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Multigram synthesis of an orthogonally protected pentasaccharide for use as glycan precursor in a *Shigella flexneri* 3a conjugate vaccine: application to a ready-for-conjugation decasaccharide

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The rapidly growing interest for carbohydrate-based bioactive molecules calls for strategies enabling appropriate design and large scale delivery of the glycan moiety. Here, we described a robust and high-yielding chemical synthesis of an orthogonally protected pentasaccharide intended for use as central building block in vaccine development against *Shigella flexneri* 3a. Elaborated from advanced crystalline intermediates and fine-tuned catalytic processes facilitating regio- and stereoselective conversions, a robust [2+3] strategy was designed, which avoided several tedious purifications and efficiently delivered multigram amounts of the target pentasaccharide. Conversion of this intermediate into a donor and a linker-equipped acceptor, next merged in the frame of a [5+5] glycosylation step furnished a decasaccharide encompassing one trichloroacetamide moiety per repeat. Chemoselective delevulation and subsequent Pd(OH)₂-mediated hydrogenolysis enabling concomitant hydrodechlorination and azide reduction gave the ready-for-conjugation dimer of the repeating unit of the O-antigen from *S. flexneri* 3a featuring the natural stoichiometric O-acetylation. The proof-of-concept was established, opening the way to larger *S. flexneri* 3a oligosaccharides and to fine-tuned glycoconjugates.

Introduction

Understanding the importance of carbohydrates as mediators of biological processes has substantiated major advances in oligosaccharide synthesis to overcome limitations associated to isolates from natural sources. Various strategies are being explored, among which enzymatic, chemo-enzymatic and chemical routes. The latter feature the largest versatility in providing an access to natural as well as non-natural oligosaccharides. Programmable, one-pot solution phase and methods for solid phase and HPLC-assisted automated strategies exemplify some of the major ongoing investigations to accelerate the chemical synthesis of complex glycans. Significant developments have facilitated the expedited synthesis of diverse well-defined oligosaccharides, including homopolymers of increasing chain length, and the total synthesis of the largest chemically assembled polysaccharide to date, paving the way to useful probes for investigating further yet poorly understood carbohydrate-mediated vital biological events. When considering large heteropolymers and highly branched targets, solution phase iterative block synthesis has remained an attractive strategy. In particular, the successful delivery of glycans featuring several repeats strongly relies on the identification of building blocks empowering iterative homologation with high and reproducible glycosylation yields, while also obeying regio- and stereoselectivity criteria in addition to qualifying for efficient full deprotection. Another significant challenge for relevant building block design stems from the need for a synthesis enabling the large-scale production of these essential intermediates to subsequently deliver usable amounts of the extended glycan targets. Herein, we tackle this relevant issue in the context of vaccine development.

*Shigella* are Gram negative bacteria and the cause of shigellosis, a major diarrheal disease responsible for a high burden notably among children aged 1-5 years living in low- and middle-income settings. *Shigella* is on the WHO pathogen priority list. Epidemiological data, among which the increasing antimicrobial resistance observed among field isolates, call for the development of a multivalent *Shigella* vaccine. Toward this goal, conjugate vaccines based on the bacterial polysaccharide antigens, or surrogates thereof, have been the subject of major interest. As part of the ongoing developments, we have proposed the first synthetic glycan-protein conjugate vaccine candidate against endemic shigellosis. While many antibacterial glycovaccine candidates use haptenes corresponding to one repeating unit of the homologous natural polysaccharide antigens, the selected glycoconjugate prototype comprises a chemically synthesized pentadecasaccharide corresponding to a three core repeating unit portion from the *Shigella flexneri* 2a O-antigen (O-Ag). It was produced according to good manufacturing practice and...
demonstrated to be safe and immunogenic in adult volunteers in the frame of first-in-human clinical trial. These achievements have provided strong support to serotype broadening. In this context, our current efforts aim for a vaccine candidate against *S. flexneri* 3a (SF3a), another prevalent *Shigella* serotype for which a vaccine is in high demand.

The SF3a O-Ag is made up of a branched pentasaccharide repeat (E)AB₆C₆D (Figure 1), featuring (1→2)-trans-linked l-rhamnoses (A, B, C) and a N-acetyl-β-glucosamine residue (D). Rhamnose A is 3-O-α-D-glucosylated (E). Acetylation at position 2C is stoichiometric. In contrast, position 6D is O-acetylated to a 40% extent only. Epitope mapping has revealed the immunodominant 2C-O-acetyl (Ac) moiety and the importance of chain length for protective antibody recognition. Molecular modeling simulations supported by NMR analysis of O-Ag segments from 12 *S. flexneri* 2a and SF3a, suggested similar backbone conformational behavior. This study also revealed a dynamic behavior of the end-chain α-D-glucopyranosyl residue (1→3)-linked to rhamnose A differing from that predicted for glucose side-chains located on internal repeats. Overall, convincing evidences support the assumption that oligosaccharides achieving SF3a O-Ag functional mimicry encompass at least two repeating units. Otherwise, the role of the non-stoichiometric 6D-O-acetylation remains undisclosed.

Aiming at establishing a lead hapten candidate for SF3a vaccination, we report a straightforward multi-step chemical synthesis of pentasaccharide 1, as the lead common precursor to the (E)AB₆CD and (E)AB₆C₆D modules, their combinations and oligomers thereof, as found in the native SF3a O-Ag (Scheme 1). Going beyond our previous disclosures while aiming at scalability and robustness, the orthogonally protected pentasaccharide building block was produced in several 10-gram amounts. Emphasis was put on (i) restraining the handling of toxic and poor user-friendly reagents, in particular by circumventing the notoriously questionable tin chemistry and by avoiding concerns related to hydrazine and its derivatives especially when involved at an advanced stage of a multi-step synthesis, (ii) limiting the repeated use of low abundant catalysts despite their remarkable potential as exemplified with iridium-based compounds, and (iii) reducing the number of demanding purification steps involving column chromatography by promoting crystalline intermediates and fine-tuning of reaction parameters, while (iv) achieving high yielding conversions fulfilling regio- and stereoselectivity criteria. It is well-appreciated that concern for the latter increases when addressing glycosylation steps involved in large oligosaccharide blockwise synthesis.

Herein, significant inputs feature handy metal-catalyzed protecting group manipulation, advanced crystalline intermediates, fine-tuned 1,2-cis block glycosylation steps, and a meaningful reduction of the number of column chromatography, the latter being known to qualify as a bottleneck when aiming at large-scale synthesis. Furthermore, the proof-of-concept is established as the potential of the selected pentasaccharide building block is next demonstrated in the synthesis of a ready-for-conjugation linker-equipped decasaccharide corresponding to a dimer of the repeating unit of the SF3a O-Ag.

### Results and discussion

Building block 1 was designed as an allyl glycoside, allowing easy conversion into a donor or an acceptor. It is 2C-O-acetylated as in the SF3a O-Ag. In contrast, the second site of natural O-acetylation is masked as a 4 β-(O)-benzylidene (Bzl) acetal, allowing for the chemoselective late stage modification at OH-6A. Non-interfering hydroxyl groups are benzylated, and the site of elongation (OH-2D) features a levulinoyl ester, which fulfills criteria for stability, anchimeric assistance and orthogonality, in particular to the 2C-acetate. Relying on the imidate chemistry,

![Scheme 1 Pentasaccharide 1 and its retrosynthetic analysis. All: allyl; Lev: levulinoyl.](image-url)
pentasaccharide 1 is readily accessible from the known B₆CD and EA allyl glycosides, 2,17 and 3,35 respectively. These key intermediates are obtained from carbohydrate precursors in bulk amounts. Substantiating our previous report,36 diol 5 is routinely obtained in at least 90% yield in four steps and over 80% yield (Scheme 1). It performs as an exquisite common precursor to the known acceptor A/C (14) and donor B (13).35

Synthesis of the BCD trisaccharide 2.

Going beyond the original tin-mediated regioselective benzylation of 1,2-cis diols37 and the inherent toxicity of tin reagents used in stoichiometric amount, elegant procedures enabling the site-selective modification of carbohydrates have been developed.38, 39 The recently reported iron(III)-based catalysts, Fe(dibm), offering high regioselectivity, broad scope and high reactivity,40 and its cheaper although equally efficient analog Fe(dipm),41 called our attention (Scheme 2). Readily obtained from the inexpensive FeCl₃·6H₂O, these reagents are considered non-air sensitive, non-toxic and environmentally benign.42 Gratifyingly, Fe(dibm)-promoted benzylation of diol 5 in the absence of additive proceeded at 80 °C in acetonitrile to give the desired alcohol 7 (92%) together with its regiosomer 7a (5%). Satisfactorily, Fe(dipm), performed as well. The 19:1 regioselectivity compares nicely with the 87% yield achieved using tin chemistry.36 Advantageously, purification is simpler. Next, instead of using a large excess of levulinic anhydride prepared up front, Steglich esterification of alcohol 7 gave levulinate 8,35 which was in turn deallylated into hemicetal 9.35

As an attempt to avoid the previously adopted efficient, albeit expensive, 19[(COD)(PCH₂C₆H₄)₂]PF₆ catalyst and its necessary hydrogen-mediated activation (Table 1, Entry 1),17 we have favoured the use of more Earth-abundant metal catalysts, focusing primarily on well-explored palladium derivatives (Table 1) amid numerous possible reagents.43-45 to complete the anomeric deallylation step.46 Unexpectedly, Pd(PPh₃)₄ used in combination with mild acids47 led at best in partial conversion to propen-1-yl 10 (Entries 2 and 3). Therefore, established protocols involving Pd(II) catalysts, which are generally more stable and less expensive than Pd(0) derivatives, were considered instead. Diverging from previous observations,48 PdCl₂ in buffered AcOH/ACONa was low-yielding (Entry 4). Although the phenomenon was barely reported, methyl glycoside 11 was repeatedly isolated when using PdCl₂ in methanol (Entries 5 and 6), while the Wacker-type products49 12a/12b were formed in DMF (Entries 7 and 8). We reasoned that changing DMF to a non-polar solvent used in combination with water as the proton source would prevent side-oxidation. Indeed, conversion to propen-1-yl 10 was slow, but oxidized 12a/12b were not observed in DCM/H₂O (Entry 9). Otherwise, changing DMF for THF led to low conversion (Entry 10). Gratifyingly, heating hemicetal 8 to 50 °C for 2 h in DCM/H₂O (3:1) containing PdCl₂ (4 mol%) allowed faster completion and provided hemicetal 9 in quantitative yield post iodine addition (Entry 11). These yet unreported easy-to-handle conditions were adopted on the large scale (Entries 12 and 13).

