211}N_5O_{61}$)+Na^+: 1540.1674[1/2*M*+Na]$^+$; found: 1540.1673.

Ganglioside X1 (1): A solution of sodium methoxide (28% in MeOH, 0.4 mg) was added to a solution of **32** (22 mg, 7.25 μmol) in MeOH (500 μL) and THF (500 μL). The mixture was stirred for 45 h at RT, whilst monitoring the reaction by TLC ($CHCl_3$/MeOH/12 mM $MgCl_2$ aq, 5:4:1). The mixture was then heated at 40 °C and stirring was continued for 32 h at 40 °C. Water (200 μL) was added and stirring was continued for 35 h at 40 °C. After neutralization with Dowex-50 (H^+), the mixture was filtered through cotton wool, and the removed resin was washed with mixed solvent ($CHCl_3$/MeOH, 1:1). The combined filtrate and washings were concentrated and the residue was purified by column chromatography on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and column chromatography on silica gel ($CHCl_3$/MeOH/H_2O, 5:4:0.6→5:4:0.7) to give **1** (13.5 mg, 95%). $[\alpha]_D=+1.4$ ($c=0.7$, MeOH); ^{1}H NMR (600 MHz, CD_3OD): $\delta=5.67$ (dt, $J_{4,5}=15.1$ Hz, $J_{5,6}=J_{5,6'}=7.2$ Hz, 1H; H-5^{Cer}), 5.44 (dd, $J_{4,5}=15.1$ Hz, $J_{3,4}=7.8$ Hz, 1H; H-4^{Cer}), 4.88–4.85 (m, 2H; 2 anomeric H), 4.60 (d, $J_{1,2}=8.9$ Hz, 1H; anomeric H), 4.40 (d, $J_{1,2}=8.3$ Hz, 1H; anomeric H), 4.31 (d, $J_{1,2}=7.6$ Hz, 1H; anomeric H), 4.28 (d, $J_{1,2}=7.5$ Hz, 1H; anomeric H), 4.26 (d, $J_{3,4}=2.0$ Hz, 1H; H-4), 4.19 (dd, $J=9.9$ Hz, $J=4.5$ Hz, 1H), 4.12 (d, $J_{3,4}=2.8$ Hz, 1H; H-4), 2.75 (dd, $J_{gem}=12.8$ Hz, $J_{3eq,4}=5.2$ Hz, 1H; H-3g_{eq}), 2.03–2.00 (m, 14H; H-6^{Cer}, H-6′^{Cer}, 4 Ac), 2.16 (t, $J=7.6$ Hz, 2H; $NHCOCH_2$), 1.89 (t, $J_{3ax,4}=12.0$ Hz, 1H; H-3g_{ax}), 1.57 (m, 2H; $NHCOCH_2CH_2$),1.39–1.28 (m, 50H; 25 CH_2), 0.90 ppm (2×t, $J=7.3$ Hz, 6H; 2 CH_2CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta=175.9$, 175.6, 175.0, 174.7, 174.7, 174.5, 135.1, 131.4, 105.2, 105.0, 104.6, 104.4, 104.3, 103.4, 103.4, 101.3, 84.7, 83.5, 78.9, 77.7, 76.9, 76.5, 76.5, 76.3, 76.1, 75.8, 75.7, 75.1, 74.9, 74.2, 73.8, 73.4, 73.0, 71.3, 70.7, 70.4, 70.3, 70.0, 69.9, 69.7, 65.4, 63.0, 62.9, 61.8, 61.8, 56.2, 54.7, 54.3, 54.1, 53.8, 38.8, 37.4, 33.5, 33.1, 30.9, 30.8, 30.8, 30.8, 30.7, 30.6, 30.5, 30.5, 30.4, 27.2, 23.7, 23.6, 23.6, 23.5, 22.6, 14.5 ppm; HRMS: *m/z* calcd for $C_{89}H_{157}N_5O_{41}$−H: 1951.0281 [*M*−H]$^-$; found: 1951.0280.

TLC with immunostaining: The novel GM2-epitope-containing gangliosides, X1 and X2, and authentic gangliosides GM2, GM1, GD1a, GD1b, and GT1b were prepared from bovine brain gangliosides as described elsewhere.[3,22] These gangliosides and the synthesized X1 were layered on precoated Silica Gel 60 plates (Merck, Darmstadt, Germany). The plates were developed with a solvent system of chloroform/methanol/12 mM magnesium chloride in water (5:4:1, by volume), dipped in *n*-hexane containing 0.4% > polyisobutylmethacrylate for 1 min, then dried under an air stream. The TLC plate was overlaid with serum from the patient with ALS-like disorder (1:50 dilution with phosphate-buffered saline/0.5% casein) and kept at 4 °C overnight. The plates were washed and overlaid with peroxidase-conjugated anti-human *m*-chain-specific antibodies (Dako, Glostrup, Denmark; 1:250 dilution with phosphate-buffered saline/0.5% casein), kept at 20 °C for 2 h, then washed. Binding activities were made visible with 4-chloro-1-naphtol.

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