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Highly Efficient Syntheses of Hyaluronic Acid Oligosaccharides

Lijun Huang and Xuefei Huang*[a]

Abstract: Highly efficient syntheses of hyaluronic acid oligosaccharides have been accomplished through the pre-activation based iterative one-pot strategy. A series of oligosaccharides ranging from di- to hexasaccharides were rapidly assembled using only near stoichiometric amounts of the building blocks without aglycon adjustment or purifications of intermediate oligosaccharides. Deprotection and oxidation protocols were developed for protective group removal and oxidation-state adjustment. The availability of such structurally well defined synthetic hyaluronic acid oligosaccharides will greatly facilitate the establishment of detailed structure–function relationships.

Keywords: carbohydrates · glycosylation · hyaluronic acid · oligosaccharides

Introduction

Glycosaminoglycans (GAG) are a family of linear and negatively charged oligosaccharides, which are involved in many important biological processes such as lymphocyte trafficking, tumor metastasis, inflammatory response, and neuron growth.[1–6] As the only unsulfated GAG, hyaluronic acid (HA) is comprised of tandem disaccharide repeats of β-1,4-D-glucuronic acid-β-1,3-D-*N*-acetylglucosamine. Biological studies of HA are receiving increased interests due to their mediatory roles in cell adhesion, cell migration, innate immunity and wound healing.[2,7]

Endogenous HA can be transformed into hyaluronan oligosaccharides (sHA) in vivo. sHA have novel biological properties[8–18] distinct from high molecular weight ($\sim 2\times 10^6$ D) and low molecular weight ($\sim 8\times 10^4$ D) HA polymers. It has been reported that sHA can not only suppress tumor cell growth[12] but also sensitize multidrug resistant tumor cells towards a variety of chemotherapeutic drugs,[9] while HA is ineffective. In addition, sHA have been shown to be angiogenic, whereas their high molecular weight counterparts suppress angiogenesis.[17,18] It has become evident that the biological activities of sHA are sequence specific,[10,14] an example of which is the report that sHA tetrasaccharides up-regulate a heat-shock protein expression and suppress cell death under stress conditions while the corresponding di-, hexa- and octasaccharides are inactive.[10] However, in most cases, detailed understandings of structure–activity relationships remain to be established due to the lack of structurally defined sHA. Access to synthetic sHA with distinct length and sequence can greatly facilitate a systematic investigation of its structure–activity relationship, providing exciting opportunities for the development of novel therapeutics for a variety of diseases.

Chemical syntheses of sHA are typically carried out following two general approaches.[5,19] In the first method, glucuronic acid building blocks are directly utilized to react with a glucosamine derivative.[20] The newly formed disaccharide was transformed into either an acceptor by selective deprotection or a glycosyl donor through aglycon adjustment. Repetition of the glycosylation, selective deprotection, aglycon adjustment and glycosylation processes led to the impressive synthesis of a sHA octasaccharide.[20] Due to the low reactivity of glucuronic acid, as an alternative, more reactive glucose can be used as building blocks.[21–27] With this strategy, a selectively removable protective group must be installed on the 6-hydroxyl group of glucose to allow for oxidation-state adjustment. The complete deprotection and oxidation of 6-OH on all glucose units can present significant challenges.

All current sHA syntheses are characterized by their stepwise nature, which require multiple protective group adjustment, aglycon modification and purification of intermediate oligosaccharides.[5,19–27] To facilitate biological studies of sHA, a robust method needs to be developed so that fast and efficient assembly of multiple sHA can be achieved.

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Results and Discussion

Recently, we have developed a new reactivity independent pre-activation based iterative one-pot oligosaccharide synthesis method, where multiple sequential glycosylations can be carried out in the same reaction vessel without intermediate purification, allowing construction of oligosaccharides within a few hours.[28,29] Moreover, unlike the traditional reactivity based one-pot approach where glycosylation reactions must be performed in the order of decreasing donor anomeric reactivity values,[30,31] it is not necessary to fine-tune anomeric reactivities using the pre-activation method.[29] This is particularly advantageous for syntheses of oligosaccharides, which consist of repeating units, as the same building blocks can be used repeatedly without resorting to anomeric reactivity adjustments.[28,29] Herein, we explore the applicability of the iterative one-pot method in construction of the highly challenging sHA.

Building block evaluations: Previous syntheses of sHA have been dominated by the usage of powerful glycosyl trichloroacetimidates.[5,19] We plan to examine the alternative of using thioglycosides as building blocks, because thioglycosides are stable to most functional group transformation and yet can be easily activated by a wide range of thiophilic promoters.[32,33] Moreover, they can be conveniently stored on bench top for months without decomposition. Despite the fact that thioglycosides have been one of the most popular glycosyl donors in syntheses of complex oligosaccharides,[32,33] they have been rarely used for sHA assembly.[34,35]

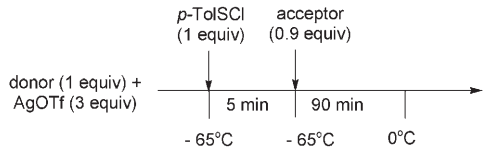
The direct usage of glucuronic acid as the glycosyl donor was investigated first. Thioglucuronic acid **1** was activated in the absence of an acceptor by *p*-TolSOTf, formed in situ by reacting *p*-TolSCl with AgOTf at −65 °C,[29] which was followed by addition of thioglycosyl acceptor **2** (Table 1, entry 1). However, even after the reaction temperature was raised to room temperature, only donor hydrolysis product was isolated without the desired disaccharide **3**. In order to improve the nucleophilicity of the acceptor, we replaced the bulky phthalimido (Phth) moiety in **2** with trichloroethyl carbamate (Troc) (acceptor **4**).[36] However, this did not result in any improvements. Glycosylation of acceptor **2** with glycosyl bromide **5** using promoter AgOTf or reaction of acceptor **6**[37] with donor **1** promoted by *N*-iodosuccinimide (NIS)/AgOTf did not produce significant amounts of disaccharides. These results demonstrated low reactivities of these glucuronic acid donors and pointing to the necessity of using more reactive glucosyl donors.

Peracetylated thioglucosyl donor **7**[31] and *p*-methoxybenzylidene containing donor **8** were examined next with glucosamine acceptors **2**, **4**, **9**, and **10** under the pre-activation condition, which did not produce much desired disaccharides in dichloromethane, diethyl ether or a mixed solvent dichloromethane/diethyl ether system (Table 1, entries 2–8). Finally, we decided to introduce the *tert*-butyldimethylsilyl (TBS) moiety into the glucosyl donor (donor **11**), due to beneficial effects of TBS on glycosylation in our previous chitotetraose syntheses.[28]

Thioglucosyl donor **11** was prepared from *p*-tolyl-1-thio-β-D-glucopyranoside (**16**),[31] which was first protected with *p*-methoxybenzylidene (Scheme 1). Dibutyltin oxide mediated selective benzylation[38] followed by benzoylation gave thioglycoside **8** in 66% yield over three steps. Regioselective opening of the *p*-methoxybenzylidene group and TBS protection produced donor **11** (90% yield for two steps). The *p*-methoxybenzyl (PMB) moiety in **11** can be selectively removed by oxidation in the presence of other protective groups, which is crucial for future oxidation-state adjustments.

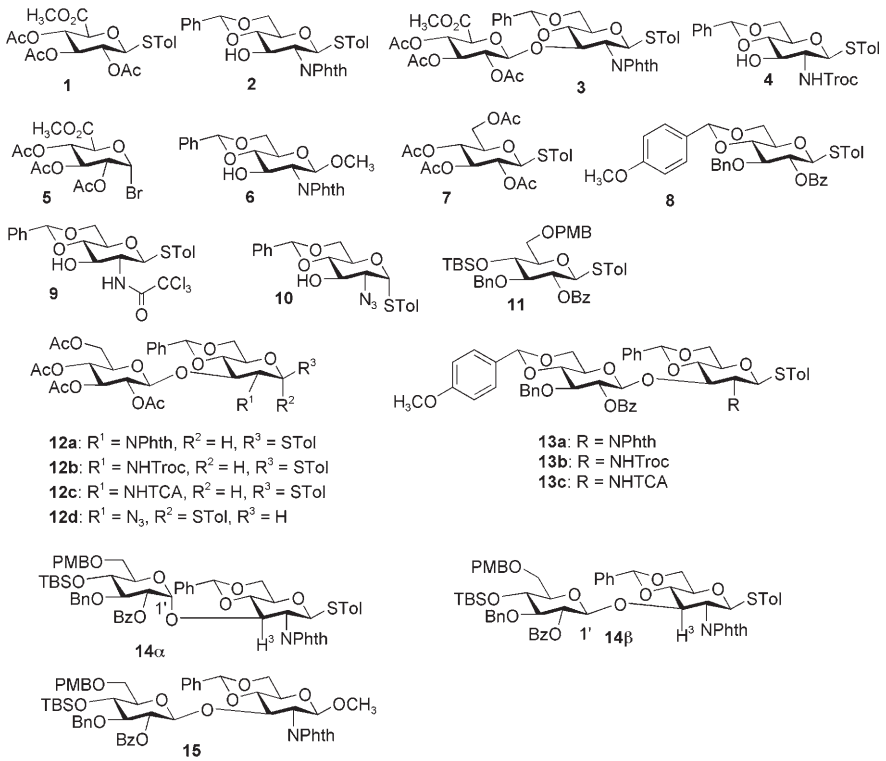
The glycosylation of thioglucoside donor **11** with acceptor **2** was carried out first in diethyl ether (Table 1, entry 9). The desired thioglycosyl disaccharide **14**β (^{1}H NMR: $\delta_{H1'}$ = 4.73 ppm, $^3J_{H1',H2'}$ = 8.4 Hz) was obtained in 75% yield along with 5% of the corresponding α disaccharide **14**α (^{1}H NMR: $\delta_{H1'}$ = 5.48 ppm, $^3J_{H1',H2'}$ = 4.2 Hz). The structure of **14**α was confirmed by the presence of Bz carbonyl in the ^{13}C NMR spectrum with a chemical shift of δ_{Bz} = 164.9 ppm and a strong HMBC correlation between C1′ and H3. The decrease in the stereospecificity of this reaction despite the presence of participating benzoyl moiety on O2′ is due to the competing α-directing effect of ethereal solvents,[39–44] presumably through stabilization of the oxo-carbenium ion by the oxygen lone pair of diethyl ether from the β-face, leading to α-glycoside. To enhance the stereoselectivity, we discovered that by simply adding the acceptor dissolved in dichloromethane to the pre-activated donor in diethyl ether, the formation of disaccharide **14**α can be suppressed to negligible amount without affecting the yield of the desired β-disaccharide (Table 1, entry 10).[45] Disaccharide **15** bearing a methoxy group at the reducing end was also synthesized in good yield by glycosylating methyl glycoside **6** with donor **11** (Table 1, entry 11).

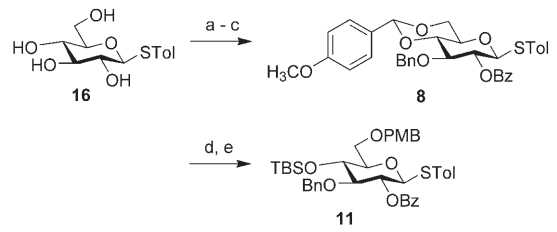
Table 1. Evaluations of building blocks.



Entry	Donor	Acceptor	Disaccharide (yield/%)
1	**1**	**2**	**3** (< 5)
2	**7**	**2**	**12a** (< 5)
3	**7**	**4**	**12b** (< 10)
4	**7**	**9**	**12c** (< 5)
5	**7**	**10**	**12d** (< 20)
6	**8**	**2**	**13a** (< 5)
7	**8**	**4**	**13b** (< 5)
8	**8**	**9**	**13c** (< 5)
9	**11**	**2**	**14**β (75) **14**α (5)[a]
10	**11**	**2**	**14**β (75)[b]
11	**11**	**6**	**15** (88)[c]

[a] Acceptor **2** was added as a solution in diethyl ether to pre-activated donor in diethyl ether. [b] Acceptor **2** was added as a solution in CH_2Cl_2 to pre-activated donor in diethyl ether. Final ratio of CH_2Cl_2/diethyl ether 1:1. [c] Reaction was carried out by mixing donor and acceptor together in CH_2Cl_2/diethyl ether 1:1 followed by addition of the promoter.

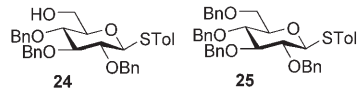




Scheme 1. Building block **11**: a) *p*-methoxybenzylidene dimethyl acetal, camphorsulfonic acid, DMF, 60–70 °C; b) nBu$_2$SnO, toluene, reflux; BnBr, CsF, DMF, 140 °C; c) BzCl, *N*,*N*-dimethylaminopyridine, 66% for three steps; d) NaCNBH$_3$, TFA, MS-AW 300, DMF; e) TBSOTf, 2,6-lutidine, CH$_2$Cl$_2$, 90% for two steps.