Table 1 Pd-mediated isomerization of hemicetal 8.

| Entry | Conditions | Products (Yield) |
|-------|------------|-----------------|
| 1a    | [(COD)(PCH₂C₆H₄)₂]PF₆ | 9 (93%) |
| 2     | Pd(PPh₃)₄, TsOH    | 8            |
| 3     | Pd(PPh₃)₄, AcOH     | 10 (70%)     |
| 4     | PdCl₂, AcOH/ACONa   | 10 (58%)     |
| 5     | PdCl₂, MeOH         | 11           |
| 6     | PdCl₂, MeOH/THF     | 11           |
| 7     | PhCl, CuCl, DMF     | 12a/12b (85%)|
| 8     | PdCl₂, DMF/H₂O      | 12a/12b (74%)|
| 9     | PdCl₂, DCM/H₂O      | 10 (74%)     |
| 10    | PdCl₂, THF/H₂O      | 8, 10        |
| 11a   | PdCl₂, DCM/H₂O      | 9 (full conversion) |
| 12a   | 2-4 h, 50 °C        | 9 (88%), 12% |
| 12b   | 2-4 h, 50 °C        | 9 (88%), 12% |

* 60 mg scale and rt unless stated otherwise. a Post hydrolysis. b From alcohol 7 (30 g).

Remarkably, trichloroacetimidate 13 easily obtained by reacting hemicetal 9 and trichloroacetotrinitol in the presence of a base,40 is now routinely prepared on the 40 g scale (92%) from alcohol 7 in three steps and no intermediate purification (Scheme 3). Donor 13 is stable for at least a month at -20 °C despite being isolated as a syrup.

The stepwise conversion of diol 5 into the BC donor 17 is a robust process (Scheme 3), reaching 69% over four steps on a 5-10 g scale.41 Herein, this conversion was achieved without intermediate purification reaching an overall yield of 86%, which was proven reproducible upon scaling up. Indeed, donor 17 was isolated in 30 g amount (84%) starting from 13 g of diol 5. Noteworthy features in doing so include the reaction of acceptor 14, readily obtained from diol 5 as a 95:5 mixture of regiosomers, with a reduced excess of donor 13 (1.1 instead of 1.2 equiv.) and the use of the newly established Pd(II)-mediated anomeric deallylation protocol without any yield loss, as demonstrated for the independent conversion of hennobioside 15 into trichloroacetimidate 17 (91%).

Otherwise, acceptor 18 (88%) was achieved from tetracetate 4 in four steps as described.42 In line with expectation,17 the TMSOTf-promoted [18-17] glycosylation proved to be highly efficient (Scheme 4). Crystalline B₆CD 2 of acceptable purity for the next step was isolated in 97% yield from 11 g of crystalline 18 and a slight excess (1.15 equiv.) of crude 17 obtained from disaccharide 15.
achievements, favored glucosyl donor stereoselectivity has guided several reports on strategies.

Synthesis of disaccharides EA from diol

Scheme 5 Synthesis of disaccharides EA from diol 5 and tetrabenzyl glucosyl donors. (i) MeC(OOMe), PTSA·H₂O, MeCN, rt then 80% aq. AcOH, 0 °C.

Next, the possible remote anchimeric assistance of the protecting groups masking on the [E+A] glycosylation outcomes was examined. In view of their orthogonal properties and easy access by means of the selective 6-O-debenzylation of hemiacetal 6 (Scheme 6), 59 glucosyl donors bearing a temporary 6-O-acetyl ester or 6-O-tert-butyldiphenylsilyl ether (TBDDS), respectively, were considered. However, the enhanced α/β ratio expected from long-range 6-O-acyl-assistance using donors 28 and 29 or from a foreseeable steric hindrance- controlled α-glucosylation by means of the silylated analogs 31 and 32 was not observed in our hands providing the condensation products in at best a 7:3 α/β ratio (Schemes S2, not described). Leaving aside promising albeit more demanding strategies involving specific protecting group manipulation, 32 we turned to investigate the potential of exogenous nucleophiles to control stereoselectivity 52 when considering solely the more readily available tetrabenzyl donors, and in particular imidates 21 and 22, as the simplest possible E precursors. We prioritized the DMF-modulated glycosylation
strategy introduced by the Mong laboratory, a highly attractive approach as valuably demonstrated by Codée and coworkers. Satisfactorily, an improved α/β ratio was observed when rhamnoside 14 was reacted with trichloroacetimidate 21 in DCM containing DMF and stoichiometric TFOH (Table 2, Entry 8). This tendency was independent of the imidate donor (Entry 12). Besides strengthening further the potential of DMF as an external modulator of glycosylation reactions, the observed enhanced stereoselectivity was compatible with the subsequent transesterification step, therefore facilitating isolation of the product of α-glucosylation as alcohol 24 (Entries 9 and 13). To our utmost satisfaction, scaling up did not interfere with stereoselectivity (Entry 10). Indeed, as a clear step forward to a robust high yielding process, these conditions delivered 25 g of the glycosylation products 24 and 25 in an excellent 95:5 α/β ratio and 81% yield over three steps from diol 5 and trichloroacetimidate 21, both of which are easily accessible crystalline materials. Whereas Steglich levulination at OH-2 of disaccharide 24 hardly reached completion, full conversion into the key intermediate 3 was achieved when substituting DCC by the

| Entry | Conditionsa | Productsb,c (α:β ratio, yield from 5) | Productsd,c (α:β ratio, yield from 5) |
|-------|-------------|---------------------------------------|---------------------------------------|
| 1b    | TMSOTf (0.02), toluene/DCM, -78 °C to rt | 23 (85:15), 24 (61%) | 26 |
| 2     | TMSOTf (0.3), EtOAc, -78 °C | 23 (80:20, 60%) | 26 |
| 3     | TFOH (0.3), EtOAc, -78 °C | 23 (80:20, 60%) | 26 |
| 4     | Bi(OIT) (0.3), EtOAc, -78 °C | 23 (85:15) | 26 |
| 5     | TMSOTf (0.07), EtOAc, -105 °C | 23 (85:15) | 26 |
| 6     | TMSOTf (0.3), toluene, -78 °C | 23 (80:20, 60%) | 26 |
| 7     | TMSOTf (0.07), toluene, -78 °C | 23 (85:15) | 26 |
| 8     | TFOH (1.0), DMF (20), DCM, -78 °C to rt | 23 (90:10, 76%) | 24 (88%) |
| 9†,f  | TFOH (1.0), DMF (20), DCM, -78 °C to rt | 24 (90:10) | 24 (88%) |
| 10†,f | TMSOTf (0.3), EtOAc, -78 °C | 23 (90:10) | 24 (88%) |
| 11a   | TFOH (1.0), DMF (20), DCM, rt | 23 (90:10) | 24 (88%) |
| 12a   | TFOH (1.0), DMF (20), DCM, rt | 23 (90:10) | 24 (88%) |

a Reactions were run on 70-80 mg of diol 5, using 1.3 equivalents of donor 21 and 0.3 promotor equivalent, at -78 °C unless stated otherwise. b α/β ratio based on NMR data of the crude. c Isolated yields for 23 and 24 are from diol 5, over two steps and over three steps, respectively. d 1.0 g scale. e 11 g scale. f Post transesterification. g Use of donor 22. h Use of donor 19. i Use of donor 20.

Scheme 6 Synthesis of the 6-O-modified glucopyranosyl donors from hemiacetal 6. i) TFA, AcO, 0 °C; ii) NH₂NH₂.H₂O, AcOH, DMF, 87% over two steps; iii) CCl₃CN, K₂CO₃, DCM, 85% for 28, 78% for 31; iv) PTFAC, K₂CO₃ acetone, 90% for 29, 40% over 4 steps for 32; v) MeONa, MeOH; vi) TBDPSI, Imidazole, DMAP, DMF.

