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FULL PAPER

Synthesis of fucosylated chondroitin sulfate glycoclusters: a robust route to novel anticoagulant agents

Xiao Zhang[a], Wang Yao[a], Xiaojiang Xu[a], Huifang Sun[b], Jinhua Zhao[b], Xiangbao Meng[a], Mingyi Wu*[b] and Zhongjun Li*[a]

Abstract: *Fucosylated chondroitin sulfate (FuCS) is a structurally distinct glycosaminoglycan with excellent anticoagulant activity. Studies show that FuCS and its depolymerized fragments exhibit different anticoagulant mechanism from heparin derivatives, with decreased risks of adverse effects and bleeding. However, further exploitation has been hindered by the scarcity of structurally defined oligosaccharides. Herein we report a facile method to synthesize the repeating trisaccharide unit of FuCS based on the degradation of chondroitin sulfate polymers. A series of simplified FuCS glycomimetics that have highly tunable structures, controllable branches, and defined sulfation motifs were generated by CuAAC. Remarkable improvement in APTT assay activities was observed as the branches increased, while no significant influences were observed for PT and TT assay activities. Further FXase inhibition tests suggested glycoclusters* ***33b*~*40b*** *selectively inhibited intrinsic anticoagulant activities while showed little effect on the extrinsic and common coagulation pathways. Notably, glycoclusters bearing 2,4-di-O-sulfated Fuc residue displayed the most potency, which was in consistent with natural polysaccharides. These FuCS clusters demonstrated potency to mimic linear glycosaminoglycans and offer a new framework for the development of novel anticoagulant agents.*

Introduction

Thrombotic disease is one of the most serious threats to human health [1]. The available antithrombotic drugs have a common side effect: the risk of bleeding during therapy [2]. In addition, structural heterogeneity of unfractionated heparin and LMWH often leads to adverse effects and variable dose–response relationships [3], leading to potential side effect and inconvenience in clinical use. Shorter fragments, such as Arixtra prepared through chemical approaches, is difficult and expensive to synthesize [4], which, to a certain extent, limits its application. Thus, alternatives with broad anticoagulation activity, improved safety profile and well-defined structure are greatly needed.

Fucosylated chondroitin sulfate (FuCS) is a structurally distinct glycosaminoglycan isolated from sea cucumber. Natural FuCSs show a very high variety of structures. Heterogeneity comes not only from different sulfation patterns on Fuc, but also on GalNAc. Furthermore, Fuc branches could be sometimes found not only on GlcA units, but also on GalNAc [5]. Among these structures, FuCS with repeating unit depicted in Figure 1 is the most common one. This kind of marine polysaccharide has recently attracted considerable attention because of its various biological activities, such as coagulation and thrombosis [5a, 6~10], anti-inflammatory [7, 11, 12], anti-HIV [13], and angiogenesis activities [14], among which anticoagulant activity is particularly promising due to its satisfactory activity, low risk of bleeding [9, 10] and oral efficacy [15]. It is noteworthy that the presence of sulfated Fuc branches is strictly required, and most of these biological activities significantly decrease when the Fuc residues are selectively released [6, 16].

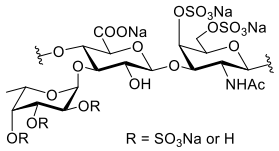

Figure 1. Repeating trisaccharide unit of FuCS

However, similar to heparin-like drugs, the native polysaccharide also has drawbacks, such as side effects of platelet aggregation, factor XII activation and bleeding [17], together with high variability of sulfation patterns. Efforts have been devoted to develop mild and selective depolymerization methods aimed to shorten the FuCS polymer chain without cleaving the sulfate groups as well as the labile Fuc branches [9, 18]. Results show that the anticoagulant activity increases in proportion to the molecular weight [9], and the depolymerized fragments indeed display reduced adverse effects [9, 10, 19], nevertheless remaining heterogeneous.

To avoid the use of animal sourced molecules, a total synthesis of FuCS trisaccharide unit has been accomplished by Tamura and Nifantiev groups [20]. In addition, a modular approach to a library of semi-synthetic FuCS species has very recently been proposed by Bedini group via chemical fucosylation of a microbial sourced chondroitin polysaccharide [21].

To the best of our knowledge, the anticoagulant evaluation of FuCS derivatives with precise structure has only been reported by Zhao et al, elucidating FuCS and its depolymerized fragments selectively inhibit the intrinsic coagulation pathway, and nonasaccharide is the minimum structural unit that retains the potent activity [10, 22]. Nevertheless, further exploitation of their structure–activity relationship has been hindered by the scarcity of structurally defined oligosaccharides whether from natural sources or synthetic approaches, thus new methods are greatly needed for the efficient preparation of well-defined FuCS-like molecules.

"Cluster effect" is known to enhance the interaction between carbohydrates and proteins [23], and previous studies have shown that synthetic glycopolymers or clusters could mimic the activity of natural glycosaminoglycans in neurite outgrowth [24],

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anticoagulant action [25] and inhibition of Alzheimer's disease protease BACE-1 [26]. More importantly, a series of glycoclusters with different valences, orientations and molecular weights are readily accessible simply by adjustment of frameworks, which makes it easy to summarize the structure–activity relationship and guide the effort to make better targets. As for FuCS, we expected to know how many trisaccharide repeating units are necessary for potent activity and whether the sulfation patterns would influence its anticoagulant behavior or not, then screen a structurally defined minimal active fragment *via* glycocluster assembly. Herein, we report the synthesis of multivalent FuCS trisaccharide glycoclusters and their ability to mimic natural polysaccharide chains in anticoagulant activity (Figure 2).

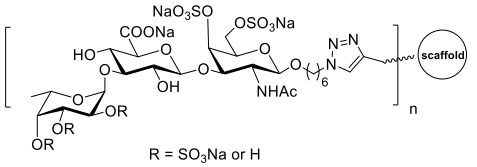
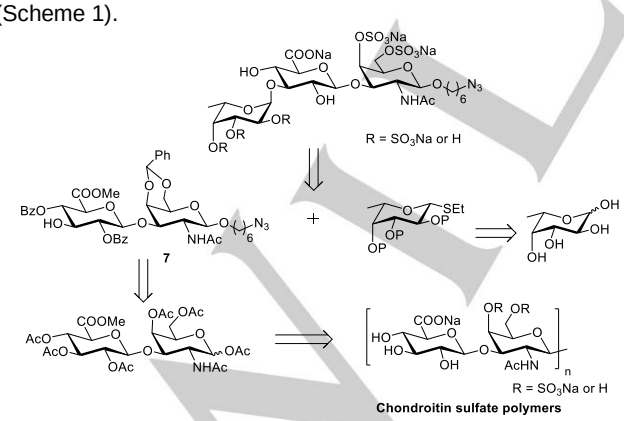


Figure 2. Synthetic targets.

Results and Discussion

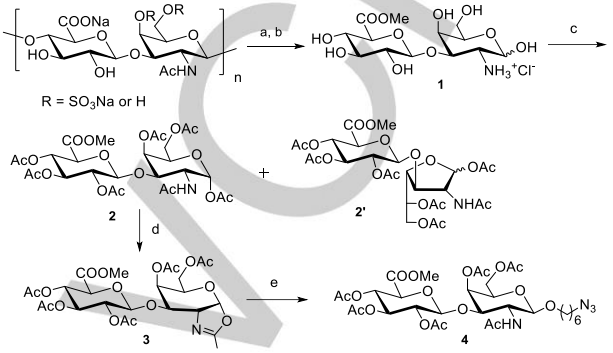
Chemical synthesis of FuCS in a homogenous and structurally defined way has recently been disclosed by Tamura et al, who utilized monosaccharide as the starting material to construct protected building blocks, then coupled the GlcA, GalNAc and Fuc moiety sequentially, followed by the sulfation and deprotecion process. Nevertheless, the conventional synthetic approach based on the coupling of the monosaccharide building blocks always faces great challenges in GAG oligosaccharide synthesis due to the low reactivity of both GlcA and hexosamine building blocks. Decade ago, Jacquinet and co-workers developed an acidic controlled degradation method to obtain protected CS disaccharide building blocks from the natural abundant CS polysaccharide. Inspired by their work, we designed a [2+1] synthetic strategy toward FuCS trisaccharide, which contains a properly protected CS disaccharide acceptor **7** and Fuc donors (Scheme 1).



Scheme 1. Retrosynthetic analysis of fucosylated chondroitin sulfate trisaccharides.

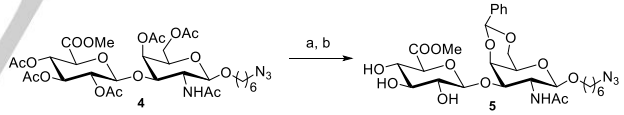
Synthesis of CS disaccharide acceptor

Using CS-A polysaccharide (~€280/Kg) as the starting material, disaccharide **1** was obtained following the previous acidic degradation procedure (Scheme 2). Acetylation of **1** led to a mixture of pyranoside **2** in 31% yield from the CS-A polysaccharide along with undesired furanoside **2'** [27a]. Considering further protecting group manipulation and cluster assembling, a linear spacer with an azido group was introduced via an oxazoline intermediate **3** [28], which was generated from pyranose **2** in 79% and achieved exclusive β configuration and almost quantitative yield of **4** by Copper(II)-mediated ring-opening reaction [29].



Scheme 2. Reaction conditions: a) IR-120 [H^+] resin, H_2O; then 0.5 M H_2SO_4, 100 ºC; b) HCl/MeOH, 0 ºC; c) Ac_2O, pyridine, 0 ºC~rt, 31% for **2**, 16% for **2'** from the polymer; d) TMSOTf, CH_2Cl_2, 0 ºC~rt, 79%; e) 6-azido-hexanol, $CuCl_2$, $CHCl_3$, reflux, 94%. TMSOTf = trimethylsilyl trifluoromethanesulfonate.

In order to protect the hydroxyl groups selectively, traditional Zemplen deacetylation led to a large amount of Δ4, 5- GlcA, while the acidic hydrolysis [30] condition resulted in partial cleavage of the azido spacer. After careful condition screening, the acetyl groups were cleaved smoothly with K_2CO_3/MeOH [31] in high yield with little α, β-elimination on GlcA. The resulting crude product was directly benzilidenated in the presence of CSA catalyst to yield compound **5** in 83% yield from **4** (Scheme 3).



Scheme 3. Reaction conditions: a) K_2CO_3, MeOH, -10 ºC~rt; b) $PhCH(OMe)_2$, CSA, CH_3CN, rt, 83% for 2 steps. CSA= (+)-10-Camphorsulfonic acid.

With compound **5** in hand, in order to build the acceptor **7** with a free 3-OH on GlcA, several strategies were tested to protect the 2-OH and 4-OH on the GlcA moiety selectively. Tin-mediated selective benzoylation [27a] reported by Jacquinet et al required the protection of the 2-OH and 3-OH firstly by acetelidine before the levulinoylation of 4-OH. To simplify the manipulation of the protecting groups, the strategy using GlcA 6, 3-lactone as key intermediate [32] was preferred (Scheme 4). Saponification of ester **5** in LiOH/MeOH/H_2O [33] gave free uronic acid, then lactonization with Bz_2O in DMF, followed by complete benzoylation with pyridine/DMAP yielding 6,3-lactone **6**, and final methanolysis of the lactone ring in the presence of NaOAc afforded the desired disaccharide acceptor **7** in 64% yield for three steps from **5**.

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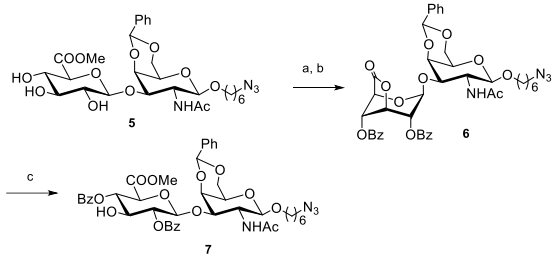
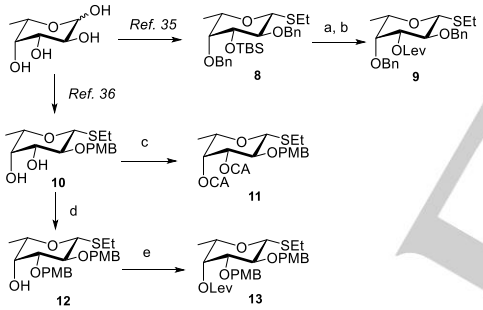


Scheme 4. Preparation of acceptor **7**. Reaction conditions: a) LiOH, MeOH/H_2O, 0 ºC~rt; b) Bz_2O, DMF, 85 ºC, then DMAP, pyridine, rt; c) NaOAc, MeOH/ CH_2Cl_2, rt, 64% for 3 steps. DMAP=4-(N,N-dimethylamino) pyridine.

Synthesis of Fuc donors

Sulfation patterns of the fucose branches isolated from different sea cucumbers vary from species to species [8a, 8b], among which 2, 4-O-disulfation, 3, 4-O-disulfation and 4-O-sulfation are the main forms, thus thioglycoside **9**, **11** and **13** were designed as the Fuc donors, considering the reactivity and α-selectivity during the glycosylation process (Scheme 5). Donor **9** was readily obtained by sequential de-O-silylation and levulinoylation in 95% yield for two steps from the known compound **8** [34]. From the same diol **10** [35], donor **11** was straightly obtained by chloroacetylation in 85% yield. Tin-mediated selective *p*-methoxybenzylation afforded alcohol **12**, which was transformed into donor **13** by levulinoylation in 90% yield.

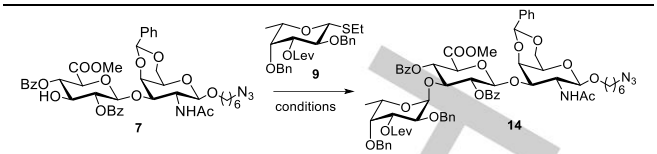


Scheme 5. Reaction conditions: a) TBAF, THF, rt; b) LevOH, EDCl, DMAP, CH_2Cl_2, rt, 95% for 2 steps; c) $ClAc_2O$, pyridine/CH_2Cl_2, 0 ºC~rt, 85%; d) Bu_2SnO, Tol, 110 ºC, then PMBCl, CsF, TBAI, DMF, 60 ºC, 89%; e) LevOH, DCC, DMAP, CH_2Cl_2, rt, 90%. TBAF= Tetrabutylammonium fluoride, LevOH= Levulinic acid; EDCl= 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; TBAI= Tetrabutylammonium Iodide; DCC= Dicyclohexylcarbodiimide.

Glycosylation and deprotection

With Fuc donors **9, 11**, **13** and disaccharide acceptor **7** in hand, the glycosylation conditions were investigated. Unexpectedly, fucosylation with thioglycoside **9** or **13** under NIS/AgOTf [36] activation led to the desired trisaccharide, but together with a less polar byproduct by TLC monitoring. The amount of the latter was not reduced after a series of optimization attempts by changing thioglycoside activation system, varying the temperature, donor equivalent, or prolonging reaction time (Table 1). Notably, this byproduct which was successfully isolated by chromatography showed complicated ^{1}H NMR spectrum and lack of the N*H* signal.

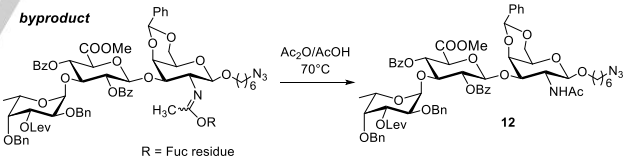
Table 1. Fucosylation of acceptor **7**



entry	donor	promoter	Temp/ ºC	yield[a]
1	1.5 eq	NIS/AgOTf	-10 ºC~ rt	40%[b]
2	1.5 eq	NIS/AgOTf	-20 ºC~ rt	48%[b]
3	1.2 eq	NIS/AgOTf	-20 ºC~ rt	*n.d.*[c]
4	1.5 eq	NIS/TfOH	-20 ºC~ rt	*n.d.*[b]
5	1.5 eq	MeOTf	rt	*n.d.*[b]
6	1.8 eq	$CuBr_2$/TBAB	rt	*n.d.*[c]

[a] Isolated yield; [b] Target trisaccharide together with a byproduct; [c] Incomplete consumption of acceptor; *n.d*. not determined.

Careful literature searches revealed that a similar phenomenon had been reported by Auzanneau et al when a rhamnoside donor was coupled to the 4-OH of the LacNAc acceptor under the promotion of TESOTf [37], which yielded a glycosylated imidate on the GlcNAc moiety. Tamura et al had also reported the glycosylated imidate structure during their synthetic attempts toward the chondroitin sulfate octasaccharide, in which prolonged reaction time facilitated the decrease of the undesired imidate product [38]. In consideration of the previous results and the ^{1}H analysis of the byproduct, we proposed that a fucosylated imidate compound was formed during the glycosylation reaction (Scheme 6). Thus, in order to enhance the yield of the desired trisaccharide **14**, a mixture of Ac_2O/AcOH was used to convert the imidate into NHAc. Fortunately, after the crude glycosylation mixture was treated with Ac_2O/AcOH at 70 °C, the desired trisaccharide **14** and **16** were successfully isolated in 84% and 77% yield, respectively, which in turn support the formation of an imidate byproduct during the glycosylation process.



Scheme 6. The structure of the imidate byproduct and transformation into the target trisaccharide.

Interestingly, the coupling of less reactive donor **11** with the same acceptor **7** gave directly trisaccharide **15** in 78% yield without the acidic treatment. The α configuration of the fucosyl unit was confirmed by ^{1}H NMR with coupling constant between H-1 and H-2 = 3.4~3.6 Hz. Sequential de-O-p-methoxybenzylation and benzoylation of **15** afforded **17** in 89% yield for two steps, and **18** in 91% yield from **16**. Acid hydrolysis of the benzylidene acetal, followed by de-O-benzylation [39], de-O-chloroacetylation or de-O-levulinoylation generated the corresponding tetraol **19**, **20** and triol **21** in 65%~76% yield for two steps. Finally, sulfation of the free hydroxy groups of **19**~**21** with $SO_3 \cdot NMe_3$ afforded **22**~**24**, respectively, in almost quantitative yield. Their ^{1}H NMR spectra showed the expected downfield shifts for GalNAc H-4, H-6a,b and the corresponding ^{1}H signal on the fucosyl residue. Deprotection of **22**~**24** was then achieved through a two-step saponification

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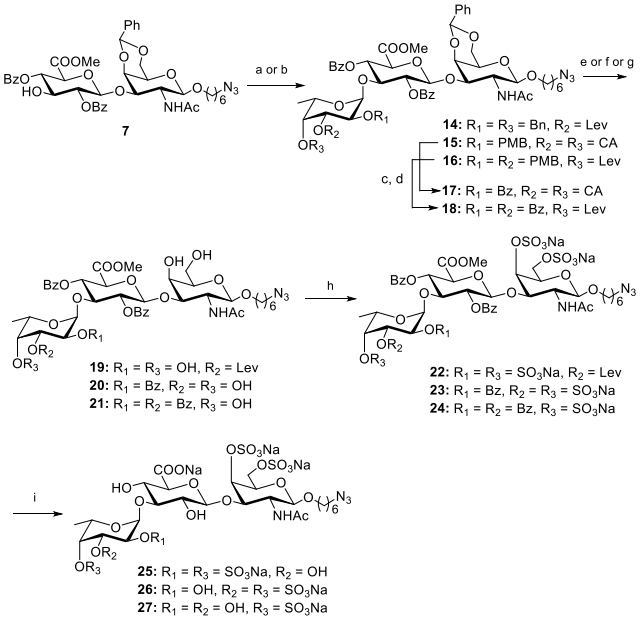
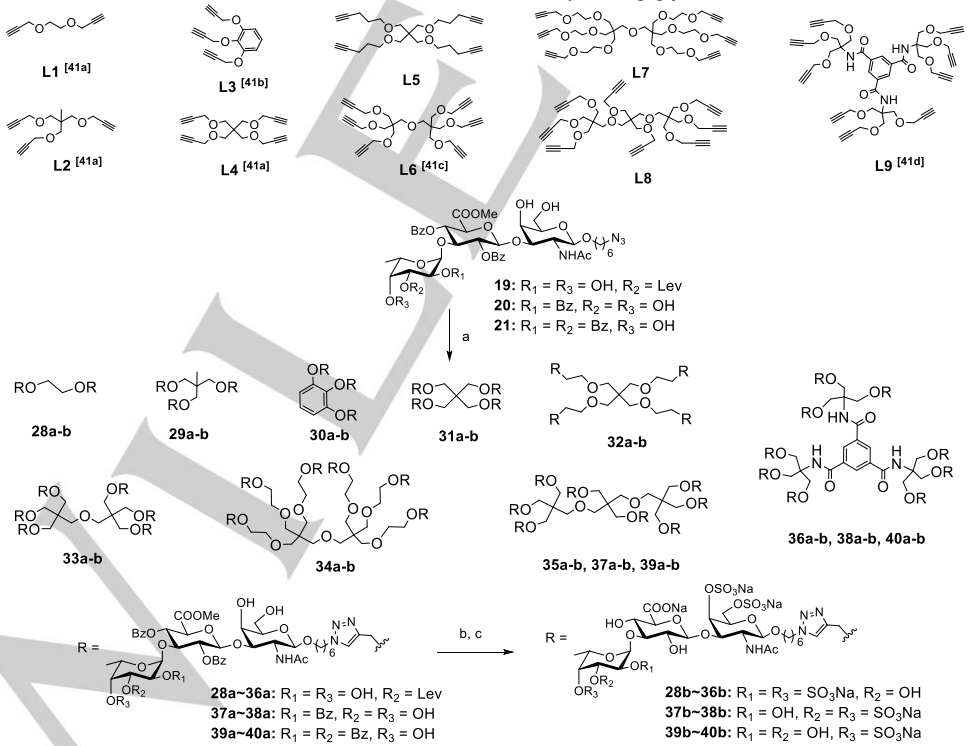
process, avoiding α, β-elimination at the GlcA units with lithium hydroperoxide [27c] and sodium hydroxide, to afford the target trisaccharides **25**~**27** in more than 90% yield (Scheme 7).



