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A convergent chemoenzymatic strategy to deliver a diversity of *Shigella flexneri* serotype-specific O-antigen segments from a unique lightly protected tetrasaccharide core

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ABSTRACT

Progress in glycoscience is strongly dependent on the availability of broadly diverse tailored-made, well-defined and often complex oligosaccharides. Herein, going beyond natural resources and aiming to circumvent chemical boundaries in glycochemistry, we tackle the development of an *in vitro* chemoenzymatic strategy holding great potential to answer the need for molecular diversity characterizing microbial cell-surface carbohydrates. The concept is exemplified in the context of *Shigella flexneri*, a major cause of diarrheal disease. Aiming at a broad serotype coverage *S. flexneri* glycoconjugate vaccine, a non-natural lightly protected tetrasaccharide was designed for compatibility with (i) serotype-specific glucosylations and O-acetylations defining *S. flexneri* O-antigens, (ii) recognition by suitable α -transglucosylases, and (iii) programmed oligomerization post enzymatic α -D-glucosylation. The tetrasaccharide core was chemically synthesized from two crystalline monosaccharide precursors. Six α -transglucosylases found in the Glycoside Hydrolase family 70 were shown to transfer glucosyl residues on the non-natural acceptor. The successful proof-of-concept is achieved for a pentasaccharide featuring the glucosylation pattern from the *S. flexneri* type IV O-antigen. It demonstrates the potential of appropriately planned chemo-enzymatic pathways involving non-natural acceptors and low-cost donor/transglucosylase systems to achieve the demanding regioselective α -D-glucosylation of large substrates, paving the way to microbial oligosaccharides of vaccinal interest.

INTRODUCTION

Shigella are Gram negative enteroinvasive bacteria frequently associated with diarrhoeal disease. They are responsible for shigellosis, or bacillary dysentery, and represent a significant cause of mortality and morbidity worldwide.¹ Recent estimates emphasize the importance of shigellosis burden in the pediatric populations living in low and middle income countries.²⁻³ They also point to *Shigella* as being the second leading cause of diarrhoeal mortality among all ages.⁴ Disease burden combined to increasing antibiotic-resistance call for a vaccine ensuring protection against *Shigella*,⁵⁻⁶ and in particular against the numerous *S. flexneri* serotypes identified amongst the prevalent isolates found in *Shigella* infection.⁷ *Shigella* lipopolysaccharides (LPSs), and in particular the O-antigen (O-Ag) moieties, are prime targets for the host's protective immunity against infection, and serum IgG antibodies to *Shigella* LPS have emerged as a correlate of protection against shigellosis.⁸ Over the past decades, conjugates derived from *Shigella* surface polysaccharides have been extensively investigated as parenteral vaccines.⁹ Synthetic carbohydrate conjugates encompassing well-defined oligosaccharides acting as functional O-Ag surrogates are actively explored as an attractive option.¹⁰⁻¹² Proposed by L.A. Mulard and collaborators against *S. flexneri* 2a,¹³⁻¹⁴ the most advanced vaccine candidate in this category – SF2a-TT15 – was found safe and immunogenic in young naïve adults in a phase I clinical trial.¹⁵ To answer the need in the field,⁷ investigation towards broadening *S. flexneri* serotype coverage is ongoing. For that purpose, synthetic routes for straightforward access to well-defined O-Ag fragments featuring serotype-specific substitutions are essential.¹⁶⁻¹⁸ However, the multiple *S. flexneri* types and subtypes identified so far have revealed a tremendous O-Ag structural diversity built from a unique core.¹⁹ Indeed, while most *S. flexneri* O-Ags share the same linear backbone composed of 1,2-*trans* linked L-rhamnose (A, B, C) and *N*-acetyl-D-glucosamine (D) residues, the repeating units of the different serotype-specific O-Ags differ by their α -D-glucosylation (E) pattern, as well as by their O-acetyl and phosphoethanolamine substitutions (Figure 1). One main synthetic roadblock resides in the regio- and stereo-specific control of the 1,2-*cis* glucosylation step as concluded from the numerous reports on the chemical synthesis of oligosaccharides representative of *S. flexneri* O-Ag glucosylation patterns. Early attempts at the chemical grafting of the α -D-glucosyl residue on protected di-, tri-, and tetrasaccharide acceptors met issues,²⁰ resulting in tedious purifications and poor yields of the α -linked glucosylated products. As a general trend in subsequent work, the glucosyl residue was introduced first to form a disaccharide building block featuring the branching point.^{13, 17-18, 21} While this strategy remains in use,²² *S. flexneri* target diversity and complexity call for improvements. However, although several stereoselective methods for the chemical synthesis of 1,2-*cis* glycosides were reported over the last decade,²³⁻²⁹ there is still no general strategy enabling versatile 1,2-*cis* glucosylation, especially when considering complex acceptors.

