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A cyclic glucosyl ceramide acceptor as a versatile building block for complex ganglioside synthesis

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ABSTRACT

A cyclic glucosyl ceramide (GlcCer) acceptor has been developed as a versatile building block for the synthesis of complex ganglioside. A macrocyclic GlcCer acceptor, which was the product of intramolecular glycosylation between glucose and ceramide, exhibited high reactivity during the coupling reactions with a variety of complex oligosaccharyl donors, thereby furnishing the corresponding ganglioside frameworks in high yields.

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Among glycolipids, which cover the surface of animal cells, gangliosides are distinguished by the one or more sialic acid residues that are positioned at the termini of their glycan chains. It is well known that gangliosides function as receptors for toxins, viruses and bacteria and mediate the targeting of lymphocytes to inflammatory tissue.[1,2] Furthermore, gangliosides involved in raft such as GM3 and GM1 are considered to be important mediators in the signal transduction.[3] In addition, many gangliosides have been reported to be tumor-specific antigens.[4] Therefore, the elucidation and medicinal application of the biological functions of gangliosides have been intensively investigated. However, gangliosides are only a minor constituent of glycolipids, which has been an impediment to functional research into gangliosides; thus, a large and readily available supply of pure gangliosides is highly desirable.

To date, our group has completed the first syntheses of various gangliosides including sialyl Lewis X,[5] sialyl Lewis A,[6] and GQ1b.[7] However, to advance the biological and medicinal application of gangliosides, we must approach the synthesis of gangliosides systematically and on large scale. In our previous syntheses of gangliosides, a fully assembled glycan segment was coupled with azide-sphingosine to construct the glycolipid framework. This was followed by the reduction of the azide group and amide formation via reaction with fatty acid such as stearic acid, yielding the protected ganglioside.[5–8] However, the final assembly of the ganglioside framework was not always satisfactory in terms of total yield, and required considerable effort and expertise, which prevented a large supply of fine gangliosides from being produced. In this context, direct coupling of the full-length glycan and ceramide segments effectively reduces the total steps required for the synthesis of the final structure. However, the yield of the direct coupling tends to diminish as the length of the oligosaccharide increases.[9]

Recently, we have proposed a new strategy for the synthesis of gangliosides that uses cyclic glucosyl ceramide as a key building block. In this approach, saturated phytoceramide was used instead of unsaturated ceramide.[10] In the present Letter, we demonstrate the power of this new strategy by presenting representative examples of the efficient assembly of complex ganglioside frameworks using a cyclic glucosyl ceramide acceptor.

In this new strategy, ganglioside is disconnected at the β(1,4)-linkage between the Gal-Glc sequence, which is a common sequence in all mammalian gangliosides, thus providing an oligosaccharyl galactosyl donor and a glucosyl ceramide (GlcCer) acceptor. Glucose is first glycosidated with the C1 hydroxyl group of ceramide, and then the GlcCer unit serves as a common coupling partner for oligosaccharide donors (Scheme 1). To accept a variety of branched and complex oligosaccharide donors, the C4 hydroxyl group of the GlcCer acceptor is required to be highly reactive. In addition, the efficiency of the ceramide introduction, which is generally low even in the case of glucose, should be further improved with respect to total efficiency. To meet these criteria, we have envisaged the intramolecular glycosidation[11] of glucose and cera-