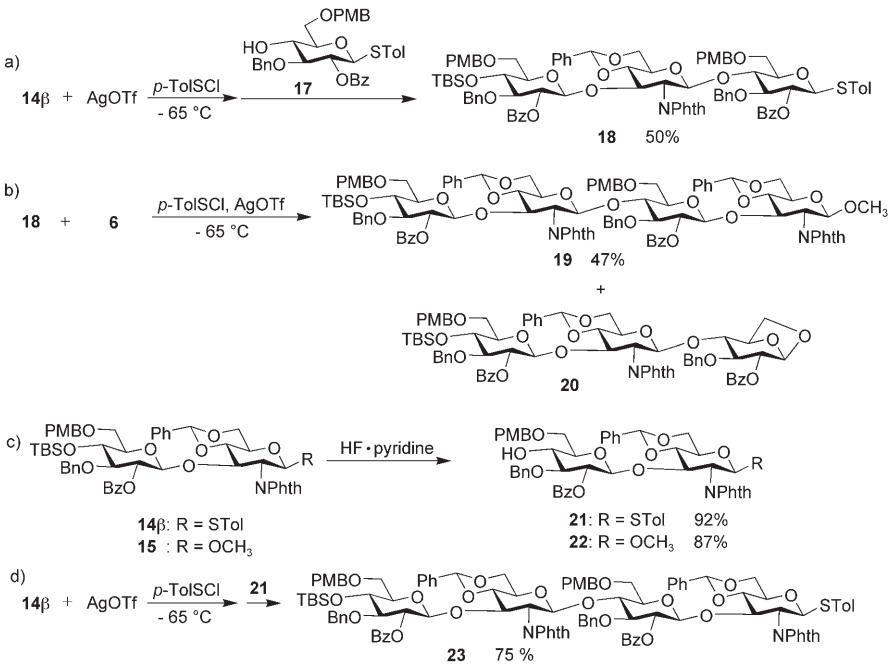
With the thiotolyl moiety at its reducing end, disaccharide **14**β was directly used as a glycosyl donor without any aglycon adjustments. Glycosylation of acceptor **17** by disaccharide **14**β formed trisaccharide **18** in 50% yield with 20% of the hydrolyzed donor as the major side product (Scheme 2a). Reaction of trisaccharide **18** with the *o*-glycoside acceptor **6** led to the desired tetrasaccharide **19** in 47% yield (Scheme 2b). Small amount of trisaccharide **20** could also be isolated from the reaction mixture, which was formed due to the assistance of electron rich 6-OPMB in stabilization of the oxo-carbenium ion followed by removal of the *p*-methoxybenzyl moiety. This long range participation by 6-O protective group has been reported when the glycosyl donor contains a participating group on 6-OH.[39,46,47] Although we have obtained the tetrasaccharide, the yields for formation of tri- and tetrasaccharides are not sufficiently high enough to enable one-pot synthesis. We investigated next the usage of disaccharide as the acceptor. The disaccharides **14**β and **15** were deprotected with HF·pyridine producing thioglycoside **21** and disaccharide **22** in 92 and 87% yield, respectively (Scheme 2c). Gratifyingly, glycosylation of **21** by **14**β formed tetrasaccharide **23** in 75% yield (Scheme 2d).

From results of these glycosylations, we conclude that it is advantageous to perform donor pre-activation in diethyl ether particularly for building blocks having high anomeric reactivities (e.g. **11**). This corroborates with our observation that glycosylation of glucoside **24** by the reactive perbenzylated thioglucoside **25** proceeded cleanly in diethyl ether in 75% yield, while the corresponding reaction in dichloromethane led to a lower yield (~48%) with several side products isolated resulting from donor decomposition. This may be due to stabilization of the reactive oxocarbenium ion intermediate by diethyl ether.



Another important factor to consider in the synthesis is solubilities of the building blocks because the reactions are carried out at low temperatures. The TBS moiety in thioglycoside donor **11** renders it more soluble in diethyl ether compared with donor **8**, leading to significantly improved yields in reaction with acceptor **2**. Higher glycosylation yields obtained with disaccharide **21** (Scheme 2d) versus monosaccharide acceptor **17** (Scheme 2a) can also be partially attributed to better solubility of disaccharide **21** in the reaction medium.

One-pot syntheses of sHA: With suitable building blocks and reaction conditions identified, one-pot syntheses were performed. Pre-activation of donor **11** (1 equiv) by *p*-TolSOTf was followed by addition of **2** (0.9 equiv) and a sterically hindered non-nucleophilic base 2,4,6-tri-*t*ert-butylpyrimidine (TTBP).[48] The reaction was allowed to proceed for 90 minutes resulting in the complete disappearance of acceptor **2** based on TLC analysis. The reaction mixture was then warmed up to 0 °C for 15 minutes to decompose the slight excess of activated donor, and cooled back down to −65 °C. Addition of the second acceptor **22** (0.81 equiv) and



Scheme 2.

TTBP to the reaction mixture followed by activation with *p*-TolSOTf produced sHA tetrasaccharide **19** in 64–75% overall yield in just three hours (Table 2, entry 1), which was the only compound needed purification in this three component one-pot synthesis.

sHA sequences containing odd number of monosaccharide units can also be synthesized. Sequential one-pot reactions of **26**,[28] **21** and **22** following the reaction protocol A produced pentasaccharide **28** (65% yield) (Table 2, entry 2, protocol A). The other possible pentasaccharide sequence **29** with glucose at the reducing end was rapidly synthesized through consecutive condensations of four components, that is, **11**, **2**, **21** and **27**[25] (Table 2, entry 3, protocol B), in 55% overall yield for the one-pot reactions.

Because it is unnecessary to achieve the precise anomeric reactivities with our pre-activation based one-pot strategy,[29] building blocks can be pooled to generate various sHA without anomeric reactivity adjustment. Following the exact reaction sequence for the one-pot synthesis of **19** except for the insertion of **21** as the second acceptor,

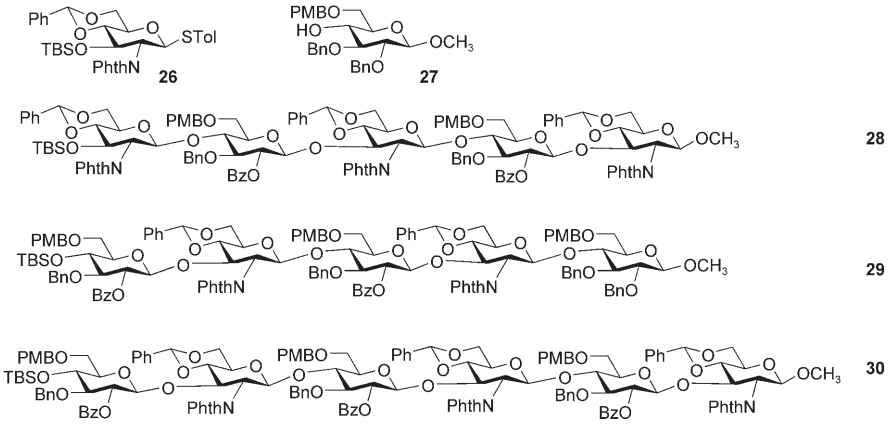
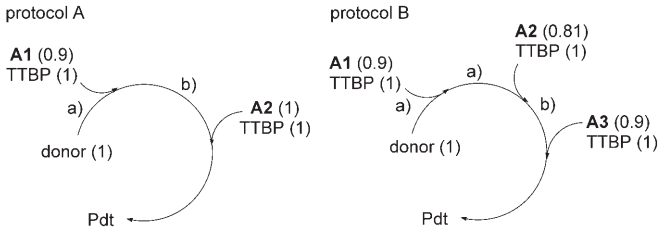


Table 2. Iterative one-pot synthesis of sHA. The values in the parenthesis denote the number of equivalents of each reagent.[a]



	Protocol	Donor	Acceptor **1** (A1)	Acceptor **2** (A2)	Acceptor **3** (A3)	Product	Yield/%
1	A	**11**	**2**	**22**		**19**	64–75
2	A	**26**	**21**	**22**		**28**	65
3	B	**11**	**2**	**21**	**27**	**29**	55
4	B	**11**	**2**	**21**	**22**	**30**	54–60

[a] Reagents and conditions: a) AgOTf, *p*-TolSCl, −65 °C, 10 min; then acceptor, TTBP, 90 min to 0 °C; 15 min, 0 °C; b) acceptor, TTBP, AgOTf, *p*-TolSCl, −65→ 0 °C in 90 min.

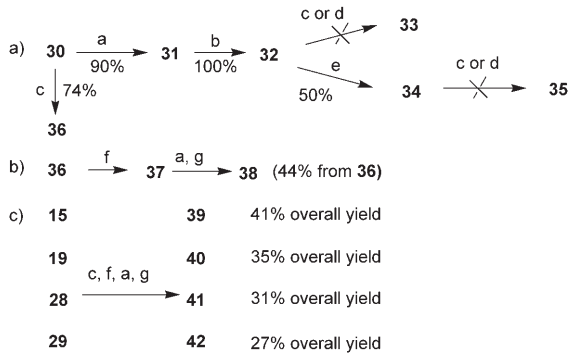
sHA hexasaccharide **30** was produced in 54–60% yield (Table 2, entry 4, protocol B), which corresponded to an average of 90% yield for the five synthetic steps carried out in a one-pot reaction.

With close to stoichiometric amounts of building blocks used for each glycosylation reaction and no oligosaccharide intermediate purification involved, these syntheses can be easily scaled up. Near gram quantity of sHA hexasaccharide **30** was obtained within several

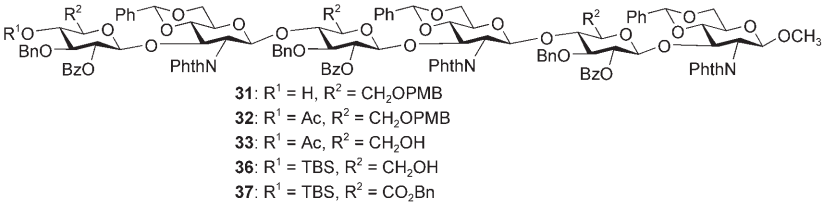
hours in similar yields as the smaller scale. In comparison, the overall yield for the traditional stepwise synthesis of a sHA hexasaccharide was 27% for five synthetic steps starting from an advanced disaccharide building block.[22] In addition, it required multiple chromatography separations of synthetic intermediates. The scalability coupled with the speed of glycoassembly and higher overall yields highlights the advantages of using the iterative one-pot approach for complex oligosaccharide synthesis.

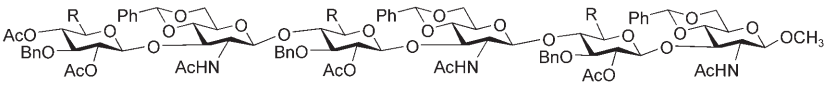
Deprotection of sHA: Deprotection of large complex oligosaccharides can be a highly challenging task due to the presence of large number of protective groups. This is further compounded by the necessity of oxidation-state adjustment in sHA synthesis and thus the need to differentiate 6-hydroxyl groups of the glucose units from others. Treatment of hexasaccharide **30** with HF·pyridine cleanly removed the TBS moiety and the resulting free hydroxyl group was protected by acetate. Although it has been reported that a PMB group was easily removed by cerium ammonium nitrate (CAN) or 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) oxidation from hyaluronan di- and trisaccharides,[21,25] cleavage of all three PMB groups in **32** turned out to be very problematic. Treatment of **32** with CAN or DDQ both failed to produce the desired triol **33** in good yields with multiple decomposition products formed. Simultaneous removal of the phthalimide, acetate and benzoyl groups in **32** followed by per-acetylation led to hexasaccharide **34** in 50% yield. Unfortunately, CAN or DDQ oxidation of **34** again did not generate the desired triol **35** either. Surprisingly, we discovered that the presence of TBS moiety was beneficial for deprotection of PMB, as CAN oxidation of hexasaccharide **30** produced triol **36** in 74% yield (Scheme 3a).

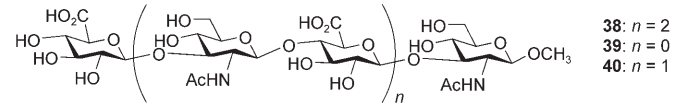
Subsequent oxidation-state adjustment of **36** to tricarboxylic acid proves to be non-trivial. In contrast to literature results, 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO)/NaOCl[21,49–56] or TEMPO/iodobenzene diacetate[57] oxidation led to a mixture of partially oxidized products, which is probably due to the hydrophobic nature of our fully protected oligosaccharides.[58] A two-step process of Dess–Martin oxidation and subsequent treatment with $NaClO_2$[59] also gave inconsistent results. Through extensive experimentation, we discovered that with a two-step one-pot oxidation protocol of NaOCl/TEMPO followed by addition of $NaClO_2$,[58] we were able to carry out this transformation cleanly, which was followed by benzyl ester formation with phenyl diazomethane producing ester **37**.[60] Removal of TBS, catalytic hydrogenation, transamidation with methyl amine and selective acetylation led to fully deprotected sHA hexasaccharide **38** in 44% overall yield from **36** (Scheme 3b). Identical deprotection sequences on sHA **15**, **19**, **28**, and **29** provided free sHA oligosaccharides **39**–**42** in good overall yields (Scheme 3c). sHA sequences obtained through enzymatic degradation are limited to oligosaccharides containing even number of monosaccharide units.[11,13] This is the first time that the sHA pentasaccharide sequence **41** has been acquired through either chemical synthesis or degradation.