Scheme 7 Synthesis of donors EA from diol 5 and tetrabenzyl glucosyl donors 21 or 22. (i) Me(OCH)₃, PTSA/H₂O, MeCN, rt then 80% eq. AcOH, 0 °C; (ii) TFOH, DMF, 4 Å MS, DCM, -78 °C to rt; (iii) MeONa, MeOH/DCM, from 21 (1.15 equiv.), 88% (over two steps) and from 22 (1.3 equiv.), 79% (over three steps); (iv) nLevOH, EDC, DMAP, DMF, 90% (30 g scale); (v) [Ir(COD)PCH₂(C₆H₅)₂]PF₆ , THF, then i), THF/H₂O, 85% (29 g scale); (vi) CCl₃CN, DBU, DCE, -5 °C, 93% (23 g scale), also from 24, 67%, (42 g scale, 3 steps) together with 25 (30%); (vii) PdCl₂, DCM/H₂O then ii), THF, 50 °C; (viii) PTFAC, K₂CO₃ acetone, 90% (34 g scale, 3 steps).
more reactive EDC (Scheme 7). This successful in situ activation of levulinic acid advantageously replaced the formerly adopted conditions.\textsuperscript{35} Deallylation, whether conventional\textsuperscript{35} or using the newly established aforementioned PdCl\textsubscript{2} protocol, delivered the known hemiacetal 33\textsuperscript{35} quantitatively for direct conversion into imidates 34\textsuperscript{35} and 35. Alternatively, the fully protected 3 was evolved into these same donors without intermediate purification. Scaling up this efficient three-step conversion provided trichloroacetimidate 34 in 67% yield in combination with hemiacetal 33 (30%), post chromatography. Obviously, the recovery of a meaningful amount of 33 was attributable to donor hydrolysis on the column, suggesting that careful consideration be given to the purification step for large scale development. Satisfactorily, the more stable PFTA donor 35 was isolated in 40 g amount in an excellent 90% yield over three steps.

Synthesis of the EAB\textsubscript{3}CD pentasaccharide building block 1.

Restraining the number of column chromatographies, the two-step conversion of the fully protected 2 (\(-25\) g) into pentasaccharide 1 employed the crude acceptor 36\textsuperscript{17} (Scheme 8). Satisfactorily, the independent use of donor 34 or 35 (1.15 equiv.) ensured a good 80% yield from the fully protected 2, or rather a 88-94% corrected yield based on recovered 36. Adding to the overall improved strategy of the (E)AB\textsubscript{3}CD building block (1), this compares favourably with original stepwise achievements.\textsuperscript{17} In particular, the proof of concept having been established, we are confident that additional fine-tuning on the two-step conversion on a large scale will contribute to increase further the isolated yield pentasaccharide 1. On the way forward toward this aim, we also envisioned alternatives to hydrazine acetate involving less toxic reagents for the selective delevulination at OH-2 of the 2-O-acetyl B\textsubscript{3}CD precursor (2). While the former remains from far the method most frequently encountered, it is not without drawback. In particular, we have previously observed the partial reduction of the olefinic bond of the allyl aglycon in pentasaccharide 1 concomitant to hydrazinolysis of the 2\textsubscript{\alpha}-O-levulinoyl ester.\textsuperscript{17} Inspiration from earlier findings,\textsuperscript{66} encouraged the investigation of sulfoxide as a handy reagent. However, resulting at best in incomplete conversion despite prolonged reaction time (not described), neither the original conditions nor their modified version implemented in the context of oligonucleotide synthesis\textsuperscript{67} fulfilled our expectations. Optimization was not attempted. Instead, implementation of user-friendly conditions enabling the high-yielding delevulination of trisaccharide 2 took advantage of a previous report from R. Adamo’s group.\textsuperscript{68} Indeed, replacing hydrazine acetate by the more acceptable ethylenediamine provided alcohol 36 in a selective manner suggesting that these conditions could be adopted in the future (Scheme 8).
pentasaccharide 45 in a satisfactory 70% yield post RP-HPLC chromatography.

Interestingly, transferring the most promising [5+5] glycosylation conditions to the 2-azidoethyl-equipped acceptor revealed that the [39+37] glycosylation was somewhat sensitive to both solvent and temperature (not described). In agreement with original findings, running the condensation in non-polar toluene at -40 °C were identified as the best conditions providing decasaccharide 43 in a reproducible 90% average yield (Scheme 10). Subsequent delevulination gave alcohol 44, which was next submitted to a one-step full deprotection. While enabling the concomitant cleavage of the two 4-O-benzyldiene acetal and 16 benzyl ethers in addition to the simultaneous reduction of the two 2-trichloroacetamides and azide moiety, the Pd(OH)₂-catalyzed hydrogenation/hydrogenolysis of the azidoethyl glycoside 44 in tBuOH/DCM/H₂O into the aminoethyl decasaccharide 46 was more demanding than that of its counterpart 42 into pentasaccharide 45. The use of a higher Pd(OH)₂ amount combined to a longer reaction time at ambient temperature and pressure furnished the conjugation-ready 46 in a good 52% yield post RP-HPLC (Scheme 10). Nevertheless, the observed drop in the yield of the two O-Ag repeating unit segment 46 versus the one repeating unit oligosaccharide 45 suggested that improvement might be needed for the full deprotection of larger oligomers featuring an aminoalkyl aglycon and a higher number of trichloroacetamide groups.

Table 3 [5+5] Glycosylation to reach decasaccharide 40.

| Entry | Conditions* | Product (yield) |
|-------|-------------|----------------|
|       | Acceptor    | 39 equiv.      | Solvent | Temperature |     |
| 1     | 37          | 1.3            | DCM     | -40 °C      | 40 (90%) |
| 2     | 37          | 1.3            | DCM     | rt          | 40 (81%) |
| 3     | 37          | 1.3            | DCM     | 0 °C        | 40 (86%) |
| 4     | 37          | 1.1            | DCM     | -78 °C      | 40 (83%), 37 |
| 5     | 37          | 1.1            | DCM     | -40 °C      | 40 (85%), 37 (10%) |
| 6     | 37          | 1.2            | DCM     | -40 °C      | 40 (87%), 37 |
| 7     | 37          | 1.3            | toluene | -40 °C      | 40 (91%) |

* Reactions were run on 100 mg of acceptor 37 using TMSOTf (0.2 equiv.) as promoter.
Conclusions
This study aimed at achieving a robust process enabling the large-scale synthesis of pentasaccharide 1, and at demonstrating that this orthogonally protected building block could serve as a suitable precursor to a donor and an acceptor, whose combination would provide ready-for-conjugation oligosaccharides for use in the development of a synthetic carbohydrate-based conjugate vaccine candidate against SF3a. A robust and convenient 26-step synthesis, orthogonally protected building block could serve as a suitable candidate against SF3a. A robust and convenient 26-step synthesis, orthogonally protected building block could serve as a suitable concept for building block selection enabling a robust [5+5] chain from crystalline 1,3,4-6-tetra-O-acyl tetrasaccharide was efficiently transformed into donor 1

Experimental
Iron(III) dipivaloylmethane (Fe(dipm))41,42 To a biphasic mixture of 2,2,6,6-tetramethyl-heptane-3,5-dione (10.2 g, 55.5 mmol, 3.0 equiv.) and NaOAc (4.6 g, 55.5 mmol, 3.0 equiv.) in ETOH/H2O (1:1, 140 mL) was added FeCl3 · 6H2O (5.0 g, 18.5 mmol). A red slurry formed and the mixture was heated at 60 °C for 2 h. The reaction was cooled down to rt then to 0 °C for 15 min. Filtration gave an orange powder, which was washed with water and crystallized using EtOH/H2O (90:10, 70 mL). After cooling to 0 °C, crystals were filtered and rinsed using -78 °C cooled EtOH/H2O (90:10) furnishing Fe(dipm)3 as a red solid (10.9 g, 97%).

Allanyl 3,4-di-O-benzyl-a-L-rhamnopyranoside (7). Route 1. To a solution of diol 5 (5.0 g, 17 mmol, 1.0 equiv.) in MeCN (150 mL) and benzyl bromide (2.22 mL, 19 mmol, 1.1 equiv.) were added at rt K2CO3 (3.52 g, 25.5 mmol, 1.5 equiv.) and Fe(dibm)3 (440 mg, 0.85 mmol, 5 mol%). The reaction mixture was stirred for 4 h at 80 °C. Additional benzyl bromide (1.5 equiv.) and Fe(dibm)3 (2 mol%) were added and the mixture was stirred for an additional 24 h. A TLC control (Tol/EtOAc, 80:20) indicated the total conversion of 5 into less polar products. Solids were filtered over a pad of Celite® and washed generously with DCM. Purification by flash column chromatography (Tol/EtOAc, 100:0 to 90:10 to 80:20) gave the known 2-O-benzyl isomer70 7a as a yellow oil (350 mg, 5%) along with the desired 7 (6.0 g, 92%). Regioisomer 7a had Rf = 0.75 (cHex/EtOAc, 70:30). 1H NMR (400 MHz, CDCl3) δ = 7.47 – 7.32 (m, 10H, HAr), 5.94 (m, 1H, CH=CH2), 5.34 (dq, J = 17.2, 1.5 Hz, 1H, CH=CH2), 5.24 (m, 1H, CH=CH2), 4.98 (d, J = 11.2 Hz, 1H, HNHTCA), 4.94 (d, J = 1.5 Hz, 1H, H-1), 4.80 (d, J = 11.8 Hz, 1H, H3), 4.73 (d, J = 11.2 Hz, 1H, H2), 4.68 (d, J = 11.8 Hz, 1H Hz), 4.22 (m, 1H, CH2=CH), 4.08 (dd, Japp = 9.0, 3.8 Hz, 1H, H-3), 4.02 (m, 1H, CH2=CH), 3.83 (dd, J = 3.8, 1.5 Hz, 1H, H-2), 3.33 (t, Japp = 9.2 Hz, 1H, H-4), 2.53 (brs, 1H, OH-1), 1.42 (d, J = 6.3 Hz, 3H, H-6). 13C NMR (100 MHz, CDCl3) δ 138.7 (C6a), 137.9 (C6a), 133.9 (CH=CH2), 128.6 – 127.7 (10CH2), 117.2 (CH=CH2), 96.3 (C-1), 82.4 (C-4), 78.8 (C-2), 75.1 (CH2=CH), 73.1 (CH2=CH), 71.8 (C-3), 67.8 (CH2=CH), 67.5 (C-5), 18.1 (C-6). HRMS (ESI®): m/z 407.1865 (calcd for C23H30O12Na [M+Na]+: m/z 407.1820).