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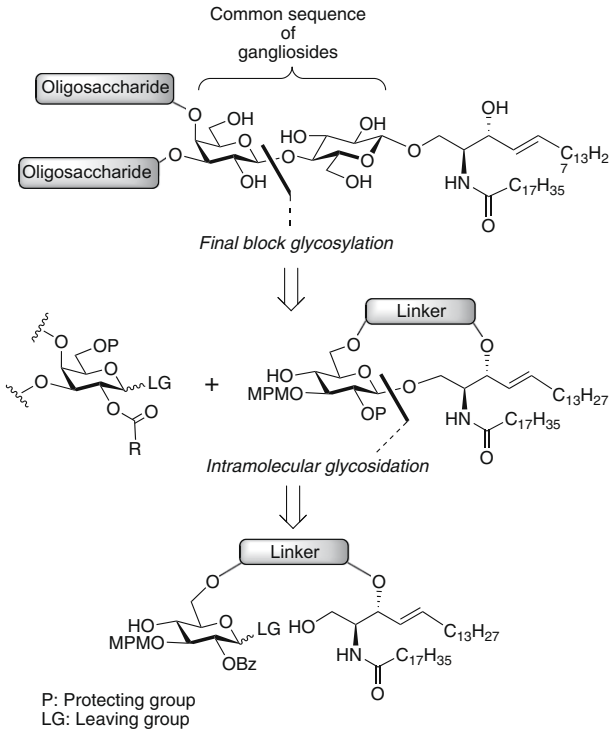
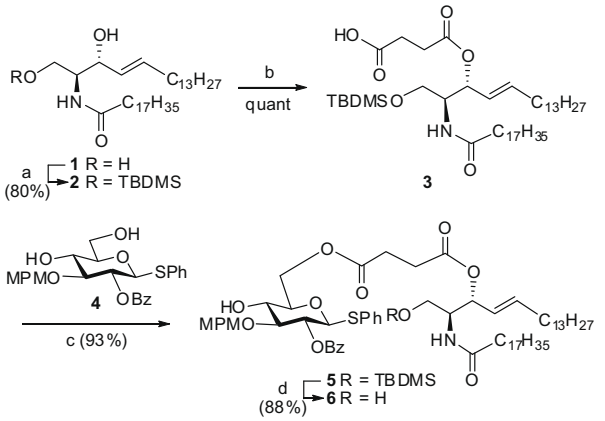


Scheme 1. Key concept of new synthetic strategy toward gangliosides.

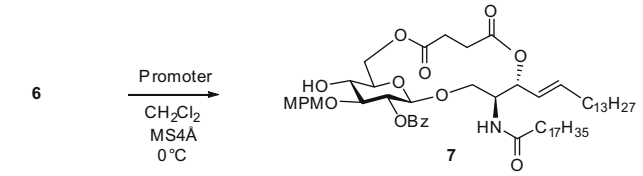
mide as a critical step. The tethering of glucose and ceramide was expected to increase the likelihood of the attack of C1 hydroxyl group of ceramide to a glucosyl oxocarbenium ion, thus allowing the C4 hydroxyl group of the glucose donor to remain unprotected. Furthermore, the generated cyclic GlcCer acceptor is expected to have a highly reactive hydroxyl group at C4 because the C6–O6 bond may not adopt a gauche/trans configuration due to ring strain. In the design of the glucose moiety, a benzoyl group was mounted at the C2 hydroxyl group to impart β-selectivity during intramolecular glycosidation, and the C3 hydroxyl group was protected with an electron-donating *p*-methoxybenzyl group.

For the tethering, the C1 hydroxyl group of ceramide **1**[12] was selectively protected as the silyl ether, and then succinylated at the C3 position by reaction with succinic anhydride and DMAP in pyridine to afford **3** in high yield (80%, two steps) (Scheme 2). Next,



Scheme 2. Reagents and conditions: (a) TBDMSCl, DMAP, Et_3N/CH_2Cl_2, rt, 80%; (b) succinic anhydride, DMAP/pyr, 40 °C, quant; (c) **4**, EDC·HCl, DMAP/CH_2Cl_2, rt, 93%; (d) TBAF, AcOH/THF, rt, 88%. TBDMSCl = *tert*-butyldimethylsilyl chloride, DMAP = 4-dimethylaminopyridine, EDC·HCl = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, TBAF = tetrabutylammonium fluoride.

Table 1
Examination of intramolecular glycosidation

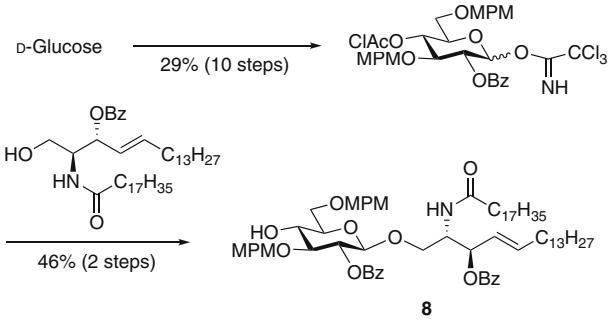


Entry	Concn of **6** (mM)	Promoter	Time (h)	Yield (%)
1	20	NIS (1.1 equiv), TfOH (0.2 equiv)	2	Trace
2	5.0	DMTST (3.0 equiv)	2	92
3	20	DMTST (3.0 equiv)	2	88
4	5.0	DMTSF (3.0 equiv)	6	50