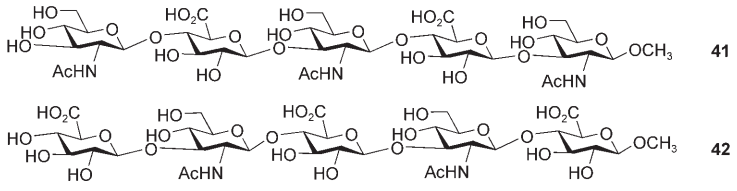


Scheme 3. a) HF·pyridine; b) Ac_2O, pyridine; c) CAN, CH_3CN, H_2O; d) DDQ, H_2O, CH_2Cl_2; e) $NH_2CH_2CH_2NH_2$, *n*BuOH, 80 °C then Ac_2O, pyridine; f) TEMPO, NaOCl, NaBr, Bu_4NBr, $NaHCO_3$, then $NaClO_2$, 2-methy-2-butene, NaH_2PO_4; $PhCHN_2$; g) H_2, $Pd(OH)_2$; CH_3NH_2 then Ac_2O, MeOH.









Conclusion

We have developed highly efficient syntheses of sHA oligosaccharides through the pre-activation based iterative one-pot strategy. Glycoassemblies were accomplished rapidly using only near stoichiometric amounts of building blocks without aglycon adjustment or purification of intermediate oligosaccharides. Reaction solvents and protective groups are important to ensure high yielding glycosylations. The sequence of deprotection is found to be crucial for oxidation-state adjustments and protective group removal. Preliminary studies indicate that this methodology can also be applied to syntheses of other GAGs, such as chondroitin and heparin oligosaccharides. We anticipate that the availability of these structurally well defined sHA will greatly facilitate our understanding of the versatile biological roles of sHA.

Experimental Section

General conditions: Chemicals used were reagent grade as supplied except where noted. All reactions were performed under a nitrogen atmosphere unless specified otherwise. Analytical thin-layer chromatography was performed using silica gel 60 F254 glass plates (EM Science); compound spots were visualized by UV light (254 nm) and/or by staining with a solution containing $Ce(NH_4)_2(NO_3)_6$ (0.5 g) and $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (24.0 g) in 6% H_2SO_4 (500 mL) or a solution of $KMnO_4$ (3 g), K_2CO_3 (20 g) and NaOH (0.25 g) in water (300 mL). Flash column chromatography was performed on silica gel 60 (230–400 mesh, EM Science). ^1H NMR and ^{13}C NMR spectra were recorded on a Varian VXRS-400 or Inova-600 instrument and were referenced using Me_4Si (0 ppm), residual $CHCl_3$ (δ ^1H NMR 7.26 ppm) $CDCl_3$ (δ ^{13}C NMR 77.0 ppm), residual $CHDCl_2$ (δ ^1H NMR 5.32 ppm), CD_2Cl_2 (δ ^{13}C NMR 54.0 ppm). ESI mass spectra were recorded on an ESQUIRE LC-MS operated in both positive and negative ion mode. High-resolution mass spectra were recorded on a Micromass electrospray Tof II (Micromass, Wythenshawe, UK) mass spectrometer equipped with an orthogonal electrospray source (*Z* spray) operated in positive ion mode, which is located at the Mass Spectrometry and Proteomics Facility, the Ohio State University.

General procedure for pre-activation based single-step glycosylation: The reaction mixture of donor (0.321 mmol) and freshly activated MS 4 Å (500 mg) in anhydrous Et_2O (10 mL) was stirred for 1 h at room temperature, then cooled to −65 °C. A solution of AgOTf (250 mg, 0.96 mmol) in Et_2O (10 mL) was added directly to the solution without touching the wall of the reaction flask. After 10 min at −65 °C, *p*-TolSCl (50 μL, 0.321 mmol) was added via a syringe. The mixture was stirred for 5 min until the yellow color dissipated and the donor was completely consumed judged by TLC. A solution of acceptor **1** (0.289 mmol) and TTBP (80 mg, 0.321 mmol) in anhydrous CH_2Cl_2 (10 mL) was added slowly to the pre-activated donor along the wall of the reaction flask. The reaction was continued for 90 min from −65 to 0 °C and quenched with triethylamine (500 μL). The obtained reaction mixture was concentrated under reduced pressure, re-suspended in CH_2Cl_2 (50 mL) and filtered. The filtrate was concentrated and purified by flash column chromatography (hexanes, EtOAc and CH_2Cl_2) to afford the desired product.

General procedures for one-pot synthesis of oligosaccharide

Protocol A: The mixture of donor (0.321 mmol) and freshly activated MS 4 Å (500 mg) in anhydrous Et_2O (10 mL) was stirred for 1 h at room temperature, then cooled to −65 °C. A solution of AgOTf (250 mg, 0.96 mmol) in Et_2O (10 mL) was added directly to the solution without touching the wall of the reaction flask. After 10 min at −65 °C, *p*-TolSCl (50 μL, 0.321 mmol) was added via a syringe. The mixture was stirred for 5 min until the yellow color dissipated and the donor was completely consumed judged by TLC. A solution of acceptor **1** (0.289 mmol) and TTBP (80 mg, 0.321 mmol) in anhydrous CH_2Cl_2 (10 mL) was added slowly to the pre-activated donor along the wall of the reaction flask. The reaction was continued for 90 min from −65 to 0 °C. A solution of acceptor **2** (0.261 mmol) and TTBP (71 mg, 0.289 mmol) in anhydrous CH_2Cl_2 (10 mL) was added and the resulted solution was cooled down to −65 °C followed by the addition of AgOTf (200 mg, 0.783 mmol) in Et_2O (8 mL) and *p*-TolSCl (45 μL, 0.289 mmol). The reaction was stirred for 90 min from −65 to 0 °C and quenched with triethylamine (300 μL). The obtained reaction mixture was concentrated under reduced pressure, re-suspended in CH_2Cl_2 (50 mL) and filtered. The filtrate was concentrated and purified by flash column chromatography (hexanes, EtOAc and CH_2Cl_2) to afford the desired product.

Protocol B: The mixture of donor (230 mg, 0.321 mmol) and freshly activated MS 4 Å (500 mg) in anhydrous Et_2O (10 mL) was stirred for 1 h at room temperature, then cooled to −65 °C. A solution of AgOTf (250 mg, 0.96 mmol) in Et_2O (10 mL) was added directly to the solution without touching the wall of the reaction flask. After 10 min at −65 °C, *p*-TolSCl (50 μL, 0.321 mmol) was added via a syringe. The mixture was stirred for 5 min until the yellow color dissipated and donor was consumed judged by TLC. A solution of acceptor **1** (0.289 mmol) and TTBP (71 mg, 0.289 mmol) in anhydrous CH_2Cl_2 (10 mL) was added slowly to the pre-activated donor along the wall of the reaction flask. The reaction was continued for 90 min from −65 to 0 °C and cooled back down to −65 °C. To the reaction mixture, AgOTf (220 mg, 0.866 mmol) in Et_2O (8 mL) and *p*-TolSCl (45 μL, 0.289 mmol) were sequentially added. The mixture was stirred for 15 min until no donor was left when a solution of acceptor **2** (0.255 mmol) and TTBP (71 mg, 0.289 mmol) in anhydrous CH_2Cl_2 (10 mL) was added slowly. The reaction was kept for 90 min from −65 to 0 °C. Then acceptor **3** (0.261 mmol) and TTBP (63 mg, 0.253 mmol) in anhydrous CH_2Cl_2 (10 mL) was added. The mixture was cooled down to −65 °C followed by the sequential addition of AgOTf (200 mg, 0.783 mmol) in Et_2O (8 mL) and *p*-TolSCl (41 μL, 0.261 mmol). The reaction was stirred for 90 min from −65 to 0 °C and quenched with triethylamine (400 μL). The obtained reaction mixture was concentrated under reduced pressure, re-suspended in CH_2Cl_2 (100 mL) and filtered. The filtrate was concentrated and purified by flash column chromatography (hexanes, EtOAc and CH_2Cl_2) to afford the desired product.

General procedures for deprotection and oxidation-state adjustment

Deprotection of PMB: An aqueous solution (2.5 mL) of CAN (1.16 g, 2.14 mmol) was added at 0 °C to a solution of the PMB-protected compound (0.42 mmol) in acetonitrile (10 mL). The reaction mixture was stirred for 1 h from 0 °C to room temperature then diluted with EtOAc (100 mL) and washed with water (30 mL) three times. The organic phases were collected and dried over $MgSO_4$. The obtained residue was purified by flash column chromatography to give the desired product.

Oxidation of alcohols to carboxylic acids: A 1 M aqueous solution of NaBr (25 μL), 1 M aqueous solution of tetrabutylammonium bromide (50 μL), TEMPO (2.2 mg, 0.014 mmol, 0.3 equiv per hydroxyl group) and a saturated aqueous solution of $NaHCO_3$ (125 μL) were added to a solution of the alcohol (0.045 mmol) in CH_2Cl_2 (1 mL) and H_2O (170 μL) cooled with an ice-water bath. The resulted mixture was treated with an aqueous solution of NaOCl (150 μL, chlorine content not less than 4%) and continuously stirred for 1 h from 0 °C to RT. The reaction media was neutralized with 1 N HCl (about 50 μL) to pH 6–7. It is important to keep the acidity of the reaction close to neutral as lower pH had resulted in formation of large amount of hemiacetal side product. After neutralization, *t*BuOH (0.7 mL), 2 M 2-methyl-but-2-ene (1.4 mL) in THF and a solution of $NaClO_2$ (50 mg, 0.44 mM) and NaH_2PO_4 (40 mg, 0.34 mM) in water (200 μL) were added. The reaction mixture was kept at room temperature for 1–2 h, diluted with saturated aqueous NaH_2PO_4 solution (5 mL) and extracted with EtOAc (10 mL) three times. The organic layers were combined and dried over $MgSO_4$. After removal of the solvent, the desired compound was purified by flash column chromatography (hexanes, EtOAc + 1% AcOH).

Benzyl ester formation: The crude product from the oxidation reaction was dissolved in dichloromethane (5 mL) and treated with phenyl diazomethane solution in diethyl ether (~2 equiv per acid) for 2–3 h until the

disappearance of all starting material as judged by TLC. The residue after evaporation was purified by flash column chromatography to provide the benzyl ester.

Removal of TBS: The TBS-protected compound (0.5 mmol) was dissolved in pyridine (4 mL) in a plastic flask followed by the addition of 65–70 % HF·pyridine solution (4 mL, diluted with 2 mL pyridine) at 0 °C. The solution was stirred for 48 h until complete disappearance of the starting material as judged from TLC analysis. The reaction mixture was diluted with EtOAc (30 mL) and washed with 10 % aqueous $CuSO_4$ solution (20 mL). The aqueous phase was extracted with EtOAc (30 mL) twice and the combined organic layers were washed with saturated aqueous $NaHCO_3$ solution (3×50 mL). After drying over $MgSO_4$ and concentrated, the obtained residue was purified by flash column chromatography (hexanes/EtOAc) to give the desired product.

Hydrogenation: $Pd(OH)_2$ (80 mg) was added to a solution of the oligosaccharide (0.045 mmol) in THF (1.5 mL), MeOH (2 mL) and acetic acid (1.5 mL). The reaction flask was evacuated using a water aspirator and filled with hydrogen. The process was repeated two times and the reaction mixture was stirred under hydrogen atmosphere until a single desired peak was observed by ESI mass spectrum (~48 h). The solution was filtered and concentrated to provide the crude product.

Removal of benzoyl and phthalimido groups by $MeNH_2$: The crude product from hydrogenation was treated with 33 % methyl amine solution in ethanol (1 mL per μmol of compound) until only the desired peak was observed by ESI mass spectrum (~72 h).

Acetylation: The crude reaction product from methyl amine treatment (0.05 mmol) was dissolved in methanol (2 mL) followed by addition of acetic anhydride (5 equiv) and triethylamine at 0 °C. The reaction was run for 2–3 h, and treated with Amberlite IR-120 for 30 min. After filtration, the solvents were removed and the residue was dissolved in water (5 mL), washed with Et_2O (3 mL) and purified by Sephadex G-15 size-exclusion chromatography and anion-exchange chromatography (DE 52, Whatman) to give the desired product.

***p*-Tolyl-2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranuronic acid methyl ester (1)**: *p*-TolSH (312 mg, 2.52 mmol) and tetrabutylammonium iodide (465 mg, 1.26 mmol) at room temperature were added to a solution of bromo-2,3,4-tri-*O*-acetyl-α-D-glucopyranuronic acid methyl ester (**5**; 500 mg, 1.26 mmol) in EtOAc (5 mL) and 1 M aqueous Na_2CO_3 (5 mL). The reaction was stirred for 1 h until no starting material was observed by TLC. Extraction by EtOAc and washing of the organic layer with saturated $NaHCO_3$ followed by flash column chromatography gave compound **1** (499 mg, 90 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.99 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.35 (s, 3H, OCH_3), 3.76 (s, 3H, CO_2CH_3), 4.10 (d, ^3J(H,H) = 10.0 Hz, 1H), 4.66 (d, ^3J(H,H) = 10.0 Hz, 1H), 4.93 (t, 1H, ^3J(H,H) = 9.6 Hz, 1H), 5.14 (t, ^3J(H,H) = 9.6 Hz, 1H), 5.25 (t, ^3J(H,H) = 9.6 Hz, 1H), 7.13 (d, ^3J(H,H) = 12 Hz, 2H), 7.39 (d, ^3J(H,H) = 12 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.7, 20.8, 21.0, 21.4, 53.1, 69.4, 69.9, 73.5, 76.4, 86.4, 127.3, 130.0, 134.2, 139.2, 167.1, 169.3, 169.5, 170.3; ESI-MS: *m*/*z*: calcd for $C_{21}H_{24}NaO_6S$: 463.1, found: 463.2 $[M+Na]^+$.