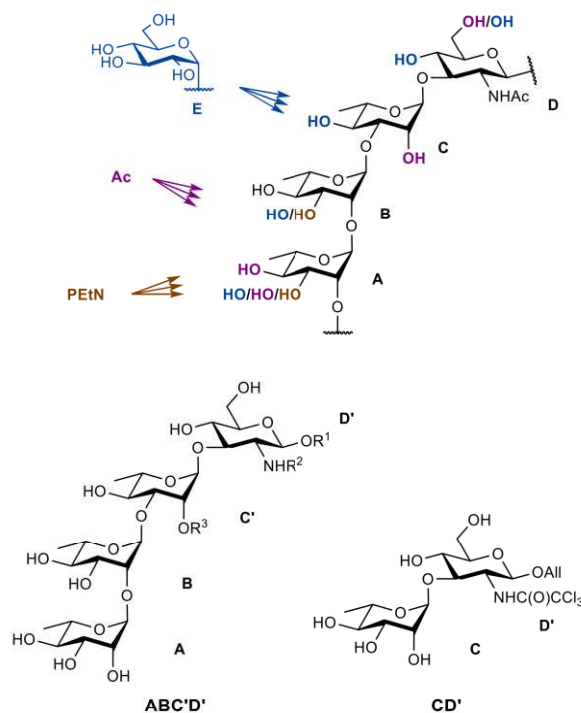


Figure 1: *Top:* The linear ABCD tetrasaccharide featuring the core of the O-Ag repeating units characterizing all *S. flexneri* serotypes and subtypes, except *S. flexneri* type 6. Type-specific substitutions include α -D-glucopyranosyl residue (E, blue), stoichiometric and/or non-stoichiometric O-acetyl groups (Ac, purple) and phosphoethanolamine residue (PEtN, brown). *Bottom left:* ABC'D', the lightly protected ABCD-like tetrasaccharide showing the generic protection pattern. All hydroxyl groups are identified as potential sites of enzymatic glucosylation with modification at 3_A, 3_B, 4_C, 4_D and 6_D featuring *S. flexneri* type-specific branching points. R¹, R², R³: protecting groups. *Bottom right:* the CD' disaccharide previously used as acceptor in the chemoenzymatic synthesis of a *S. flexneri* 2a 10mer and 15mer oligosaccharides.³⁰

The chemoenzymatic synthesis of complex oligosaccharides has found wide interest over the past decades.³¹⁻³⁵ In recent years, the use of synthetic core intermediates and their modification upon action of a range of glycosyltransferases has contributed to tremendous progress in the field. In this context, achievement from the Boons' group on the synthesis of libraries of asymmetrical multi-antennary N-glycans based on a scaffold compatible with customized modifications³⁶ has paved the way to novel developments. Obviously, the feasibility of protecting group-controlled enzymatic glycosylation by use of partially protected acceptors represents a powerful strategy to guide glycodiversification from a core precursor.³⁷⁻³⁹ As part of this ongoing trend supporting innovation in the field of chemoenzymatic synthesis, we have explored in recent years the potential of chemical synthesis and regioselective enzymatic α -D-glucosylation working in synergy to access various *S. flexneri* serotype-specific carbohydrate precursors^{30, 40} as a way to overcome the poor α/β stereoselectivity of the chemical glucosylation process.²³ Undeniably, the successful chemoenzymatic synthesis of the 15mer oligosaccharide hapten involved in SF2a-TT15 from a chemically synthesized disaccharide precursor³⁰ has paved the way to novel challenges. However and of special concern, these elegant and promising strategies still required intensive chemistry efforts as distinct synthetic routes have to be developed depending on the targeted *S. flexneri* serotype.