Scheme 7. Synthesis of trisaccharide **25**~**27**. Reaction conditions: a) **9** or **13**, NIS, AgOTf, 4 Å MS, CH_2Cl_2, -20 ºC~rt, then Ac_2O/AcOH, 70 ºC, **14**, 84%, **16**, 77%; b) **11**, NIS, AgOTf, 4 Å MS, CH_2Cl_2, -20 ºC~rt, 78%; c) DDQ, CH_2Cl_2/H_2O, rt; d) BzCl, pyridine, -10 ºC~rt, **17**, 89% for 2 steps, **18**, 91% for 2 steps; e) AcOH(aq), 50 ºC, then $NaBrO_3$, $Na_2S_2O_4$, EtOAc/H_2O, rt, 65% for 2 steps; f) AcOH(aq), 50 ºC, then thiourea, lutidine, MeOH, 60 ºC, 70% for 2 steps; g) AcOH(aq), 50 ºC, then N_2H_4·AcOH, pyridine/CH_2Cl_2, 0 ºC~rt, 76% for 2 steps; h) SO_3·NMe_3, DMF, 50 ºC, 95%~97%; i) LiOH, H_2O_2, THF/H_2O, -10 ºC~rt, then 4 M NaOH(aq), MeOH, 0 ºC~rt, 90%~quant. DDQ= 2,3-dicyano-5,6-dichlorobenzoquinone.

Assembly of glycoclusters

The sulfated fucose branches were essential for the anticoagulant activity [40]. The difference in their activities could be attributed to the sulfation patterns of the fucose branches, and especially 2, 4-O-disulfation was shown to be important [8a, 8b]. Therefore, trisaccharide **25** was selected as the single-entity for preliminary cluster assembling, and a series of scaffolds with different valences, orientations and lengths were designed [41] (Scheme 8). Unfortunately, attempts toward the direct coupling of **25** with different scaffolds using the Copper(I)-catalyzed azide alkyne cycloaddition reaction [41a] failed, probably due to the interference of anionic sulfo groups with the copper catalyst [41c]. Therefore, the coupling reaction was conducted before sulfation, and this strategy successfully afforded multivalent clusters **28a**~**36a** in almost quantitative yields. Finally, corresponding glycoclusters **28b**~**36b** were obtained upon sequential sulfation and deprotection in 60%~86% yield for two steps. In order to verify whether the anticoagulant behavior of FuCS glycoclusters is similar or not to natural polysaccharides concerning the sulfation patterns, conjugations between trisaccharides **20**, **21** and the most promising multivalent scaffolds (**L8**, **L9**) were also performed following the same procedure, which afforded corresponding glycoclusters **37b**~**40b**.



Scheme 8. Synthesis of **28b**~**40b**. Reaction conditions: a) scaffolds, $CuSO_4$, Na Ascorbate, $CHCl_3$/MeOH/H_2O, 45 ºC, 88%~quant; b) SO_3·NMe_3, DMF, 50 ºC; c) LiOH, H_2O_2, THF/H_2O, -10 ºC~rt, then 4 M NaOH(aq), MeOH, 0 ºC~rt, 60%~86% for 2 steps.

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Anticoagulant activity evaluation

The anticoagulant activities of the trisaccharides **25**~**27** and glycoclusters **28b**~**40b** were evaluated using the activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) of plasma clotting assays (Table 2; Figure 3), which are used to determine the ability to inhibit the intrinsic, extrinsic, and common pathways of the coagulation pathways, respectively [10]. The results demonstrated that trisaccharides **25**~**27** and glycoclusters with low valence **28b**~**32b**, had no significant influences on APTT at concentrations as high as 128 μg/mL, and a weak effect was observed for hexamer **34b**, octa-/nona-glycoclusters bearing 3, 4-O-disulfation or 4-O-sulfation **37b**~**40b** at the same concentration. Notably, hexamer **33b**, which has a "shorter-armed" scaffold compared with **34b**, together with octamer **35b** and nonamer **36b** bearing 2, 4-O-disulfation, strongly prolonged human plasma APTT. Concentrations between 7.12 and 11.87 μM of **33b**, **35b**, and **36b** were required to double the APTT. In general, the anticoagulant activities of these compounds increased with increasing molecular weights, at least 6 trischaride repeating units were required for remarkable prolongation of APTT activity, and the sulfation patterns of Fuc residues really made a difference [9]. With regard to PT and TT assays, no significant influences were observed for all the compounds at concentrations as high as 128 μg/mL (Figure 3), thus these trisaccharides or glycoclusters had little effect on the extrinsic and common coagulation pathways. Anticoagulant activities of these multivalent glycoclusters were slightly lower than that of LMWH (2.35 μM was required to double the APTT). The results may be attributed to differences in their structures and anticoagulant mechanisms [8c, 22, 42], since the FuCS derivatives are remarkably different from LMWH, which is a linear glycosaminoglycan containing the heparin pentasaccharide sequences and simultaneously has strong effect on the extrinsic and common coagulation pathways [42]. Meanwhile, natural FuCS [22] displayed the most potent anticoagulant activity, probably due to its higher molecular weight and 2, 4-O-disulfation patterns.

Furthermore, **33b~40b** potently inhibited the intrinsic tenase complex (FXase), which converts zymogen factor X (FX) to FXa [1, 43] (Table 2 and Figure 4). FXase is the final and rate-limiting enzyme complex in the intrinsic pathway, so the glycoclusters could suppress the generation of FXa activated by FXase, thus inhibited the intrinsic coagulation pathway, in a manner consistent with the clotting assays. The half maximal inhibitory concentrations (IC_{50}) for **33b~40b** were all in the nanomolar range, especially for glycocluster **35b**, with an IC_{50} value of 17.45 nM, which was in close proximity to that of LMWH (15.02 nM). Notably, possessing the same scaffold (octamer or nonamer), glycoclusters bearing mono 4-O-sulfated Fuc residue (**39b**, **40b**) showed significantly weaker inhibitory activity than ones with disulfated Fuc residues (**35b~38b**), and clusters bearing 2, 4-O-disulfation (**35b**, **36b**) displayed the most potency, which was in consistent with natural polysaccharides [8b,22]. Interestingly, the inhibitory activity clearly increased from "long-armed" hexamer **34b** to "short-armed" hexamer **33b**, and nonamer **36b** also showed a weaker inhibition of FXase compared with octamer **35b.** These results along with the APTT assays indicated that apart from molecular weight, the scaffolds, which determine the arrangement of the repeating units, might play a certain role.

Conclusions

To meet the great needs of safer anticoagulant drugs, increasing interests and attentions have been focused on the FuCS polysaccharides which present high anticoagulant activities and limited adverse effects. An efficient synthetic approach toward the azido linked FuCS trisaccharide repeating unit bearing diverse sulfation patterns on fucosyl moiety is reported in this paper. To amplify the bio-activity of the single trisaccharide unit, a series of glycoclusters were synthesized and compound **35b** bearing 8 trisaccharides epitopes presented the most promising anticoagulant activities by selective activiating the intrinsic anticoagulant pathway. The influences of molecular weight, scaffold length and orientation, together with the sulfation patterns were also explored. Our work provided a readily synthetic method to the FuCS repeating unit and furthermore, we indicated that the assembly of the single trisaccharide unit into multivalent glycoclusters would not change the action-mode of the FuCS, which could be further developed to novel anticoagulant agents.

Table 2. APTT assays and FXase inhibition activities of **33b**~**40b**.

Compounds [a]	Mw (Da)	2APTT [b]		IC_{50} [c]	
		(μg/mL)	(μM)	(ng/mL)	(nM)
33b	7070	83.92	11.87	262.25 ± 17.72	37.09 ± 2.51
34b	7334	>128 (1.40) [e]	-	929.78 ± 79.54	126.78 ± 1.08
35b	9460	**67.37**	**7.12**	165.09 ± 9.79	**17.45 ± 1.03**
36b	10743	90.97	8.47	260.21 ± 26.83	24.22 ± 2.50
37b	9460	>128 (1.83) [e]	-	195.14 ± 30.26	20.63 ± 3.20
38b	10743	>128 (1.31) [e]	-	707.90 ± 82.96	65.89 ± 7.72
39b	8644	>128 (1.11) [e]	-	1389.11 ± 155.13	160.70 ± 17.95
40b	9825	>128 (1.36) [e]	-	2516.18 ± 188.85	256.10 ± 19.22
LMWH	~4500	10.58	2.35	67.58 ± 6.78	15.02 ± 1.51
FuCS [d]	53700	3.89	0.07	13.6 ±1.4	0.25 ±0.03

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[a] The final concentrations of each agent were between 0~128 μg/mL; [b] The concentration of each agent that is required to double the APTT; [c] Concentration required to inhibit 50% activity of FXase; [d] Natural FuCS with about 92% 2,4-di-O-sulfated fucoses was purified from the sea cucumber *Stichopus monotuberculatus* and its chemical structure was in detail described in our previous publication [22]; [e] Ratio of clotting time to control in the presence of compound at 128 μg/mL.

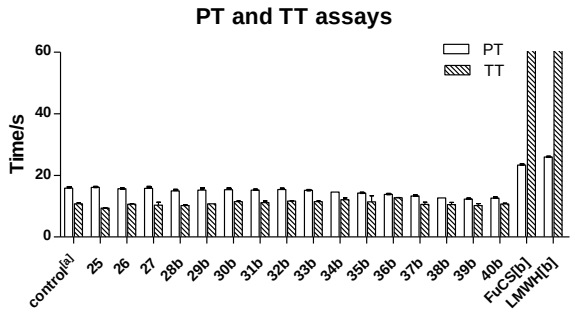

Figure 3. PT and TT assays of the compounds at concentration of 128 μg/mL. [a] no sample in 0.02 M Tris-HCl buffer (pH = 7.4); [b] TT >300 s for FuCS and LMWH.

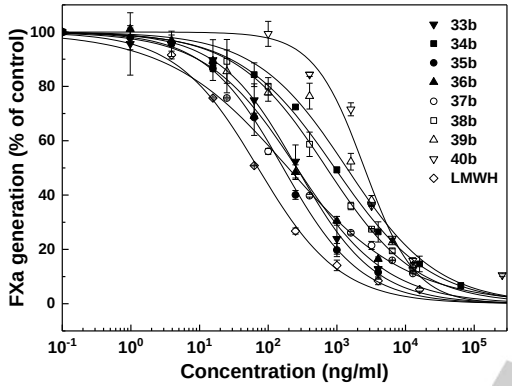

Figure 4. Factor Xa generation indicating the inhibition of FXase.

Experimental Section

General synthetic procedures

All chemicals were purchased as reagent grade and used without further purification unless otherwise noted. Dry dichloromethane was distilled over calcium hydride prior to use. Dry DMF (Extra dry) and TMSOTf were purchased from Acros Co. The boiling range of petroleum ether (PE) used as fluent in column chromatography was 65-80 °C. Analytical thin layer chromatography (TLC) was performed on silica gel 60 F_{254} aluminum supported plate (layer thickness 0.2 mm). Visualization of the spots was achieved by exposure to UV light (254nm) and/or charring with a solution of 5 % (v/v) sulfuric acid in EtOH or ceric ammonium molybdate, then gently heated. Column chromatography on SiO_2 was carried out with silica gel (200-300 mesh). High resolution mass spectra (HRMS) were obtained by Electro Spray Ionization (ESI). For ^{1}H nuclear magnetic resonance (NMR) spectra, chemical shifts were reported in parts per million (ppm) calibrated with tetramethylsilane (δ = 0. 00 ppm) in $CDCl_3$. ^{13}CNMR spectra were calibrated with tetramethylsilane (δ = 0.0 ppm) in $CDCl_3$. Coupling constants (*J*) were given in Hertz (Hz).

O-(Methyl 2, 3, 4-tri-O-acetyl-*β*-D-glucopyranosyluronate)-(1→3)-2-acetamido-1, 4, 6-tri-O-acetyl-2-deoxy-α-D-galactopyranose (2).

A solution of chondroitin sulfate A (20 g) in water (200 mL) was adjusted to pH = 1.6 with Amberlite IR-120 [H^+] resin, and filtered. The resin was washed with water, and the volume was adjusted to 390 mL. Concentrated H_2SO_4 (18 M, 11.1 mL, 0.5 M final concentration) was added, and the mixture was stirred at 100ºC for 6 h, folllowed by cooling to room temperature. The mixture was adjusted to pH = 3.5 with $Ba(OH)_2{\cdot}8H_2O$ under vigorous stirring, and the slurry was allowed to subside overnight. The solids were filtered off through a Celite pad, washed with water, and the yellow filtrate was concentrated to approximately 200 mL and slowly applied to a column of Amberlite IR-120 [H^+] resin (200 mL, settled volume). The column was washed with water (400 mL), AcOH/water (3:1, 400 mL), then with aqueous 1 M HCl until there was no effluent. The fractions containing ninhydrin-positive material were pooled, concentrated, and dried under vacuum overnight. The residue was treated with methanolic HCl (0.02 M, 200 mL) for 4 d at 4ºC, then was concentrated. Repeated additions of absolute EtOH and concentration gave a pale yellow solid. The crude product was treated with Ac_2O (70 mL) and pyridine (180 mL) at 0 ºC, then was allowed to warm to room temperature and stirred overnight. The reaction was quenched by addition of MeOH at 0 ºC, then was concentrated, washed with 1 M HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. Flash silica chromatography (DCM/MeOH 70:1~50:1, 0.1% Et_3N) gave first the pyranose **1** (8.25 g, 31% from the polymer). R_f = 0.30 (DCM/MeOH 40:1); $[\alpha]_D^{25}$ = +40 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) *δ* 6.40 (1H, m, N*H*), 6.34 (1H, d, H_A-1, *J* = 3.3 Hz), 5.28-5.24 (2H, m, H_A-4, H_B-3), 5.18-5.13 (2H, m, H_B-2, H_B-3), 4.87 (1H, d, H_B-1, *J* = 8.1 Hz), 4.55 (1H, m, H_A-2), 4.24-4.20 (2H, m, H_A-3, H_A-5), 4.13-4.06 (2H, m, H_A-6a, H_B-5), 4.00 (1H, m, H_A-6b), 3.76 (3H, s, COOC*H*$_3$), 2.19-2.04 (18H, m, C*H*$_3$CO), 1.91 (3H, s, C*H*$_3$CONH); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) *δ* 170.6, 170.4, 169.7, 169.5, 169.0, 167.4, 97.5, 91.4, 72.1, 71.9, 69.0, 68.9, 68.6, 68.3, 67.9, 61.1, 53.2, 48.0, 22.9, 21.0, 20.8, 20.7, 20.6, 20.5, 20.4; HRMS (ESI-FT-ICR) m/z caled. for $C_{27}H_{38}NO_{18}$ $[M+H]^+$ 664.2083, found 664.2092. Next eluted was the furanose (4.26 g, 16.0% from the polymer).

O-(Methyl 2, 3, 4-tri-O-acetyl-*β*-D-glucopyranosyluronate)-(1→3)-2-methyl-(4, 6-di-O-acetyl-1, 2-dideoxy-α-D-galactopyrano)[2, 1-d] 2-oxazoline (3).

To a solution of **2** (5.00 g, 7.54 mmol) in anhydrous CH_2Cl_2 (180 mL) was added dropwise TMSOTf (2.73 mL, 15.08 mmol, 2.0 eq) at 0 °C under argon atmosphere. The reaction mixture was stirred at room temperature until the starting material was totally consumed, quenched with triethylamine at 0 °C, and evaporated. The residue was purified by silica gel chromatography (petroleum ether/ethyl acetate 1:1.7~1:2.0, containing 0.1% triethylamine) to give pure **3** (4.55 g, 79%) as white amorphous. R_f = 0.50 (DCM/MeOH 30:1); $[\alpha]_D^{25}$ = +35 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) *δ* 5.93 (1H, d, H_A-1, *J* = 6.5 Hz), 5.40 (1H, d, H_A-4, *J* = 3.5 Hz), 5.30-5.20 (2H, m, H_B-3, H_B-4), 5.07 (1H, d, H_B-1, *J* = 8.0 Hz), 4.99 (1H, dd, H_B-2, *J* =*J* = 8.0 Hz), 4.18-4.12 (3H, m, H_A-6a, H_A-6b, H_A-5), 4.08 (1H, d, H_B-5, *J* = 9.5 Hz), 3.93 (1H, dd, H_A-3, *J* = 6.5 Hz, *J* = 3.5 Hz), 3.83 (1H, dd, H_A-2, *J* = *J* = 6.5 Hz), 3.76 (3H, s, COOC*H*$_3$), 2.08-2.02 (18H, m, C*H*$_3$CO, C*H*$_3$C of oxazoline); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) *δ* 170.6, 170.0, 169.6, 169.3, 169.0, 167.2, 165.6, 101.5, 98.8, 75.8, 72.5, 71.7, 71.2, 70.1, 69.3, 66.3, 65.9, 62.1, 52.8, 20.8, 20.6, 20.6, 20.5, 20.5, 14.4; HRMS (ESI-FT-ICR) m/z caled. for $C_{25}H_{34}NO_{16}$ $[M+H]^+$ 604.1872, found 604.1882.

6-Azidohexyl O-(Methyl 2, 3, 4-tri-O-acetyl-*β*-D-glucopyranosyluronate)-(1→3)-2-acetamido-4, 6-di-O-acetyl-2-deoxy-β-D-galactopyranoside (4).

To a solution of **3** (3.564 g, 5.91 mmol) in anhydrous $CHCl_3$ (40 mL) was added linker (6.623 g, 46.3 mmol, 7.8 eq), anhydrous Cupric (II) chloride (874 mg, 6.50 mmol, 1.1 eq) under argon atmosphere. The mixture was refluxed overnight, quenched by saturated aqueous $NaHCO_3$ and the solids were filtered off through a Celite pad. The filtrate was extracted with DCM and the organic phase was dried over Na_2SO_4, concentrated, and purified by silica gel chromatography (petroleum ether/ethyl acetate

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1:1.5~1:2.0, containing 0.1% triethylamine) to give pure **4** (4.142 g, 94%) as white amorphous. R_f = 0.30 (DCM/MeOH 40:1); $[\alpha]_D^{25}$ = +5 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) *δ* 5.80 (1H, d, N*H*, *J* = 7.0 Hz), 5.39 (1H, d, H_A-4, *J* = 3.2 Hz), 5.23-5.15 (2H, m, H_B-3, H_B-4), 5.00-4.95 (2H, m, H_A-1, H_B-2), 4.71 (1H, d, H_B-1, *J* = 7.8 Hz), 4.66 (1H, dd, H_A-3, *J* = 3.2 Hz, *J* = 10.8 Hz), 4.15-3.99 (3H, m, H_A-6a, H_A-6b, H_B-5), 3.87-3.83 (2H, m, H_A-5, OC*H*$_2$ of linker), 3.75 (3H, s, COOC*H*$_3$), 3.48 (1H, m, OC*H*$_2$ of linker), 3.37 (1H, m, H_A-2), 3.27 (2H, t, C*H*$_2$N$_3$, *J* = 6.7 Hz), 2.08-1.96 (18H, m, C*H*$_3$CO), 1.60 (4H, m, C*H*$_2$ of linker), 1.38 (4H, m, C*H*$_2$ of linker); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) *δ* 170.8, 170.4, 170.0, 169.9, 169.3, 166.9, 99.4, 99.1, 74.4, 72.3, 72.2, 71.1, 71.1, 69.9, 69.0, 67.8, 62.2, 54.9, 52.8, 51.3, 29.3, 28.7, 26.4, 25.5, 23.5, 20.7, 20.6, 20.5, 20.5; HRMS (ESI-FT-ICR) m/z caled. for $C_{31}H_{47}N_4O_{17}$ $[M+H]^+$ 747.2931, found 747.2924.