Route 2. To a solution of diol 5 (400 mg, 1.36 mmol, 1.0 equiv.) in MeCN (7 mL) and benzyl bromide (0.19 mL, 1.63 mmol, 1.2 equiv.) were added at rt K2CO3 (282 mg, 2.04 mmol, 1.5 equiv.) and Fe(dipm)3 (440 mg, 0.85 mmol, 5 mol%). The reaction mixture was stirred for 4 h at 80 °C. Additional benzyl bromide (1.5 equiv.) and
iron catalyst (2 mol%) were added and the mixture was stirred for an additional 10 h. A TLC control (tol/EtOAc, 80:20) indicated the total conversion of diol 5 into less polar products. Solids were filtered over a pad of Celite® and washed generously with DCM. Purification by flash column chromatography (tol/EtOAc, 100:0 to 90:10 to 80:20) gave the known 3-O-benzyl isomer as a yellow oil (480 mg, 92%). The target 7 had Rf = 0.55 (chex/EtOAc, 70:30). ¹H NMR (400 MHz, CDCl₃, δ 7.46 – 7.30 (m, 10H, HAr), 5.91 (dd, δ = 17.2, 10.4, 6.1, 5.1 Hz, 1H, CH=CH₂), 5.30 (dd, δ = 17.2, 1.6 Hz, 1H, CH=CH₂), 5.21 (dd, δ = 10.4, 4.7 Hz, 1H, CH=CH₂), 4.92 (dd, δ = 10.9 Hz, 1H, H₂Bn), 4.88 (dd, δ = 1.7 Hz, 1H, H-1), 4.72 (s, 2H, H₂Bn), 4.67 (dd, δ = 10.9 Hz, 1H, H₂Bn), 4.18 (dd, δ = 10.4, 4.7 Hz, 1H, CH=CH₂), 7.29 (dd, δ = 9.5, 6.2 Hz, 1H, H-5), 3.39 (dd, δ = 9.3, 1.4 Hz, 1H, CH₃₆), 3.10 (s, 9.3, 1.4 Hz, H-3), 3.79 (s, 9.3, 1.4 Hz, 1H, CH₃₆), 2.51 (brs, 1H, OH), 1.34 (s, 9.3, 1.4 Hz, 3H, H-6). ¹³C NMR (100 MHz, CDCl₃) δ 138.4 (C₅), 138.0 (C₅), 133.8 (C₅), 128.5 – 127.7 (10HAr), 117.4 (CH=CH₂), 98.2 (C-1, JCH=CH₂ = 168.7 Hz), 80.1 (C-3), 80.0 (C-4), 75.4 (CH₃₆), 69.3 (CH₂Bn), 66.2 (C-2), 67.9 (CH₂Bn), 67.4 (C-5), 17.9 (C-6). HRMS (ESI⁺): m/z 470.1809 (calcd for C₂₀H₁₅O₂Na [M+Na⁺]: m/z 470.1820).

**3,4-Di-O-benzyl-2-O-levulinoyl-α/β-l-rhamnopyranosyl (9).** ⁵PDCl₂ (769 mg, 2.6 mmol, 0.05 equiv., 60% purity) was added to a solution of allyl glycoside 8 (251 g, 520 mmol) in DCM/H₂O (3:1, 260 mL). After stirring for 4 h at 50 °C, a TLC control (tol/EtOAc, 80:20) showed the presence of a major less polar product in addition to some remaining 8. Nevertheless, the reaction mixture was allowed to cool to rt and le (26.4 g, 104.1 mmol, 2.0 equiv.) was added. After 40 min, a TLC control (tol/EtOAc, 80:20) showed the formation of two more polar products. Sat. aq. Na₂SO₄ was added and the biphasic solution was filtered on Celite® and the organic phase was washed with sat. aq. NaHCO₃. The mixture was filtered through a plug of silica gel and the organic phase was washed with sat. aq. NaHCO₃. The mixture was stirred for 4 h at 50 °C. A TLC control showed the starting material had been converted to a less polar product. DCU was filtered by passing through a pad of Celite®, and the solids were washed extensively with DCM. The organic phase was washed with water, sat. aq. NaHCO₃, sat. aq. CuSO₄, then with water and finally with brine. The organic layer was dried on Na₂SO₄, filtered, and concentrated under reduced pressure. To a solution of the crude 8 in DCM/H₂O (3:1, 600 mL) was added PdCl₂ (920 mg, 3.0 mmol, 0.04 equiv., 60% purity). The mixture was stirred for 3 h at 50 °C. A TLC control showed the conversion of the fully protected 8 into a less polar product. After cooling the solution to 0 °C, a solution of iodine (19.8 g, 156 mmol, 2.0 equiv.) in THF (140 mL) was added slowly and the mixture was stirred at rt for 2.5 h. A TLC control showed the complete disappearance of the intermediate and the presence of more polar products. Excess iodine was destroyed by adding a solution of sat. aq. Na₂SO₄. The biphasic mixture was filtered on Celite® and the organic phase was washed with sat. aq. NaHCO₃, water and brine. The organic phase was dried on Na₂SO₄, filtered, and concentrated under reduced pressure. To a solution of the crude hemiacetal 9 in 1,2-DCE (260 mL) stirred under Ar at 0 °C were added trichloroacetonitrile (39.1 mL, 390 mmol, 5.0 equiv.) and DBU (3.3 mL, 21.8 mmol, 0.28 equiv.) dropwise. The brown mixture was stirred at 0 °C for 3 h. A TLC control showed the conversion of the intermediate 9 into less polar products. After incomplete concentration under reduced pressure, the mixture was purified by column chromatography on neutralized silica gel (chex/EtOAc, 100:0 to 60:40 + 1% Et₃N) to give the known donor 13.
Acetyl-4-[(3,4-di-O-benzyl-2-O-levulinoyl-α-L-rhamnopyranosyl)-(1→3)-2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl] trichloroacetimidate (17). Route 1. To a solution of disaccharide 15 (43.7 g, 57.4 mmol) in DCM/H₂O (3:1, 575 mL) was added PdCl₂ (850 mg, 2.87 mmol, 0.05 equiv., 60% purity). The mixture was stirred for 3 h at 50 °C. TLC (Tol/ETOAc, 80:20) showed the disappearance of the starting 15 and the presence of a less polar product. Iodine (14.6 g, 115 mmol, 2.0 equiv.) in THF (100 mL) was added slowly at 0 °C and the mixture was stirred at rt for 2.5 h. TLC (Tol/ETOAc, 70:30) showed the complete disappearance of the intermediate and the presence of a single more polar product. Excess iodine was destroyed by adding sat. aq. Na₂SO₃. The biphasic mixture was filtered on cotton and the two layers were separated. The organic phase was washed with sat. aq. NaHCO₃, water and brine, dried on Na₂SO₄, filtered, and concentrated under reduced pressure. Trichloroacetoneitrile (29 mL, 287 mmol, 5.0 equiv.) and DBU (2.5 mL, 16.1 mmol, 0.28 equiv.) were added dropwise to the obtained crude hemiacetal in anhyd. 1,2-DCE (215 mL) at rt. After stirring at rt for 1 h, the mixture was stirred at this temperature for 30 min. TLC (Tol/ETOAc, 80:20) showed the complete consumption and the presence of a major less polar product. EtOAc was added along with water and the two layers were separated. The aq. phase was neutralized with cold toluene gave crude disaccharide 16 as yellow syrup along with recovered hemiacetal (1.8 g, 5%).

Route 2. Trimethyl orthoacetate (10.9 mL, 85.5 mmol, 1.9 equiv.) and monohydrated PTSA (128 mg, 0.68 mmol, 0.015 equiv.) were added to diol 5 (13.2 g, 45.0 mmol, 1.0 equiv.) in anhyd. MeCN (26 mL) at rt. After stirring at rt for 1.5 h, 80% aq. AcOH (26.5 mL) was added at 0 °C and the mixture was stirred at this temperature for 30 min, then at rt for 1 h. TLC (Tol/ETOAc, 80:20) showed the presence of more polar products. DCM was added along with water and the two layers were separated. The aq. phase was extracted with DCM and the combined organic layers were washed successively with sat. aq. NaHCO₃ and brine, dried over Na₂SO₄, evaporated and evaporated under reduced pressure to give crude acceptor 14. TMSOTf (2.33 mL, 13.9 mmol, 0.25 equiv.) was slowly added dropwise to a solution of the latter (45.0 mmol, 1.0 equiv.) and trichloroacetimidate 13 (27.8 g, 47.3 mmol, 1.05 equiv.) in toluene (540 mL) containing activated 4 Å MS (450 mg) at -78 °C. After stirring for 45 min at -78 °C, TLC (Tol/ETOAc, 80:20) indicated conversion of the intermediate into a more polar product. Excess iodine was destroyed by adding a solution of sat. aq. Na₂SO₃. The mixture was filtered on cotton, and the two layers were separated. The organic phase was washed with sat. aq. NaHCO₃, water and brine, dried on Na₂SO₄, filtered and concentrated under reduced pressure. To a solution of crude 16 in anhyd. 1,2-DCE (215 mL) under inert atmosphere, at 0 °C, were added dropwise trichloroacetoneitrile (21.6 mL, 215 mmol, 5.0 equiv.) and DBU (1.84 mL, 12.0 mmol, 0.28 equiv.). After stirring at 0 °C for 1 h and at rt for 15 h, TLC (cHex/ETOAc 70:30, 1% Et₃N) showed the conversion of hemiacetal 16 into less polar products. The solution was concentrated to a minimum of solvent and purified by flash chromatography on neutralized silica gel (cHex/ETOAc 100:0 to 80:20, 1% Et₃N) to give trichloroacetimidate 17 (31.5 g, 84% over 4 steps) as an orange oil.