1 With the aim of alleviating efforts and offering novel and easy-to-implement synthetic alternatives, we
2 came up with the idea of developing a highly convergent chemoenzymatic strategy opening the way to
3 a large panel of *S. flexneri* type-specific oligosaccharides. Taking advantage of the unique structure of
4 the ABCD tetrasaccharide defining the backbone repeating unit of most *S. flexneri* O-Ags (Figure 1),
5 the strategy is centered on the use of a common non-natural ABCD-like tetrasaccharide precursor. The
6 latter is designed to be compatible with a regioselective ~~site-selective~~ enzymatic 1,2-*cis* glucosylation
7 and subsequent chain elongation, allowing to deliver a portfolio of well-defined modular building blocks
8 that could then be easily assembled into *S. flexneri* O-Ag ~~mimics~~-surrogates relevant for vaccine design.
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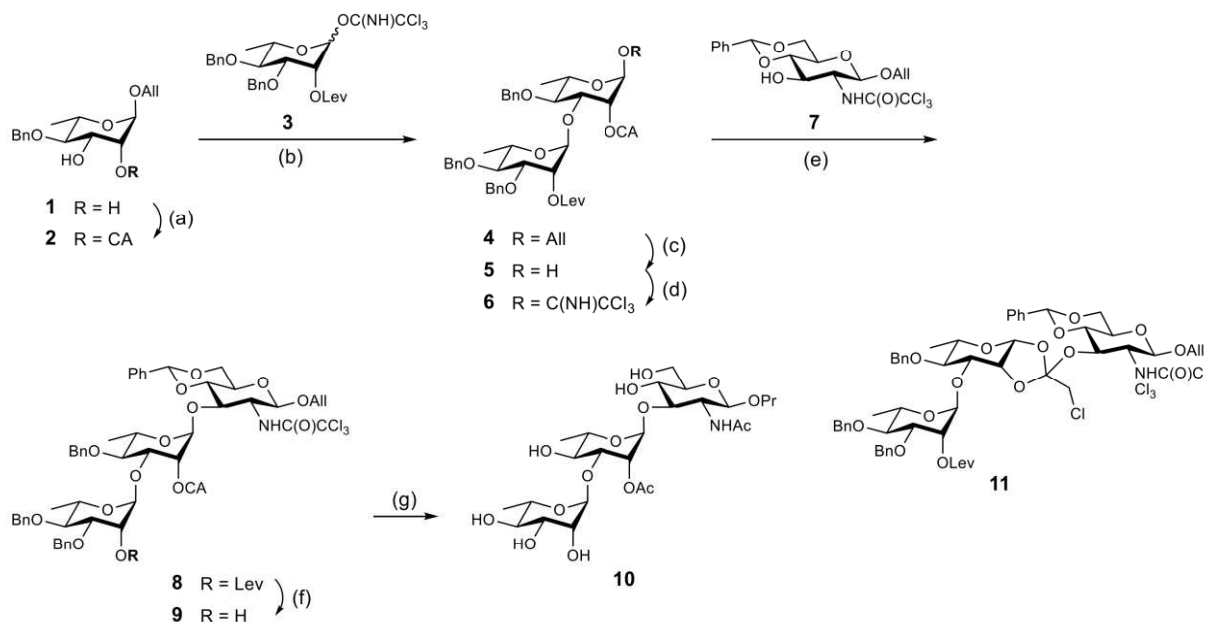
10 Herein, we present the design and chemical synthesis of ABC'D', an original lightly protected ABCD-
11 like tetrasaccharide (Figure 1), and its subsequent glucosylation using highly promiscuous sucrose-
12 active α -transglucosylases found in the Glycoside Hydrolase family 70.⁴¹ This unprecedented concept,
13 which avoids the use of poorly accessible glucosyltransferases, in particular those predicted to
14 encompass multiple transmembrane regions such as non-Leloir glucosyltransferases found in *S.*
15 *flexneri*,⁴² and complex nucleotide- or polyprenol phosphate-activated donors, is exemplified in this
16 study to produce a pentasaccharide specific for *S. flexneri* serotypes 4a/4b.
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29 RESULTS AND DISCUSSION