6-Azidohexyl O-(Methyl-*β*-D-glucopyranosyluronate)-(1→3)-2-acetamido-4, 6-O-benzylidene-2-deoxy-β-D-galactopyranoside (5).

To a solution of **4** (4.106 g, 5.50 mmol) in anhydrous methanol (220 mL) was added potassium carbonate (380 mg, 2.75 mmol, 0.5 eq) at -10 °C under argon atmosphere, the mixture was stirred for 1h at this temperature and allowed to slowly warm to room temperature. After completion of the reaction as monitored by TLC, 1 M HCl was added to neutralize the base, and the solution was concentrated to dryness. Then the residue was dissolved in anhydrous acetonitrile (60 mL), (+)-10-camphorsulfonic acid (256 mg, 1.1 mmol, 0.2 eq) and benzaldehyde dimethyl acetal (3.8 mL, 27.5 mmol, 5.0 eq) were added successively. The mixture was stirred at room temperature for 2 h, quenched with triethylamine and evaporated. Flash silica chromatography (DCM/MeOH 14:1) gave pure **5** (2.843 g, 83% for 2 steps) as white amorphous. R_f = 0.30 (DCM/MeOH 12:1); $[\alpha]_D^{25}$ = -12 (*c* = 1.0, CH_3OH); ^{1}H NMR (400 MHz, MeOD-*d*$_4$) *δ* 7.56-7.54, 7.38-7.36 (5H, m, aromatic), 5.61 (1H, s, PhC*H*), 4.61 (1H, d, H_A-1, *J* = 8.4 Hz), 4.45 (1H, d, H_B-1, *J* = 7.6 Hz), 4.36 (1H, d, H_A-4, *J* = 3.1 Hz), 4.23-4.08 (3H, m, H_A-6a, H_A-6b, H_A-2), 3.99 (1H, dd, H_A-3, *J* = 3.3 Hz, *J* = 11.0 Hz), 3.93 (1H, m, OC*H*$_2$ of linker), 3.87 (1H, d, H_B-5, *J* = 9.8 Hz), 3.80 (3H, s, COOC*H*$_3$), 3.57-3.51 (3H, m, H_B-4, H_A-5, OC*H*$_2$ of linker), 3.37 (1H, m, H_B-3), 3.30-3.25 (3H, m, H_B-2, C*H*$_2$N$_3$), 1.98 (3H, s, C*H*$_3$CO), 1.62 (4H, m, C*H*$_2$ of linker), 1.43 (4H, m, C*H*$_2$ of linker); ^{13}C NMR (100 MHz, MeOD-*d*$_4$) *δ* 172.7, 169.8, 138.3, 128.5, 127.5, 126.3, 105.0, 101.0, 101.0, 77.5, 75.7, 75.6, 75.4, 73.0, 71.6, 68.9, 68.8, 66.5, 51.6, 51.4, 51.0, 29.1, 28.5, 26.1, 25.2, 21.8; HRMS (ESI-FT-ICR) m/z caled. for $C_{28}H_{41}N_4O_{12}$ $[M+H]^+$ 625.2716, found 625.2725.

6-Azidohexyl O-(2, 4-di-O-benzoyl-β-D-glucopyranosylurono-6, 3-lactone)-(1→3)-2-acetamido-4, 6-O-benzylidene-2-deoxy-β-D-galactopyranoside (6).

Lithium hydroxide monohydrate (336 mg 8.0 mmol) was added to a solution of **5** (2.843 g, 4.55 mmol) in MeOH-H_2O (70 mL/10mL) at 0 °C. The mixture was stirred for 5 h and neutralized with Amberlite IR-120 $[H^+]$ resin, then concentrated to dryness, treated with a solution of DCM-AcOH (20 mL/4mL) and stirred for 30 min. The solution was coevaporated with toluene and the crude product (2.676 g, 96%) was used in next step without further purification. R_f = 0.60 (n-BuOH/EtOH/H_2O 1:1:1);

To a solution of the crude product (381 mg, 0.624 mmol) in anhydrous DMF (11 mL), Bz_2O (3.53 g, 15.60 mmol, 25 eq) was added and the reaction mixture was kept at 85 °C for 4 h, cooled to room temperature. Pyridine (3 mL) and DMAP (46 mg, 0.376 mmol, 0.6 eq) were added and the solution was stirred for 36 h, then was concentrated to dryness. The residue was washed with Et_2O to remove excess Bz_2O and the crude product **6** was used in next step without further purification. One fraction was purified by silica gel chromatography (petroleum ether/ethyl acetate 3:2~1:1) and characterized. R_f = 0.40 (DCM/MeOH 50:1); $[\alpha]_D^{25}$ = -30 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) *δ* 8.11, 7.68-7.57, 7.45-7.26, 6.99 (15H, m, aromatic), 6.23 (1H, d, N*H*, *J* = 6.7 Hz), 5.67 (1H, s, PhC*H*), 5.40 (1H, d, H_B-2, *J* = 3.2 Hz), 5.26 (1H, m, H_B-4), 5.21-5.18 (2H, m, H_A-1, H_B-1), 5.16 (1H, m, H_B-3), 4.88 (1H, dd, H_A-3, *J* = 3.3 Hz, *J* = 11.0 Hz), 4.41 (1H, d, H_B-5, *J* = 3.2 Hz), 4.34-4.31 (2H, m, H_A-4, H_A-6a), 4.11 (1H, d, H_A-6b, *J* = 12.1 Hz), 3.92 (1H, m, OC*H*$_2$ of linker), 3.54-3.44 (3H, m, H_A-2, H_A-5, OC*H*$_2$ of linker), 3.24 (2H, t, C*H*$_2$N$_3$, *J* = 6.8 Hz), 1.90 (3H, s, C*H*$_3$CO), 1.57 (4H, m, C*H*$_2$ of linker), 1.36 (4H, m, C*H*$_2$ of linker); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) *δ* 172.0, 169.0, 165.8, 165.3, 138.3, 134.0, 133.6, 130.2, 129.7, 128.7, 128.5, 128.2, 127.9, 126.6, 100.7, 100.4, 98.7, 75.5, 73.9, 72.3, 69.8, 69.5, 69.1, 68.0, 67.7, 66,7, 55,2, 51.4, 29.3, 28.8, 26.5, 25.5, 23.7; HRMS (ESI-FT-ICR) m/z caled. for $C_{41}H_{45}N_4O_{13}$ $[M+H]^+$ 801.2978, found 801.2975.

6-Azidohexyl O-(Methyl-2, 4-di-O-benzoyl-*β*-D-glucopyranosyl-uronate)-(1→3)-2-acetamido-4, 6-O-benzylidene-2-deoxy-β-D-galactopyranoside (7).

The crude product **6** was dissolved in a solution of MeOH-DCM (15 mL/11mL) at room temperature, Sodium acetate anhydrous (46 mg, 0.56 mmol, 0.9 eq) was added and the solution was stirred overnight. After completion of the reaction as monitored by TLC, the mixture was concentrated. Flash silica chromatography (petroleum ether/ethyl acetate 1:1.5~1:2) gave pure **7** (333 mg, 64% for 2 steps) as white amorphous. R_f = 0.25 (DCM/MeOH 50:1); $[\alpha]_D^{25}$ = +23 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) *δ* 8.04, 7.60-7.52, 7.47-7.41, 7.35-7.31 (15H, m, aromatic), 5.87 (1H, d, N*H*, *J* = 6.7 Hz), 5.52 (1H, s, PhC*H*), 5.45 (1H, dd, H_B-4, *J* = *J* = 9.6 Hz), 5.30 (1H, dd, H_B-2, *J* = *J* = 8.0 Hz), 5.14 (1H, d, H_B-1, *J* = 7.3 Hz), 5.06 (1H, d, H_A-1, *J* = 8.1 Hz), 4.82 (1H, dd, H_A-3, *J* = 3.3 Hz, *J* = 11.2 Hz), 4.43 (1H, d, H_A-4, *J* = 3.3 Hz), 4.26-4.22 (2H, m, H_B-5, H_A-6a), 4.10 (1H, dd, H_B-3, *J* = *J* = 8.7 Hz), 4.00 (1H, m, H_A-6b), 3.82 (1H, m, OC*H*$_2$ of linker), 3.62 (3H, s, COOC*H3*), 3.41-3.36 (3H, m, H_A-2, H_A-5, OC*H*$_2$ of linker), 3.21 (2H, t, C*H*$_2$N$_3$, *J* = 6.9 Hz), 1.59 (3H, s, C*H*$_3$CO), 1.51 (4H, m, C*H*$_2$ of linker), 1.30 (4H, m, C*H*$_2$ of linker); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) *δ* 171.4, 168.0, 166.0, 165.6, 138.0, 133.6, 133.5, 129.9, 129.8, 129.4, 129.2, 128.7, 128.6, 128.5, 128.0, 126.3, 100.8, 100.7, 98.9, 76.1, 75.4, 74.4, 73.3, 72.6, 72.1, 69.4, 69.2, 66.4, 54.5, 52.7, 51.3, 29.2, 28.7, 26.4, 25.4, 23.2; HRMS (ESI-FT-ICR) m/z caled. for $C_{42}H_{49}N_4O_{14}$ $[M+H]^+$ 833.3240, found 833.3215.

Ethyl 2, 4-di-O-benzyl-3-O-levulinoyl-1-thio-β-L-fucopyranoside (9).

A solution of the known building block **8** (2.34 g, 4.67 mmol) in THF (30 mL) was treated with a solution of 0.7 M TBAF in THF (10 mL, 7.0 mmol, 1.5 eq) and the reaction was stirred overnight, then concentrated and directly purified by silica gel chromatography (petroleum ether/EtOAc 5:1). A mixture of the imidiate, LevOH (1.64 g, 14.1 mmol, 3.0 eq), EDCI (4.5 g, 23.5 mmol, 5.0 eq) and 4-DMAP (170 mg, 1.41 mmol, 0.3 eq) in CH_2Cl_2 (30 mL) was stirred overnight at room temperature. Then the solution was diluted with DCM, washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. Flash silica chromatography (petroleum ether/EtOAc 4:1) gave **9** (2.21 g, 95% for 2 steps) as colorless oil. R_f = 0.30 (petroleum ether/EtOAc 4:1); $[\alpha]_D^{25}$ = -56 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) *δ* 7.39-7.26 (10H, m, aromatic), 4.95 (1H, dd, H-3, *J* = 2.9 Hz, *J* = 9.7 Hz), 4.89 (1H, d, PhC*H*$_2$, *J* = 11.0 Hz), 4.76 (1H, d, PhC*H*$_2$, *J* = 11.8 Hz), 4.64-4.61 (2H, m, PhC*H*$_2$), 4.44 (1H, d, H-1, *J* = 9.6 Hz), 3.82 (1H, dd, H-2, *J* = *J* = 9.7 Hz), 3.71 (1H, d, H-4, *J* = 2.9 Hz), 3.62 (1H, q, H-5, *J* = 6.4 Hz), 2.83-2.30 (6H, m, C*H*$_2$ of SEt, C*H*$_2$ of Lev), 2.14 (3H, s, C*H*$_3$CO), 1.30 (3H, t, C*H*$_3$ of SEt, *J* = 7.4 Hz), 1.22 (3H, d, H-6, *J* = 6.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) *δ* 206.2, 172.3, 138.4, 138.3, 128.3, 128.2, 128.0, 128.0, 127.7, 127.6, 85.0, 77.6, 77.5, 76.4, 75.3, 74.3, 37.7, 29.8, 27.9, 24.9, 16.9, 15.0; HRMS (ESI-FT-ICR) m/z caled. for $C_{27}H_{38}NO_6S$ $[M+NH_4]^+$ 504.2414, found 504.2418.

Ethyl 3, 4-di-O-chloroacetyl-2-O-(4-methoxybenzyl)-1-thio-β-L-fucopyranoside (11).

A solution of the known building block **10** (640 mg, 1.95 mmol) in CH_2Cl_2-pyridine (8 mL/2mL) was treated with chloroacetic anhydride (1.0 g, 5.85 mmol, 3.0 eq) at 0 °C, the mixture was allowed to warm to room temperature and stirred overnight. The reaction was diluted with DCM, washed with 1 M HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. Flash silica chromatography (petroleum

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ether/EtOAc 7:1~6:1) gave **11** (805 mg, 85%) as pale yellow oil. R_f = 0.25 (petroleum ether/EtOAc 6:1); $[\alpha]_D^{25}$ = -40 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ 7.27, 6.87 (4H, m, aromatic), 5.26 (1H, m, H-4), 5.06 (1H, dd, H-3, *J* = 3.5 Hz, *J* = 9.7 Hz), 4.77 (1H, d, PhCH_2, *J* = 10.5 Hz), 4.56 (1H, d, PhCH_2, *J* = 10.5 Hz), 4.51 (1H, d, H-1, *J* = 9.8 Hz), 4.15 (2H, s, CH_2 of CA), 3.89 (2H, m, CH_2 of CA), 3.79 (3H, s, OCH_3), 3.70 (1H, q, H-5, *J* = 6.5 Hz), 3.64 (1H, dd, H-2, *J* = *J* = 9.6 Hz), 2.78 (2H, m, CH_2 of SEt), 1.34 (3H, t, CH_3 of SEt, *J* = 7.4 Hz), 1.20 (3H, d, H-6, *J* = 6.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) δ 167.5, 166.4, 159.5, 130.0, 113.8, 85.0, 75.9, 75.3, 75.2, 72.7, 72.3, 55.3, 40.6, 40.5, 25.1, 16.4, 14.9; HRMS (ESI-FT-ICR) m/z caled. for $C_{20}H_{27}Cl_2O_7S$ $[M+H]^+$ 481.0849, found 481.0846.

Ethyl 2, 3-di-O-(4-methoxybenzyl)-1-thio-β-L-fucopyranoside (12).

A mixture of the known building block **10** (804 mg, 2.45 mmol) and di-n-butyltin oxide (763 mg, 3.07 mmol, 1.25 eq) in toluene (35 mL) was stirred at reflux for 4 h. Solvent was then distilled at reduced pressure to approximately 10 mL, and the mixture was cooled to room temperature under argon. DMF (15 mL), 4-Methoxybenzyl chloride (0.5 mL, 3.68 mmol, 1.5 eq), CsF (745 mg, 4.9 mmol, 2.0 eq), TBAI (272 mg, 0.74 mmol, 0.3 eq) were added successively, and the mixture was stirred for 12 h at 60 °C, then diluted with EtOAc, washed with brine. Flash silica chromatography (petroleum ether/EtOAc 3:1) gave **12** (980 mg, 89%) as pale yellow oil. R_f = 0.30 (petroleum ether/EtOAc 5:2); $[\alpha]_D^{25}$ = -25 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ 7.32, 7.27, 6.86 (8H, m, aromatic), 4.78 (1H, d, PhCH_2, *J* = 9.8 Hz), 4.68 (1H, d, PhCH_2, *J* = 9.8 Hz), 4.64 (2H, s, PhCH_2), 4.36 (1H, d, H-1, *J* = 9.6 Hz), 3.79 (6H, m, OCH_3), 3.76 (1H, m, H-4), 3.58 (1H, dd, H-2, *J* = *J* = 9.2 Hz), 3.53-3.49 (2H, m, H-3, H-5), 2.80-2.67 (2H, m, CH_2 of SEt), 2.44 (1H, m, O*H*), 1.34-1.28 (6H, m, CH_3 of SEt, H-6); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) δ 159.4, 159.3, 130.5, 130.0, 129.5, 113,9, 113.7, 84.7, 82.4, 75.4, 74.1, 71.8, 69.5, 55.3, 24.6, 16.7, 15.0; HRMS (ESI-FT-ICR) m/z caled. for $C_{24}H_{36}NO_6S$ $[M+NH_4]^+$ 466.2258, found 466.2272.

Ethyl 4-O-levulinoyl-2, 3-di-O-(4-methoxybenzyl)-1-thio-β-L-fuco-pyranoside (13).

A mixture of **12** (845 mg, 1.89 mmol), LevOH (650 mg, 5.66 mmol, 3.0 eq), DCC (1.55 g, 7.54 mmol, 4.0 eq) and 4-DMAP (70 mg, 0.57 mmol, 0.3 eq) in CH_2Cl_2 (15 mL) was stirred overnight at room temperature. Then the solution was filtered through a pad of Celite, diluted with DCM, washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. Flash silica chromatography (petroleum ether/EtOAc 4:1~5:2) gave **13** (930 mg, 90%) as pale yellow oil. R_f = 0.45 (DCM/MeOH 60:1); $[\alpha]_D^{25}$ = -33 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ 7.32-7.24, 6.87-6.83 (8H, m, aromatic), 5.34 (1H, m, H-4), 4.75-4.62 (3H, m, PhCH_2), 4.45-4.40 (2H, m, PhCH_2, H-1), 3.80 (6H, m, OCH_3), 3.64 (1H, q, H-5, *J* = 6.6 Hz), 3.59-3.49 (2H, m, H-3, H-2), 2.83-2.65 (6H, m, CH_2 of SEt, CH_2 of Lev), 2.18 (3H, s, CH_3CO), 1.31 (3H, t, CH_3 of SEt, *J* = 7.5 Hz), 1.22 (3H, d, H-6, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) δ 206.2, 172.6, 159.3, 159.3, 130.5, 130.0, 129.7, 113.7, 113.7, 84.9, 80.7, 75.4, 73.0, 71.4, 70.2, 55.3, 55.2, 38.1, 29.8, 28.1, 24.8, 16.7, 15.0; HRMS (ESI-FT-ICR) m/z caled. for $C_{29}H_{42}NO_8S$ $[M+NH_4]^+$ 564.2626, found 564.2639.

6-Azidohexyl O-(2, 4-di-O-benzyl-3-O-levulinoyl-α-L-fucopyranosyl)-(1→3)-(Methyl-2, 4-di-O-benzoyl-β-D-glucopyranosyluronate)-(1→3) - 2-acetamido-4, 6-O-benzylidene-2-deoxy-β-D-galactopyranoside (14).

A mixture of acceptor **7** (298 mg, 0.358 mmol), donor **9** (261 mg, 0.537 mmol, 1.5 eq), and 4 Å powdered molecular sieves (700 mg) in dry CH_2Cl_2 (7.5 mL) was stirred for 2 h at room temperature under dry argon. Then the mixture was cooled to -20 °C and treated successively with NIS (161 mg, 0.716 mmol, 2.0 eq) and AgOTf (36.8 mg, 0.143 mmol, 0.4 eq) and kept for 2 h at this temperature, then allowed to slowly warm to room temperature and stirred overnight. The reaction was quenched with triethylamine and filtered through a pad of Celite. The filtrate was concentrated to dryness and treated with AcOH/Ac_2O (1:3) at 70 °C for 2 h, then was coevaporated with toluene. Flash silica chromatography (petroleum ether/Acetone 3:1~2:1) gave pure trisaccharide **14** (381 mg, 85%) as white solid. R_f = 0.40 (DCM/MeOH 50:1); $[\alpha]_D^{25}$ = +24 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ 7.99-7.95, 7.57-7.52, 7.44-7.29, 7.24-7.16, 7.05-7.04 (25H, m, aromatic), 5.80 (1H, d, N*H*, *J* = 6.4 Hz), 5.60 (1H, dd, H_B-4, *J* = *J* = 9.2 Hz), 5.50 (1H, s, PhC*H*), 5.45 (1H, dd, H_B-2, *J* = *J* = 7.2 Hz), 5.15 (1H, d, H_B-1, *J* = 6.8 Hz), 5.10-5.07 (2H, m, H_A-1, H_C-3), 5.04 (1H, d, H_C-1, *J* = 3.5 Hz), 4.73 (1H, dd, H_A-3, *J* = 3.5 Hz, *J* = 11.1 Hz), 4.54 (1H, d, PhCH_2, *J* = 11.5 Hz), 4.41 (1H, d, H_A-4, *J* = 3.3 Hz), 4.37 (1H, d, PhCH_2, *J* = 11.5 Hz), 4.30-4.21 (4H, m, H_B-3, H_B-5, H_A-6a, PhCH_2), 4.07-4.02 (2H, m, H_A-6b, PhCH_2), 3.90-3.79 (2H, m, H_C-5, OCH_2 of linker), 3.76 (1H, dd, H_C-2, *J* = 10.6 Hz, *J* = 3.5 Hz), 3.54 (3H, s, COOCH_3), 3.48-3.39 (4H, m, H_A-2, H_A-5, H_C-4, OCH_2 of linker), 3.23 (2H, t, CH_2N$_3$, *J* = 6.9 Hz), 2.71-2.22 (4H, m, CH_2 of Lev), 2.12 (3H, s, CH_3CO), 1.61 (3H, s, CH_3CO), 1.55 (4H, m, CH_2 of linker), 1.34 (4H, m, CH_2 of linker), 0.69 (3H, d, H_C-6, *J* = 6.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) δ 206.3, 171.9, 171.2, 168.0, 165.2, 164.4, 138.4, 138.1, 138.1, 133.3, 129.8, 129.7, 129.4, 128.5, 128.4, 128.2, 128.2, 128.1, 127.9, 127.7, 127.6, 127.4, 126.3, 100.5, 100.1, 99.1, 98.9, 78.3, 78.3, 75.9, 75.4, 75.1, 73.7, 73.2, 72.7, 72.4, 72.1, 70.9, 69.3, 69.2, 66.8, 66.5, 54.4, 52.7, 51.4, 37.7, 29.8, 29.3, 28.8, 27.8, 26.4, 25.5, 23.3, 15.8; HRMS (ESI-FT-ICR) m/z caled. for $C_{67}H_{77}N_4O_{20}$ $[M+H]^+$ 1257.5126, found 1257.5143.