the 4,6-unprotected phenylthioglycoside of glucose **4**[10] and the ceramide derivative **3** were condensed in the presence of EDC·HCl and DMAP in CH_2Cl_2 at room temperature to produce the C6-tethered compound **5** exclusively in 93% yield. For the next intramolecular glycosidation, the tethered compound **5** was then desilylated by treatment with TBAF and AcOH in THF to afford **6** in 88% yield.

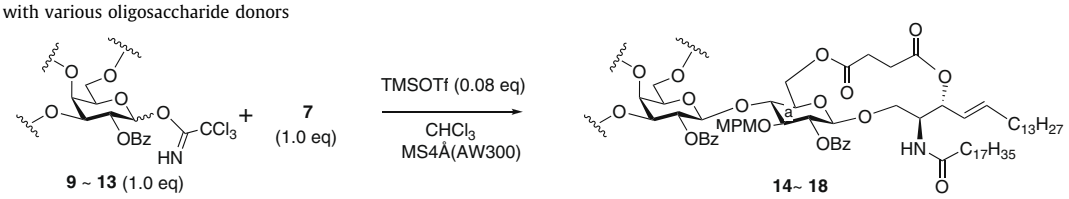
Intramolecular glycosidation[13] of **6** was examined under various conditions (Table 1). When using an NIS–TfOH system[14] to promote the reaction, the olefin moiety at the C4–C5 position reacted with iodonium cation, eventually affording the hydroxylated byproduct after aqueous work-up along with other unknown byproducts (entry 1). Next, a thiophilic promoter, namely, methylsulfonium cation, was examined. In entry 2, DMTST[15] (3.0 equiv) was used with **6**. This reaction produced the glycosidated product **7**[16] in 92% yield. Under highly concentrated conditions (**6**; 20 mM), **7** was also produced in high yield (88%) (entry 3). When the counter-anion was replaced with tetrafluoroborate, the reaction became sluggish and the yield decreased to 50% (entry 4). The best result was obtained in entry 2, which could also be reproduced on gram scale. Finally, based on a free glucose, the cyclic GlcCer acceptor **7** was synthesized in a total yield of 36% over 10 steps (compound **4** was synthesized from glucose in 48% over seven steps[10]). In contrast, the acyclic GlcCer acceptor **8**,[7b] which was previously reported in the synthesis of GQ1b, was obtained in 13% over 12 steps (Scheme 3).