***p*-Tolyl-2-deoxy-2-*N*-Troc-4,6-*O*-benzylidene-β-D-glucopyranoside (4)**: The solution of compound **2** (1.25 g, 2.48 mmol) and ethylenediamine (2 mL) in *n*-butanol (15 mL) was refluxed for 1.5 h until no starting material was observed by TLC. The solution was dried under reduced pressure followed by chromatography to afford free amine (881 mg, 95 %). The free amine (750 mg, 2.16 mmol) was dissolved in a biphasic system of aqueous saturated $NaHCO_3$ (15 mL) and THF (15 mL) followed by addition of 2,2,2-trichloroethoxy chloroformate (349 μL, 2.60 mmol) at 0 °C. The reaction was stirred for 1 h and diluted with dichloromethane followed by extraction and flash chromatography to provide the desired product **4** (1.40 g, 99 %). ^{1}H NMR (400 MHz, $CDCl_3$ + 1 drop of CD_3OD): δ = 2.26 (s, 3H, CH_3), 3.36–3.48 (m, 3H), 3.71 (t, ^3J(H,H) = 10.0 Hz, 1H), 3.77 (t, ^3J(H,H) = 9.2 Hz, 1H), 4.25 (dd, ^3J(H,H) = 4.4, 10.0 Hz, 1H), 4.62 (d, ^3J(H,H) = 12.0 Hz, 1H), 4.75 (d, ^3J(H,H) = 10.0 Hz, 1H), 4.77 (d, ^3J(H,H) = 12.0 Hz, 1H), 5.47 (s, 1H, PhCH), 7.03–7.42 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$ + 1 drop of CD_3OD): δ = 21.2, 57.4, 68.7, 70.5, 72.3, 74.7, 81.4, 87.8, 95.7, 101.9, 126.4–138.5 (aromatic carbons), 155.2; ESI-MS: *m*/*z*: calcd for $C_{23}H_{25}Cl_3NNaO_6S$: 571.1, found: 571.0 $[M+Na]^+$.

***p*-Tolyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-*p*-methoxybenzylidene-1-thio-β-D-glucopyranoside (8)**: The solution of *p*-tolyl-1-thio-β-D-glucopyranoside **16** (10 g, 35 mmol), *p*-anisaldehyde dimethyl acetal (7.1 mL, 42 mmol) and camphorsulfonic acid (1.6 g, 7 mmol) in anhydrous DMF (80 mL) was swirled on a rotary evaporator under aspirator pressure at 50 °C for 3 h, then was warmed up to 70 °C to remove methanol generated until the reaction was completed judging by TLC. The resulting solution was diluted with diethyl ether (150 mL) followed by washing with saturated $NaHCO_3$ and drying over $MgSO_4$. The residue was purified by flash column chromatography (hexanes/EtOAc 5:5 → 3:7) to afford the desired diol *p*-tolyl 4,6-*O*-*p*-methoxybenzylidene-1-thio-β-D-glucopyranoside (13 g, 92 %) after purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.36 (s, 3H, CH_3), 2.71 (br, 1H, OH), 2.81 (br, 1H, OH), 3.42 (t, ^3J(H,H) = 8.8 Hz, 1H), 3.46–3.49 (m, 2H), 3.74 (t, ^3J(H,H) = 10.0 Hz, 1H), 3.77 (s, 3H, OCH_3), 3.82 (t, ^3J(H,H) = 8.8 Hz, 1H), 4.36 (dd, ^3J(H,H) = 4.0, 10.0 Hz, 1H), 4.54 (d, ^3J(H,H) = 10.0 Hz, 1H), 5.47 (s, PhCH), 6.87–7.44 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.4, 55.2, 68.6, 70.6, 72.8, 74.8, 80.4, 88.8, 102.0, 114.0–138.8 (aromatic carbons); ESI-MS: *m*/*z*: calcd for $C_{21}H_{25}O_6S$: 405.3, found: 405.2 $[M+H]^+$.

The diol *p*-tolyl 4,6-*O*-*p*-methoxybenzylidene-1-thio-β-D-glucopyranoside (8.5 g, 21 mmol) was heated under reflux with Bu_2SnO (5.7 g, 23 mmol) in a flask equipped with a Dean–Stark device in anhydrous toluene (400 mL) for 3 h and concentrated to approximate 100 mL. After cooling the reaction mixture down to room temperature, anhydrous DMF (150 mL dried over 5 % w/v MS 4 Å twice overnight) was added followed by addition of CsF (3.5 g, 23 mmol) and BnBr (2.76 mL, 23.1 mmol) and the obtained solution was stirred for 4 h at 140 °C. After the reaction was complete, DMF was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and extracted by 1 M aqueous solution of KF. The organic phase was dried over $MgSO_4$ and purified by flash column chromatography (hexanes/EtOAc/CH_2Cl_2 5:1:1) to offer the desired product *p*-tolyl 3-*O*-benzyl-4,6-*O*-*p*-methoxybenzylidene-1-thio-β-D-glucopyranoside (8.4 g, 80 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.35 (s, 3H, CH_3), 2.52 (br, 1H, OH), 3.44–3.51 (m, 2H), 3.61 (t, ^3J(H,H) = 9.2 Hz, 1H), 3.67 (t, ^3J(H,H) = 8.8 Hz,, 1H), 3.76 (t, ^3J(H,H) = 8.4 Hz, 1H), 3.81 (s, 3H, OCH_3), 4.36 (dd, ^3J(H,H) = 4.2, 10.8 Hz, 1H), 4.55 (d, ^3J(H,H) = 9.6 Hz, 1H), 4.77 (d, ^3J(H,H) = 11.6 Hz, 1H), 4.94 (d, ^3J(H,H) = 11.6 Hz, 1H), 5.52 (s, 1H, PhCH), 6.88–7.43 (m, 13H); ESI-MS: *m*/*z*: calcd for $C_{28}H_{30}NaO_6S$: 517.6, found: 517.7 $[M+Na]^+$.

p-Tolyl 3-*O*-benzyl-4,6-*O*-*p*-methoxybenzylidene-1-thio-β-D-glucopyranoside (7.5 g, 15.2 mmol) was treated with benzoyl chloride (2.12 mL, 18.2 mmol), *N*,*N*-dimethylaminopyridine (DMAP, 4.4 g, 36.4 mmol) in anhydrous CH_2Cl_2 (50 mL) overnight at room temperature. The reaction mixture was washed with an aqueous NH_4Cl solution (30 mL) and an aqueous saturated $NaHCO_3$ solution (30 mL) followed by drying over $MgSO_4$. The desired product **8** was obtained (8.2 g, 90 %) after purification by flash column chromatography (hexanes/EtOAc/CH_2Cl_2 4:1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.32 (s, 3H, CH_3), 3.75 (dt, ^3J(H,H) = 4.8, 9.2 Hz, 1H), 3.78 (t, ^3J(H,H) = 9.2 Hz, 1H), 3.82 (s, 3H, OCH_3), 3.78–3.84 (m, 1H), 3.86 (t, ^3J(H,H) = 9.2 Hz, 1H), 4.39 (dd, ^3J(H,H) = 4.8, 10.0 Hz, 1H), 4.64 (d, ^3J(H,H) = 12.0 Hz, 1H), 4.77 (d, ^3J(H,H) = 10.0 Hz, 1H), 4.78 (d, ^3J(H,H) = 12.0 Hz, 1H), 5.25 (dd, ^3J(H,H) = 9.2, 10.0 Hz, 1H), 5.56 (s, 1H, PhCH), 6.90–8.05 (m, 18H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.4, 55.5, 68.8, 70.8, 72.3, 74.4, 79.6, 81.6, 87.4, 101.5, 113.9–138.8 (aromatic carbons), 160.3, 165.2 (carbonyl groups); ESI-MS: *m*/*z*: calcd for $C_{35}H_{35}O_7S$: 599.2, found: 599.0 $[M+H]^+$.

***p*-Tolyl 4,6-*O*-benzylidene-2-deoxy-2-*N*-trichloroacetyl-β-D-glucopyranoside (9)**: A solution of compound **2** (1.25 g, 2.48 mmol) and ethylenediamine (2 mL) in *n*-butanol (15 mL) was heated under reflux for 1.5 h until no starting material was observed by TLC. The solution was dried under reduced pressure followed by chromatography to afford free amine (881 mg, 95 %). The free amine (534 mg, 1.43 mmol) was dissolved in anhydrous THF (3 mL) followed by addition of triethylamine (216 mg, 2.14 mmol) and trichloroacetyl chloride (167 μL, 1.50 mmol) at 0 °C. The reaction was stirred for 1 h and diluted with dichloromethane followed by extraction and flash chromatography (hexanes/EtOAc 3:1) to provide

desire product **9** (740 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$ + 1 drop of CD_3OD): $\delta = 2.25$ (s, 3H, CH_3), 3.42–3.45 (m, 2H), 3.61 (t, $^3J(H,H) = 10.2$ Hz, 1H), 3.69 (t, $^3J(H,H) = 10.2$ Hz, 1H), 3.93 (t, $^3J(H,H) = 9.6$ Hz, 1H), 4.24 (dd, $^3J(H,H) = 4.2$, 10.2 Hz, 1H), 4.93 (d, $^3J(H,H) = 10.2$ Hz, 1H), 5.45 (s, 1H, PhCH), 7.03–7.39 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$ + 1 drop of CD_3OD): $\delta = 21.3$, 57.3, 68.7, 70.6, 71.4, 81.5, 86.8, 101.9, 126.4–138.8 (aromatic carbons), 165.6 (carbonyl group); ESI-MS: *m*/*z*: calcd for $C_{22}H_{22}Cl_3NNaO_5S$: 541.8, found: 542.1 $[M+Na]^+$.

***p*-Tolyl-2-deoxy-2-azido-4,6-*O*-benzylidene-1-thio-α-D-glucopyranoside (10)**: Borontrifluoride etherate (15 mL, 120 mmol) was added slowly at 0°C under an atmosphere of N_2 to a solution of 2-deoxy-2-azido-3,4,6-tri-*O*-acetyl-α/β-D-glucopyranosyl acetate[61] (7.6 g, 20 mmol) and *p*-TolSH (3.72 g, 30 mmol) in dichloromethane (50 mL). The reaction was kept at room temperature for 5 d and carefully quenched with saturated aqueous solution of $NaHCO_3$. The aqueous layer was then separated and washed twice with dichloromethane. The organic layers were combined and dried over Na_2SO_4. *p*-Tolyl-2-deoxy-2-azido-3,4,6-tri-*O*-acetyl-1-thio-α/β-D-glucopyranoside (6.0 g, 13.7 mmol, 70%) was isolated by flash chromatography (hexanes/EtOAc 2:1).

Sodium methoxide in MeOH (5M, 4 mL) was added at room temperature to a solution of *p*-tolyl-2-deoxy-2-azido-3,4,6-tri-*O*-acetyl-1-thio-α/β-D-glucopyranoside (6.0 g, 13.7 mmol) in MeOH (20 mL). After 2 d, the solution was neutralized with Amberlite IR-120, filtered and evaporated. The crude mixture of *p*-tolyl 2-deoxy-2-azido-1-thio-D-glucopyranosides obtained was azotropically dried with anhydrous toluene and then dissolved in anhydrous CH_3CN (30 mL). Benzaldehyde dimethyl acetal (4.2 mL, 27.4 mmol) and camphorsulfonic acid (0.9 g, 4.1 mmol) were added and the reaction mixture was stirred at room temperature overnight. The reaction was quenched with Et_3N and evaporated. Silica gel column chromatography (hexanes/EtOAc 3:1) afforded compound **10** (3.2 g) and the corresponding β-isomer (1.6 g) in 90% total yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.37 (s, 3H), 2.88 (br s, 1H, OH), 3.60 (t, $^3J(H,H) = 9.2$ Hz, 1H), 3.78 (t, $^3J(H,H) = 10.2$ Hz, 1H), 3.92 (dd, $^3J(H,H) = 5.4$, 10.0 Hz, 1H), 4.10 (t, $^3J(H,H) = 9.6$ Hz, 1H), 4.25 (dd, $^3J(H,H) = 5.0$, 10.2 Hz, 1H), 4.44 (dt, $^3J(H,H) = 5.0$, 10.0 Hz, 1H), 5.58 (d, $^3J(H,H) = 5.4$ Hz, 1H), 5.58 (s, 1H), 7.05–7.56 (m, 9H, ArH); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 21.2, 63.3, 63.9, 68.5, 70.7, 81.7, 88.1, 102.2, 126.3, 128.4, 129.0, 129.5, 130.0, 133.1, 136.8, 138.5; ESI-MS: *m*/*z*: calcd for $C_{20}H_{21}N_3NaO_4S$: 422.1, found: 422.2 $[M+Na]^+$.