6-Azidohexyl O-[3, 4-di-O-chloroacetyl-2-O-(4-methoxybenzyl)-α-L-fucopyra-nosyl]-(1→3)-(Methyl-2, 4-di-O-benzoyl-β-D-glucopyranos-yluronate)-(1→3)-2-acetamido-4, 6-O-benzylidene-2-deoxy-β-D-galactopyranoside (15).

A mixture of acceptor **7** (314 mg, 0.383 mmol), donor **11** (276 mg, 0.575 mmol, 1.5 eq), and 4 Å powdered molecular sieves (600 mg) in dry CH_2Cl_2 (7.5 mL) was stirred for 2 h at room temperature under dry argon. Then the mixture was cooled to -20 °C and treated successively with NIS (172 mg, 0.766 mmol, 2.0 eq) and AgOTf (34.4 mg, 0.143 mmol, 0.35 eq) and kept for 2 h at this temperature, then allowed to slowly warm to room temperature and stirred overnight. The reaction was quenched with triethylamine and filtered through a pad of Celite. The filtrate was concentrated to dryness. Flash silica chromatography (petroleum ether/Acetone 3:1~2:1) gave pure trisaccharide **15** (367 mg, 78%) as white solid. R_f = 0.45 (DCM/MeOH 50:1); $[\alpha]_D^{25}$ = +34 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ 8.03-7.96, 7.60-7.30, 6.92, 9.73 (19H, m, aromatic), 5.79 (1H, d, N*H*, *J* = 6.8 Hz), 5.61 (1H, dd, H_B-4, *J* = *J* = 9.2 Hz), 5.52 (1H, s, PhC*H*), 5.43 (1H, dd, H_B-2, *J* = *J* = 7.3 Hz), 5.23-5.18 (2H, m, H_B-1, H_C-3), 5.10 (1H, d, H_A-1, *J* = 8.2 Hz), 5.02 (1H, m, H_C-4), 4.88 (1H, d, H_C-1, *J* = 3.4 Hz), 4.78 (1H, dd, H_A-3, *J* = 3.4 Hz, *J* = 11.1 Hz), 4.43 (1H, d, H_A-4, *J* = 3.3 Hz), 4.30-4.14 (4H, m, H_A-6a, H_B-3, H_B-5, PhCH_2), 4.07-4.01 (2H, m, H_A-6b, H_C-5), 3.93-3.84 (4H, m, CH_2 of CA, OCH_2 of linker, PhCH_2), 3.80-3.77 (5H, m, CH_2 of CA, OCH_3), 3.59-3.39 (7H, m, COOCH_3, OCH_2 of linker, H_C-2, H_A-2, H_A-5), 3.24 (2H, t, CH_2N$_3$, *J* = 7.0 Hz), 1.66 (3H, s, CH_3CO), 1.56 (4H, m, CH_2 of linker), 1.34 (4H, m, CH_2 of linker), 0.68 (3H, d, H_C-6, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) δ 171.3, 167.9, 167.1, 165.9, 165.1, 164.6, 159.3, 138.0, 133.4, 133.3, 129.8, 129.7, 129.5, 129.3, 128.7, 128.6, 128.5, 128.0, 126.3, 113.7, 100.6, 100.1, 99.2, 99.1, 79.6, 75.9, 75.1, 73.2, 72.1, 72.0, 71.9, 71.3, 71.0, 69.3, 69.2, 66.5, 65.1, 55.3, 54.3, 52.8, 51.4, 40.4, 40.4, 29.3, 28.8, 26.4, 25.5, 23.3, 15.2; HRMS (ESI-FT-ICR) m/z caled. for $C_{60}H_{72}Cl_2N_5O_{21}$ $[M+NH_4]^+$ 1268.4091, found 1268.4119.

6-Azidohexyl O-[4-O-levulinoyl-2, 3-di-O-(4-methoxybenzyl)-α-L-fucopyranosyl]-(1→3)-(Methyl-2, 4-di-O-benzoyl-β-D-glucopyranosyl-uronate)-(1→3)-2-acetamido-4, 6-O-benzylidene-2-deoxy-β-D-galactopyranoside (16).

From donor **13** (243 mg, 0.445 mmol) as described for the preparation of **14**. Flash silica chromatography (petroleum ether/Acetone 3:1~1.5:1) gave pure trisaccharide **16** (287 mg, 77%) as white solid. R_f = 0.45 (DCM/MeOH 50:1); $[\alpha]_D^{25}$ = +15 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ 8.04, 7.94, 7.59-7.32, 7.11, 6.95, 6.81, 6.69 (23H, m, aromatic), 5.72 (1H, m,

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N*H*), 5.59 (1H, dd, H_B-4, *J* = *J* = 9.5 Hz), 5.51 (1H, s, PhC*H*), 5.48 (1H, dd, H_B-2, *J* = *J* = 7.7 Hz), 5.13 (1H, d, H_B-1, *J* = 7.2 Hz), 5.10 (1H, d, H_A-1, *J* = 8.6 Hz), 4.99 (1H, m, H_C-4), 4.81 (1H, d, H_C-1, *J* = 3.1 Hz), 4.75 (1H, dd, H_A-3, *J* = 2.8 Hz, *J* = 11.0 Hz), 4.41 (2H, m, H_A-4, PhCH_2), 4.30-4.17 (5H, m, H_A-6a, H_B-3, H_B-5, PhCH_2), 3.98 (2H, m, H_A-6b, PhCH_2), 3.88 (2H, m, H_C-5, OCH_2 of linker), 3.79 (3H, s, OCH_3), 3.77-3.73 (4H, m, OCH_3, H_C-3), 3.55 (3H, s, COOCH_3), 3.47-3.36 (4H, m, H_A-5, H_A-2, H_C-2, OCH_2 of linker), 3.23 (2H, t, CH_2N$_3$, *J* = 6.7 Hz), 2.64-2.41 (4H, m, CH_2 of Lev), 2.02 (3H, s, CH_3CO), 1.60 (3H, s, CH_3CO), 1.55 (4H, m, CH_2 of linker), 1.33 (4H, m, CH_2 of linker), 0.70 (3H, d, H_C-6, *J* = 6.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) *δ* 206.2, 172.2, 171.2, 167.9, 165.3, 164.6, 159.1, 159.0, 138.1, 133.4, 133.3, 130.7, 130.5, 130.0, 129.8, 129.6, 129.6, 129.3, 128.7, 128.5, 128.0, 126.3, 113.6, 113.5, 100.6, 100.3, 99.5, 99.1, 78.9, 76.0, 75.6, 75.1, 73.9, 73.2, 72.1, 72.0, 71.9, 71.4, 71.0, 69.4, 69.2, 66.5, 65.7, 55.2, 54.5, 52.7, 51.4, 38.0, 29.7, 29.3, 28.8, 28.0, 26.4, 25.5, 23.3, 15.5; HRMS (ESI-FT-ICR) m/z caled. for $C_{69}H_{84}N_5O_{22}$ $[M+NH_4]^+$ 1334.5603, found 1334.5558.

6-Azidohexyl O-(2-O-benzoyl-3, 4-di-O-chloroacetyl-*α*-L-fucopyran-osyl)-(1→3)-(Methyl-2, 4-di-O-benzoyl-*β*-D-glucopyranosyluronate)-(1→3)-2-acetamido-4, 6-O-benzylidene-2-deoxy-β-D-galactopyranoside (17).

DDQ (6.0 mg, 0.0265 mmol, 1.5 eq) was added to a solution of **15** (22.1 mg, 0.0177 mmol) in CH_2Cl_2 (0.4 mL)/H_2O (40 µL). The suspension was stirred for 4 h at room temperature. Completion of the reaction was confirmed by TLC. The mixture was diluted with CH_2Cl_2, and the organic layer was washed with saturated aqueous $NaHCO_3$/$Na_2S_2O_3$ solution and brine, dried over Na_2SO_4, and concentrated. The residue was coevaporated with toluene to remove trace solvent, then dissolved in dry pyridine (0.5 mL) under Argon. The mixture was cooled to -10 °C and benzoyl chloride (3.0 µL, 0.0265 mmol, 1.5 eq) was added, the reaction was kept at -10 °C for 1 h and 0 °C for 30 min, then diluted with EtOAc, washed with 1 M HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. Flash silica chromatography (DCM/MeOH 60:1) gave **17** (19.4 mg, 89% for 2 steps) as white solid. R_f = 0.50 (DCM/MeOH 40:1); $[\alpha]_D^{25}$ = -8 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) *δ* 8.03, 7.79, 7.74, 7.63-7.46, 7.36-7.30 (20H, m, aromatic), 5.70 (1H, dd, H_B-4, *J* = *J* = 9.3 Hz), 5.59 (1H, d, N*H*, *J* = 6.4 Hz), 5.50 (1H, d, H_C-1, *J* = 3.7 Hz), 5.48-5.44 (2H, m, PhCH_2, H_C-3), 5.27 (1H, dd, H_B-2, *J* = *J* = 6.4 Hz), 5.20 (1H, dd, H_C-2, *J* = 3.7 Hz, *J* = 10.9 Hz), 5.12-5.07 (3H, m, H_A-1, H_B-1, H_C-4), 4.71 (1H, dd, H_A-3, *J* = 3.4 Hz, *J* = 11.1 Hz), 4.35-4.31 (2H, m, H_B-3, H_A-4), 4.26-4.24 (2H, m, H_B-5, H_A-6a), 4.05 (2H, s, CH_2 of CA), 4.02-3.99 (2H, m, H_C-5, H_A-6b), 3.88-3.81 (3H, m, OCH_2 of linker, CH_2 of CA), 3.56 (3H, s, COOCH_3), 3.41-3.37 (2H, m, H_A-5, OCH_2 of linker), 3.25-3.21 (3H, m, H_A-2, CH_2N$_3$), 1.58 (3H, s, CH_3CO), 1.55 (4H, m, CH_2 of linker), 1.33 (4H, m, CH_2 of linker), 0.71 (3H, d, H_C-6, *J* = 6.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) *δ* 171.2, 168.0, 167.1, 166.1, 165.6, 165.2, 164.1, 138.1, 133.6, 133.5, 129.9, 129.7, 129.1, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 127.9, 126.3, 100.5, 99.7, 98.9, 96.6, 75.9, 74.9, 73.8, 72.7, 71.9, 70.5, 69.3, 69.3, 69.1, 67.9, 66.4, 65.0, 54.6, 53.9, 52.7, 51.4, 40.4, 40.2, 29.3, 28.8, 26.4, 25.5, 23.2, 15.3; HRMS (ESI-FT-ICR) m/z caled. for $C_{59}H_{68}Cl_2N_5O_{21}$ $[M+NH_4]^+$ 1252.3778, found 1252.3761.

6-Azidohexyl O-(2, 3-di-O-benzoyl-4-O-levulinoyl-*α*-L-fucopyranosyl)-(1→3)-(Methyl-2, 4-di-O-benzoyl-*β*-D-glucopyranosyluronate)-(1→3)-2-acetamido-4, 6-O-benzylidene-2-deoxy-β-D-galactopyranoside (18).

From trisaccharide **16** (193.1 mg, 0.147 mmol) as described for the preparation of **17.**. Flash silica chromatography (DCM/MeOH 70:1~60:1) gave **18** (172.1 mg, 91% for 2 steps) as white solid. R_f = 0.45 (DCM/MeOH 45:1); $[\alpha]_D^{25}$ = -22 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) *δ* 8.06, 7.78, 7.72, 7.63-7.26 (25H, m, aromatic), 5.73 (1H, dd, H_B-4, *J* = *J* = 9.4 Hz), 5.65 (1H, d, N*H*, *J* = 6.5 Hz), 5.58-5.53 (2H, m, H_C-3, H_C-1), 5.48 (1H, s, PhC*H*), 5.41 (1H, dd, H_C-2, *J* = 3.8 Hz, *J* = 10.8 Hz), 5.32 (1H, dd, H_B-2, *J* = *J* = 6.6 Hz), 5.21 (1H, m, H_C-4), 5.13 (1H, d, H_B-1, *J* = 6.4 Hz), 5.10 (1H, d, H_A-1, *J* = 8.2 Hz), 4.71 (1H, dd, H_A-3, *J* = 3.3 Hz, *J* = 11.1 Hz), 4.39 (1H, dd, H_B-3, *J* = *J* = 7.1 Hz), 4.31 (1H, d, H_A-4, *J* = 3.2 Hz), 4.29-4.24 (2H, m, H_B-5, H_A-6a), 4.07-4.00 (2H, m, H_C-5, H_A-6b), 3.85 (1H, m, OCH_2 of linker), 3.57 (3H, s, COOCH_3), 3.42-3.37 (2H, m, H_A-5, OCH_2 of linker), 3.24-3.21 (3H, m, H_A-2, CH_2N$_3$), 2.65-2.48 (4H, m, CH_2 of Lev), 2.04 (3H, s, CH_3CO), 1.58 (3H, s, CH_3CO), 1.55 (4H, m, CH_2 of linker), 1.33 (4H, m, CH_2 of linker), 0.72 (3H, d, H_C-6, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) *δ* 205.9, 171.9, 171.2, 168.1, 165.8, 165.3, 165.2, 164.0, 138.0, 133.6, 133.4, 133.2, 133.0, 129.9, 129.8, 129.7, 129.5, 129.3, 129.2, 129.0, 128.9, 128.6, 128.4, 128.2, 128.2, 127.9, 126.3, 100.5, 99.9, 98.9, 96.9, 75.9, 75.0, 73.9, 72.0, 71.3, 70.5, 69.4, 69.2, 68.4, 68.2, 66.4, 65.5, 54.6, 52.8, 51.4, 37.7, 29.7, 29.3, 28.8, 27.7, 26.4, 25.5, 23.3, 15.5; HRMS (ESI-FT-ICR) m/z caled. for $C_{67}H_{76}N_5O_{22}$ $[M+NH_4]^+$ 1302.4977, found 1302.4955.

6-Azidohexyl O-(3-O-levulinoyl-*α*-L-fucopyranosyl)-(1→3)-(Methyl-2, 4-di-O-benzoyl-*β*-D-glucopyranosyluronate)-(1→3)-2-acetamido-2-deoxy-β-D-galacto-pyranoside (19).

80 % aqueous AcOH (1.0 mL) was added to a flask containing compound **14** (52.5 mg, 0.0418 mmol). The mixture was stirred for 1.5 h at 50 °C, then concentrated and co-evaporated with toluene. The residue was washed with Et_2O and used in next step without further purification (43.3 mg, 89%).To a solution of diol (43.3 mg, 0.037 mmol) in ethyl acetate (1.0 mL) was added a solution of $NaBrO_3$ (39.1 mg, 0.26 mmol, 7.0 eq) in water (0.5 mL). A solution of $Na_2S_2O_4$ (85 %, 45.5 mg, 0.22 mmol, 6.0 eq) in water (0.6 mL) was added over 10 min and the mixture was vigorously stirred for 6 h at room temperature. The mixture was quenched with saturated sodium thiosulphate, diluted with ethyl acetate and washed with water. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuum. The remainder was purified by flash chromatography (DCM/MeOH 20:1~15:1) to afford **19** (26.8 mg, 73%). R_f = 0.30 (DCM/MeOH 15:1); $[\alpha]_D^{25}$ = +13 (*c* = 1.0, CH_3OH); ^{1}H NMR (400 MHz, MeOD-*d*$_4$) *δ* 8.14-8.01, 7.70-7.45 (10H, m, aromatic), 5.49 (1H, dd, H_B-4, *J* = *J* = 9.1 Hz), 5.42 (1H, dd, H_B-2, *J* = *J* = 8.2 Hz), 5.10 (1H, d, H_C-1, *J* = 4.0 Hz), 5.05 (1H, d, H_B-1, *J* = 7.7 Hz), 4.74 (1H, dd, H_C-3, *J* = 3.0 Hz, *J* = 10.7 Hz), 4.54 (1H, d, H_B-5, *J* = 9.1 Hz), 4.49 (1H, dd, H_B-3, *J* = *J* = 8.7 Hz), 4.43 (1H, d, H_A-1, *J* = 8.3 Hz), 4.19 (1H, m, H_A-4), 3.95 (1H, m, H_A-3), 3.87-3.74 (5H, m, H_A-2, H_C-5, H_A-6a, H_A-6b, OCH_2 of linker), 3.65 (1H, dd, H_C-2, *J* = 3.9 Hz, *J* = 10.6 Hz), 3.61 (3H, s, COOCH_3), 3.52-3.50 (2H, m, H_A-5, H_C-4), 3.42 (1H, m, OCH_2 of linker), 3.26 (2H, t, CH_2N$_3$, *J* = 6.8 Hz), 2.78-2.74, 2.56-2.52 (4H, m, CH_2 of Lev), 2.16 (3H, s, CH_3CO), 1.53 (4H, m, CH_2 of linker), 1.34 (4H, m, CH_2 of linker), 1.27 (3H, s, CH_3CO), 0.71 (3H, d, H_C-6, *J* = 6.5 Hz); ^{13}C NMR (100 MHz, MeOD-*d*$_4$) *δ* 208.5, 172.8, 171.8, 168.5, 165.6, 164.8, 133.4, 133.0, 129.8, 129.7, 129.5, 129.4, 128.4, 128.2, 128.1, 101.5, 101.1 100.1, 80.8, 77.0, 74.7, 73.5, 73.4, 71.9, 70.6, 69.3, 68.8, 67.7, 66.6, 65.4, 61.1, 52.0, 51.9, 51.6, 51.1, 51.0, 37.3, 29.1, 28.7, 28.5, 28.3, 27.6, 26.1, 25.2, 21.2, 14.8; HRMS (ESI-FT-ICR) m/z caled. for $C_{46}H_{61}N_4O_{20}$ $[M+H]^+$ 989.3874, found 989.3897.

6-Azidohexyl O-(2-O-benzoyl-*α*-L-fucopyranosyl)-(1→3)-(Methyl-2, 4-di-O-benzoyl-*β*-D-glucopyranosyluronate)-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranoside (20).

80 % aqueous AcOH (3.0 mL) was added to a flask containing compound **17** (126.7 mg, 0.1026 mmol). The mixture was stirred for 3 h at 50 °C, then concentrated and co-evaporated with toluene. The residue was redissolved in MeOH (2.5 mL), thiourea (117 mg, 1.54 mmol, 15 eq) and lutidine (180 µL, 1.54 mmol, 15 eq) were added to the solution. The mixture was stirred for 8 h at 60 °C, evaporated and dissolved in EtOAc, then washed with 1 M HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. Flash silica chromatography (DCM/MeOH 30:1~20:1) gave **20** (70.7 mg, 70% for 2 steps) as white solid. R_f = 0.35 (DCM/MeOH 15:1); $[\alpha]_D^{25}$ = +27 (*c* = 1.0, CH_3OH); ^{1}H NMR (400 MHz, MeOD-*d*$_4$) *δ* 8.10, 7.86, 7.78, 7.70, 7.62-7.55, 7.43-7.37 (15H, m, aromatic), 5.47 (1H, dd, H_B-4, *J* = *J* = 9.4 Hz), 5.37 (1H, d, H_C-1, *J* = 3.8 Hz), 5.29 (1H, dd, H_B-2, *J* = *J* = 8.4 Hz), 5.02-4.99 (2H, m, H_C-2, H_B-1), 4.56 (1H, dd,

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H_B-3, J = J = 8.9 Hz), 4.51 (1H, d, H_B-5, J = 9.4 Hz), 4.38 (1H, d, H_A-1, J = 8.3 Hz), 4.15 (1H, m, H_A-4), 3.93-3.88 (3H, m, H_C-5, H_C-3, H_A-3), 3.82-3.72 (4H, m, H_A-6a, H_A-2, H_C-4, OCH_2 of linker), 3.59 (3H, s, COOCH_3), 3.48 (1H, m, H_A-6b), 3.42-3.36 (2H, m, H_A-5, OCH_2 of linker), 3.24 (2H, t, CH_2N$_3$, J = 6.9 Hz), 1.50 (4H, m, CH_2 of linker), 1.31 (4H, m, CH_2 of linker), 1.15 (3H, s, CH_3CO), 0.85 (3H, d, H_C-6, J = 6.5 Hz); ^{13}C NMR (100 MHz, MeOD-d_4) δ 171.7, 168.3, 166.4, 165.7, 164.4, 133.5, 133.2, 132.7, 129.7, 129.6, 129.5, 129.3, 129.1, 128.5, 128.2, 127.8, 101.3, 101.2, 96.7, 80.7, 75.3, 74.7, 73.8, 72.2, 71.7, 70.7, 68.8, 67.6, 67.2, 67.0, 61.0, 51.9, 51.0, 29.1, 28.4, 26.1, 25.2, 21.1, 14.9; HRMS (ESI-FT-ICR) m/z caled. for $C_{48}H_{62}N_5O_{19}$ $[M+NH_4]^+$ 1012.4034, found 1012.4058.