**Allyl (2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-(1→3)-4-O-benzyl-α-L-rhamnopyranoside** (24). Route 1 (1 g scale). Trimethyl orthoacetate (820 μL, 6.45 mmol, 1.9 equiv.) and monohydrated PTSA (10 mg, 0.05 mmol, 0.015 equiv.) were added to diol 5 (1.0 g, 3.4 mmol, 1.0 equiv.) in anhyd. MeCN (2.3 mL) at rt. After stirring at rt for 1 h, 80% aq. AcOH (2.3 mL) was added at 0 °C and the mixture was stirred at this temperature for 30 min. TLC (Tol/ETOAc, 70:30) showed total consumption of the intermediate orthoester. DCM was added along with water and the two layers were separated. The aq. phase was extracted with DCM and the combined organic phases were washed successively with sat. aq. NaHCO₃ and brine, dried over Na₂SO₄, filtered and concentrated to dryness to give crude acceptor 14. TIOH (300 μL, 3.4 mmol, 1.0 equiv.) was slowly added to a solution of the latter and trichloroacetimidate 21 (2.68 g, 3.91 mmol, 1.15 equiv.) in DCM (39 mL) containing DMF (5.26 mL, 68 mmol, 20 equiv.) and activated 4 Å MS (450 mg) at -78 °C. The suspension was stirred overnight while the temperature reached rt. TLC (Tol/ETOAc, 70:30) indicated acceptor consumption and the presence of less polar products. Et₃N (345 μL) was added and after 15 min the suspension was filtered over a pad of Celite™. Solids were washed thoroughly with DCM and the organic phase was washed with sat. aq. NaHCO₃, water and brine. The combined organic phases were dried over Na₂SO₄, and concentrated to dryness. The crude was solubilized in DCM/MeOH (11.8, 48 mL), 25% methanolic MeONa (1.16 mL, 5.1 mmol, 1.5 equiv.) was added and the solution was stirred overnight. Dowex H⁺ resin was added to the solution under gentle stirring until neutralisation. Filtration, concentration of the filtrate to dryness, and purification of the crude by flash chromatography (Tol/ETOAc, 90:10) gave the known α anomeric 24 (2.45 g, 88%).

Route 2. To a solution of rhamnoside 5 (1.0 g, 3.40 mmol, 1.0 equiv) in MeCN (2.0 mL) were added trimethyl orthoacetate (0.7 mL, 6.45 mmol, 1.9 equiv.) and monohydrated PTSA (10 mg, 0.05 mmol, 0.015 equiv.) at rt. The orange mixture was stirred at rt for 1.5 h, and 80% aq. AcOH (2.0 mL) was added at 0 °C. After stirring for 30 min at 0 °C and at rt for 1 h, TLC (cHex/ETOAc, 80:20) indicated total consumption of the intermediate orthoester. DCM and water were added and the two layers were separated. The aq. phase was...
extracted with DCM and the combined organic phases were washed successively with sat. aq. NaHCO₃ and brine, dried over Na₂SO₄, filtered and concentrated to dryness to give the crude acceptor 14. DMF (5.28 mL, 67.9 mmol, 20 equiv.) and activated 4 Å MS (0.5 g) were added to a mix of the latter and the PTFDA donor 22 (3.14 g, 4.42 mmol, 1.3 equiv.) in anhyd. DCM (44 mL) and the suspension was stirred at rt under Ar for 30 min, then at -78 °C for 15 min. TIOH (0.3 mL, 3.40 mmol, 1.0 equiv.) was added very slowly at -78 °C. The reaction mixture was then stirred for 1 h while slowly warming up to rt. TLC (cHex/EtOAc, 70:30) showed the complete disappearance of rhomnose 14 and the presence of less polar products. The reaction mixture was neutralized with Et₃N. EtOAc was added along with water and the two layers were separated. The aq. phase was extracted with EtOAc and the combined organic layers were washed successively with sat. aq. NaHCO₃ and brine, dried over Na₂SO₄, filtered and evaporated. MeONa (25% in MeOH, 1.17 mL, 5.1 mmol, 1.5 equiv.) was added to the obtained crude in DCM/MeOH (11.8, 50 mL). After stirring at rt overnight, TLC (Tol/EtOAc, 80:20) revealed that the glycosylation products had reacted and more polar products were present. DOWEX H⁺ resin was added and the mixture was stirred 30 minutes before filtering and washing thoroughly with MeOH. Et₃N (few drops) were added and volatiles were evaporated.

Purification by flash column chromatography (Tol/EtOAc, 100:0 to 90:10) gave the desired α anomer 24 as a pale yellow oil (2.2 g, 79% over 3 steps) and the commercially available hemiacetal 6 (345 mg). The expected 24 had Rf = 0.45 (cHex/EtOAc, 75:25). ¹H NMR (400 MHz, CDCl₃) δ 7.47 – 7.17 (m, 25H, H₂ Ar), 5.97 (m, 1H, CH=CH₂), 5.36 (dq, J = 17.2 Hz, 1.5 Hz, 1H, CH=CH₂), 5.27 (dd, J = 10.4 Hz, 1.6 Hz, 1H, CH=CH₂), 5.06 – 4.97 (m, 4H, H-1a, H-2a, 2H, 2H), 4.91 (d, J = 11.0 Hz, 1H, H-6a), 4.90 (d, J = 11.6 Hz, 1H, H-6a), 4.84 (d, J = 10.7 Hz, 1H, H-6a), 4.78 (d, J = 11.6 Hz, 1H, H-6a), 4.72 (d, J = 10.7 Hz, 1H, H-6a), 4.59 (d, J = 12.2 Hz, 1H, H-6a), 4.57 (d, J = 11.0 Hz, 1H, H-6b), 4.38 (d, J = 12.2 Hz, 1H, H-6b), 4.23 (ddt, J = 13.1 Hz, 5.5 Hz, 1.5 Hz, 1H, CH₃Ar), 4.14 (t, J = 9.3 Hz, 1H, H-3a), 4.12 (dd, J = 9.0 Hz, 3.2 Hz, 1H, H-3a), 4.08 – 4.00 (m, 3H, H-3a, H-4a, H-2a), 3.86 (dd, J = 9.6 Hz, 6.1 Hz, 1H, H-5a), 3.81 (t, J = 9.3 Hz, 1H, H-4a), 3.69 (dd, J = 9.6 Hz, 3.7 Hz, 1H, H-6a), 3.59 (t, J = 9.3 Hz, 1H, H-4a), 3.54 (dd, J = 11.0 Hz, 2.9 Hz, 1H, H-6a), 3.47 (brs, 1H, OH-2a), 3.43 (dd, J = 10.9 Hz, 2.0 Hz, 1H, H-6b), 1.45 (d, J = 6.3 Hz, 3H, H-6b). ¹³C NMR (100 MHz, CDCl₃) δ 138.7 – 137.6 (SC₆), 133.9 (CH=CH₂), 128.7 – 127.6 (25CH Ar), 117.3 (CH=CH₂), 98.3 (C-1a), 170.1 Hz), 94.0 (C-1a), 82.5 (C-3a), 39.4 (C-4a), 79.1 (C-2a), 77.9 (C-4a), 76.7 (C-3a), 75.6 (2CH₃Ben), 75.0 (CH₂=CH₂), 74.3 (CH₂=CH₂), 73.4 (CH₂=CH₂), 70.8 (C-5a), 68.0 (C-6a), 67.9 (CH₂=CH₂), 67.5 (C-2a), 67.3 (C-5a), 18.0 (C-6a). HRMS (ESI⁺): m/z 839.3749 (calcd for C₆₀H₅₅O₄Na [M+Na⁺]: m/z 839.3766).

6-O-Acetyl-2,3,4-tri-O-benzyl-α/β-o-glucopyranosyl (N-phenyl)trifluoroacetimidate (29). K₂CO₃ (0.53 g, 3.86 mmol, 2.0 equiv.) and (N-phenyl)trifluoroacetimidoyl chloride (0.46 mL, 2.89 mmol, 1.5 equiv.) were added to hemiacetal 27 (950 mg, 1.93 mmol, 1.0 equiv.) in acetone (20 mL) stirred at rt. The suspension was stirred at this temperature for 2 h. TLC (cHex/EtOAc, 80:20) revealed the conversion of the starting 27 into less polar products. The suspension was filtered over a pad of Celite®, generously washed with DCM, and volatiles were evaporated. Purification of the residue by flash column chromatography on silica gel (cHex/EtOAc, 100:0 to 95:5) gave (N-phenyl)trifluoroacetimidate 29 as a 1:1 mix of α/β anomers (1.1 g, 90%). An analytical sample was obtained by means of a second purification. Donor 29 had Rf = 0.6 (cHex/EtOAc, 90:10). ¹H NMR (400 MHz, CDCl₃) δ 7.44 – 7.21 (m, 17H, HAr), 7.12 (m, 1H, HAr), 6.78 – 6.73 (m, J = 7.7 Hz, 2H, HAr), 6.45 (brs, 1H, H-1), 5.04 (d, J = 10.8 Hz, 1H, H-1a), 4.92 (d, J = 10.8 Hz, 1H, H-1a), 4.85 (brs, 1H, H-1b), 4.82 (d, J = 10.8 Hz, 1H, H-1b), 4.38 – 4.25 (m, 2H, H-6), 4.08 (t, J = 9.3 Hz, 1H, H-3), 4.02 (m, 1H, H-5), 3.73 (dd, J = 9.3, 3.4 Hz, 1H, H-2), 3.61 (t, J = 9.5 Hz, 1H, H-2), 2.06 (s, 3H, CH₃Ar₂). ¹³C NMR (100 MHz, CDCl₃) δ 170.5 (CO₂Na), 143.5 – 137.6 (4αC₂), 128.7 – 127.7 (19CH Ar), 124.2 (CF₂), 119.4 (CH₂=CH₂), 81.5 (C-3), 79.3 (C-2), 77.4 (C-5), 75.3 (CH₂=CH₂), 73.4 (CH₂=CH₂), 71.5 (C-5S), 62.6 (C-6), 20.8 (CH₃Ar). (C and CN could not be detected due to relaxation issues). HRMS (ESI⁺): m/z 681.2778 (calcd for C₄₀H₂₆F₄N₂O₂ [M+H⁺]: m/z 681.2782).