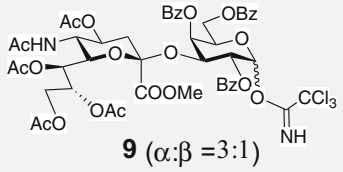
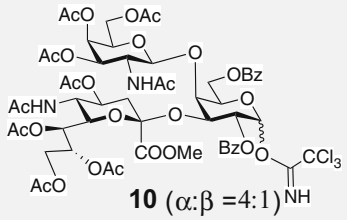
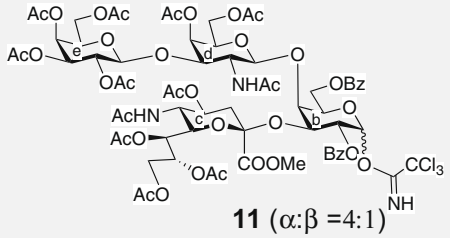
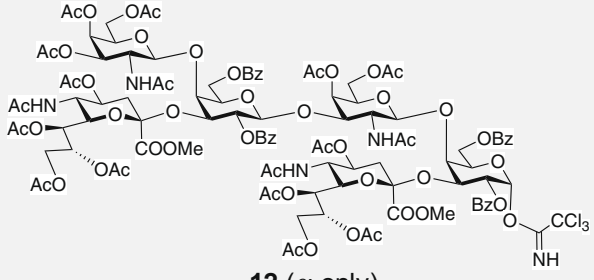
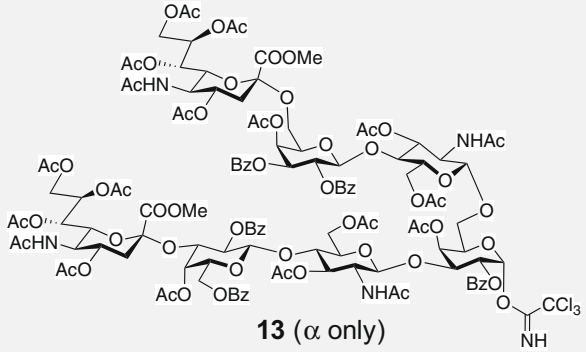
With the cyclic GlcCer acceptor **7** in hand, glycosylation with various oligosaccharide donors, including linear and branched donors, was performed (Table 2). The sialyl galactosyl imidate **9**,[17] upon treatment with a catalytic amount of TMSOTf in $CHCl_3$ at 0 °C, was successfully glycosidated with **7** to afford the GM3 ganglioside skeleton **14** in 85% yield (entry 1). For GM2 epitope and GM1 epitope donors, **10**[18] and **11**,[19] which have a branch at C3 and C4 of galactose, GlcCer **7** served as an excellent coupling partner, producing GM2 and GM1 ganglioside skeletons **15** and **16**[20] in high yield, respectively (entries 2 and 3). A more complex donor,



Scheme 3. Overview of the synthesis of GlcCer acceptor **8** in the previous Letter.

Table 2
Glycosylations of 7 with various oligosaccharide donors

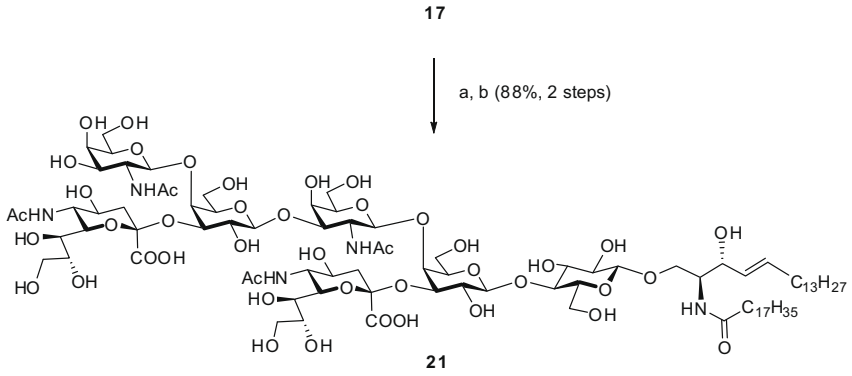


Entry	Donor	Temp (°C)	Time (h)	Product	Yield (%)
1	 **9** (α:β =3:1)	0	2	**14**	85
2	 **10** (α:β =4:1)	0	2	**15**	70
3	 **11** (α:β =4:1)	0	2	**16**	73
4	 **12** (α only)	rt	2	**17**	60
5	 **13** (α only)	rt	1	**18**	49

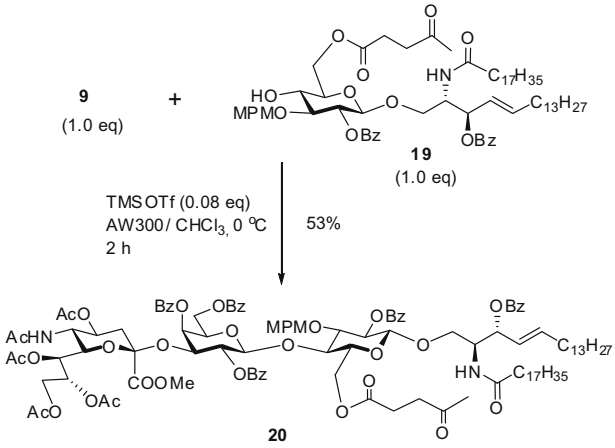
[a] 0.1 equiv of TMSOTf was used.