6-Azidohexyl O-(2, 3-di-O-benzoyl-α-L-fucopyranosyl)-(1→3)-(Methyl-2, 4-di-O-benzoyl-β-D-glucopyranosyluronate)-(1→3)-2-acetamido-2-deoxy-β-D-galacto-pyranoside (21).

80 % aqueous AcOH (3.0 mL) was added to a flask containing compound **18** (172.1 mg, 0.134 mmol). The mixture was stirred for 3 h at 50 °C, then concentrated and co-evaporated with toluene. The residue was redissolved in CH_2Cl_2 (2 mL), and a freshly prepared mixture of 0.5 M hydrazine hydrate in pyridine/acetic acid 3:2 (0.8 mL 0.40 mmol, 3.0 eq) were added to the solution at 0 °C. The mixture was allowed to warm to room temperature and stirred for 5 h, then diluted with EtOAc, washed with 1 M HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. Flash silica chromatography (DCM/MeOH 30:1~25:1) gave **21** (112.2 mg, 76% for 2 steps) as white solid. R_f = 0.30 (DCM/MeOH 25:1); $[\alpha]_D^{25}$ = -15 (c = 1.0, CH_3OH); ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.14, 7.84, 7.76, 7.68, 7.59-7.45, 7.36-7.26 (20 H, m, aromatic), 5.56 (1H, dd, H_B-4, J = J = 9.3 Hz), 5.47-5.44 (2H, m, H_C-1, H_C-2), 5.40-5.36 (2H, m, H_B-2, H_C-3), 5.05 (1H, dd, H_B-1, J = J = 7.8 Hz), 4.62 (1H, dd, H_B-3, J = J = 8.9 Hz), 4.56 (1H, d, H_B-5, J = 9.3 Hz), 4.42 (1H, d, H_A-1, J = 8.3 Hz), 4.18 (1H, m, H_A-4), 4.04 (1H, q, H_C-5, J = 6.5 Hz), 3.94 (1H, m, H_A-3), 3.83-3.75 (5H, m, H_C-4, H_A-2, H_A-6a, H_A-6b, OCH_2 of linker), 3.61 (3H, s, COOCH_3), 3.51 (1H, m, H_A-5), 3.40 (1H, m, OCH_2 of linker), 3.23 (2H, t, CH_2N$_3$, J = 6.8 Hz), 1.50 (4H, m, CH_2 of linker), 1.30 (4H, m, CH_2 of linker), 1.18 (3H, s, CH_3CO), 0.85 (3H, d, H_C-6, J = 6.5 Hz); ^{13}C NMR (100 MHz, MeOD-d_4) δ 171.7, 168.3, 165.9, 165.8, 165.8, 164.4, 133.5, 133.2, 132.8, 129.7, 129.6, 129.5, 129.5, 129.2, 129.0, 129.0, 128.5, 128.2, 127.9, 127.8, 101.3, 101.1, 97.1, 80.7, 76.2, 74.7, 73.7, 71.8, 71.2, 70.8, 69.7, 68.8, 68.1, 67.6, 67.0, 61.1, 52.0, 50.9, 29.1, 28.4, 26.1, 25.2, 21.1, 14.8; HRMS (ESI-FT-ICR) m/z caled. for $C_{55}H_{66}N_5O_{20}$ $[M+NH_4]^+$ 1116.4296, found 1116.4275.

General Procedure A: O-Sulfation

Sulfur trioxide trimethylamine complex (5 equiv. per hydroxyl group) was added to the starting materials in dry DMF (1.0 mL for 40 mg). The mixture was heated at 50 ºC under argon for 72 h, then was cooled, and directly eluted from a column of Sephadex LH-20 with CH_2Cl_2/MeOH 1:1 to give O-sulfated products.

6-Azidohexyl O-(3-O-levulinoyl-2, 4-di-O-sulfo-α-L-fucopyranosyl)-(1→3)-(Methyl-2, 4-di-O-benzoyl-β-D-glucopyranosyluronate)-(1→3) - 2-acetamido-2-deoxy-4, 6-di-O-sulfo-β-D-galactopyranoside (22).

From compound **19** (53.5 mg, 0.0541 mmol) following general procedure **A** to give **22** (86.2 mg, 95%) as white solid. R_f =0.65 ($CHCl_3$/MeOH/H_2O 10:10:3); $[\alpha]_D^{25}$ = +4 (c = 1.0, CH_3OH); ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.14, 8.08, 7.67, 7.60-7.52, 7.46 (10H, m, aromatic), 5.67 (1H, d, H_C-1, J = 3.3 Hz), 5.60 (1H, dd, H_B-4, J = J = 9.1 Hz), 5.51 (1H, dd, H_B-2, J = J = 7.3 Hz), 5.13 (1H, d, H_B-1, J = 6.7 Hz), 5.05 (1H, d, H_A-4, J = 2.4 Hz), 4.79 (1H, dd, H_C-3, J = 3.2 Hz, J = 10.9 Hz), 4.63 (1H, dd, H_B-3, J = J = 8.1 Hz), 4.55 (1H, dd, H_C-2, J = 3.3 Hz, J = 10.9 Hz), 4.49 (1H, d, H_B-5, J = 9.1 Hz), 4.41-4.38 (3H, m, H_A-1, H_A-6a, H_C-4), 4.26 (1H, dd, H_A-6b, J = 7.6 Hz, J = 11.5 Hz), 4.09-4.02 (2H, m, H_A-2, H_C-5), 3.97-3.91 (2H, m, H_A-3, H_A-5), 3.84 (1H, m, OCH_2 of linker), 3.67 (3H, s, COOCH_3), 3.47 (1H, m, OCH_2 of linker), 3.27 (2H, t, CH_2N$_3$, J = 6.9 Hz), 2.73-2.68, 2.48-2.44 (4H, m, CH_2 of Lev), 2.13 (3H, s, CH_3CO), 1.55 (4H, m, CH_2 of linker), 1.43 (3H, s, CH_3CO), 1.35 (4H, m, CH_2 of linker), 1.05 (3H, d, H_C-6, J = 6.4 Hz); ^{13}C NMR (100 MHz, MeOD-d_4) δ 208.9, 172.8, 172.4, 169.5, 166.3, 165.5, 133.3, 133.0, 130.5, 129.4, 129.2, 128.4, 128.0, 101.3, 100.9, 96.2, 78.1, 76.1, 75.8, 75.4, 72.6, 72.4, 72.0, 71.6, 71.3, 69.0, 68.4, 67.4, 66.1, 52.3, 51.0, 44.3, 44.2, 37.5, 29.1, 28.5, 28.3, 27.9, 26.1, 25.2, 21.3, 15.4; HRMS (ESI-FT-ICR) m/z caled. for $C_{46}H_{58}N_4NaO_{32}S_4^-$ $[M+2H+Na]^-$ 1329.1809, found 1329.1794.

6-Azidohexyl O-(2-O-benzoyl-3, 4-di-O-sulfo-α-L-fucopyranosyl)-(1→3)-(Methyl-2, 4-di-O-benzoyl-β-D-glucopyranosyluronate)-(1→3)-2-acetamido-2-deoxy-4, 6-di-O-sulfo-β-D-galactopyranoside (23).

From compound **20** (42.8 mg, 0.043 mmol) following general procedure **A** to give **23** (65.0 mg, 97%) as white solid. R_f =0.65 ($CHCl_3$/MeOH/H_2O 10:10:3); $[\alpha]_D^{25}$ = +11 (c = 1.0, CH_3OH); ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.11, 7.93, 7.77, 7.71-7.53, 7.46-7.40 (15H, m, aromatic), 5.60 (1H, dd, H_B-4, J = J = 9.5 Hz), 5.36 (1H, dd, H_B-2, J = J = 8.2 Hz), 5.33 (1H, d, H_C-1, J = 3.9 Hz), 5.11 (1H, dd, H_C-2, J = 3.9 Hz, J = 10.9 Hz), 5.02-4.97 (2H, m, H_B-1, H_A-4), 4.77 (1H, dd, H_C-3, J = 3.0 Hz, J = 10.9 Hz), 4.67 (1H, m, H_C-4), 4.57 (1H, dd, H_B-3, J = J = 9.2 Hz), 4.46 (1H, m, H_B-5), 4.40 (1H, m, H_A-6a), 4.27 (1H, d, H_A-1, J = 8.0 Hz), 4.26 (1H, m, H_A-6b), 3.98-3.76 (5H, m, H_A-5, H_C-5, H_A-3, H_A-2, OCH_2 of linker), 3.61 (3H, s, COOCH_3), 3.40 (1H, m, OCH_2 of linker), 3.25 (2H, t, CH_2N$_3$, J = 6.9 Hz), 1.49 (4H, m, CH_2 of linker), 1.31 (4H, m, CH_2 of linker), 1.23 (3H, s, CH_3CO), 0.85 (3H, d, H_C-6, J = 6.4 Hz); ^{13}C NMR (100 MHz, MeOD-d_4) δ 171.7, 168.0, 166.3, 165.8, 165.8, 164.1, 133.5, 133.2, 132.9, 132.8, 130.2, 129.8, 129.6, 129.2, 128.5, 128.2, 127.9, 101.8, 101.8, 97.0, 78.0, 77.5, 76.3, 75.8, 75.7, 74.4, 72.9, 71.9, 71.8, 70.5, 69.1, 67.9, 66.6, 51.8, 51.8, 51.0, 29.1, 28.4, 26.1, 25.1, 21.2, 21.1, 15.8; HRMS (ESI-FT-ICR) m/z caled. for $C_{48}H_{55}N_4O_{31}S_4^{3-}$ $[M+H]^{3-}$ 437.0572, found 437.0575.

6-Azidohexyl O-(2, 3-di-O-benzoyl-4-O-sulfo-α-L-fucopyranosyl)-(1→3)-(Methyl-2, 4-di-O-benzoyl-β-D-glucopyranosyluronate)-(1→3)-2-acetamido-2-deoxy-4, 6-di-O-sulfo-β-D-galactopyranoside (24).

From compound **21** (50.8 mg, 0.046 mmol) following general procedure **A** to give **24** (59.5 mg, 95%) as white solid. R_f =0.70 ($CHCl_3$/MeOH/H_2O 10:10:3); $[\alpha]_D^{25}$ = +21 (c = 1.0, CH_3OH); ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.15, 7.88, 7.76, 7.70, 7.60-7.41, 7.33-7.22 (20H, m, aromatic), 5.66 (1H, dd, H_B-4, J = J = 9.5 Hz), 5.53-5.46 (3H, m, H_C-1, H_B-2, H_C-3), 5.38 (1H, dd, H_C-2, J = 3.3 Hz, J = 10.7 Hz), 5.10 (1H, d, H_B-1, J = 7.8 Hz), 5.05 (1H, m, H_A-4), 4.65-4.60 (2H, m, H_C-4, H_B-3), 4.54 (1H, d, H_B-5, J = 9.9 Hz), 4.40-4.34 (2H, m, H_A-1, H_A-6a), 4.27 (1H, m, H_A-6b), 4.17 (1H, q, H_C-5, J = 6.5 Hz), 3.97 (2H, m, H_A-2, H_A-3), 3.88 (1H, m, H_A-5), 3.77 (1H, m, OCH_2 of linker), 3.61 (3H, s, COOCH_3), 3.40 (1H, m, OCH_2 of linker), 3.23 (2H, t, CH_2N$_3$, J = 6.8 Hz), 1.48 (4H, m, CH_2 of linker), 1.29 (4H, m, CH_2 of linker), 1.25 (3H, s, CH_3CO), 0.97 (3H, d, H_C-6, J = 6.2 Hz); ^{13}C NMR (100 MHz, MeOD-d_4) δ 172.2, 169.0, 166.3, 166.0, 165.8, 165.3, 133.6, 133.3, 132.8, 132.5, 129.9, 129.6, 129.5, 129.4, 128.9, 128.5, 128.2, 127.9, 127.7, 101.6, 101.5, 97.2, 78.7, 77.0, 76.3, 76.0, 73.7, 72.4, 71.9, 70.9, 69.1, 68.8, 68.5, 67.3, 66.6, 52.2, 51.0, 44.5, 29.0, 28.4, 26.1, 25.1, 21.2, 15.6; HRMS (ESI-FT-ICR) m/z caled. for $C_{55}H_{59}N_4O_{29}S_3^{3-}$ $[M]^{3-}$ 445.0804, found 445.0817.

General Procedure B: Saponification

A solution of ester in THF/water (3:1, 2 mL) was treated at -10ºC with a freshly prepared mixture of hydrogen peroxide (35 wt% in water, 0.25 mL) and LiOH (1 M, 0.5 mL), and the mixture was kept for 1 h at -10ºC and allowed to warm to room temperature and stirred overnight. Then the solution was cooled to 0ºC, Methanol (0.5 mL) and NaOH (4 M, 1.3 mL) were added, and the mixture was stirred for 10 h at room temperature, then was adjusted to pH 7 with Amberlite IR-120 [H^+] resin, filtered, concentrated, and directly purified by Sephadex LH-20 with water to give the sodium salt.

6-Azidohexyl O-(2, 4-di-O-sulfo-α-L-fucopyranosyl)-(1→3)-(β-D-glucopyranosyl-uronic acid)-(1→3)-2-acetamido-2-deoxy-4, 6-di-O-sulfo-β-D-galactopyranoside (25).

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From compound **22** (50.1 mg, 0.0325 mmol) following general procedure **B** to give the sodium salt **25** (32.1 mg, 90%) as a white solid. R_f=0.40 (EtOAc/EtOH/H_2O 2:1:1); $[\alpha]_D^{25}$ = -25 (*c* = 1.0, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 5.61 (1H, d, H_C-1, *J* = 3.7 Hz), 4.87 (1H, m, H_A-4), 4.71 (1H, m, H_C-4), 4.60-4.53 (3H, m, H_C-5, H_A-1, H_B-1), 4.48 (1H, dd, H_C-2, *J* = 3.7 Hz, *J* = 10.5 Hz), 4.34 (1H, dd, H_A-6a, *J* = 2.7 Hz, *J* = 11.4 Hz), 4.25-4.17 (2H, m, H_C-3, H_A-6b), 4.11-4.02 (3H, m, H_A-2, H_A-3, H_A-5), 3.94 (1H, m, OCH_2 of linker), 3.80 (1H, d, H_B-5, *J* = 9.3 Hz), 3.75-3.60 (4H, m, H_B-2, H_B-3, H_B-4, OCH_2 of linker), 3.35 (2H, t, CH_2N$_3$, *J* = 6.9 Hz), 2.06 (3H, s, CH_3CO), 1.62 (4H, m, CH_2 of linker), 1.40 (4H, m, CH_2 of linker), 1.28 (3H, d, H_C-6, *J* = 6.5 Hz); ^{13}C NMR (100 MHz, D_2O) δ 174.8, 103.6, 101.2, 96.9, 81.8, 81.0, 76.4, 75.8, 75.4, 73.1, 72.5, 70.7, 70.1, 68.2, 66.7, 66.3, 51.9, 51.3, 28.6, 28.1, 25.7, 24.7, 22.5, 15.8; HRMS (ESI-FT-ICR) m/z caled. for $C_{26}H_{42}N_4O_{28}S_4^{2-}$ $[M+3H]^{2-}$ 493.0429, found 493.0446.

6-Azidohexyl O-(3, 4-di-O-sulfo-α-L-fucopyranosyl)-(1→3)-(β-D-glucopyranosyl-uronic acid)-(1→3)-2-acetamido-2-deoxy-4, 6-di-O-sulfo-β-D-galactopyranoside (26).

From compound **23** (52.7 mg, 0.034 mmol) following general procedure **B** to give the sodium salt **26** (34.5 mg, 92%) as a white solid. R_f=0.45 (EtOAc/EtOH/H_2O 2:1:1); $[\alpha]_D^{25}$ = -17 (*c* = 1.0, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 5.36 (1H, d, H_C-1, *J* = 3.9 Hz), 4.89 (1H, m, H_C-4), 4.83 (1H, m, H_A-4), 4.62 (1H, dd, H_C-3, *J* = 2.7 Hz, *J* = 10.5 Hz), 4.55-4.48 (3H, m, H_C-5, H_A-1, H_B-1), 4.30 (1H, dd, H_A-6a, *J* = 2.6 Hz, *J* = 11.3 Hz), 4.20 (1H, m, H_A-6b), 4.08-4.01 (3H, m, H_A-2, H_A-3, H_A-5), 3.97-3.89 (2H, m, H_C-2, OCH_2 of linker), 3.74-3.57 (5H, m, H_B-2, H_B-3, H_B-4, H_B-5, OCH_2 of linker), 3.32 (2H, t, CH_2N$_3$, *J* = 6.9 Hz), 2.03 (3H, s, CH_3CO), 1.59 (4H, m, CH_2 of linker), 1.37 (4H, m, CH_2 of linker), 1.25 (3H, d, H_C-6, *J* = 6.5 Hz); ^{13}C NMR (100 MHz, D_2O) δ 174.8, 103.6, 101.2, 96.9, 81.8, 81.0, 76.4, 75.8, 75.4, 73,1, 72.5, 70.7, 70.0, 68.2, 66.7, 66.3, 51.9, 51.3, 28.6, 28.1, 25.7, 24.7, 22.5, 15.8; HRMS (ESI-FT-ICR) m/z caled. for $C_{26}H_{42}N_4O_{28}S_4^{2-}$ $[M+3H]^{2-}$ 493.0429, found 493.0442.

6-Azidohexyl O-(4-O-sulfo-α-L-fucopyranosyl)-(1→3)-(β-D-glucopyranosyluronic acid)-(1→3)-2-acetamido-2-deoxy-4, 6-di-O-sulfo-β-D-galactopyranoside (27).

From compound 24 (46.0 mg, 0.0328 mmol) following general procedure B to give the sodium salt 27 (32.1 mg, 98%) as a white solid. R_f=0.45 (EtOAc/EtOH/H_2O 2:1:1); $[\alpha]_D^{25}$ = -23 (*c* = 1.0, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 5.30 (1H, d, H_C-1, *J* = 3.9 Hz), 4.84 (1H, m, H_A-4), 4.62 (1H, m, H_C-4), 4.57-4.47 (3H, m, H_A-1, H_B-1, H_C-5), 4.32 (1H, dd, H_A-6a, *J* = 2.8 Hz, *J* = 11.4 Hz), 4.21 (1H, m, H_A-6b), 4.11-4.01 (4H, m, H_A-2, H_A-3, H_A-5, H_C-3), 3.93 (1H, m, OCH_2 of linker), 3.81 (1H, dd, H_C-2, *J* = 3.9 Hz, *J* = 10.5 Hz), 3.76 (1H, d, H_B-5, *J* = 9.7 Hz), 3.70-3.63 (2H, m, H_B-4, OCH_2 of linker), 3.60-3.58 (2H, m, H_B-2, H_B-3), 3.34 (2H, t, CH_2N$_3$, *J* = 6.9 Hz), 2.05 (3H, s, CH_3CO), 1.60 (4H, m, CH_2 of linker), 1.38 (4H, m, CH_2 of linker), 1.25 (3H, d, H_C-6, *J* = 6.5 Hz); ^{13}C NMR (100 MHz, D_2O) δ 174.7, 103.3, 101.1, 99.1, 81.6, 80.8, 76.3, 75.1, 73.1, 72.3, 70.6, 70.2, 68.6, 68.6, 68.0, 66.2, 51.7, 51.2, 28.4, 28.0, 25.6, 24.6, 22.3, 15.8; HRMS (ESI-FT-ICR) m/z caled. for $C_{26}H_{42}N_4O_{25}S_3^{2-}$ $[M+2H]^{2-}$ 453.0645, found 453.0658.