6-O-Acetyl-2,3,4-tri-O-benzyl-α/β-o-glucopyranosyl (N-phenyl)trifluoroacetimidate (29). A mixture of Ac₂O/TFA (4:1, 30 mL) was added...
at 0 °C to hemiacetal 6 (2.5 g, 5.0 mmol, 1.0 equiv.) under Ar and the suspension was stirred at rt for 3 h, at which time TLC (cHex/EtOAc, 60:40) indicated conversion of the starting 6 into less polar products. Cold water (50 mL) was added at 0 °C and the mixture was stirred for 15 min at this temperature, then neutralized with 4 M aq. NaOH. EtOAc was added and the two layers were separated. The aq. layer was extracted with EtOAc and the combined organic phases were washed with brine and dried over Na2SO4. Volatiles were evaporated and MeONa (25% in MeOH, 5 mL) was added to the crude intermediate stirred in MeOH (20 mL) at rt. After stirring overnight at this temperature, TLC (cHex/EtOAc, 60:40) showed the complete disappearance of the intermediate and the presence of more polar products. Dowex H+ resin was added portionwise while the suspension was gently stirred until neutralisation. The suspension was filtered over a pad of Celite and concentrated in vacuo. The residue was extracted with diethyl ether and the combined organic phases were washed successively with sat. aq. NaHCO3, water and then with brine. The organic phase was washed with sat. aq. NaHCO3, brine and dried over Na2SO4. Volatiles were evaporated under reduced pressure, two successive purifications by flash column chromatography on silica gel (cHex/EtOAc, 100:0 to 95:5) gave hemiacetal 30 as a colorless oil (mostly α/β mixture, 1.7 g) contaminated with tert-butylidiphenylsilanol (10 mol%). Only the major isomer is described.

1H NMR (400 MHz, DMSO-d6) δ 7.85 – 7.09 (m, 25H, HAr), 7.21 – 7.11 (m, 5H, HAr), 7.14 – 7.04 (m, 1H, H-2α), 6.52 (t, J = 7.3 Hz, 1H, H-1a), 6.20 (brs, 0.89H, H-1b), 4.69 (d, J = 11.1 Hz, 1H, H-5a), 4.50 (d, J = 9.2 Hz, 1H, H-5b), 3.69 (m, 3H, H-2β), 2.11 (s, 3H, CH3), 1.94 (s, 3H, CH3), HRMS (ESI+): m/z 706.3548 (calcd for C43H62NO2Si [M+Na]+; m/z 706.3558).

2,3,4-Tri-O-benzyl-6-O-tert-butylidiphenylsilyl-,α-β-glucopyranosyl (N-phenyl)trifluoroacetimidate (32). K2CO3 (0.32 g, 2.32 mmol, 2.0 equiv.) and (N-phenyl)trifluoroacetimidoyl chloride (PTFAI, 0.28 mL, 1.74 mmol, 1.5 equiv.) were added to hemiacetal 30 (800 mg, 1.16 mmol, 1.0 equiv.) in acetonitrile (11.6 mL) at rt. After stirring at rt for 20 h, TLC (cHex/EtOAc, 90:10) indicated conversion of hemiacetal 30 into less polar products. The suspension was filtered over a pad of Celite and the solids were washed successively with sat. aq. NaHCO3, water and then with brine. The organic phase was dried on Na2SO4, filtered and concentrated in vacuo. The residue was then dissolved in DMF (40 mL) at rt. To this solution under Ar and the mixture was stirred overnight at rt, at which time more imidazole (2.0 mmol, 0.2 equiv.) and DMAP (3.4 g, 17 mmol, 0.4 equiv.) were added to alcohol 24 (34 g, 42 mmol, 1.0 equiv.) in anhyd. DCM (210 mL). The mixture was stirred at rt for 60 h, at which time TLC (Tol/EtOAc, 80:20) showed the full consumption of the starting 24 and the presence of a more polar product. The reaction mixture was diluted with water and DCM. The two layers were separated and the aq. phase was extracted with DCM repeatedly. The combined organic layers were washed successively with sat. aq. NaHCO3, water and finally brine. The organic layer was dried on Na2SO4, filtered and concentrated in vacuo. The residue was dissolved in DCM (3:1, 420 mL). The mixture was stirred and monitored by TLC until no starting 23 was detected. Volatiles were evaporated and the residue was extracted with EtOAc (35 mL) and the combined organic phases were washed successively with sat. aq. NaHCO3, water and then with brine. The organic phase was dried on Na2SO4, filtered and concentrated in vacuo. The residue was then dissolved in DCM (210 mL) at rt. To this solution under Ar and the mixture was gently stirred at 50 °C for 3 h. At this temperature, TLC (Tol/EtOAc, 80:20) showed conversion of the starting 23 into a less polar product. Iodine (10.7 g, 84 mmol, 2.0 equiv.) in THF (50 mL) was added slowly to the solution at rt. After stirring at this temperature for 2.5 h, TLC (Tol/EtOAc, 8:2) showed conversion of the intermediate into a more polar product. Sat. aq. NaHCO3 was added and the mixture was washed vigorously. The organic phase was washed with sat. aq. NaHCO3, water and brine. The organic phase was dried on Na2SO4, filtered and concentrated in vacuo. The residue was then dissolved in DCM (3:1, 420 mL). To this solution under Ar and the mixture was gently stirred at 50 °C for 3 h. After cooling to rt, TLC (Tol/EtOAc, 80:20) showed conversion of the intermediate into a less polar product.
Route 2. (1.6 g, 1.38 mmol, 1.0 equiv.) in pyridine (42 mL). The reaction mmol, 5.0 equiv.) were successively added at 0 °C to trisaccharide a white foam. The latter had R
(CH₃(OAc)(300 mL), dried on Na₂ΟSO₄ (750 µL, 4.12 mmol, 0.2 equiv.) was added very slowly at rt.
mL) and the suspension was stirred at rt, under Ar, for 30 min. TLC showed reaction completion. The solution was diluted with DCM (600 mL) and washed with water (300 mL). The aqueous phase was extracted with DCM (300 mL) and the combined organic phases were washed with brine (300 mL), dried over sodium sulfate, filtered and concentrated under vacuum. The crude product was purified by flash column chromatography (Tol/EtOAc, 80:20) to give oil of elution the remaining 2 (208 mg, 13%) and the acceptor 36 (1.08 g, 74%) as a white foam.

Allyl (2,3,4,6-tetra-O-benzyl-α-L-glucopyranosyl)-(1→3)-(4-O-benzyl-2-O-leuvinoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(4-O-acetyl-2-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(4,6-benzylidene-2-deoxy-2-trichloroacetamidob-β-D-glucopyranoside (37). Hydrazine hydrate (50 µL, 1.0 mmol, 2 equiv.) was added to the pentasaccharide 1 (1.0 g, 0.52 mmol) in pyridine/AcOH (3:2, 10 mL) and the solution was stirred at rt under Ar for 30 min. TLC showed complete disappearance of the starting material into a less polar compound. Water (100 mL) and EtOAc (100 mL) were added and the phases were separated. The aqueous layer was extracted with EtOAc (50 mL twice) and the combined organic layers were washed with sat. aq. NaHCO₃ and brine, then dried on Na₂SO₄, filtered and concentrated under reduced pressure. Flash chromatography of the crude residue (Tol/EtOAc, 90:10 to 50:50) gave alcohol 37 (865 mg, 92%) as a white foam.