***p*-Tolyl 2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-1-thio-β-D-glucopyranoside (17)**: Acetal **8** (6.5 g, 10.8 mmol) in anhydrous DMF (80 mL) was cooled down to 0°C followed by sequential addition of solid $NaCNBH_3$ (5.44 g, 86.4 mmol) and TFA (12.4 g, 108 mmol). The resulting suspension was stirred for 48 h until no starting material was found. After neutralization by solid $NaHCO_3$, the solution was filtered and diluted with EtOAc followed by washing with a saturated aqueous $NaHCO_3$ solution. The organic phase was collected and applied to flash column to afford alcohol **17** (6.1 g, 95%) containing trace amount of regioisomer *p*-tolyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-1-thio-β-D-glucopyranoside. Pure **17** was obtained after removal of TBS from compound **11** following the general procedure for TBS deprotection. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.31$ (s, 3H, CH_3), 3.52–3.60 (m, 1H), 3.69 (t, $^3J(H,H) = 8.8$ Hz, 1H), 3.76–3.81 (m, 3H), 3.82 (s, 3H, OCH_3), 4.49 (d, $^3J(H,H) = 11.6$ Hz, 1H), 4.52 (d, $^3J(H,H) = 11.6$ Hz, 1H), 4.65 (d, $^3J(H,H) = 11.6$ Hz, 1H), 4.69 (d, $^3J(H,H) = 11.6$ Hz, 1H), 4.72 (d, $^3J(H,H) = 9.6$ Hz, 1H), 5.22 (dd, $^3J(H,H) = 8.8$, 9.6 Hz, 1H), 6.87–8.06 (m, 18H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 21.4$, 55.5, 70.4, 72.3, 72.4, 73.7, 74.9, 78.5, 83.9, 86.9, 114.1, 128.0–138.3 (aromatic carbons), 159.6, 165.4 (carbonyl groups); ESI-MS: *m*/*z*: calcd for $C_{35}H_{36}NaO_7S$: 623.2, found: 623.3 $[M+Na]^+$.

***p*-Tolyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-6-*O*-*p*-methoxybenzyl-1-thio-β-D-glucopyranoside (11)**: A solution of alcohol **17** (5.56 g, 9.26 mmol) in anhydrous CH_2Cl_2 (50 mL) was cooled down to −20°C followed by sequential addition of 2,6-lutidine (1.98 g, 18.5 mmol) and TBSOTf (3.67 g, 13.9 mmol). The resulting solution was warmed to room temperature slowly until no starting material was left judged by TLC. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with saturated $NaHCO_3$ solution. The organic phase was collected and dried followed by separation by flash column chromatography (hexanes/EtOAc/CH_2Cl_2 20:1:1) to give target molecule **11** (6.27 g, 90%) along with a 1:1 mixture (~5%) of compound **11** with its regioisomer *p*-tolyl 2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-4-*O*-*p*-methoxybenzyl-1-thio-β-D-glucopyranoside. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = -0.05$ (s, 3H, CH_3Si), 0.00 (s, 3H, CH_3Si), 0.85 (s, 9H, $(CH_3)_3CSi$), 2.30 (s, CH_3, 3H), 3.57–3.69 (m, 4H), 3.80–3.84 (m, 1H), 3.82 (s, 3H, OCH_3), 4.45 (d, $^3J(H,H) = 11.6$ Hz, 1H), 4.56 (d, $^3J(H,H) = 11.6$ Hz, 1H), 4.61–4.64 (m, 2H), 4.75 (d, $^3J(H,H) = 10.0$ Hz, 1H), 5.24 (t, $^3J(H,H) = 10.0$ Hz, 1H), 6.87–7.99 (m, 18H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = -4.5$, −3.5, 18.2, 21.4, 26.2, 55.5, 69.5, 71.3, 73.2, 73.4, 75.4, 81.2, 85.0, 86.6, 113.9–138.0 (aromatic carbons), 159.4, 165.4 (carbonyl groups); HRMS: *m*/*z*: calcd for $C_{41}H_{50}NaO_7SSi$: 737.2944, found: 737.2953 $[M+Na]^+$.

***p*-Tolyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-1-thio-β-D-glucopyranoside (14β)**: The mixture of donor **11** (714 mg, 1 mmol) and freshly activated MS 4 Å (800 mg) in diethyl ether (20 mL) was stirred for 1 h at room temperature, and cooled down to −65°C followed by the addition of AgOTf (771 mg, 3 mmol) in anhydrous Et_2O (10 mL) directly to the solution without touching the wall of the reaction flask. After 5 min, *p*-TolSCl (157 μL, 1 mmol) was added via a syringe to activate the donor. The yellow color of the reaction disappeared quickly and TLC analysis showed the donor was completely consumed. A solution of acceptor **2** (451 mg, 0.9 mmol) and TTBP (248 mg, 1 mmol) in CH_2Cl_2 (3 mL) was then slowly added to the reaction mixture along the wall of the reaction flask. It was stirred for 90 min until the temperature reached 0°C, and triethylamine (300 μL) was added. The reaction mixture was concentrated to remove most Et_2O, re-suspended in CH_2Cl_2 (100 mL), and filtered. The filtrate was concentrated and purified by flash column chromatography using a three solvent system (CH_2Cl_2, hexanes and EtOAc) to give the desired disaccharide **14**β (740 mg, 0.675 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = -0.16$ (s, 3H, CH_3Si), −0.09 (s, 3H, CH_3Si), 0.74 (s, 9H, $(CH_3)_3CSi$), 2.28 (s, 3H, *p*-CH_3ArS), 3.12 (m, 1H), 3.29 (m, 1H), 3.38 (t, $^3J(H,H) = 8.4$ Hz, 1H), 3.42 (dd, $^3J(H,H) = 2.0$, 8.4 Hz, 1H), 3.57–3.67 (m, 3H), 3.83 (s, 3H, *p*-CH_3OAr), 3.87 (t, $^3J(H,H) = 8.8$ Hz, 1H), 4.28–4.44 (m, 5H), 4.58 (d, $J =$ 12 Hz, 1H), 4.66 (t, $^3J(H,H) = 8.8$ Hz, 1H), 4.73 (d, $^3J(H,H) = 8.4$ Hz, 1H), 5.03 (t, $^3J(H,H) = 8.4$ Hz, 1H), 5.33 (s, 3H, PhCH), 5.44 (d, $^3J(H,H) = 10.8$ Hz, 1H), 6.90–7.95 (m, 27H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = -4.7$, −3.7, 18.2, 21.4, 26.1, 54.7, 55.6, 68.7, 70.8, 71.0, 73.1, 74.4, 74.6, 76.2, 76.5, 81.2, 83.4, 84.7, 99.6, 101.6, 114.0, 126.7–138.5 (aromatic carbons), 159.5, 164.7 (carbonyl groups); HRMS: *m*/*z*: calcd for $C_{62}H_{67}NNaO_{13}SSi$: 1116.4000, found: 1116.3998 $[M+Na]^+$.

***p*-Tolyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-6-*O*-*p*-methoxybenzyl-α-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-1-thio-β-D-glucopyranoside (14α)**: When acceptor **2** was added as a solution in diethyl ether, **14**α (~5%) was separated from the reaction along with **14**β (75%). ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = -0.20$ (s, 3H, CH_3Si), −0.14 (s, 3H, CH_3Si), 0.70 (s, 9H, $(CH_3)_3CSi$), 2.30 (s, 3H, *p*-CH_3ArS), 2.74 (dd, $^3J(H,H) = 2.4$, 10.8 Hz, 1H), 2.78 (dd, $^3J(H,H) = 3.0$, 10.8 Hz, 1H), 3.04–3.08 (m, 1H), 3.54–3.62 (m, 2H), 3.66 (t, $^3J(H,H) =$ 9.0 Hz, 1H), 3.74 (dd, $^3J(H,H) = 9.0$, 10.2 Hz, 1H), 3.81 (s, 3H, *p*-CH_3OAr), 4.21 (dd, $^3J(H,H) = 4.8$, 10.2 Hz, 1H), 4.22–4.28 (m, 2H), 4.46 (t, $^3J(H,H) = 10.2$ Hz, 1H), 4.60 (d, $^3J(H,H) = 11.4$ Hz, 1H), 4.64 (s, 1H), 4.69 (d, $^3J(H,H) = 11.4$ Hz, 1H), 4.80 (dd, $^3J(H,H) = 9.0$, 9.6 Hz, 1H), 5.09 (dd, $^3J(H,H) = 4.2$, 9.6 Hz, 1H), 5.48 (d, $^3J(H,H) = 4.2$ Hz, 1H), 5.59 (d, $^3J(H,H) = 10.8$ Hz, 1H), 6.90–7.92 (m, 27H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = -5.1$, −3.5, 18.2, 21.4, 26.3, 54.5, 55.6, 67.2, 68.6, 73.1, 73.1, 73.2, 74.0, 75.0, 79.7, 83.0, 85.1, 96.8, 101.3, 113.9, 123.7, 124.2, 126.2–138.7 (aromatic carbons), 159.3, 164.9, 167.4; ESI-MS: *m*/*z*: calcd for $C_{62}H_{67}NNaO_{13}SSi$: 1116.4, found: 1116.2 $[M+Na]^+$.

Methyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-1-thio-β-D-glucopyranoside (15): The mixture of donor **7** (714 mg, 1 mmol), acceptor **10** (370 mg, 0.9 mmol) and freshly activated MS 4 Å (800 mg) in diethyl ether (20 mL) and CH_2Cl_2 (10 mL) was stirred for 1 h at room temperature, and cooled down to −65°C. A solution of AgOTf (771 mg, 3 mmol) in anhydrous Et_2O (10 mL) was added di-

rectly to the solution without touching the wall of the reaction flask. After 10 min, *p*-TolSCl (157 μL, 1 mmol) was added via a syringe. The reaction mixture was stirred for 1.5 h until the temperature reached 0 °C, and triethylamine (300 μL) was added. The reaction mixture was concentrated to remove most Et_2O, resuspended in CH_2Cl_2 (100 mL), and filtered. The filtrate was concentrated and purified by flash column chromatography (hexanes/EtOAc 4:1 → 2.5:1) to give the desired disaccharide **15** (800 mg, 0.792 mmol, 88 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = −0.16 (s, 3H, CH_3Si), −0.08 (s, 3H, CH_3Si), 0.75 (s, 9H, $(CH_3)_3CSi$), 3.07 (m, 1H), 3.29 (dd, ^3J(H,H) = 6.0, 10.4 Hz, 1H), 3.36 (s, 3H, CH_3O), 3.38–3.44 (m, 2H), 3.56–3.61 (m, 2H), 3.68 (t, ^3J(H,H) = 10.4 Hz), 3.83 (s, 3H, *p*-CH_3OAr), 3.87 (m, 1H), 4.27–4.33 (m, 4H), 4.35 (d, ^3J(H,H) = 11.2 Hz, 1H), 4.44 (d, ^3J(H,H) = 11.2 Hz, 1H), 5.58 (d, ^3J(H,H) = 11.2 Hz, 1H), 4.67 (dd, ^3J(H,H) = 8.4, 10.0 Hz, 1H), 4.73 (d, ^3J(H,H) = 8.4 Hz, 1H), 5.01 (d, ^3J(H,H) = 8.4 Hz, 1H), 5.04 (t, ^3J(H,H) = 8.4 Hz, 1H), 5.33 (s, 1H, PhCH), 6.91–7.46 (m, 23H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = −4.7, −3.7, 18.1, 21.4, 26.1, 55.4, 55.5, 57.1, 66.7, 68.6, 68.9, 70.9, 73.0, 74.5, 74.6, 75.6, 76.1, 81.8, 83.4, 99.7, 99.7, 101.6, 114.0, 126.7–138.0 (aromatic carbons), 159.5, 164.7 (carbonyl groups); HRMS: *m*/*z*: calcd for $C_{56}H_{63}NNaO_{14}Si$: 1024.3916, found: 1024.3879 $[M+Na]^+$.