Scaffold L5

A solution of pentaerythritol (48 mg, 0.35 mmol) in 7 mL of dry DMF was cooled to 0 °C, NaH (60% w/w in mineral oil, 112 mg, 2.8 mmol) and 4-Pentyn-1-(4-methylbenzenesulfonate) [44a] (500 mg, 2.1 mmol) was added. The reaction mixture was stirred under Ar atmosphere at room temperature for 20 h and quenched with methanol. The solvent was removed under reduced pressure, then dissolved with AcOEt and washed with brine. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuum. The remainder was purified by flash chromatography (petroleum ether/EtOAc 15:1) to afford **L5** (91 mg, 65%) as a colorless oil. R_f = 0.30 (petroleum ether/EtOAc 15:1); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ 3.49 (8H, t, *J* = 6.1 Hz), 3.40 (8H, s), 2.30 (8H, dt, *J* = 2.6 Hz, *J* = 7.2 Hz), 1.98 (4H, t, *J* = 2.6 Hz), 1.79 (8H, m); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) δ 84.2, 69.6, 69.5, 69.3, 45.5, 28.7, 15.3; HRMS (ESI-FT-ICR) m/z caled. for $C_{25}H_{36}NaO_4$ $[M+Na]^+$ 423.2506, found 423.2587.

Scaffold L7

A solution of Dipentaerythritol (30 mg, 0.118 mmol) in 2 mL of dry DMF was cooled to 0 °C, NaH (60% w/w in mineral oil, 57 mg, 1.4 mmol) and Ethanol-2-(2-propyn-1-yloxy)-1-(4-methylbenzenesulfonate) [44b] (300 mg, 1.2 mmol) was added. The reaction mixture was stirred under Ar atmosphere at room temperature for 20 h and quenched with methanol. The solvent was removed under reduced pressure, then dissolved with AcOEt and washed with brine. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuum. The remainder was purified by flash chromatography (petroleum ether/EtOAc 3:1) to afford **L7** (65 mg, 74%) as a colorless oil. R_f = 0.40 (petroleum ether/EtOAc 2:1); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ 4.19 (12H, m), 3.66 (12H, m), 3.58 (12H, m), 3.44 (12H, s), 3.36 (4H, s), 2.45 (6H, t, *J* = 2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) δ 79.9, 74.4, 70.9, 70.2, 70.0, 69.0, 58.3, 45.7; HRMS (ESI-FT-ICR) m/z caled. for $C_{40}H_{58}NaO_{13}$ $[M+Na]^+$ 769.3770, found 769.3743.

Scaffold L8

A solution of Tripentaerythritol (50 mg, 70%+, 0.094 mmol) in 1.5 mL of dry DMF was cooled to 0 °C, NaH (60% w/w in mineral oil, 86 mg, 2.2 mmol) and 80% Propargyl bromide in toluene (240 µL, 2.2 mmol) was added. The reaction mixture was stirred under Ar atmosphere at 50 °C for 48 h and quenched with methanol. The solvent was removed under reduced pressure, then dissolved with AcOEt and washed with brine. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuum. The remainder was purified by flash chromatography (petroleum ether/EtOAc 4:1) to afford **L8** (41 mg, 65%) as a colorless oil. R_f = 0.40 (petroleum ether/EtOAc 4:1); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ 4.13 (16H, m), 3.52 (16H, m), 3.39 (8H, m), 2.44 (8H, m); ^{13}C NMR (100 MHz, $CDCl_3$, TMS) δ 80.3, 80.1, 74.2, 74.1, 69.9, 69.8, 69.3, 69.2, 58.7, 45.5, 45.1; HRMS (ESI-FT-ICR) m/z caled. for $C_{39}H_{48}NaO_{10}$ $[M+Na]^+$ 699.3140, found 699.3098.

General procedure C: CuAAC reaction

Scaffolds and trisaccharides **19~21** (~20 mg, 1.2 eq per alkynyl group) were dissolved in a mixture of $CHCl_3$/MeOH/H_2O (3:1:1, 400 µL). $CuSO_4$ (0.12 eq per alkynyl group) and Na ascorbate (1.00 eq per alkynyl group) were added. The mixture was stirred at 45 °C overnight, and directly eluted from a column of Sephadex LH-20 with CH_2Cl_2/MeOH 1:1 to give the clusters.

Compound 28a

From trisaccharide **19** (15.5 mg, 15.7 µmol) and **L1** (0.902 mg, 6.53 µmol) following general procedure **C** to give the dimer **28a** (14.5 mg, quant) as white solid. R_f=0.40 (CH_2Cl_2/MeOH 7:1). ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.13-7.99, 7.69-7.57, 7.56-7.43 (22H, m, aromatic), 5.52-5.38 (4H, m), 5.10 (4H, m), 4.74 (2H, d, *J* = 10.4 Hz), 4.68-4.63 (4H, m), 4.58-4.49 (4H, m), 4.41-4.36 (6H, m), 4.21 (2H, m), 3.82-3.75 (8H, m), 3.73-3.65 (6H, m), 3.61 (8H, m), 3.54-3.50 (8H, m), 3.43-3.36 (2H, m), 2.78-2.73 (4H, m), 2.55-2.52 (4H, m), 2.16 (6H, s), 1.87-1.84 (4H, m), 1.51-1.45 (4H, m), 1.34-1.22 (12H, m), 1.11 (2H, m), 0.71 (6H, d, *J* = 6.3 Hz); ^{13}C NMR (100 MHz, MeOD-d_4) δ 208.5, 172.8, 171.7, 171.4, 168.6, 168.3, 165.7, 164.8, 133.4, 133.0, 129.9, 129.7, 129.6, 129.4, 128.4, 128.3, 128.1, 123.8, 101.5, 101.3, 100.1, 82.4, 81.0, 77.1, 74.7, 73.5, 73.4, 73.1, 71.8, 70.9, 70.8, 70.6, 69.3, 68.7, 67.6, 66.6, 66.2, 65.3, 63.6, 61.1, 52.0, 51.4, 49.9, 37.3, 29.8, 28.9, 28.4, 27.6, 25.7, 25.1, 21.3, 14.8; ESI-Q-TOF (positive mode) calcd for $C_{100}H_{131}N_8NaO_{42}$ $[M+Na+H]^{2+}$ m/z 1069.9141, found 1069.9139.

Compound 29a

From trisaccharide **19** (21.9 mg, 22.2 µmol) and **L2** (1.44 mg, 6.15 µmol) following general procedure **C** to give the trimer **29a** (19.1 mg, 97%) as white solid. R_f=0.40 (CH_2Cl_2/MeOH 7:1). ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.11-8.07, 7.69-7.60, 7.56-7.47 (33H, m, aromatic), 5.52-5.42 (6H, m),

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5.12 (6H, m), 4.76 (3H, d, *J* = 10.4 Hz), 4.61-4.54 (12H, m), 4.41-4.37 (9H, m), 4.22 (3H, m), 3.95 (6H, m), 3.82-3.77 (12H, m), 3.69-3.65 (3H, m), 3.61 (12H, m), 3.51-3.40 (12H, m), 2.75 (6H, m), 2.53 (6H, m), 2.15 (9H, s), 1.86 (6H, m), 1.47 (6H, m), 1.32 (12H, m), 1.27 (9H, s), 0.91 (3H, s), 0.72 (9H, d, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, MeOD-*d*$_4$) *δ* 208.5, 172.8, 171.7, 168.6, 165.7, 164.8, 133.4, 133.0, 129.8, 129.6, 129.4, 128.4, 128.3, 101.5, 100.2, 81.1, 77.2, 74.7, 73.5, 73.4, 72.5, 71.8, 70.7, 69.3, 68.8, 67.7, 66.6, 65.4, 61.2, 52.0, 51.3, 50.0, 37.3, 29.8, 29.0, 28.4, 27.6, 25.7, 25.0, 24.8, 21.3, 16.7, 14.8; ESI-Q-TOF (positive mode) calcd for $C_{152}H_{200}N_{12}O_{63}$ $[M+2H]^{2+}$ m/z 1601.1419, found 1601.1331.

Compound 30a

From trisaccharide **19** (19.4 mg, 19.6 µmol) and **L3** (1.30 mg, 5.5 µmol) following general procedure **C** to give the trimer **30a** (19.1 mg, quant) as white solid. R$_f$=0.30 (CH_2Cl_2/MeOH 6:1). ^{1}H NMR (400 MHz, MeOD-*d*$_4$) *δ* 8.18-8.00, 7.69-7.44, 7.06, 6.84 (36H, m, aromatic), 5.51-5.39 (6H, m), 5.19-5.14 (10H, m), 4.77-4.75 (3H, d, *J* = 10.5 Hz), 4.61-4.55 (6H, m), 4.40-4.38 (6H, m), 4.23 (4H, m), 4.00-3.93 (6H, m), 3.78 (12H, m), 3.69-3.66 (3H, m), 3.59 (12H, m), 3.51-3.48 (3H, m), 3.40 (3H, m), 2.76-2.72 (6H, m), 2.55-2.51 (6H, m), 2.14 (9H, s), 1.84-1.74 (6H, m), 1.43 (6H, m), 1.31-1.19 (17H, m), 0.71 (9H, d, *J* = 6.3 Hz); ^{13}C NMR (100 MHz, MeOD-*d*$_4$) *δ* 208.6, 172.7, 168.6, 164.8, 133.5, 133.0, 129.9, 129.6, 129.4, 128.4, 128.3, 101.5, 100.2, 77.2, 74.6, 73.5, 73.4, 71.7, 70.7, 69.3, 68.9, 67.6, 66.6, 65.4, 61.3, 52.1, 37.3, 29.7, 29.0, 28.4, 27.6, 25.7, 25.0, 21.4, 14.8; ESI-Q-TOF (positive mode) calcd for $C_{153}H_{194}N_{12}O_{63}$ $[M+2H]^{2+}$ m/z 1604.1184, found 1604.0677.

Compound 31a

From trisaccharide **19** (19.7 mg, 20.0 µmol) and **L4** (1.20 mg, 4.15 µmol) following general procedure **C** to give the tetremer **31a** (18.5 mg, quant) as white solid. R$_f$=0.40 (CH_2Cl_2/MeOH 6:1). ^{1}H NMR (400 MHz, MeOD-*d*$_4$) *δ* 8.11-8.03, 7.96, 7.69-7.60, 7.56-7.47 (44H, m, aromatic), 5.52-5.43 (8H, m), 5.13 (8H, m), 4.76 (4H, d, *J* = 10.5 Hz), 4.62-4.51 (16H, m), 4.40-4.36 (12H, m), 4.23 (4H, m), 3.95 (8H, m), 3.78 (16H, m), 3.67 (4H, m), 3.60 (16H, m), 3.51-3.44 (16H, m), 2.74 (8H, m), 2.53 (8H, m), 2.15 (12H, s), 1.84 (8H, m), 1.45 (8H, m), 1.31 (16H, m), 1.26 (12H, s), 0.71 (12H, d, *J* = 6.3 Hz); ^{13}C NMR (100 MHz, MeOD-*d*$_4$) *δ* 208.5, 172.7, 164.9, 133.4, 133.0, 129.8, 129.6, 129.4, 128.4, 128.3, 123.6, 101.5, 100.2, 77.2, 74.7, 73.5, 73.4, 71.8, 70.7, 69.3, 68.9, 67.7, 66.6, 65.4, 61.2, 52.1, 49.9, 37.3, 29.8, 29.0, 28.4, 27.6, 25.7, 25.0, 21.4, 14.8; ESI-Q-TOF (positive mode) calcd for $C_{201}H_{262}N_{16}NaO_{84}$ $[M+Na+2H]^{3+}$ m/z 1422.8890, found 1422.8743.

Compound 32a

From trisaccharide **19** (21.2 mg, 21.4 µmol) and **L5** (1.80 mg, 4.47 µmol) following general procedure **C** to give the tetramer **32a** (22.1 mg, quant) as white solid. R$_f$=0.50 (CH_2Cl_2/MeOH 6:1). ^{1}H NMR (400 MHz, MeOD-*d*$_4$) *δ* 8.11-8.08, 7.79, 7.69-7.60, 7.55-7.47 (44H, m, aromatic), 5.52-5.42 (8H, m), 5.12 (8H, m), 4.76 (4H, d, *J* = 10.5 Hz), 4.60-4.56 (8H, m), 4.41 (4H, m), 4.32 (8H, m), 4.22 (4H, m), 3.95 (8H, m), 3.79 (16H, m), 3.67 (4H, m), 3.60 (16H, m), 3.51 (4H, m), 3.44-3.40 (20H, m), 2.74 (16H, m), 2.53 (8H, m), 2.15 (12H, s), 1.96-1.83 (16H, m), 1.46 (8H, m), 1.31-1.26 (28H, m), 0.71 (12H, d, *J* = 6.3 Hz); ^{13}C NMR (100 MHz, MeOD-*d*$_4$) *δ* 208.5, 172.7, 171.7, 168.6, 165.7, 164.9, 133.4, 133.0, 129.8, 129.6, 129.4, 128.4, 128.3, 101.5, 100.2, 81.2, 77.2, 74.7, 73.4, 71.8, 70.7, 70.1, 69.3, 68.8, 67.7, 66.6, 65.4, 61.2, 52.0, 49.9, 45.3, 37.3, 29.8, 29.0, 28.4, 27.6, 25.8, 25.0, 21.9, 21.4, 14.8; ESI-Q-TOF (positive mode) calcd for $C_{209}H_{279}N_{16}O_{84}$ $[M+3H]^{3+}$ m/z 1452.9368, found 1452.9291.

Compound 33a

From trisaccharide **19** (25.3 mg, 25.6 µmol) and **L6** (1.71 mg, 3.56 µmol) following general procedure **C** to give the hexamer **33a** (21.8 mg, 96%) as white solid. R$_f$= 0.25 (CH_2Cl_2/MeOH 6:1). ^{1}H NMR (400 MHz, MeOD-*d*$_4$) *δ* 8.10, 7.98, 7.67-7.50 (66H, m, aromatic), 5.52-5.45 (12H, m), 5.20-5.14 (12H, m), 4.77 (6H, d, *J* = 10.5 Hz), 4.64-4.58 (12H, m), 4.50 (12H, m), 4.42-4.35 (18H, m), 4.25 (6H, m), 3.96-3.94 (12H, m), 3.79 (24H, m), 3.68 (6H, m), 3.59 (24H, m), 3.42 (12H, m), 2.73 (12H, m), 2.52 (12H, m), 2.14 (18H, s), 1.83 (12H, m), 1.44 (12H, m), 1.31-1.25 (36H, m), 0.71 (18H, d, *J* = 6.3 Hz); ^{13}C NMR (100 MHz, MeOD-*d*$_4$) *δ* 208.5, 172.7, 171.5, 168.7, 165.8, 164.9, 144.8, 133.4, 133.0, 129.9, 129.6, 129.4, 128.4, 128.3, 123.8, 101.5, 100.3, 77.3, 74.6, 73.5, 71.8, 70.8, 69.4, 68.9, 67.7, 66.6, 65.4, 61.3, 52.1, 51.1, 50.0, 37.3, 29.8, 29.0, 28.4, 27.6, 25.8, 25.0, 23.8, 21.4, 14.8; ESI-Q-TOF (positive mode) calcd for $C_{304}H_{397}N_{24}NaO_{127}$ $[M+Na+3H]^{4+}$ m/z 1610.3830, found 1610.3635.

Compound 34a

From trisaccharide **19** (20.3 mg, 20.5 µmol) and **L7** (2.13 mg, 2.85 µmol) following general procedure **C** to give the hexamer **34a** (16.7 mg, 88%) as white solid. R$_f$= 0.30 (CH_2Cl_2/MeOH 5:1). ^{1}H NMR (400 MHz, MeOD-*d*$_4$) *δ* 8.11-8.07, 7.97, 7.69-7.47 (66H, m, aromatic), 5.52-5.42 (12H, m), 5.17-5.13 (12H, m), 4.77 (6H, d, *J* = 10.5 Hz), 4.62-4.56 (24H, m), 4.42-4.34 (18H, m), 4.23 (6H, m), 3.96 (12H, m), 3.80-3.77 (24H, m), 3.70-3.51 (66H, m), 3.43 (18H, m), 2.75 (12H, m), 2.53 (12H, m), 2.15 (18H, s), 1.84 (12H, m), 1.46 (12H, m), 1.31-1.28 (42H, m), 0.71 (18H, d, *J* = 6.3 Hz); ^{13}C NMR (100 MHz, MeOD-*d*$_4$) *δ* 208.5, 172.8, 171.6, 168.6, 165.8, 164.9, 133.4, 133.1, 129.9, 129.6, 129.4, 128.4, 128.3, 101.5, 100.2, 81.3, 77.2, 74.6, 73.5, 71.8, 70.8, 69.8, 69.4, 69.3, 68.9, 67.7, 66.6, 65.4, 63.8, 61.2, 52.1, 51.2, 49.9, 45.6, 37.3, 29.8, 29.0, 28.4, 27.6, 25.8, 25.0, 21.4, 14.8; ESI-Q-TOF (positive mode) calcd for $C_{316}H_{421}N_{24}NaO_{133}$ $[M+Na+3H]^{4+}$ m/z 1676.4623, found 1676.4055.

Compound 35a

From trisaccharide **19** (25.1 mg, 25.4 µmol) and **L8** (1.8 mg, 2.65 µmol) following general procedure **C** to give the octamer **35a** (23.8 mg, quant) as white solid. R$_f$= 0.10 (CH_2Cl_2/MeOH 6:1). ^{1}H NMR (400 MHz, MeOD-*d*$_4$) *δ* 8.12-8.00, 7.69-7.49 (88H, m, aromatic), 5.52-5.40 (16H, m), 5.24-5.16 (16H, m), 4.78 (8H, d, *J* = 10.5 Hz), 4.67-4.64 (16H, m), 4.51-4.28 (48H, m), 4.11-4.06 (8H, m), 3.98-3.93 (8H, m), 3.80 (32H, m), 3.71-3.68 (16H, m), 3.59 (24H, m), 3.52 (12H, m), 3.43 (16H, m), 2.75-2.70 (16H, m), 2.53-2.50 (16H, m), 2.14 (24H, s), 1.82 (16H, m), 1.44 (16H, m), 1.31-1.24 (48H, m), 0.71 (24H, d, *J* = 6.3 Hz); ^{13}C NMR (100 MHz, MeOD-*d*$_4$) *δ* 208.6, 172.8, 171.5, 168.8, 165.9, 164.9, 144.8, 133.5, 133.1, 129.9, 129.6, 129.4, 128.5, 123.8, 101.6, 100.3, 77.4, 74.5, 73.5, 71.7, 70.8, 69.3, 69.0, 67.7, 66.6, 65.4, 64.1, 63.3, 61.4, 52.2, 51.1, 50.0, 37.3, 29.9, 29.1, 28.5, 27.6, 25.8, 25.0, 23.9, 21.5, 14.9; ESI-Q-TOF (positive mode) calcd for $C_{407}H_{537}N_{34}Na_2O_{170}$ $[M+2Na+2NH_4+H]^{5+}$ m/z 1734.0865, found 1733.8549.

Compound 36a

From trisaccharide **19** (20.0 mg, 20.2 µmol) and **L9** (1.60 mg, 1.85 µmol) following general procedure **C** to give the nonamer **36a** (18.1 mg, quant) as white solid. R$_f$= 0.15 (CH_2Cl_2/MeOH 6:1). ^{1}H NMR (400 MHz, MeOD-*d*$_4$) *δ* 8.11-7.98, 7.68-7.45 (102H, m, aromatic), 5.52-5.40 (18H, m), 5.22-5.14 (18H, m), 4.78 (9H, d, *J* = 10.5 Hz), 4.59 (36H, m), 4.41-4.25 (36H, m), 4.04-3.97 (27H, m), 3.79 (36H, m), 3.71-3.67 (18H, m), 3.59 (36H, m), 3.52 (9H, m), 3.41 (9H, m), 2.73 (18H, m), 2.51 (18H, m), 2.13 (27H, s), 1.79 (18H, m), 1.41 (18H, m), 1.30-1.25 (54H, m), 0.71 (27H, d, *J* = 6.3 Hz); ^{13}C NMR (100 MHz, MeOD-*d*$_4$) *δ* 208.6, 172.8, 171.5, 168.7, 165.8, 164.9, 144.8, 133.5, 133.1, 129.9, 129.6, 129.4, 128.5, 128.4, 101.5, 100.3, 77.3, 74.5, 73.5, 71.8, 70.8, 69.4, 69.0, 68.1, 67.7, 66.7, 65.4, 64.0, 61.3, 60.9, 52.1, 51.1, 50.0, 37.3, 29.8, 29.0, 28.5, 27.6, 25.8, 25.0, 21.5, 14.9; ESI-Q-TOF (positive mode) calcd for $C_{462}H_{595}N_{39}NaO_{192}$ $[M+Na+4H]^{5+}$ m/z 1957.7606, found 1957.7356.