Route 2. Acetic acid (10.5 mL) and ethylenediamine (583 µL, 5.2 mmol, 1.0 equiv.) were successively added at 0 °C to pentasaccharide 1 (1.0 g, 520 µmol, 1.0 equiv.) in pyridine (15.7 mL). The reaction mixture was heated to 70 °C and for 24 h. At this time, a ¹H NMR control showed reaction completion. The solution was diluted with DCM (400 mL) and washed with water (200 mL). The aqueous phase was extracted with DCM (200 mL) and the combined organic phases were washed with brine (300 mL), dried over sodium sulfate, filtered and concentrated under vacuum. The crude product was purified by flash column chromatography (Tol/EtOAc, 80:20) to give pentasaccharide 37 (841 mg, 89%) as a white foam. The latter had Rₘ 0.3 (Tol/EtOAc, 70:30). ¹H NMR (400 MHz, CDCl₃) δ 7.52 – 7.12 (m, 45H, H₂), 7.02 (d, J = 7.4 Hz, 1H, NHCO), 5.93 – 5.82 (m, 1H, CH₃(OAc)), 5.56 – 5.18 (m, 2H, CH₂(C₆H₅)), 5.16 (dd, J = 3.3, 1.9 Hz, 1H, H-5'), 5.13 (brs, 1H, H-4'), 5.12 (d, J = 8.5 Hz, 1H, H-1'), 4.99 – 4.81 (m, 8H, H-1a, H-1b, H-1c, H-1d), 4.78 – 4.28 (m, 14H, H-3a, H-6b, CH₂(OAc), 1Hb), 4.14 – 4.07 (m, 1H, CH₃(OAc)), 4.07 – 3.91 (m, 2H, H-7, H-3c, H-2a, H-3a, H-3b, H-2b, H-3c, H-5'), 3.86 – 3.55 (m, 8H, H-5a, H-5b, H-5c, H-5d, H-5e, H-5f, H-5g), 3.64 – 3.36 (m, 3H, H-7, H-3d, H-4b, H-6a, H-6b), 3.28 (t, J = 9.5 Hz, 1H, H-4d), 2.06 (s, 3H, OCO₂CH₃), 1.68 (bs, 1H, OH), 1.26 (d, J = 6.2 Hz, 3H, H-6b), 1.25 (d, J = 6.2 Hz, 3H, H-6b), 0.73 (d, J = 6.2 Hz, 3H, H-6b). ¹C NMR (100 MHz, CDCl₃) δ 169.9 (OCO₂CH₃), 162.3 (CONH), 133.4 (CH=CH₂), 138.7 – 137.1 (C₆H₅), 129.2 – 126.6 (45CH₃), 118.5 (CH₂(C₆H₅)), 102.1 (C₆H₅), 101.5 (C-1a, JCH = 170.7 Hz), 100.8 (C-1c, JCH = 173.8 Hz), 98.3 (C-1b, JCH = 168.9 Hz), 97.6 (C-1c, JCH = 173.5 Hz), 94.1 (C-1a, JCH = 170.2 Hz), 92.2 (C₆H₅), 82.6 (C-3c), 80.4 (C-4a), 80.3 (C-4a), 80.1 (C-4a), 79.8 (C-4a), 79.4 (C-3a), 79.0 (C-2a), 78.5 (C-3c), 77.9 (C-4c), 76.6 (C-2a), 75.8

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(2C, CH₂CO₂H), 75.4 (CH₂CO₂H), 75.3 (CH₂CO₂H), 75.1 (CH₂CO₂H), 75.0 (C-3a), 74.6 (CH₂CO₂H), 74.0 (C-3a), 73.6 (CH₂CO₂H), 72.5 (CH₂CO₂H), 72.3 (C-2), 71.1 (CH₂), 70.9 (C-5a), 69.1 (C-5a), 68.9 (C-6a), 68.1 (C-5c), 68.0 (C-6b), 67.9 (C-5c), 67.4 (C-4a), 66.4 (C-5a), 60.4 (C-3a), 21.2 (OOCCH₃), 18.0 (C-2c, C-6a), 17.4 (C-6c), HRMS (ESI^+): m/z 1813.6810 (calcd for C₁₀H₁₃ClO₂N₂O₂H₄ [M+NH₄]^+: m/z 1813.6822.

(2,3,4,6-Tetra-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(4-O-benzyl-2-O-levulinyl-α-L-rhamnopyranosyl)-(1→2)-[3,4-di-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranose (38), 1.5-Cyclooctadiene-

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2-Azidoethyl (2,3,4,6-tetra-O-benzyl-a-L-glucopyranosyl)-(1→3)-(4-O-benzyl-a-L-rhamnopyranosyl)-(1→3)-(2-O-acetyl-4-O-benzyl-a-L-rhamnopyranosyl)-(1→3)-(4-O-benzylidene-2-deoxy-2-R-trichloroacetamido-β-D-glucopyranoside) (41). To a solution of 2-azidoethanol (68 mL, 897 µmol, 3.1 equiv.) and glycosyl donor 39 (600 mg, 293 µmol, 1.0 equiv.) in anhyd. toluene (2.9 mL) was added activated 4 Å MS (470 mg) and the suspension was stirred at rt under an argon atmosphere for 15 min, then for 10 min at -50 °C. TMSOTf (21 µL, 0.12 mmol, 0.2 equiv.) was added rapidly at -50 °C. The reaction mixture was stirred at -40 °C for 30 min, at which time a TLC (cHex/EtOAc, 70:30) follow up showed consumption of the donor. Et3N was added at -40 °C and the suspension was stirred for another 10 min. Solids were filtered over a pad of Celite and washed generously with DCM. The combined filtrates were concentrated to dryness and the residue was purified by flash column chromatography (cHex/EtOAc, 100:50 to 50:50) to give decasarccarhide 40 (184 mg, 91%) as a white foam. The latter had Rf = 0.3 (cHex/EtOAc, 70:30). 1H NMR (400 MHz, CDCl3) δ 7.50 – 7.02 (m, 9H, H2A, H2B) 6.94 (d, J = 9.0 Hz, 1H, H2C), 5.94 – 5.83 (m, 1H, C2H=CH2), 5.56 (s, 1H, H2D) 5.53 (dd, J = 3.0, 1.9 Hz, 1H, H2E), 5.33 – 5.20 (m, 4H, H3A, H3B, C2H=CH2), 5.01 (brs, 1H, H3D), 4.95 (brs, 1H, H3D), 4.94 – 4.78 (m, 9H, 1H, H3C), 4.78 – 4.66 (m, 20H, CH2CONa). 13C NMR (100 MHz, CDCl3) δ 206.3 (COOC2H5), 171.8 (COOC2H5), 170.0, 169.6 (2C, COO2H5), 163.2, 162.0 (2C, COOC2H5), 139.0 – 137.1 (18C, Na+, Cl−), 133.4 (CH2CONa), 129.5 – 126.5 (90C, CH2), 118.5 (CH2CONa), 102.2, 101.8 (2C, Na+, Cl−), 101.4 (C-1B, JCH = 171.4 Hz), 101.2 (C-1B, JCH = 164.6 Hz), 100.8 (C-1C, JCH = 176.9 Hz), 100.6 (C-1C, JCH = 170.7 Hz), 99.1 (C-1C, JCH = 173.8 Hz), 98.4 (C-1C, JCH = 167.0 Hz), 97.7 (2C, C-2C, C1C, JCH = 173.0 Hz), 97.4 (C-1C, JCH = 168.2 Hz), 93.1 (2C, C-1C, JCH = 170.2 Hz), 93.2 (CCH2CONa), 92.3 (CCH2), 83.3 (C-3C), 82.3 (C-3C), 80.7 (C-4C), 80.6 (C-4C), 80.4 (C-4C), 80.3 (C-4C), 80.1 (2C, C-4C, C-4C), 80.0 (C-4C), 79.9 (C-4a), 79.7 (C-4a), 79.6 (C-4C), 79.3 (C-4a), 78.8 (C-4a), 78.5 (C-3C), 77.9 (C-3C), 77.7 (C-3C), 77.4 (C-3C), 76.9 (C-3C), 76.4 (C-2C), 76.3 (CH2CONa), 76.1 (CH2CONa), 75.7 (CH2CONa), 75.5 (CH2CONa), 75.2 (CH2CONa), 75.1 (CH2CONa), 75.0 (CH2CONa), 74.7 (C-3C), 74.3 (C-3C), 74.0 (C-3C), 73.7 (C-2C), 73.5 (CH2CONa), 73.4 (C-2C), 72.9 (C-2C), 72.6 (C-2C), 72.5 (C-2C), 72.3 (C-2C), 72.1 (CH2CONa), 71.0 (C-2C), 70.4 (C-2C), 70.2 (C-2C), 69.1 (C-5c), 69.2 (C-5c), 69.0 (C-5c), 68.9 (C-5c), 68.8 (C-5c), 68.5 (C-5c), 68.3 (C-5c), 68.2 (C-6c), 68.1 (C-6c), 68.0 (C-6c), 67.9 (C-6c), 66.4 (C-6c), 66.1 (C-5c), 65.1 (C-5c), 65.0 (C-5c), 57.7 (C-2C), 53.1 (CH2CONa), 29.9 (CH2CONa), 28.3 (CH2CONa), 21.2 (OCOC2H5), 21.1 (OCOC2H5), 18.1 – 18.0 (4C, CH3-6c, CH3-6c, CH3-6c, CH3-6c). HRMS (EI*): m/z 1854.1809 (calcd for C230H224Cl2NaO45[M+2Na]2+: m/z 1854.1814).
crude (To/EtOAc, 90:10 to 70:30) gave alcohol 42 (589 mg, 92%) as a white foam. The latter had Rf = 0.3 (cHex/EtOAc, 70:30). 1H NMR (400 MHz, CDCl3) δ 7.52 – 7.06 (m, 5CH, 6H), 5.56 (s, 1H, H-6b), 5.17 (d, J = 3.5 Hz, 1H, H-1c), 5.16 (dd, J = 3.3, 1.9 Hz, 1H, A-2c), 5.13 (brs, 1H, A-1b), 4.97 (d, J = 1.9 Hz, 1H, H-4c), 4.89 – 4.81 (m, 7H, H-5c, 4c, 3c, 2c, 1c, H-1b), 4.78 – 4.28 (m, 13H, H-3a, H-6b, 11H), 4.09 – 3.91 (m, 8H, H-2a, H-3a, 4H, OCH2CH2NCH2CH2CH3), 3.87 – 3.56 (m, 5H, H-5a, H-4a, 2H, H-6a, OCH2CH2NCH2CH2CH3, 5H, H-6a, H-5b, H-6b), 3.54 – 3.33 (m, 3H, H-3g, H-4g, 2H, H-6a, 6b, 2H, CH2NH3), 3.28 (t brs, J = 9.5 Hz, 1H, H-1e), 1.68 (brs, 1H, OH), 1.26 (d, J = 6.2 Hz, 3H, 3c-6c, 2H), 1.25 (d, J = 6.2 Hz, 3H, 3c-6c, 2H), 0.70 (d, J = 6.2 Hz, 3H, 3c-6c, 2H). 13C NMR (100 MHz, CDCl3) δ 169.0 (C-1), 153.6 (C-2), 146.2 (C-3), 142.5 (C-4), 136.8 (C-5), 131.8 (C-6a), 129.2 – 127.6 (45CH2), 102.1 (C-5g), 101.4 (C-1b, ICN = 170.7 Hz), 100.1 (C-1c, ICN = 173.8 Hz), 99.1 (C-1b, ICN = 168.9 Hz, 97.5 (C-1c, ICN = 173.5 Hz, 94.0 (C-1b, ICN = 170.2 Hz, 92.9 (C-1c), 92.7 (C-4a), 82.5 (C-3a), 80.4 (C-4a), 80.2 (C-4a), 80.0 (C-4a), 79.7 (C-4a), 79.3 (C-3a), 79.0 (C-2a), 78.4 (C-3a), 77.8 (C-2a), 76.5 (C-2a), 75.7 (C2, CH2=CH2), 75.3 (CH2), 72.4 (CH2=CH2), 72.0 (CH2=CH2), 72.2 (CH2=CH2), 70.8 (C-3c), 69.1 (C-3a), 69.0 (OCH2CH2NCH2CH2CH3), 68.7 (C-6a), 68.1 (C-6a), 68.0 (C-6a), 67.9 (C-5a), 67.4 (C-5a), 66.4 (C-6a), 60.4 (C-2c), 59.0 (OCH2CH2NCH2CH2CH3), 21.1 (OCOCH3), 18.0 (2C, C-6c, C-6a), 17.3 (C-6c). HRMS (ESI+): m/z 1856.6378 (calcd for C50H52Cl2N4O4Na [M+Na]+: m/z 1856.6359).