GalNAc-GD1a hexasaccharide epitope **12**, was also successfully combined with **7** in 60% yield (entry 4). To our delight, even heptasaccharyl donor **13**, which has heavy branching at C3 and C6 of galactose, reacted with GlcCer to afford octaosyl ceramide **18** in 49% yield (entry 5).

On the other hand, the glycosylation of the acyclic GlcCer acceptor **19** with reactive donor **9** produced the corresponding GM3 skeleton **20** in 53% yield (Scheme 4). The distinct difference between the cyclic and the acyclic is in accordance with the aforesaid initial expectation in the design of GlcCer acceptor.



Scheme 5. Reagents and conditions: (a) TFA/CH_2Cl_2, 0 °C, quant; (b) NaOMe/MeOH–THF (1:1), then H_2O, rt, 88%. TFA = trifluoroacetic acid.



Scheme 4. Glycosylation of acyclic GlcCer acceptor **19** with donor **9**.

The obtained protected gangliosides **14**–**18** were successfully deprotected to afford the corresponding gangliosides in pure form. For example, **17** was treated with TFA in CH_2Cl_2 at 0 °C to remove the MPM group, followed by the deprotection of the acyl groups and hydrolysis of the methyl ester, furnishing ganglioside GalNAc-GD1a[21] **21** in 88% yields over two steps (Scheme 5).

In conclusion, we have developed a cyclic GlcCer acceptor **7** which is a versatile building block for intricate ganglioside syntheses. The cyclic GlcCer acceptor **7** was synthesized with high efficiency via regioselective tethering of glucose and ceramide, and subsequent intramolecular glycosidation in high yield. The results of couplings with complex oligosaccharide donors have successfully demonstrated the high utility of **7**.

Acknowledgements

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Supplementary data

Supplementary data (^1H and ^{13}C NMR spectra of compounds **5**–**7**, **14**–**18** and **21**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.12.121.