Compound 37a

From trisaccharide **20** (20.7 mg, 20.8 µmol) and **L8** (1.45 mg, 2.2 µmol) following general procedure **C** to give the nonamer **37a** (18.7 mg, quant) as white solid. R$_f$= 0.30 (CH_2Cl_2/MeOH 6:1). ^{1}H NMR (400 MHz, $CDCl_3$/MeOD-*d*$_4$ = 1:1) *δ* 8.20-8.19, 7.97-7.95, 7.83-7.73, 7.65, 7.49-7.44 (128H, m, aromatic), 5.65-5.60 (8H, m), 5.48 (8H, m), 5.44-5.40 (8H, m), 5.17-5.15 (8H, m), 5.06 (8H, m), 4.60-4.52 (32H, m), 4.41 (32H, m), 4.23 (8H, m), 4.16 (8H, m), 4.05-3.96 (20H, m), 3.85 (24H, m), 3.70 (32H, m),

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3.55 (32H, m), 3.49-3.45 (32H, m), 2.55-2.52 (4H, m), 1.93 (24H, s), 1.53 (24H, m), 1.42-1.36 (48H, m), 1.27 (24H, m), 0.97 (24H, m); DEPT-135 (100 MHz, $CDCl_3$/MeOD-d_4 = 1:1) δ 133.1, 132.8, 132.3, 129.1, 128.0, 127.7, 127.3, 100.7, 100.0, 96.3, 79.6, 74.8, 73.7, 73.2, 71.4, 70.0, 68.8, 68.3, 67.1, 66.8, 66.3, 63.7, 61.6, 60.6, 51.9, 51.3, 50.0, 29.3, 28.4, 25.3, 24.4, 21.1, 14.8, 7.8; ESI-Q-TOF (positive mode) calcd for $C_{423}H_{514}N_{32}Na_3O_{162}$ $[M+3Na+2H]^{5+}$ m/z 1741.2553, found 1741.2602.

Compound 38a

From trisaccharide **20** (22.1 mg, 22.2 μmol) and **L9** (1.60 mg, 1.85 μmol) following general procedure **C** to give the nonamer **38a** (16.5 mg, 91%) as white solid. R_f= 0.15 (CH_2Cl_2/MeOH 6:1). ^{1}H NMR (400 MHz, $CDCl_3$/MeOD-d_4 = 1:1) δ 8.15-8.13, 7.90-7.88, 7.75-7.69, 7.61-7.59, 7.45-7.38 (147H, m, aromatic), 5.59-5.54 (9H, m), 5.43 (9H, m), 5.39-5.35 (9H, m), 5.12-5.09 (9H, m), 5.01 (9H, m), 4.64-4.49 (54H, m), 4.34 (45H, m), 4.18-4.11 (27H, m), 3.99-3.97 (27H, m), 3.91 (18H, m), 3.79 (36H, m), 3.65 (45H, m), 3.56 (9H, m), 3.50 (9H, m), 3.44 (9H, m), 3.40 (27H, m), 1.85 (27H, s), 1.46 (27H, m), 1.34-1.25 (54H, m), 1.21 (27H, m), 0.90 (27H, m); DEPT-135 (100 MHz, $CDCl_3$/MeOD-d_4 = 1:1) δ 133.2, 132.9, 132.4, 129.2, 129.1, 128.1, 127.8, 127.3, 122.7, 100.7, 99.4, 96.4, 79.1, 74.9, 73.6, 73.2, 71.3, 70.0, 68.9, 68.4, 67.8, 67.2, 66.9, 66.3, 63.7, 60.6, 52.1, 49.5, 29.3, 28.9, 25.3, 24.4, 21.4, 14.9; ESI-Q-TOF (positive mode) calcd for $C_{480}H_{576}N_{39}Na_3O_{183}$ $[M+3Na+3H]^{6+}$ m/z 1647.9465, found 1648.0503.

Compound 39a

From trisaccharide **21** (15.3 mg, 13.9 μmol) and **L8** (0.98 mg, 1.45 μmol) following general procedure **C** to give the nonamer **39a** (13.7 mg, quant) as white solid. R_f= 0.35 (CH_2Cl_2/MeOH 8:1). ^{1}H NMR (400 MHz, $CDCl_3$/MeOD-d_4 = 1:1) δ 8.23-8.22, 8.06, 7.94-7.92, 7.77, 7.71, 7.63, 7.54-7.53, 7.40-7.38 (168H, m, aromatic), 5.67 (8H, m), 5.59 (8H, m), 5.48 (8H, m), 5.10 (8H, m), 4.58 (24H, m), 4.42 (16H, m), 4.25 (12H, m), 4.11 (16H, m), 3.90-3.85 (32H, m), 3.74-3.55 (56H, m), 3.48-3.44 (32H, m), 1.93 (24H, s), 1.51 (16H, m), 1.37-1.25 (72H, m), 0.95 (24H, m); DEPT-135 (100 MHz, $CDCl_3$/MeOD-d_4 = 1:1) δ 133.2, 132.9, 132.5, 129.2, 128.9, 128.1, 127.8, 127.6, 127.4, 100.8, 100.3, 96.8, 80.0, 77.2, 75.7, 73.7, 73.1, 71.7, 71.4, 70.8, 70.2, 69.1, 68.5, 67.5, 67.3, 66.5, 60.8, 60.1, 52.1, 29.3, 29.0, 28.2, 28.1, 25.4, 21.2, 14.8; ESI-Q-TOF (positive mode) calcd for $C_{479}H_{545}N_{32}Na_4O_{170}$ $[M+4Na+H]^{5+}$ m/z 1912.2943, found 1912.2901.

Compound 40a

From trisaccharide **21** (18.3 mg, 16.7 μmol) and **L9** (1.20 mg, 1.39 μmol) following general procedure **C** to give the nonamer **40a** (14.1 mg, 95%) as white solid. R_f= 0.30 (CH_2Cl_2/MeOH 8:1). ^{1}H NMR (400 MHz, $CDCl_3$/MeOD-d_4 = 1:1) δ 8.26-8.24, 8.10-8.05, 7.97-7.95, 7.82-7.80, 7.66-7.64, 7.59-7.51, 7.42-7.38 (192H, m, aromatic), 5.72-5.67 (9H, m), 5.61 (9H, m), 5.52-5.47 (9H, m), 5.12 (9H, m), 4.71 (18H, m), 4.60 (18H, m), 4.40 (18H, m), 4.27 (9H, m), 4.14-4.13 (18H, m), 4.04 (18H, m), 3.94 (9H, m), 3.87 (27H, m), 3.77-3.73 (36H, m), 3.65 (9H, m), 3.51-3.47 (18H, m), 1.91 (27H, s), 1.52 (18H, m), 1.44-1.29 (63H, m), 0.98 (27H, m); DEPT-135 (100 MHz, $CDCl_3$/MeOD-d_4 = 1:1) δ 133.2, 132.9, 132.5, 129.2, 128.8, 128.1, 127.8, 127.6, 127.4, 100.8, 100.1, 96.8, 79.8, 77.2, 75.6, 75.4, 73.8, 73.4, 73.1, 71.7, 71.4, 70.8, 70.2, 69.1, 68.9, 68.4, 67.8, 67.5, 67.2, 66.5, 63.7, 60.7, 52.1, 49.6, 28.9, 25.3, 24.5, 24.1, 21.2, 14.8; ESI-Q-TOF (positive mode) calcd for $C_{543}H_{615}N_{40}Na_3O_{192}$ $[M+3Na+NH_4+2H]^{6+}$ m/z 1806.8236, found 1806.5319.

Compound 28b.

From **28a** (14.5 mg) successively following general procedure **A** and **B** to give the dimer **28b** (9.1 mg, 60% for 2 steps) as white solid. R_f=0.30 (EtOAc/EtOH/H_2O 2:1:1). ^{1}H NMR (600 MHz, D_2O) δ 8.11 (2H, s), 5.52 (2H, d, J = 3.7 Hz), 4.78 (2H, m), 4.62 (2H, m), 4.58 (2H, m), 4.49-4.45 (6H, m), 4.40-4.38 (6H, m), 4.26-4.23 (2H, m), 4.15-4.09 (4H, m), 4.02-3.93 (4H, m), 3.82-3.79 (2H, m), 3.73-3.63 (8H, m), 3.59-3.52 (6H, m), 1.95 (6H, s), 1.85 (4H, m), 1.47 (4H, m), 1.26 (6H, m), 1.19 (8H, m); ^{13}C NMR (100 MHz, D_2O) δ 174.6, 103.6, 101.0, 96.8, 81.5, 81.3, 80.8, 76.2, 75.8, 75.1, 73.3, 72.9, 72.2, 70.4, 70.3, 70.1, 70.0, 69.8, 68.7, 67.9, 66.4, 66.1, 61.0, 51.6, 50.9, 50.6, 29.2, 28.3, 25.1, 24.3, 22.2, 15.6; ESI-Q-TOF (negative mode) calcd for $C_{60}H_{96}N_8O_{58}S_8^{2-}$ $[M-10Na+8H]^{2-}$ m/z 1056.6309, found 1056.6293; calcd for $C_{60}H_{94}N_8O_{55}S_7^{4-}$ $[M-9Na-SO_3Na+6H]^{4-}$ m/z 507.5718, found 507.5697.

Compound 29b.

From **29a** (19.1 mg) successively following general procedure **A** and **B** to give the trimer **29b** (14.7 mg, 70% for 2 steps) as white solid. R_f=0.30 (EtOAc/EtOH/H_2O 2:1:1). ^{1}H NMR (600 MHz, D_2O) δ 8.16 (3H, s), 5.52 (3H, d, J = 3.7 Hz), 4.78 (3H, m), 4.63 (3H, m), 4.58 (2H, m), 4.49-4.46 (12H, m), 4.41-4.35 (9H, m), 4.25-4.23 (3H, m), 4.15-4.09 (6H, m), 4.01-3.95 (9H, m), 3.79-3.78 (6H, m), 3.66 (3H, m), 3.60-3.53 (9H, m), 3.32 (3H, m), 1.95 (9H, s), 1.82 (6H, m), 1.45 (6H, m), 1.24 (6H, m), 1.20-1.15 (15H, m), 0.78 (3H, s); ^{13}C NMR (100 MHz, D_2O) δ 174.7, 103.6, 101.0, 96.8, 81.5, 80.8, 76.2, 76.0, 75.1, 72.9, 72.2, 70.4, 69.7, 67.9, 67.4, 66.4, 66.1, 51.6, 50.5, 29.3, 28.3, 25.2, 24.3, 22.3, 15.6; ESI-Q-TOF (negative mode) calcd for $C_{92}H_{144}N_{12}Na_3O_{87}S_{12}^{3-}$ $[M-12Na+9H]^{3-}$ m/z 1087.4535, found 1087.4937.

Compound 30b.

From **30a** (19.1 mg) successively following general procedure **A** and **B** to give the trimer **30b** (13.7 mg, 71% for 2 steps) as white solid. R_f=0.10 (EtOAc/EtOH/H_2O 2:1:1). ^{1}H NMR (600 MHz, D_2O) δ 8.11 (3H, s), 7.13 (1H, m), 6.85 (2H, m), 5.57 (3H, m), 4.82 (3H, m), 4.67 (3H, m), 4.55-4.49 (6H, m), 4.47-4.43 (12H, m), 4.29 (6H, m), 4.24-4.13 (8H, m), 4.02-4.01 (9H, m), 3.82-3.78 (5H, m), 3.67 (7H, m), 3.61-3.51 (12H, m), 1.99 (10H, m), 1.87 (5H, m), 1.71 (2H, m), 1.46 (8H, m), 1.34-1.24 (17H, m), 1.18 (7H, m), 0.99 (2H, m); ^{13}C NMR (100 MHz, D_2O) δ 174.6, 151.5, 108.6, 103.5, 101.1, 96.7, 81.6, 80.9, 76.2, 76.0, 75.5, 75.2, 73.0, 72.2, 70.5, 70.1, 68.0, 66.4, 51.6, 50.5, 48.9, 44.7, 29.3, 28.3, 25.1, 24.3, 22.3, 15.6; ESI-Q-TOF (negative mode) calcd for $C_{93}H_{147}N_{14}O_{87}S_{12}^{3-}$ $[M-15Na+10H+2NH_4]^{3-}$ m/z 1078.8069, found 1078.8077.

Compound 31b.

From **31a** (18.5 mg) successively following general procedure **A** and **B** to give the tetremer **31b** (16.8 mg, 86% for 2 steps) as white solid. R_f=0.30 (EtOAc/EtOH/H_2O 2:1:1). ^{1}H NMR (600 MHz, D_2O) δ 8.06 (4H, s), 5.57 (4H, d, J = 3.7 Hz), 4.83 (4H, m), 4.67 (4H, m), 4.55-4.49 (16H, m), 4.46-4.27 (4H, m), 4.38 (8H, m), 4.30-4.27 (4H, m), 4.20-4.18 (4H, m), 4.16-4.13 (4H, m), 4.06-3.99 (12H, m), 3.87-3.84 (4H, m), 3.79-3.73 (4H, m), 3.70-3.67 (4H, m), 3.64-3.57 (16H, m), 2.00 (12H, s), 1.85 (8H, m), 1.53-1.49 (8H, m), 1.32-1.28 (12H, m), 1.25-1.18 (20H, m); ^{13}C NMR (100 MHz, D_2O) δ 174.7, 103.6, 101.1, 96.8, 81.6, 80.9, 76.3, 75.8, 75.2, 73.0, 72.3, 70.5, 70.0, 68.0, 66.5, 66.2, 51.7, 50.5, 44.8, 29.3, 28.4, 25.2, 24.4, 22.3, 15.7; ESI-Q-TOF (negative mode) calcd for $C_{121}H_{191}N_{16}Na_2O_{116}S_{16}^{3-}$ $[M-18Na+15H]^{3-}$ m/z 1427.4972, found 1427.5712.

Compound 32b.

From **32a** (22.1 mg) successively following general procedure **A** and **B** to give the tetramer **32b** (15.0 mg, 70% for 2 steps) as white solid. R_f=0.20 (EtOAc/EtOH/H_2O 2:1:1). ^{1}H NMR (600 MHz, D_2O) δ 7.81 (4H, s), 5.52 (4H, d, J = 3.6 Hz), 4.78 (4H, m), 4.63 (4H, m), 4.49-4.46 (12H, m), 4.41-4.38 (4H, m), 4.35-4.28 (8H, m), 4.24 (4H, m), 4.15-4.08 (8H, m), 4.01-3.97 (12H, m), 3.79-3.76 (8H, m), 3.70-3.63 (4H, m), 3.60-3.53 (12H, m), 3.41 (8H, m), 3.26 (4H, m), 1.95 (12H, s), 1.84-1.79 (16H, m), 1.54 (8H, m), 1.24 (8H, m), 1.19-1.16 (20H, m); ^{13}C NMR (100 MHz, D_2O) δ 174.5, 103.6, 101.1, 96.8, 81.6, 80.9, 76.3, 76.0, 75.2, 73.0, 72.3, 70.5, 69.9, 68.0, 66.5, 66.2, 51.7, 50.5, 29.4, 28.5, 27.9, 25.3, 24.5, 22.4, 21.4, 15.7; ESI-Q-TOF (negative mode) calcd for $C_{129}H_{202}N_{16}Na_7O_{116}S_{16}^{3-}$ $[M-13Na+10H]^{3-}$ m/z 1501.5088, found 1501.6167.

Compound 33b.

From **33a** (21.8 mg) successively following general procedure **A** and **B** to give the hexamer **33b** (18.3 mg, 76% for 2 steps) as white solid. R_f= 0.20 (EtOAc/EtOH/H_2O 4:3:3). ^{1}H NMR (600 MHz, D_2O) δ 7.93 (6H, s), 5.52 (6H,

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d, *J* = 3.7 Hz), 4.79 (6H, m), 4.63 (6H, m), 4.50-4.46 (18H, m), 4.41-4.38 (18H, m), 4.32-4.31 (12H, m), 4.24 (9H, m), 4.16-4.12 (6H, m), 4.10 (6H, m), 4.01-3.98 (18H, m), 3.84-3.80 (12H, m), 3.69-3.65 (6H, m), 3.62-3.58 (9H, m), 3.57-3.54 (12H, m), 3.31-3.29 (12H, s), 3.15-3.13 (6H, m), 1.96 (18H, s), 1.79 (12H, m), 1.45 (12H, m), 1.27-1.21 (12H, m), 1.21-1.15 (36H, m); ^{13}C NMR (100 MHz, D_2O) δ 174.7, 103.6, 101.1, 96.8, 81.4, 80.8, 76.2, 75.1, 75.0, 72.9, 72.4, 70.5, 70.0, 69.7, 68.4, 67.8, 66.4, 66.1, 63.7, 51.5, 50.4, 46.7, 29.4, 28.4, 25.3, 24.4, 22.3, 15.7, 8.3; ESI-Q-TOF (negative mode) calcd for $C_{184}H_{289}N_{26}Na_{11}O_{175}S_{24}^{4-}$ [M-19Na+13H+2NH$_4$]$^{4-}$ m/z 1671.4175, found 1671.2815.

Compound 34b.

From **34a** (16.7 mg) successively following general procedure **A** and **B** to give the hexamer **34b** (13.4 mg, 73% for 2 steps) as white solid. R_f= 0.30 (EtOAc/EtOH/H_2O 4:3:3). ^{1}H NMR (600 MHz, D_2O) δ 7.94 (6H, m), 5.53 (6H, m), 4.78 (6H, m), 4.63 (6H, m), 4.60-4.56 (12H, m), 4.49-4.48 (12H, m), 4.44 (6H, m), 4.40 (9H, m), 4.25 (6H, m), 4.15-4.10 (12H, m), 4.02-3.99 (18H, m), 3.81 (9H, m), 3.64-3.61 (24H, m), 3.58-3.53 (30H, m), 3.35 (12H, m), 3.25 (6H, m), 1.96 (18H, s), 1.81 (12H, m), 1.46 (12H, m), 1.25 (18H, m), 1.23-1.19 (30H, m); ^{13}C NMR (100 MHz, D_2O) δ 175.9, 174.7, 144.1, 124.8, 103.5, 101.2, 96.7, 81.7, 80.9, 76.9, 76.3, 75.5, 75.3, 73.1, 72.3, 70.5, 70.1, 69.7, 69.0, 68.0, 66.5, 66.2, 63.2, 51.7, 50.4, 45.3, 29.5, 28.5, 25.3, 24.5, 22.4, 15.7; ESI-Q-TOF (negative mode) calcd for $C_{196}H_{301}N_{26}Na_{22}O_{181}S_{24}^{5-}$ [M-8Na+H+2NH$_4$]$^{5-}$ m/z 1438.3249, found 1438.5393.

Compound 35b.

From **35a** (23.8 mg) successively following general procedure **A** and **B** to give the octamer **35b** (18.9 mg, 75% for 2 steps) as white solid. R_f= 0.10 (EtOAc/EtOH/H_2O 4:3:3). ^{1}H NMR (600 MHz, D_2O) δ 7.96 (8H, m), 5.62 (8H, m), 4.87 (8H, m), 4.72 (8H, m), 4.58-4.57 (16H, m), 4.53-4.47 (32H, m), 4.38-4.32 (24H, m), 4.24-4.19 (16H, m), 4.09 (24H, m), 3.89 (8H, m), 3.72-3.62 (40H, m), 3.39 (16H, m), 3.24-3.21 (8H, m), 2.06 (24H, m), 1.95 (8H, m), 1.86 (16H, m), 1.54 (16H, m), 1.31-1.26 (56H, m); ^{13}C NMR (100 MHz, D_2O) δ 175.7, 174.7, 124.7, 103.5, 101.2, 96.6, 81.6, 80.9, 76.9, 76.3, 75.5, 75.2, 73.1, 72.3, 70.6, 70.1, 68.5, 67.9, 66.5, 66.2, 63.7, 51.6, 50.4, 46.7, 29.5, 29.3, 28.5, 28.3, 25.4, 25.2, 24.5, 22.3, 15.7, 8.3; ESI-Q-TOF (negative mode) calcd for $C_{247}H_{386}N_{32}Na_6O_{234}S_{32}^{8-}$ [M-34Na+26H]$^{8-}$ m/z 1088.6226, found 1088.1543.

Compound 36b.

From **36a** (18.1 mg) successively following general procedure **A** and **B** to give the nonamer **36b** (13.8 mg, 70% for 2 steps) as white solid. R_f= 0.10 (EtOAc/EtOH/H_2O 4:3:3). ^{1}H NMR (600 MHz, D_2O) δ 8.08-7.98 (9H, m), 5.57 (9H, m), 4.83 (9H, m), 4.67 (9H, m), 4.53-4.51 (36H, m), 4.45-4.43 (9H, m), 4.29-4.27 (27H, m), 4.20-4.13 (27H, m), 4.04-4.00 (27H, m), 3.94-3.75 (36H, m), 3.70-3.57 (45H, m), 2.00-1.98 (27H, m), 1.77 (18H, m), 1.54-1.46 (18H, m), 1.35-1.16 (72H, m); ^{13}C NMR (100 MHz, D_2O) δ 174.6, 103.6, 101.3, 96.8, 81.6, 80.9, 76.3, 75.9, 75.2, 73.0, 72.3, 70.6, 70.6, 69.9, 67.9, 66.5, 66.2, 51.6, 50.5, 48.9, 44.8, 29.3, 28.5, 28.3, 25.4, 24.4, 22.3, 15.7; ESI-Q-TOF (negative mode) calcd for $C_{282}H_{437}N_{40}Na_4O_{264}S_{36}^{9-}$ [M-41Na+31H+NH$_4$]$^{9-}$ m/z 1095.0183, found 1095.1619.