Azidoethyl (2,3,4,6-tetra-O-benzyl-α-ᴅ-β-glucopyranosyl)-(1→3)-(4-O-benzyl-2-O-levulinoyl-α-ᴅ-l-rhamnopyranosyl)-(1→2)-(3,4-di-O-benzyl-α-ᴅ-l-rhamnopyranosyl)-(1→3)-(2-O-acetyl-4-O-benzyl-α-ᴅ-glucopyranosyl)-(1→3)-(4,6-di-O-benzylidine-deoxy-2-trichloroacetamido-β-ᴅ-glucopyranosyl)-(1→2)-(2,3,4,6-tetra-O-benzyl-α-ᴅ-glucopyranosyl)-(1→3)-(2,3,4,6-tetra-O-benzyl-α-ᴅ-glucopyranosyl)-(1→3)-(2,4-di-O-benzyl-α-ᴅ-l-rhamnopyranosyl)-(1→3)-(2,3,4,6-tetra-O-benzyl-α-ᴅ-glucopyranosyl)-(1→3)-(2,3,4,6-tetra-O-benzyl-α-ᴅ-glucopyranosyl)-(1→3)-(2,4-di-O-benzyl-α-ᴅ-l-rhamnopyranosyl)-(1→3)-(2,3,4,6-tetra-O-benzyl-α-ᴅ-glucopyranosyl)-(1→3)-(2,3,4,6-tetra-O-benzyl-α-ᴅ-glucopyranosyl)-(1→3)-(2,4-di-O-benzyl-α-ᴅ-l-rhamnopyranosyl)-(1→3)-(2,3,4,6-tetra-O-benzyl-α-ᴅ-glucopyranosyl)-(1→3)-(2,4-di-O-benzyl-α-ᴅ-l-rhamnopyranosyl)-(1→3)-(2,3,4,6-tetra-O-benzyl-α-ᴅ-glucopyranosyl)-(1→3)-(2,4-di-O-benzyl-α-ᴅ-l-rhamnopyranosyl)-(1→3)-(2,3,4,6-tetra-O-benzyl-α-ᴅ-glucopyranosyl)-(1→3)-(2,4-di-O-benzyl-α-ᴅ-l-rhamnopyranosyl)-(1→3)-(2,3,4,6-tetra-O-benzyl-α-ᴅ-glucopyranosyl)-(1→3)-(2,4-di-O-benzyl-α-ᴅ-l-rhamnopyranosyl)-(1→3)-(2,3,4,6-tetra-O-benzyl-α-ᴅ-glucopyranosyl)-(1→3)-(2,3,4,6-tetra-O-benzyl-α-ᴅ-glucopyranosyl)-(1→3)-(2,4-di-O-benzyl-α-ᴅ-l-rhamnopyranosyl)-(1→3)-(2,3,4,6-tetra-O-benzyl-α-ᴅ-glucopyranosyl)-(1→3)-(2,4-di-O-benzyl-α-ᴅ-l-rhamnopyranosyl).
H-3Jv, H-5cv, H-2cv, H-3cv, H-2av, H-5cv, H-3cv, H-6av, H-5av, H-3av, H-5av, H-2av, H-3av, H-2av, H-6av, OCH₂CH₂N₃, H-3av, H-5av, H-2av, H-3av, BuOH/DCM/H₂O (1→2)-α-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-(2-O-acetyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-[α-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-(2-O-acetyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranoside (46). Alcohol 44 (50 mg, 14 μmol) was dissolved in tBuOH/DCM/H₂O (7:2:1, 5 mL) and the solution was degassed repeatedly. 20 wt. % Pd(OH)₂/C (50 mg) was added and the suspension was stirred vigorously overnight under a hydrogen atmosphere. After 12 h, analytical RP-HPLC indicated the presence of several products corresponding to diversely N-chloroacetylated analogues of the desired 46. Et₃N (4 equiv.) was added, the suspension was centrifuged and the supernatant was passed through a PVDF membrane (0.2 μm). The procedure was repeated three times. The combined filtrates were freeze-dried. The residue obtained was dissolved in tBuOH/DCM/H₂O (1:4, 5.0 mL) and centrifuged (5000 min⁻¹). The supernatant was passed through a PVDF membrane (0.2 μm). This was repeated three times. The combined filtrates were concentrated by freeze-drying. The residue was dissolved in 0.5 mL H₂O and passed through a Sep-Pak C18 cartridge eluting first with 0.08% acq. TFA then with 20% MeCN in 0.08% acq. TFA. The suitable fractions were pooled, freeze-dried and the residue was purified by RP-HPLC to give pentasaccharide 45 (85 mg, 70%) as a white powder. The linker-equipped 45 had RP-HPLC (λ = 215 nm): tₚ = 10.46 min. ¹H NMR (400 MHz, D₂O) δ 6.15 (brs, 1H, H-1a), 5.09 (d, J = 3.9 Hz, H-1b), 5.02 - 4.97 (m, 2H, H-2c, H-1c), 4.87 (brs, 1H, H-1c), 4.55 (d, d = 8.5 Hz, 1H, H-1d), 4.26 (tₚ₉ = 2.4 Hz, 1H, H-2d), 4.12 - 3.99 (m, 3H, H-5c, H-2e, OCH₃CH₂NH₂), 3.98 - 3.86 (m, 4H, H-5c, H-3d, H-5d, OCH₃CH₂NH₂), 3.68 - 3.68 (m, 8H, H-3d, H-2e, H-3e, H-6a, H-6b, H-6a, H-5a, H-3a, 3.63 - 3.41 (m, 9H, H-9, H-2c, H-4c, H-5a, H-4a, H-3a, H-4a, H-4b, H-6b, H-5b, 3.26 - 3.13 (m, 2H, CH₂N₃H₂), 2.16 (s, 3H, H₃N), 2.05 (s, 3H, H₃N), 1.30 - 1.21 (m, 9H, H-6c, H-6c, H-6c, 11C NMR (100 MHz, D₂O) δ 177.4 (COO₂), 175.6 (COO₂), 104.6 (C-1a, JCH = 173.4 Hz), 103.7 (C-1b, JCH = 172.4 Hz), 103.1 (C-1c, JCH = 162.7 Hz), 101.2 (C-1d, JCH = 173.5 Hz), 98.0 (C-1e, JCH = 170.3 Hz), 84.9 (C-3a), 80.8 (C-2a), 78.8 (C-3b), 78.6 (C-5b), 77.9 (C-3c), 75.6 (C-3d), 74.9 (C-2b), 74.5 (C-4e), 74.3 (C-2c, C-4c), 74.1 (C-5c), 72.4 (C-4b), 72.6 (C-3c), 72.1 (2C, C-5c, C-5a), 71.9 (C-4c), 71.6 (C-5c), 70.9 (C-4b), 69.3 (C-2b), 68.3 (OCH₃CH₂NH₂), 63.3 (C-6b), 63.0 (C-2c), 57.7 (C-2d), 42.1 (OCH₃NH₂), 24.9 (N₃C), 22.9 (C₂), 19.4 (C-6c), 19.3 (C-6b), 18.9 (C-6c). HRMS (ESI): m/z 390.3611 (calcld for C₉H₁₄N₂O₉Na [M+Na]⁺; m/z 390.3624).
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Conflicts of interest
There are no conflicts to declare.

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