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16. Spectroscopic data of compound **7**: $[\alpha]_D$ −29.2 (*c* 0.5, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 8.03–7.45 (m, 5H, Ph), 7.19–6.78 (2 d, 4H, Ar), 5.78 (m, 1H, H-5^{Cer}), 5.70 (d, $J_{NH,2}$ = 8.9 Hz, 1H, NH), 5.26 (m, 2H, H-3^{Cer}, H-4^{Cer}), 5.17 (t, $J_{1,2}$ = $J_{2,3}$ = 7.6 Hz, 1H, H-2), 4.63 (2 d, 2H, PhCH_2), 4.54 (d, 1H, H-1), 4.41 (near t, $J_{5,6}$ = 8.9 Hz, J_{gem} = 11.7 Hz, 1H, H-6), 4.26 (m, 2H, H-6′, H-2^{Cer}), 3.88 (dd, J_{gem} = 9.6 Hz, $J_{1,2}$ = 3.4 Hz, 1H, H-1^{Cer}), 3.75 (s, 3H, OMe), 3.69–3.59 (m, 4H, H-4, H-3, H-5, H-1′^{Cer}), 2.75–2.51 (m, 4H, OCOCH_2CH_2COO), 2.50 (d, $J_{4,4\text{-OH}}$ = 2.7 Hz, 1H, 4-OH), 2.04 (t, 2H, NHCOCH_2), 1.95 (m, 2H, H-6^{Cer}, H-6′^{Cer}), 1.48 (m, 2H, NHCOCH$_2$CH_2), 1.26 (m, 50H, 25CH$_2$), 0.88 (t, 6H, 2CH$_3$); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.7, 171.9, 170.6, 165.2, 159.5, 138.3, 133.4, 129.9, 129.7, 129.6, 128.5, 124.8, 114.0, 100.0, 82.0, 73.9, 73.7, 73.4, 72.9, 70.9, 66.7, 64.1, 55.2, 50.0, 36.7, 32.3, 31.9, 29.71, 29.66, 29.62, 29.55, 29.5, 29.38, 29.36, 29.3, 29.2, 28.9, 25.6, 22.7, 14.1; HRMS (ESI) *m/z* found $[M+Na]^+$ 1056.6752, $C_{61}H_{95}NO_{12}$ calcd for $[M+Na]^+$ 1056.6751.
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20. Spectroscopic data of compound **16**: $[\alpha]_D$ = +10.0 (*c* 0.9, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 8.17–7.38 (m, 15H, 3Ph), 7.16–6.68 (2 d, 4H, Ar), 5.94 (d, $J_{NH,2}$ = 6.8 Hz, 1H, NH-d), 5.70 (m, 1H, H-5^{Cer}), 5.64 (m, 1H, H-8c), 5.60 (d, $J_{NH,2}$ = 9.6 Hz, 1H, NH^{Cer}), 5.42 (t, $J_{2,3}$ = $J_{1,2}$ = 9.7 Hz, 1H, H-2b), 5.33 (m, 2H, H-4d, H-4e), 5.24 (dd, $J_{6,7}$ = 2.8 Hz, $J_{7,8}$ = 11.6 Hz, 1H, H-7c), 5.20 (m, 2H, H-3^{Cer}, H-4^{Cer}), 5.12 (m, 3H, H-2e, H-1d, H-2a), 5.01 (d, $J_{NH,5}$ = 10.3 Hz, 1H, NH-c), 4.96 (m, 2H, H-3e, H-1b), 4.89 (dd, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 2.8 Hz, 1H, H-3d), 4.83 (m, 1H, H-4c), 4.80–4.59 (2 d, 2H, PhCH_2), 4.67 (dd, J_{gem} = 11.0 Hz, $J_{5,6}$ = 6.2 Hz, 1H, H-6b), 4.60 (m, 1H, H-1e), 4.51 (dd, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 2.8 Hz, 1H, H-3b), 4.43 (d, $J_{1,2}$ = 6.8 Hz, 1H, H-1a), 4.33 (m, 1H, H-9c), 4.21(m, 3H, H-2^{Cer}, H-6e, H-6′e), 4.05 (m, 5H, H-5e, H-6a, H-6′a, H-6′b, H-9′c), 3.80 (m, 13H, H-6d, H-6′d, H-5d, OMe, H-5b, H-3a, H-4a, H-5a, H-5c, H-4b, H-1^{Cer}), 3.62 (s, 3H, OMe), 3.52 (m, 2H, H-6c, H-1′^{Cer}), 3.41 (m, 1H, H-2d), 2.73 (dd, J_{gem} = 12.4 Hz, $J_{3eq,4}$ = 4.1 Hz, 1H, H-3c*eq*), 2.60–2.44 (m, 4H, OCOCH_2CH_2COO), 2.19–1.57 (s, 36H, 12Ac), 1.97 (m, 2H, NHCOCH_2), 1.89 (m, 2H, H-6^{Cer}, H-6′^{Cer}), 1.78 (m, 1H, H-3c*ax*), 1.45 (m,

2H, $NHCOCH_2CH_2$), 1.25 (m, 50H, $25CH_2$), 0.88 (t, 6H, $2CH_3$); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.6, 171.8, 171.1, 170.7, 170.3, 170.1, 170.0, 169.2, 168.3, 165.6, 165.1, 165.0, 158.9, 137.9, 133.33, 133.26, 133.0, 130.3, 130.2, 129.9, 129.6, 129.5, 129.4, 129.3, 128.5, 128.42, 128.35, 124.7, 113.5, 101.1, 101.0, 99.5, 99.2, 97.9, 80.7, 78.5, 77.3, 74.3, 73.9, 73.8, 73.3, 73.2, 72.6, 72.2, 71.9, 71.0, 70.9, 70.5, 68.9, 68.8, 67.3, 66.7, 66.5, 66.3, 63.4, 63.0, 62.6, 62.2, 60.8, 54.9, 52.8, 49.9, 49.3, 36.63, 36.56, 32.2, 31.9, 30.0, 29.7, 29.53, 29.45, 29.4, 29.34, 29.25, 28.8, 25.5, 23.9, 23.1, 22.7, 21.4, 20.82, 20.76, 20.70, 20.66, 20.5, 20.4, 20.3, 14.1; HRMS (ESI) m/z found $[M+Na]^+$ 2517.1294, $C_{127}H_{175}N_3O_{47}$ calcd for $[M+Na]^+$ 2517.1290.

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