Compound 37b.

From **37a** (18.7 mg) successively following general procedure **A** and **B** to give the nonamer **37b** (13.3 mg, 65% for 2 steps) as white solid. R_f= 0.10 (EtOAc/EtOH/H_2O 4:3:3). ^{1}H NMR (400 MHz, D_2O) δ 8.24-8.10 (8H, m), 5.29 (8H, m), 4.62 (16H, m), 4.55-4.49 (32H, m), 4.43 (12H, m), 4.32-4.30 (16H, m), 4.21 (16H, m), 4.07-4.01 (40H, m), 3.85-3.81 (32H, m), 3.60 (48H, m), 2.03 (24H, m), 1.90 (16H, m), 1.52 (24H, m), 1.31-1.24 (64H, m); DEPT-135 (100 MHz, D_2O) δ 102.5, 100.1, 98.2, 80.6, 79.8, 78.1, 75.3, 74.7, 72.1, 71.5, 71.2, 69.5, 69.1, 67.5, 66.8, 66.3, 65.4, 65.2, 50.6, 28.3, 27.4, 24.4, 23.5, 21.3, 14.8, 7.3; ESI-Q-TOF (negative mode) calcd for $C_{247}H_{384}N_{32}Na_8O_{234}S_{32}^{8-}$ [M-32Na+24H]$^{8-}$ m/z 1094.1181, found 1094.1917.

Compound 38b.

From **38a** (16.5 mg) successively following general procedure **A** and **B** to give the nonamer **38b** (12.2 mg, 68% for 2 steps) as white solid. R_f= 0.10 (EtOAc/EtOH/H_2O 4:3:3). ^{1}H NMR (400 MHz, D_2O) δ 8.29-8.27 (9H, m), 5.36 (9H, m), 5.20 (9H, m), 4.53 (9H, m), 4.46-4.40 (18H, m), 4.23-4.21 (9H, m), 4.12 (9H, m), 3.97-3.92 (27H, m), 3.81-3.72 (27H, m), 3.51 (27H, m), 1.92 (27H, m), 1.70 (9H, m), 1.38 (9H, m), 1.22-1.15 (45H, m); DEPT-135 (100 MHz, D_2O) δ 102.5, 100.2, 98.2, 80.7, 79.8, 79.4, 78.2, 75.4, 74.7, 74.4, 72.1, 71.3, 69.5, 69.1, 67.7, 67.6, 66.9, 65.5, 65.2, 50.7, 28.2, 27.5, 24.2, 23.5, 21.4, 14.8; ESI-Q-TOF (negative mode) calcd for $C_{282}H_{430}N_{39}Na_9O_{264}S_{36}^{8-}$ [M-36Na+28H]$^{8-}$ m/z 1243.5064, found 1243.7193.

Compound 39b.

From **39a** (13.7 mg) successively following general procedure **A** and **B** to give the nonamer **39b** (9.0 mg, 72% for 2 steps) as white solid. R_f= 0.40 (EtOAc/EtOH/H_2O 2:1:1). ^{1}H NMR (400 MHz, D_2O) δ 7.94 (8H, m), 5.24 (8H, m), 4.57 (16H, m), 4.46 (32H, m), 4.35 (12H, m), 4.27-4.24 (8H, m), 4.17-4.12 (12H, m), 4.01-3.96 (28H, m), 3.84 (12H, m), 3.77-3.74 (8H, m), 3.67-3.47 (44H, m), 3.17-3.13 (24H, m), 1.97 (24H, m), 1.82 (16H, m), 1.61 (24H, m), 1.48 (16H, m), 1.34-1.27 (40H, m), 1.20-1.19 (32H, m); DEPT-135 (100 MHz, D_2O) δ 103.1, 101.0, 98.9, 81.5, 80.6, 76.1, 73.0, 72.1, 70.4, 68.4, 67.8, 66.0, 63.4, 58.0, 51.5, 50.2, 29.3, 28.2, 25.2, 24.4, 23.0, 22.1, 19.0, 15.6, 12.8; ESI-Q-TOF (negative mode) calcd for $C_{247}H_{384}N_{32}Na_8O_{210}S_{24}^{8-}$ [M-24Na+16H]$^{8-}$ m/z 1014.1612, found 1013.9528.

Compound 40b.

From **40a** (14.1 mg) successively following general procedure **A** and **B** to give the nonamer **40b** (9.6 mg, 74% for 2 steps) as white solid. R_f= 0.30 (EtOAc/EtOH/H_2O 2:1:1). ^{1}H NMR (400 MHz, D_2O) δ 7.87 (9H, m), 5.20-5.17 (9H, m), 4.54-4.51 (18H, m), 4.42 (27H, m), 4.21-4.12 (27H, m), 3.95 (27H, m), 3.75 (27H, m), 3.59-3.49 (36H, m), 1.92 (27H, m), 1.66 (9H, m), 1.38 (18H, m), 1.16-1.14 (45H, m); DEPT-135 (100 MHz, D_2O) δ 103.2, 101.1, 99.0, 81.7, 80.7, 77.0, 76.2, 75.1, 73.1, 72.1, 70.4, 68.5, 67.8, 67.1, 66.0, 51.6, 49.3, 29.3, 28.3, 25.2, 24.4, 22.2, 15.7, 8.2; ESI-Q-TOF (negative mode) calcd for $C_{282}H_{430}N_{39}Na_9O_{237}S_{27}^{8-}$ [M-27Na+19H]$^{8-}$ m/z 1153.5550, found 1153.7568.

Anticuagulant evaluation

1. Materials

Low molecular weight heparin (LMWH, 3500–5500 Da, 0.4 ml × 4000 ml AXaIU) was purchased from Sanofi-Aventis (France). The activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT) reagents, and standard human plasma were from Teco Medical (Germany). Biophen FVIII: C kit was from Hyphen Biomed (France). Human factor VIII was from Bayer Healthcare LLC (Germany).

2. Determination of anticoagulant activities in vitro

APTT, PT, and TT were determined with a coagulometer (TECO MC-4000, Germany) using APTT, PT and TT reagents and standard human plasma as previously described [45].

3. Inhibition of human intrinsic factor Xase

Inhibition of intrinsic factor Xase (factor IXa-factor VIIIa complex) was determined as previously described [22].

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[1] N. Mackman, *Nature* **2008**, *451*, 914-918.
[2] A. Y. Y. Lee, M. N. Levine, R. I. Baker, et al. *New. Engl. J. Med.* **2003**, *349*, 146-153.
[3] J. Hirsh, T. E. Warkentin, R. Raschke, C. Granger, E. M. Ohman, J. E. Dalen, *Chest*. **1998**, *114*, 489S-510S.
[4] a) M. Petitou, C. A. A. van Boeckel, *Angew. Chem. Int. Ed*. **2004**, *43*, 3118-3133; b) C.-H. Chang, L. S. Lico, T.-Y. Huang, S.-Y. Lin, C.-L. Chang, S. D. Arco, S.-C. Hung, *Angew. Chem. Int. Ed*. **2014**, *53*, 9876-9879.
[5] a) J. Yang, Y. Wang, T. Jiang, L. Lv, B. Zhang, Z. Lv, *Int. J. Biol. Macromol*. **2014**, *72C*, 699-705; b) P. Myron, S. Siddiquee, S. A. Azad, *Carbohydr. Polym*. **2014**, *112*, 173-178; c) G. R. C Santos, B. F. Glauser, L. A. Parreiras, E. Vilanova, P. A. S Mourão, *Glycobiology* **2015**, *25*, 1043-1052; d) N. E. Ustyuzhanina, M. I. Bilan, A. S. Dmitrenok, E. A. Tsvetkova, A. S. Shashkov, V. A. Stonik, N. E. Nifantiev, A. I. Usov, *Carbohydr. Polym*. **2016**, *153*, 399-405.
[6] P. A. S. Mourão , M. S. Pereira, M. S. G. Pavão, B. Mulloy, D. M. Tollefsen, M.-C. Mowinckel, U. Abildgaard, *J. Biol. Chem*. **1996**, *271*, 23973-23984;
[7] C. G. Panagos, D. S. Thomson, C.Moss, A. D. Hughes, M. S. Kelly, Y. Liu, W. Chai, R. Venkatasamy, D. Spina, C. P. Page, J. Hogwood, R. J. Woods, B. Mulloy, C. D. Bavington, D. Uhrín, *J. Biol. Chem*. **2014**, *289*, 28284-28298;
[8] a) S. Chen, G. Li, N. Wu, X. Guo, N. Liao, X. Ye, D. Liu, C. Xue, W. Chai, *Biochim. Biophys. Acta*. **2013**, *1830*, 3054-3066; b) S. Chen, C. Xue, L. Yin, Q. Tang, G. Yu, W. Chai, *Carbohydr. Polym*. **2011**, *83*, 688-696; c) J. P. Sheehan, E. N. Walke, *Blood* **2006**, *107*, 3876-3882.
[9] M. Wu, S. Xu, J. Zhao, H. Kang, H. Ding, *Food. Chem*. **2010**, *122*, 716-723.
[10] L. Zhao, M. Wu, C. Xiao, L. Yang, L. Zhou, N. Gao, Z. Li, J. Chen, J. Chen, J. Liu, H. Qin, J. Zhao, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 8284-8289.
[11] L. Borsig, L. Wang, M. C. M. Cavalcante, L. Cardilo-Reis, P. L. Ferreira, P. A. S. Mourão, J. D. Esko, M. S. G. Pavão, *J. Biol. Chem*. **2007**, *282*,14984-14991.
[12] V. H. Pomin, *Eur. J. Med. Chem*. **2015**, *92*, 353-369.
[13] N. Huang, M. Wu, C. Zheng, L. Zhu, J. Zhao, Y. Zheng, *Carbohydr. Res*. **2013**, *380*, 64-69.
[14] J. Tapon-Bretaudiere, D. Chabut, M. Zierer, S. Matou, D. Helley, A. Bros, P. A. S. Mourao, A.-M. Fischer, *Mol. Cancer. Res*. **2002**, *1*, 96-102.
[15] R. J. C. Fonseca, P. A. S. Mourão, *Thromb. Haemost.* **2006**, *96*, 822-829.
[16]. a) M. Monteiro-Machado, M. A. Tomaz, R. J. C. Fonseca, M. A. Strauch, B. L. Cons, P. A. Borges, F. C. Patrao-Neto, M. S. Tavares- Henriques, J. M. Teixeira-Cruz, S. Calil-Elias, A. C. O. Cintra, A. M. B. Martinez, P. A. S. Mourao, P. A. Melo, *Toxicon* **2015**, *98*, 20-33; b) P. A. S. Mourao, M. A. M. Guimaraes, B. Mulloy, S. Thomas, E. Br. Gray, *J. Hamaetol*. **1998**, *101*, 647-652.
[17] R. J. C. Fonseca, M. C. G. O. Stephan-Nicollas, Vitor H. Pomin, Andre S. Mecawi, I. G. Araujo, P. A. S. Mourão, *Thromb. Haemost.* **2010**, *103*, 994-1004.
[18] a) M. Wu, S. Xu, J. Zhao, H. Kang, H. Ding, *Carbohydr. Polym*. **2010**, *80*, 1116-1124; b) L. Zhao, S. Lai, R. Huang, M. Wu, N. Gao, L. Xu, H. Qin, W. Peng, J. Zhao, *Carbohydr. Polym*. **2013**, *98*, 1514-1523; c) N. Gao, F. Lu, C. Xiao, L. Yang, J. Chen, K. Zhou, D. Wen, Z. Li, M. Wu, J. Jiang, G. Liu, J. Zhao, *Carbohydr. Polym*. **2015**, *127*, 427-437.
[19] K. Kitazato, K. T. Kitazato, H. Nagase, K. Minamiguchi, *Thromb. Res*. **1996**, *84*, 111-120.
[20] a) J.-I. Tamura, H. Tanaka, A. Nakamura, N. Takeda, *Tetrahedr. Lett*. **2013**, *54*, 3940-3943; b) N. E. Ustyuzhanina, P. A. Fomitskaya, A. G. Gerbst, A. S. Dmitrenok, N. E. Nifantiev, *Mar. Drugs* **2015**, *13*, 770-787.
[21] a) A. Laezza, A. Iadonisi, C. D. Castro, M. D. Rosa, C. Schiraldi, M. Parrilli, E. Bedini, *Biomacromolecules* **2015**, *16*, 2237-2245; b) A. Laezza, A. Iadonisi, A. V. A. Pirozzi, P. Diana, M. D. Rosa, C. Schiraldi, M. Parrilli, E. Bedini, *Chem. Eur. J*. **2016**, *22*, 18215-18226.
[22] M. Wu, D. Wen, N. Gao, C. Xiao, L. Yang, L. Xu, W. Lian, W. Peng, J. Jiang, J. Zhao, *Eur. J. Med. Chem.* **2015**, *92*, 257-269.
[23] a) M. Mammen, S.-K. Choi, G. M. Whitesides, *Angew. Chem. Int. Ed.* **1998**, *37*, 2754-2794; b) C. Müller, G. Despras, T. K. Lindhorst, *Chem. Soc. Rev.* **2016**, *45*, 3275-3302; c) V. Wittmann, R. J. Pieters, *Chem. Soc. Rev.* **2013**, *42*, 4492-4503.
[24] a) C. I Gama, S. E Tully, N. Sotogaku, P. M Clark, M. Rawat, N. Vaidehi, W. A. Goddard III, A. Nishi, L. C Hsieh-Wilson, *Nat. Chem. Biol*. **2006**, *2*, 467-473; b) M. Rawat, C. I. Gama, J. B. Matson, L. C. Hsieh-Wilson, *J. Am. Chem. Soc*. **2008**, *130*, 2959-2961; c) J. M. Brown, J. Xia, B. Zhuang, K.-S. Cho, C. J. Rogers, C. I. Gama, M. Rawat, S. E. Tully, N. Uetani, D. E. Mason, M. L. Tremblay, E. C. Peters, O. Habuchi, D. Chen, L. C. Hsieh-Wilson, *Proc. Natl. Acad. Sci. USA.* **2012**, *109*, 4768-4773; d) P. Liu, L. Chen, J. K. C. Toh, Y. Ang, J.-E. Jee, J. Lim, S. S. Lee, S.-G. Lee, *Chem. Sci*. **2015**, *6*, 450-456.
[25] Y. I. Oh, G. J. Sheng, S.-K. Chang, L. C. Hsieh-Wilson, *Angew. Chem. Int. Ed*. **2013**, *52*, 11796-11799.
[26] P. C. Tyler, S. E. Guimond, J. E. Turnbull, O. V. Zubkova, *Angew. Chem. Int. Ed*. **2015**, *54*, 2718-2723.
[27] a) C. Lopin-Bon, J.-C. Jacquinet, *Angew. Chem. Int. Ed*. **2006**, *45*, 2574-2578; b) A. Vibert, C. Lopin-Bon, J.-C. Jacquinet, *Chem. Eur. J.* **2009**, *15*, 9561-9578; c) J.-C. Jacquinet, C. Lopin-Bon, A. Vibert, *Chem. Eur. J*. **2009**, *15*, 9579-9595.
[28] S. Kobayashi, S.-I. Fujikawa, M. Ohmae, *J. Am. Chem. Soc*. **2003**, *125*, 14357-14369.
[29] P. Wipf, B. R. Eyer, Y. Yamaguchi, F. Zhang, M. D. Neal, C. P. Sodhi, M. Good, M. Branca, T. Prindle Jr., P. Lu, J. L. Brodsky, D. J. Hackam, *Tetrahedr. Lett*. **2015**, *56*, 3097-3100.
[30] I. Damager, S. Numao, H. Chen, G. D. Brayerb, S. G. Withers, *Carbohydr. Res.* **2004**, *339*, 1727-1737.
[31] S. Roy, A. E. Hadri, S. Richard, F. Denis, K. Holte, J. Duffner, F. Yu, Z. Galcheva-Gargova, I. Capila, B. Schultes, M. Petitou, G. V. Kaundinya, *J. Med. Chem*. **2014**, *57*, 4511-4520.
[32] A. V. Kornilov, E. V. Sukhova, N. E. Nifantiev, *Carbohydr. Res.* **2001**, *336*, 309-313.
[33] Y. Ma, X. Cao , B. Yu, *Carbohydr. Res.* **2013**, *377*, 63-74.
[34] Y. Hua, G. Gu, Y. Du, *Carbohydr. Res.* **2004**, *339*, 867-872.
[35] A. Mukherjee, M. M. Palcic, O. Hindsgaul, *Carbohydr. Res.* **2000**, *326*, 1-21.
[36] a) J. Zhou, L. Yang, W. Hu, *J. Org. Chem*. **2014**, *79*, 4718-4726; b) H. Tanaka, T. Ishida, N. Matoba, H. Tsukamoto, H. Yamada, T. Takahashi, *Angew. Chem. Int. Ed*. **2006**, *45*, 6349-6352.
[37] a) A. Cheng, J. L. Hendel, K. Colangelo, M. Bonin, F. I. Auzanneau, *J. Org. Chem.* **2008**, *73*, 7574-7579; b) J. L. Hendel, A. Cheng, F. I. Auzanneau, *Carbohydr. Res*. **2008**, *343*, 2914-2923.
[38] J.-I. Tamura, Y. Nakada, K. Taniguchi, M. Yamane, *Carbohydr. Res.* **2008**, *343*, 39-47.
[39] M. Niemietz, L. Perkams, J. Hoffman, S. Eller, C. Unverzagt, *Chem. Commun*. **2011**, *47*, 10485-10487.
[40] M. Wu, R. Huang, D. Wen, N. Gao, J. He, Z. Li, J. Zhao, *Carbohydr. Polym.* **2012**, *87*, 862-868.
[41] a) W. Yao, M. Xia, X. Meng, Q. Li, Z. Li, *Org. Biomol. Chem.* **2014**, *12*, 8180-8195; b) B. Li, S.-Q. Zang, R. Liang, Y.-J. Wu, T. C. W. Mak, *Organometallics*. **2011**, *30*, 1710-1718; c) P. Domínguez-Rodríguez, J. J. Reina, S. Gil-Caballero, P. M. Nieto, J. L. de Paz, J. Rojo, *Chem. Eur. J.* **2017**, *23*, 11338-11345; d) G. D'Adamio, C. Parmeggiani, A. Goti, A. J. Moreno-Vargas, E. Moreno-Clavijo, I. Robinad, F. Cardona, *Org. Biomol. Chem*. **2014**, *12*, 6250-6266.
[42] a) U. Lindahl, G. Bäckström, L. Thunberg, I. G. Leder, *Proc. Natl. Acad. Sci. USA*, **1980**, *77*, 6551-6555; b) L. Jin, J. P. Abrahams, R. Skinner, M. Petitou, R. N. Pike, R. W. Carrell, *Proc. Natl. Acad. Sci. USA* **1998**, *94*, 14683-14688.
[43] H. Brandstetter, M. Bauer, R. Huber, P. Lollar, W. Bode, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 9796-9800.
[44] a) P. Garcia, S. Moulin, Y. Miclo, D. Leboeuf, V. Gandon, C. Aubert, M. Malacria, *Chem. Eur. J.* **2009**, *15*, 2129-2139; b) A. J. McConnell, P. D. Beer, *Chem. Eur. J.* **2011**, *17*, 2724-2733.
[45] N. Gao, M. Wu, S. Liu, W. Lian, Z. Li, J. Zhao, *Mar. Drugs* **2012**, *10*, 1647-1661.

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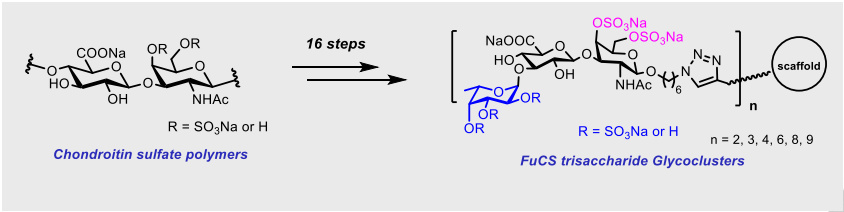

A series of glycoclusters end-capped with fucosylated chondroitin sulfate repeating trisaccharide were synthesized. These glycomimetics showed promising anticoagulant activities, displaying a new framework for the development of novel anticoagulant agents.

Xiao Zhang, Wang Yao, Xiaojiang Xu, Huifang Sun, Jinhua Zhao, Xiangbao Meng, Mingyi Wu and Zhongjun Li**

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