***p*-Tolyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-2-deoxy-2-*N*-phthalimido-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranoside (18)**: Compound **18** (100 mg, 50 %) was synthesized using donor **14**β and acceptor **17** following the general procedure for single-step pre-activation based glycosylation. ^{1}H NMR (400 MHz, $CDCl_3$): δ = −0.16 (s, 3H, CH_3Si), −0.11 (s, 3H, CH_3Si), 0.73 (s, 9H, $(CH_3)_3CSi$), 2.23 (s, 3H, CH_3), 3.10 (m, 1H), 3.16-.20 (m, 2H), 3.27–3.32 (m, 2H), 3.35–3.45 (m, 4H), 3.56–3.68 (m, 3H), 3.75 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.81 (m, 1H), 3.99–4.21 (m, 2H), 4.18 (d, ^3J(H,H) = 10.8 Hz, 1H), 4.26 (d, ^3J(H,H) = 10.8 Hz, 1H), 4.29–4.33 (m, 2H), 4.35 (d, ^3J(H,H) = 11.2 Hz, 1H), 4.43 (d, ^3J(H,H) = 11.2 Hz, 1H), 4.98 (d, ^3J(H,H) = 8.4 Hz, 1H), 4.57 (d, ^3J(H,H) = 11.6 Hz, 1H), 4.61 (d, ^3J(H,H) = 11.6 Hz, 1H), 4.67 (dd, ^3J(H,H) = 8.8, 9.2 Hz, 1H), 4.74 (d, *J* = 8.0 Hz, 1H), 4.83 (d, ^3J(H,H) = 12 Hz, 1H), 5.03 (t, ^3J(H,H) = 8.8 Hz, 1H), 5.10 (t, ^3J(H,H) = 9.2 Hz, 1H), 5.25 (d, ^3J(H,H) = 8.4 Hz, 1H), 5.29 (s, 1H, PhCH), 6.78–7.94 (m, 37H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = −4.7, −3.7, 18.1, 21.4, 26.0, 55.5, 56.1, 66.4, 68.0, 68.7, 68.8, 71.0, 72.1, 72.6, 73.1, 74.4, 74.5, 74.8, 75.5, 75.7, 76.1, 79.0, 81.4, 81.9, 83.4, 86.2, 97.9, 99.6, 101.4, 113.9–138.5 (aromatic carbons), 159.3, 159.5, 164.7, 165.3 (carbonyl groups); ESI-MS: *m*/*z*: calcd for $C_{90}H_{95}NNaO_{20}SSi$: 1592.6, found: 1592.7 $[M+Na]^+$.

Methyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-2-deoxy-4,6-*O*-benzylidene-2-*N*-phthalimido-β-D-glucopyranoside (19)

Tetrasaccharide **19** was synthesized by two methods.

Method 1: A mixture of donor **18** (100 mg, 0.063 mmol), acceptor **6** (28 mg, 0.056 mmol) and freshly activated MS 4 Å (200 mg) in diethyl ether (3 mL) and CH_2Cl_2 (3 mL) was stirred for 1 h at room temperature, and cooled down to −65 °C. A solution of AgOTf (45 mg, 0.18 mmol) in anhydrous Et_2O (1 mL) was added directly to the solution without touching the wall of the reaction flask. After 10 min, *p*-TolSCl (9 μL, 1 equiv) was added via a syringe. The reaction mixture was stirred for 1.5 h until the temperature reached 0 °C, and triethylamine (100 μL) was added. It was concentrated to remove most Et_2O, resuspended in CH_2Cl_2 (20 mL), and filtered. The filtrate was concentrated and purified by flash column chromatography (hexanes/EtOAc/CH_2Cl_2 4:1:1 → 2:1:1) to give **19** (49 mg, 0.026 mmol, 47 %), along with small amount of trisaccharide **20**. Compound **20**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = −0.16 (s, 3H, CH_3Si), −0.09 (s, 3H, CH_3Si), 0.74 (s, 9H, $(CH_3)_3CSi$), 3.02–3.08 (m, 1H), 3.22–3.76 (m, 10H), 3.78 (s, 3H, CH_3O), 4.00–4.04 (m, 2H), 4.21 (d, ^3J(H,H) = 5.2 Hz, 1H), 4.27 (d, ^3J(H,H) = 12 Hz, 1H), 4.33 (dd, ^3J(H,H) = 8.4, 10.0 Hz, 1H), 4.35 (d, ^3J(H,H) = 11.2 Hz, 1H), 4.42 (d, ^3J(H,H) = 11.2 Hz, 1H), 4.52 (dd, ^3J(H,H) = 8.4, 10.0 Hz, 1H), 4.56 (d, ^3J(H,H) = 12 Hz, 1H), 4.60 (d, ^3J(H,H) = 12 Hz, 1H), 4.69 (d, ^3J(H,H) = 7.6 Hz, 1H), 4.78 (d, ^3J(H,H) = 12 Hz, 1H), 4.84 (s, 1H), 5.05 (d, ^3J(H,H) = 8.4 Hz, 1H), 5.22–5.28 (m, 2H), 6.84–8.06 (m, 33H); ESIMS: *m*/*z*: calcd for $C_{75}H_{79}NNaO_{19}Si$: 1348.5, found: 1348.6 $[M+Na]^+$.

Method 2: Tetrasaccharide **19** (948 mg, 0.51 mmol) was synthesized in 64–75 % yield using building blocks **11** (540 mg, 0.75 mmol), **2** (342 mg, 0.68 mmol), and **22** (870 mg, 0.68 mmol) following protocol A for one-pot synthesis. ^{1}H NMR (400 MHz, $CDCl_3$): δ = −0.18 (s, 3H, CH_3Si), −0.12 (s, 3H, CH_3Si), 0.73 (s, 9H, $(CH_3)_3CSi$), 2.64 (m, 1H), 2.73 (m, 1H), 2.92 (m, 1H), 3.06 (m, 1H), 3.15 (m, 1H), 3.26–3.33 (m, 2H), 3.31 (s, 3H, CH_3O), 3.38–3.43 (m, 3H), 4.38 (m, 1H), 3.55 (t, ^3J(H,H) = 8.4 Hz, 1H), 3.60–3.65 (m, 2H), 3.75 (s, 3H, CH_3O), 3.78 (s, 3H, CH_3O), 3.91–3.95 (m, 2H), 4.02 (d, ^3J(H,H) = 12 Hz, 1H), 4.17–4.43 (m, 9H), 4.50–4.59 (m, 3H), 4.64 (m, 2H), 4.88 (m, 3H), 4.98 (s, 1H, PhCH), 5.19 (d, ^3J(H,H) = 8.4 Hz, 1H), 5.23 (s, 1H, PhCH), 6.79–7.82 (m, 46H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = −4.7, −3.7, 18.1, 26.0, 55.2, 55.5, 55.50, 55.51, 56.1, 57.0, 66.2, 66.4, 66.8, 68.7, 68.9, 71.0, 72.2, 73.1, 73.9, 73.9, 74.1, 74.4, 74.5, 75.2, 75.4, 76.0, 76.1, 80.9, 81.3, 81.6, 83.4, 97.6, 99.4, 99.7, 100.4, 101.4, 101.6, 113.9–138.7 (aromatic carbons), 159.5, 159.4, 164.5, 164.7 (carbonyl groups); HRMS: *m*/*z*: calcd for $C_{105}H_{108}N_2NaO_{27}Si$: 1879.6806, found: 1879.6783 $[M+Na]^+$.

***p*-Tolyl 2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-1-thio-β-D-glucopyranoside (21)**: Compound **21** was synthesized in 92 % yield from compound **14**β following the general procedure for TBS removal. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.28 (s, 3H, *p*-CH_3ArS), 2.70 (d, *J* = 2.4 Hz, 1H), 3.06 (m, 1H), 3.37–3.46 (m, 3H), 3.64–3.68 (m, 2H), 3.68–3.85 (m, 2H), 3.82 (s, 3H, *p*-CH_3OAr), 4.31–4.38 (m, 4H), 4.44 (d, ^3J(H,H) = 11.6 Hz, 1H), 4.50 (d, ^3J(H,H) = 11.6 Hz, 1H), 4.66–4.71 (m, 2H), 4.99 (dd, *J* = 8.4, 9.6 Hz, 1H), 5.44 (s, 1H, PhCH), 5.44 (d, ^3J(H,H) = 9.6 Hz, 1H), 6.88–7.81 (m, 27H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.4, 54.5, 55.6, 68.9, 70.4, 70.7, 72.8, 73.2, 73.5, 73.8, 74.2, 81.4, 82.4, 84.7, 100.3, 101.8, 114.1, 126.5–138.6 (aromatic carbons), 159.6, 164.7 (carbonyl groups); HRMS: *m*/*z*: calcd for $C_{56}H_{53}NNaO_{13}S$: 1002.3135, found: 1002.3140 $[M+Na]^+$.

Methyl 2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-1-thio-β-D-glucopyranoside (22): Compound **22** (500 mg) was synthesized in 87 % yield from compound **15** following the general procedure for TBS removal. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.70 (d, ^3J(H,H) = 2.4 Hz, 1H), 3.13 (m, 1H), 3.35 (s, 3H, CH_3O), 3.39–3.51 (m, 3H), 3.56- 3.71 (m, 2H), 3.78 −3.88 (m, 2H), 3.82 (s, 3H, *p*-CH_3OAr), 4.27 (dd, ^3J(H,H) = 8.8, 10.4 Hz, 1H), 4.30–4.34 (m, 3H, 1H), 4.45 (d, ^3J(H,H) = 11.2 Hz, 1H), 4.50 (d, ^3J(H,H) = 11.2 Hz, 1H), 4.68 (d, ^3J(H,H) = 8.0 Hz, 1H), 4.69 (t, ^3J(H,H) = 9.6 Hz, 1H), 5.00 (t, ^3J(H,H) = 8.8 Hz, 1H), 5.01 (d, ^3J(H,H) = 8.8 Hz, 1H), 5.45 (s, 1H), 6.88–7.47 (m, 23H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 55.2, 55.5, 57.1, 66.5, 69.0, 70.4, 72.8, 73.1, 73.5, 73.8, 74.2, 76.1, 81.7, 82.4, 99.7, 100.3, 101.8, 114.1, 126.5–138.2 (aromatic carbons), 159.6, 164.7 (carbonyl groups); HRMS: *m*/*z*: calcd for $C_{50}H_{49}NNaO_{14}$: 910.3051, found: 910.3055 $[M+Na]^+$.

***p*-Tolyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-2-deoxy-2-*N*-phthalimido-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-2-deoxy-2-*N*-phthalimido-3,6-*O*-benzylidene-β-D-glucopyranoside (23)**: Compound **23** (50 mg) was synthesized in 75 % yield using donor **14**β and acceptor **21** following the general procedure for single-step pre-activation based glycosylation. ^{1}H NMR (600 MHz, $CDCl_3$): δ = −0.19 (s, 3H, CH_3Si), −0.14 (s, 3H, CH_3Si), 0.71 (s, 9H, $(CH_3)_3CSi$), 2.23 (s, 3H, CH_3), 2.60 (m, 1H), 2.71 (m, 1H), 2.91 (m, 1H), 3.04 (m, 1H), 3.12 (m, 1H), 3.23 (m, 2H), 3.32–3.41 (m, 3H), 3.49–3.62 (m, 4H), 3.70 (m, 1H), 3.71 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.89–3.92 (m, 2H), 4.01 (m, 1H), 4.15–4.21 (m, 3H), 4.24–4.28 (m, 2H), 4.32 (d, ^3J(H,H) = 10.8 Hz, 1H), 4.36–4.41 (m, 3H), 4.82–4.56 (m, 3H), 4.63 (d, ^3J(H,H) = 11.4 Hz, 1H), 4.67 (d, ^3J(H,H) = 8.4 Hz, 1H), 4.85 (t, ^3J(H,H) = 8.4 Hz, 1H), 4.95 (t, ^3J(H,H) = 7.8 Hz, 1H), 4.96 (s, 1H, PhCH), 5.16 (d, ^3J(H,H) = 9.0 Hz, 1H), 5.21 (s, 1H, PhCH), 5.35 (d, *J* = 10.2 Hz, 1H), 6.78–7.82 (m, 50H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = −4.7, −3.6, 18.1, 21.4, 26.0, 54.4, 55.5, 55.5, 56.1, 66.2, 66.8, 68.6, 68.7, 68.8, 70.5, 71.0, 72.2, 73.1, 73.9, 74.0, 74.0, 74.4, 75.1, 75.4, 76.0, 76.8, 77.0, 80.8, 81.3, 83.4, 84.6, 97.5, 99.4, 100.3, 101.3, 101.6, 113.9–138.6 (aromatic

carbons), 159.4, 159.4, 164.5, 164.7 (carbonyl groups); ESI-MS: *m*/*z*: calcd for $C_{111}H_{112}N_2NaO_{26}SSi$: 1973.3, found: 1973.0 $[M+Na]^+$.

Methyl 4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-2-deoxy-4,6-*O*-benzylidene-2-*N*-phthalimido-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (28): Pentasaccharide **28** (350 mg, 0.156 mmol) was synthesized in 65% yield using building blocks **26** (154 mg, 0.25 mmol), **21** (220 mg, 0.22 mmol), and **22** (200 mg, 0.22 mmol) following protocol A for one-pot synthesis. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = -0.31$ (s, 3H, CH_3Si), −0.16 (s, 3H, CH_3Si), 0.54 (s, 9H, $(CH_3)_3CSi$), 2.61–2.70 (m, 3H), 2.88–2.94 (m, 2H), 3.09 (d, $^3J(H,H) = 10.4$ Hz, 1H), 3.18–3.25 (m, 2H), 3.29 (s, 3H, CH_3O), 3.33–3.51 (m, 5H), 3.64–3.69 (m, 3H), 3.73 (s, 3H, *p*-CH_3OPh), 3.76 (s, 3H, *p*-CH_3OPh), 3.92–4.15 (m, 9H), 4.24 (dd, $^3J(H,H) = 4.8$, 10.4 Hz, 1H), 4.25–4.31 (m, 4H), 4.41–4.56 (m, 6H), 4.65–4.77 (m, 4H), 4.95 (d, $^3J(H,H) = 8.4$ Hz, 1H), 5.08 (s, 3H, PhCH), 5.09 (s, 3H, PhCH), 5.15 (d, $^3J(H,H) = 8.8$ Hz, 1H), 5.22 (d, $^3J(H,H) = 8.4$ Hz, 1H), 5.41 (s, 1H, PhCH), 6.89–7.93 (m, 51H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = -5.1$, −4.2, 17.8, 25.4, 55.2, 55.4 (3C), 55.7, 56.9, 58.4, 66.0, 66.1, 66.3, 67.1, 67.4, 68.7 (2C), 68.8, 69.6, 72.2, 72.4, 73.5, 74.0, 74.2, 74.3, 74.3, 74.7, 75.0, 76.1, 76.3, 80.5, 80.6, 81.5, 81.6, 82.8, 97.4, 97.6, 99.6, 100.2, 100.3, 101.6 (2C), 102.0, 113.9–138.5 (aromatic carbons), 159.5, 159.5, 164.6 (carbonyl groups); HRMS: *m*/*z*: calcd for $C_{126}H_{125}N_3NaO_{33}Si$: 2258.7862, found: 2258.7900 $[M+Na]^+$.

Methyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranoside (29): Pentasaccharide **29** (224 mg, 0.096 mmol) was synthesized in 55% yield using building blocks **11** (156 mg, 0.218 mmol), **2** (99 mg, 0.196 mmol), **21** (173 mg, 0.176 mmol) and **27** (87 mg, 0.176 mmol) following protocol B for one-pot synthesis. ^{1}H NMR (600 MHz, CD_2Cl_2): $\delta = -0.14$ (s, 3H, CH_3Si), −0.07 (s, 3H, CH_3Si), 0.76 (s, 9H, $(CH_3)_3CSi$), 2.63 (m, 1H), 2.74 (m, 1H), 2.98 (m, 1H), 3.03–3.05 (m, 2H), 3.09 (m, 1H), 3.17–3.22 (m, 2H), 3.29–3.45 (m, 7H), 3.39 (s, 3H), 3.58 (t, $^3J(H,H) = 8.4$ Hz, 1H), 3.63 (t, $^3J(H,H) = 9.0$ Hz, 1H), 3.72 (s, 3H, *p*-CH_3OPh), 3.73 (s, 3H, *p*-CH_3OPh), 3.75 (s, 3H, *p*-CH_3OPh), 3.80–3.83 (m, 2H), 3.91 (dd, $^3J(H,H) = 4.8$, 10.8 Hz, 1H), 3.98 (t, $^3J(H,H) = 10.8$ Hz, 1H), 4.03 (dd, $^3J(H,H) = 4.8$, 10.8 Hz, 1H), 4.08 (d, $^3J(H,H) = 7.8$ Hz, 1H), 4.13–4.20 (m, 5H), 4.30–4.34 (m, 4H), 4.43 (d, $^3J(H,H) = 7.8$ Hz, 1H), 4.45 (d, $^3J(H,H) = 10.8$ Hz, 1H), 4.52–4.59 (m, 4H), 4.68–4.75 (m, 5H), 4.85–4.87 (m, 2H), 5.07 (s, 1H, PhCH), 5.17 (d, $^3J(H,H) = 8.4$ Hz, 1H), 5.25 (d, $^3J(H,H) = 8.4$ Hz, 1H), 5.31 (s, 1H, PhCH), 6.81–7.85 (m, 60H, ArH); ^{13}C NMR (150 MHz, CD_2Cl_2): $\delta = -3.8$, −4.3, 18.0, 25.8, 55.4, 55.4, 55.9, 56.0, 56.9, 66.1, 66.3, 67.3, 68.1, 68.6, 69.0, 71.0, 72.3, 72.4, 73.1, 73.5, 74.1, 74.2, 74.3, 74.4, 74.5, 74.6, 74.8, 74.9, 75.5, 75.8, 76.1, 76.2, 80.5, 81.3, 81.5, 81.8, 82.7, 83.4, 97.3, 97.8, 99.4, 100.3, 101.4, 101.6, 104.6, 113.7–139.6 (aromatic carbons), 159.4, 159.5, 164.6, 164.7 (carbonyl groups); HRMS: *m*/*z*: calcd for $C_{133}H_{138}N_2NaO_{33}Si$: 2341.8849, found: 2341.8850 $[M+Na]^+$.

Methyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (30): Hexasaccharide **30** (327 mg, 0.12 mmol) was synthesized in 54–60% yield using building blocks **11** (178 mg, 0.249 mmol), **2** (113 mg, 0.224 mmol), **21** (198 mg, 0.201 mmol) and **22** (178 mg, 0.201 mmol) following protocol B for one-pot synthesis. ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = -0.17$ (s, 3H, CH_3Si), −0.08 (s, 3H, CH_3Si), 0.75 (s, 9H, $(CH_3)_3CSi$), 2.56–2.61 (m, 2H), 2.67 (dd, $^3J(H,H) = 3.0$, 10.2 Hz, 1H), 2.71 (dd, $^3J(H,H) = 3.0$, 10.2 Hz, 1H), 2.90 (d, $^3J(H,H) = 9.6$ Hz, 1H), 2.95 (d, $^3J(H,H) = 9.6$ Hz, 1H), 3.03 (m, 1H), 3.18 (m, 1H), 3.21 (m, 1H), 3.28 (s, 3H, CH_3O), 3.31–3.34 (m, 4H), 3.36–3.40 (m, 2H), 3.42 (m, 2H), 3.47 (m, 1H), 3.55–3.60 (m, 2H), 3.63–3.66 (m, 2H), 3.69 (s, 3H, *p*-CH_3OPh), 3.72 (s, 3H, *p*-CH_3OPh), 3.74 (s, 3H, *p*-CH_3OPh), 3.78 (t, $^3J(H,H) = 9.6$ Hz, 1H), 3.90–3.96 (m, 3H), 4.02 (dd, $^3J(H,H) = 4.8$, 10.8 Hz, 1H), 4.04–4.16 (m, 4H), 4.22 (dd, $^3J(H,H) = 4.8$, 10.2 Hz, 1H), 4.26–4.33 (m, 6H), 4.39–4.52 (m, 6H), 4.56 (dd, $^3J(H,H) = 9.0$, 10.2 Hz, 1H), 4.62–4.74 (m, 5H), 4.84 (dd, $^3J(H,H) = 8.4$, 9.0 Hz, 1H), 4.93 (d, $^3J(H,H) = 8.4$ Hz, 1H), 5.01 (s, 1H, PhCH), 5.07 (s, 1H, PhCH), 5.11 (d, $J = 8.4$ Hz, 1H), 5.14 (d, $J = 8.4$ Hz, 1H), 5.30 (s, 1H, PhCH), 6.80–7.82 (m, 69H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = -4.8$, −3.7, 18.1, 26.0, 55.1, 55.4, 55.5, 57.0, 65.9, 66.2, 66.4, 68.6, 68.8, 71.0, 72.2, 72.3, 73.1, 73.8, 73.9, 74.0, 74.1, 74.3, 74.5, 75.0, 75.1, 75.4, 75.8, 75.9, 76.1, 76.7, 76.8, 80.8, 81.3, 81.4, 81.5, 83.4, 97.4, 97.5, 99.4, 99.7, 100.2, 100.3, 101.4, 101.4, 101.6, 113.9–138.6 (aromatic carbons), 159.4, 159.5, 164.4, 164.6 (carbonyl groups); HRMS: *m*/*z*: calcd for $C_{154}H_{153}N_3NaO_{40}Si$: 2734.9697, found: 2734.9697 $[M+Na]^+$.

Methyl 2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-3,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (31): Compound **31** was obtained from compound **30** in 90% yield following the general procedure for TBS removal. ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 2.59$–2.69 (m, 5H), 2.87 (d, $^3J(H,H) = 9.6$ Hz, 1H), 2.92 (d, $^3J(H,H) = 10.2$ Hz, 1H), 3.01–3.04 (m, 2H), 3.10 (m, 1H), 3.17 (t, $^3J(H,H) = 10.2$ Hz, 1H), 3.28–3.37 (m, 8H), 3.43–3.47 (m, 3H), 3.58–3.63 (m, 7H), 3.65 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.83–3.91 (m, 4H), 3.96 (d, $^3J(H,H) = 12$ Hz, 1H), 4.03–4.21 (m, 8H), 4.27–4.40 (m, 8H), 4.45 (d, $^3J(H,H) = 12$ Hz, 1H), 4.47 (t, $^3J(H,H) = 10.2$ Hz, 1H), 4.50–4.61 (m, 4H), 4.81 (t, $^3J(H,H) = 8.4$ Hz, 1H), 4.84 (d, $^3J(H,H) = 8.4$ Hz, 1H), 4.86 (d, $^3J(H,H) = 9.0$ Hz, 1H), 4.90–4.94 (m, 3H), 5.09 (s, 1H, PhCH), 5.11 (s, 1H, PhCH), 5.25 (s, 1H, PhCH), 5.35 (d, $^3J(H,H) = 10.2$ Hz, 1H), 6.78–7.82 (m, 69H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 55.1$, 55.4, 55.46, 55.48, 55.8, 55.9, 57.1, 60.6, 64.6, 65.9, 66.0, 66.3, 66.7, 67.1, 68.6, 68.8, 70.5, 72.1, 72.3, 72.8, 72.9, 73.5, 73.6, 73.7, 73.8, 73.8, 74.0, 74.2, 74.9, 75.1, 75.7, 75.8, 75.9, 80.7, 80.7, 81.3, 81.4, 81.5, 82.3, 97.3, 97.5, 99.6, 100.0, 100.2, 100.3, 101.4, 101.5, 101.6, 113.9–138.6 (aromatic carbons), 159.37, 159.39, 159.6, 164.5, 164.6 (carbonyl groups); ESI-MS: *m*/*z*: calcd for $C_{148}H_{139}N_3NaO_{40}$: 2622.6, found: 2622.7 $[M+Na]^+$.

Methyl 2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-2-deoxy-2-*N*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-2-deoxy-2-*N*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl-(1→3)-2-deoxy-2-*N*-acetyl-3,6-*O*-benzylidene-β-D-glucopyranoside (34): Acetic anhydride (0.3 mL) and DMAP (50 mg) was added to a solution of compound **31** (100 mg) in anhydrous pyridine (5 mL). The reaction mixture was stirred for 1 h at room temperature, diluted with dichloromethane and washed with aqueous solution of 1N HCl. The organic layer was dried over Na_2SO_4 and the desired compound **32** (102 mg) was obtained from flash chromatography (hexanes/EtOAc/CH_2Cl_2 1:1:1) in 100% yield. A solution of compound **32** (50 mg) and ethylenediamine (0.5 mL) in *n*-butanol (5 mL) was heated under reflux for 1.5 h until no starting material was observed by TLC. The solution was dried under reduced pressure followed by chromatography to afford free amine. The free amine was dissolved in anhydrous pyridine (3 mL) followed by addition of acetic anhydride (0.2 mL) and DMAP (100 mg). The reaction was stirred for 1 h and diluted with dichloromethane followed by extraction and flash chromatography (CH_2Cl_2/MeOH 30:1) to provide the desired product **34** (40 mg, 50%). ^{1}H NMR (600 MHz, CD_2Cl_2): $\delta = 1.74$ (s, 3H), 1.76 (s, 3H), 1.88 (s, 3H), 1.89 (s, 3H), 1.91 (s, 3H), 1.95 (s, 3H), 1.96 (s, 3H), 3.09 (m, 1H), 3.17–3.22 (m, 3H), 3.24–3.33 (m, 4H), 3.45 (s, 3H, OCH_3), 3.37–3.49 (m, 8H), 3.52–3.57 (m, 4H), 3.65 (t, $^3J(H,H) = 9.0$ Hz, 1H), 3.69–3.71 (m, 3H), 3.75 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.86–3.97 (m, 4H), 4.09 (m, 1H), 4.22–4.29 (m, 4H), 4.36–4.39 (m, 2H), 4.43–4.50 (m, 6H), 4.54–4.57 (m, 4H), 4.65 (d, $^3J(H,H) = 7.8$ Hz, 1H), 4.69–4.75 (m, 3H), 4.78–4.95 (m, 4H), 4.98 (t, $^3J(H,H) = 9.0$ Hz, 1H), 5.37 (s, 1H, PhCH), 5.40 (s, 1H, PhCH), 5.44 (s, 1H, PhCH), 6.81–

7.44 (m, 42H); ESI-MS: *m/z*: calcd for $C_{117}H_{136}N_3O_{38}$: 2190.9, found: 2190.3 $[M+H]^+$.

Methyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (36): Compound **36** (103 mg, 0.437 mmol) was synthesized in 74% yield from compound **30** (160 mg, 0.059 mmol) following the general procedure for PMB removal. ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = -0.19$ (s, 3H, CH_3Si), −0.07 (s, 3H, CH_3Si), 0.77 (s, 9H, $(CH_3)_3CSi$), 2.67–2.72 (m, 2H), 2.85–2.86 (m, 2H), 3.03 (m, 1H), 3.11–3.16 (m, 2H), 3.22 (t, ^3J(H,H) = 10.2 Hz, 1H), 3.28 (s, 3H, OCH_3), 3.29–3.42 (m, 4H), 3.47–3.57 (m, 3H), 3.63 (t, ^3J(H,H) = 9.0 Hz, 1H), 3.67 (t, ^3J(H,H) = 9.0 Hz, 1H), 3.73–3.79 (m, 3H), 3.94 (dd, ^3J(H,H) = 4.2, 10.2 Hz, 1H), 4.01 (dd, ^3J(H,H) = 4.8, 10.2 Hz, 1H), 4.03–4.08 (m, 1H), 4.14–4.18 (m, 1H), 4.28–4.37 (m, 4H), 4.42–4.50 (m, 3H), 4.5–4.65 (m, 4H), 4.83 (t, ^3J(H,H) = 8.4 Hz, 1H), 4.85 (t, ^3J(H,H) = 9.0 Hz, 1H), 4.91 (d, ^3J(H,H) = 9.0 Hz, 1H), 4.97 (t, ^3J(H,H) = 8.4 Hz, 1H), 5.08 (d, ^3J(H,H) = 8.4 Hz, 1H), 5.12 (d, ^3J(H,H) = 8.4 Hz, 1H), 5.22 (s, 1H, PhCH), 5.39 (s, 1H, PhCH), 5.41 (s, 1H, PhCH), 6.83–7.62 (m, 57H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = -4.7$, −3.8, 18.1, 26.0, 55.5, 56.1, 56.3, 57.1 (2C), 60.82, 60.83, 62.0, 66.1, 66.2, 66.5, 68.6, 68.7, 68.9, 70.6, 73.8 (2C), 74.2, 74.3, 74.5, 74.6, 74.7, 74.9, 75.3 (2C), 75.4, 75.6, 76.7, 80.7 (2C), 80.8, 83.3, 98.1 (2C), 99.76, 99.83, 100.0 (2C), 101.8, 101.9, 102.0, 123.6–138.4 (aromatic carbons), 164.8, 164.8, 164.4, 164.9 (carbonyl groups); HRMS: *m/z*: calcd for $C_{130}H_{129}N_3NaO_{37}Si$: 2374.7972, found: 2374.7881 $[M+Na]^+$.

Methyl (benzyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyluronate)-(1→3)-(2-deoxy-2-*N*-phthalimido-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-(benzyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→3)-(4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→4)-(benzyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyluronate)-(1→3)-(2-deoxy-2-*N*-phthalimido-4,6-*O*-benzylidene-β-D-glucopyranoside) (37): Compound **37** (238 mg, 0.089 mmol) was synthesized in 82% yield from compound **36** (258 mg, 0.109 mmol) following the general procedure for oxidation and benzyl ester formation. ^{1}H NMR (600 MHz, CD_2Cl_2): $\delta = -0.14$ (s, 3H, CH_3Si), −0.26 (s, 3H, CH_3Si), 0.72 (s, 9H, $(CH_3)_3CSi$), 3.04 (t, ^3J(H,H) = 10.2 Hz, 1H), 3.11–3.15 (m, 2H), 3.21–3.31 (m, 5H), 3.27 (s, 3H, OCH_3), 3.38–3.47 (m, 6H), 3.56–3.61 (m, 2H), 3.67 (t, ^3J(H,H) = 8.4 Hz, 1H), 3.84 (dd, ^3J(H,H) = 4.2, 10.2 Hz, 1H), 3.93–4.04 (m, 6H), 4.07–4.12 (m, 2H), 4.18 (dd, ^3J(H,H) = 4.2, 9.6 Hz, 1H), 4.27–4.38 (m, 6H), 4.45 (d, ^3J(H,H) = 12 Hz, 1H), 4.48 (dd, ^3J(H,H) = 8.4, 10.2 Hz, 1H), 4.52–4.58 (m, 3H), 4.62–4.68 (m, 3H), 4.97 (d, ^3J(H,H) = 8.4 Hz, 1H), 4.99 (s, 1H, PhCH), 5.01 (s, 1H, PhCH), 5.03 (d, ^3J(H,H) = 8.4 Hz, 1H), 5.10 (d, ^3J(H,H) = 12 Hz, 1H), 5.12 (s, 1H, PhCH), 5.17 (d, ^3J(H,H) = 12 Hz, 1H), 4.91–4.93 (m, 3H), 6.86–7.73 (m, 72H, ArH); ^{13}C NMR (100 MHz, CD_2Cl_2): $\delta = -5.2$, −4.3, 17.9, 25.7, 53.6, 53.9, 54.1, 55.1, 55.6, 55.7, 57.0 (2C), 65.7, 65.9, 66.3, 67.28, 67.31, 68.2, 68.4, 68.5, 71.9, 72.9, 73.9, 72.2, 74.2, 74.6, 74.7, 75.8 (2C), 76.1, 77.0, 77.5, 79.8, 79.9, 80.7, 81.0, 81.152, 82.2, 98.2 (2C), 99.5, 99.6, 99.7, 99.8, 101.0, 101.1, 101.5, 123.2–138.3 (aromatic carbons), 164.5, 164.6, 164.6, 166.9, 166.9, 168.1 (carbonyl groups); HRMS: calcd for $C_{151}H_{141}N_3NaO_{40}Si$: 2686.8758, found: 2686.8855 $[M+Na]^+$.

Methyl (β-D-glucopyranosyluronic acid)-(1→3)-(2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl)-(1→4)-(β-D-glucopyranosyluronic acid)-(1→3)-(2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl)-(1→4)-(β-D-glucopyranosyluronic acid)-(1→3)-(2-deoxy-2-*N*-acetyl-β-D-glucopyranoside) (38): Compound **38** (27 mg, 0.023 mmol) was synthesized in 53% yield from compound **37** (102 mg, 0.043 mmol) following the general procedures for hydrogenation, benzoyl/phthalimide removal and acetylation. ^{1}H NMR (400 MHz, D_2O): $\delta = 2.01$ (s, 3H, AcNH), 2.02 (s, 3H, AcNH), 2.03 (s, 3H, AcNH), 3.31–3.39 (m, 3H), 3.49–3.59 (m, 10H), 3.51 (s, 3H, MeO), 3.70–3.94 (m, 17H), 4.26 (d, ^3J(H,H) = 8.4 Hz, 1H), 4.29–4.31 (m, 3H), 4.36 (d, ^3J(H,H) = 8.4 Hz, 1H), 4.36 (d, ^3J(H,H) = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, D_2O): $\delta = 22.4$, 22.64, 22.64, 54.4, 54.4, 54.5, 57.3, 60.6, 60.7, 60.8, 68.6, 68.7, 68.8, 71.9, 72.6, 72.6, 72.9, 73.7, 73.8, 75.45, 75.49, 75.5, 75.9, 76.45, 76.54, 80.08, 80.14, 82.5, 82.6, 83.2, 100.7, 100.7, 101.9, 103.1, 103.3, 103.3, 174.38, 174.32, 174.9, 175.08, 175.08, 175.6 (carbonyl groups); HRMS: *m/z*: calcd for $C_{43}H_{67}N_3NaO_{34}$: 1192.3504, found: 1192.3550 $[M+Na]^+$.

Methyl (β-D-glucopyranosyluronic acid)-(1→3)-(2-deoxy-2-*N*-acetyl-β-D-glucopyranoside) (39): Compound **39** (2.6 mg, 6 μmol) was obtained from disaccharide **15** (13 mg, 14.6 μmol) following the general procedures for deprotection and oxidation-state adjustment in 41% overall yield. ^{1}H NMR (600 MHz, D_2O): $\delta = 2.47$ (s, 3H, AcNH), 3.80 (m, 1H), 3.91–4.03 (m, 4H), 3.96 (s, 3H, OCH_3), 4.18 (t, ^3J(H,H) = 8.4 Hz, 1H), 4.22 (dd, ^3J(H,H) = 6.0, 12.6 Hz, 1H), 4.23–4.31 (m, 2H), 4.38 (dd, ^3J(H,H) = 1.8, 12.6 Hz, 1H), 4.91 (d, ^3J(H,H) = 8.4 Hz, 1H), 4.94 (d, ^3J(H,H) = 7.8 Hz, 1H); HRMS: calcd for $C_{15}H_{25}NNaO_{12}$: 434.1274, found:434.1266 $[M+Na]^+$.

Methyl (β-D-glucopyranosyluronic acid)-(1→3)-(2-deoxy-2-*N*-Acetyl-β-D-glucopyranosyl)-(1→4)-(β-D-glucopyranosyluronic acid)-(1→3)-2-deoxy-2-*N*-acetyl-β-D-glucopyranoside (40): Compound **40** (32 mg, 39 μmol) was obtained from tetrasaccharide **19** (210 mg, 0.112 mmol) following the general procedures for deprotection and oxidation-state adjustment in 35% overall yield. ^{1}H NMR (600 MHz, D_2O): $\delta = 1.98$ (s, 3H, AcNH), 1.99 (s, 3H, AcNH), 3.11–3.15 (m, 2H), 3.25–3.48 (m, 8H), 3.47 (s, 3H, CH_3O), 3.48–3.63 (m, 8H), 4.68–4.71 (m, 2H), 4.24 (d, ^3J(H,H) = 9.0 Hz, 1H), 4.26 (d, ^3J(H,H) = 7.8 Hz, 2H), 4.33 (d, ^3J(H,H) = 8.4 Hz, 1H); ^{13}C NMR (150 MHz, D_2O): $\delta = 22.3$, 22.6, 54.3, 54.5, 57.2, 60.6, 60.8, 68.6, 68.7, 71.8, 72.5, 72.8, 73.7, 75.4, 75.45, 75.49, 75.9, 76.5, 80.1, 82.4, 83.1, 100.7, 101.9, 103.1, 103.2, 174.3, 174.9, 175.0, 175.6; HRMS: *m/z*: calcd for $C_{29}H_{46}N_2NaO_{23}$: 813.2389, found: 813.2351 $[M+Na]^+$.

Methyl (2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl)-(1→4)-(β-D-glucopyranosyluronic acid)-(1→3)-(2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl)-(1→4)-(β-D-glucopyranosyluronic acid)-(1→3)-(2-deoxy-2-*N*-acetyl-β-D-glucopyranoside) (41): Compound **41** (18 mg, 0.018 mmol) was obtained from pentasaccharide **28** (130 mg, 0.058 mmol) following the general procedures for deprotection and oxidation-state adjustment in 31% overall yield. ^{1}H NMR (600 MHz, D_2O): $\delta = 1.96$ (s, 3H, AcNH), 1.97 (s, 3H, AcNH), 1.99 (s, 3H, AcNH), 3.29–3.31 (m, 2H), 3.40–3.55 (m, 9H), 3.46 (s, 3H, CH_3O), 3.65–3.72 (m, 10H), 3.78–3.80 (m, 2H), 3.86–3.88 (m, 3H), 4.39 (d, ^3J(H,H) = 8.4 Hz, 1H), 4.42–4.44 (m, 2H), 4.47 (d, ^3J(H,H) = 8.4 Hz, 1H), 4.50 (d, ^3J(H,H) = 8.4 Hz, 1H); ^{13}C NMR (150 MHz, D_2O): $\delta = 22.3$, 22.5, 22.6, 54.4, 54.5, 55.5, 57.2, 60.59, 60.60, 60.8, 68.45, 68.7, 69.8, 72.5, 72.6, 73.6, 73.7, 74.0, 75.4, 75.5, 76.0, 76.4, 76.5, 79.9, 80.1, 82.4, 82.6, 100.7, 100.8, 101.9, 103.23, 103.24, 173.3, 174.4, 174.9, 175.0, 175.1; HRMS: calcd for $C_{37}H_{59}N_3NaO_{28}$: 1016.3183, found: 1016.3179 $[M+Na]^+$.

Methyl (β-D-glucopyranosyluronic acid)-(1→3)-(2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl)-(1→4)-(β-D-glucopyranosyluronic acid)-(1→3)-(2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl)-(1→4)-(β-D-glucopyranosyluronic acid) (42): Compound **42** (21 mg, 0.021 mmol) was obtained from pentasaccharide **29** (180 mg, 0.077 mmol) following the general procedures for deprotection and oxidation-state adjustment in 27% overall yield. ^{1}H NMR (600 MHz, D_2O): $\delta = 1.97$ (s, 3H, AcNH), 1.99 (s, 3H, AcNH), 3.26–3.31 (m, 3H), 3.43–3.57 (m, 8H), 3.36 (s, 3H, MeO), 3.65–3.74 (m, 9H), 3.75–3.80 (m, 2H), 3.85–3.87 (m, 2H), 4.34 (d, ^3J(H,H) = 8.4 Hz, 1H), 4.44 (d, ^3J(H,H) = 7.8 Hz, 2H), 4.50 (d, ^3J(H,H) = 7.8 Hz, 2H); ^{13}C NMR (150 MHz, D_2O): $\delta = 22.43$, 22.44, 54.3, 54.4, 57.6, 60.5, 68.2, 68.3, 71.2, 72.2, 72.4, 72.5, 73.3, 73.5, 73.7, 73.9, 74.2, 75.1, 75.4, 80.2, 80.3, 82.4, 82.7, 101.32, 101.32, 102.7, 102.9, 103.6, 171.11, 171.11, 172.6, 174.79, 174.84 (carbonyl groups); HRMS: *m/z*: calcd for $C_{35}H_{54}N_2NaO_{29}$: 989.2710, found: 989.2687 $[M+Na]^+$.

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