30 Design and chemical synthesis of tetrasaccharide **12** (ABC'D')

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32 Built from the ABCD backbone repeating unit common to all except one *S. flexneri* O-Ags identified to
33 date, tetrasaccharide ABC'D' was designed for compatibility with enzymatic α -D-glucosylation at all
34 possible naturally occurring glucosylation sites encountered on *S. flexneri* O-Ags (Figure 1) and with
35 inherent O-Ag O-acetylation in addition to feasibility of subsequent chemical chain elongation of the
36 products of enzymatic glucosylation at both ends. Biocatalyst compatibility imposed minimal
37 protection. On that basis, the selected ABC'D' – tetrasaccharide **12** – was synthesized as an allyl
38 glycoside encompassing a 2_D-trichloroacetamide (TCA) moiety to ensure anchimeric assistance during
39 chain elongation and recovery of the 2_D-acetamide upon final Pd/C-mediated hydrodechlorination.¹⁸
40 This protecting group combination was found appropriate to achieve the chemoenzymatic conversion
41 of a CD' disaccharide (Figure 1) into a *S. flexneri* 2a 10mer and 15mer haptens.³⁰ Owing to the 2_C-O-
42 acetylation found in the O-Ag of some of the most prevalent *S. flexneri* serotypes, for example *S. flexneri*
43 1b and 3a,⁴³ the 2_C-OH in tetrasaccharide **12** is masked by a chloroacetyl group (CA). Advantageously,
44 the CA moiety provides a direct access to a 2_C-acetate as demonstrated herein on the model trisaccharide
45 **10** or to a 2_C-hydroxyl group upon cleavage, as required.
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57 **Scheme 1: Synthesis of Trisaccharide **9** and Pd/C-mediated Hydrodechlorination Thereof to Give the BCD**
58 **trisaccharide **10**.**
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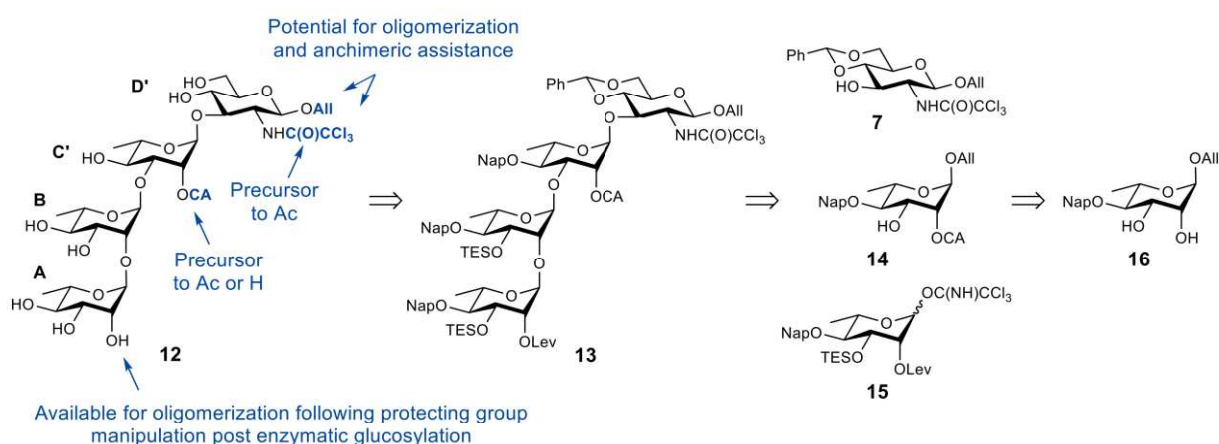


Reaction conditions: (a) (i) (MeO)₃CCH₂Cl, PTSA, CH₃CN; (ii) 80% aq. AcOH, 95% for 2 steps; (b) TMSOTf, Et₂O, -20 °C, 91%; (c) (i) [Ir], THF, (ii) I₂, THF/H₂O, 83% for 2 steps; (d) Cl₃CCN, DBU, DCE, 96%; (e) TMSOTf, DCM, -15 °C, 62%; (f) H₂NNH₂, AcOH, pyridine, 86%; (g) H₂, Pd/C, 96% aq. EtOH/DCE/Phosphate buffer, 47%. [Ir] = [Ir(THF)₂(PMePh₂)₂]PF₆, DBU: 1,8-Diazabicyclo[5.4.0]undec-7-ene, DCE: 1,2-dichloroethane, DCM: dichloromethane, PTSA: *para*-toluenesulfonic acid, THF: tetrahydrofuran.

Trisaccharide **9** features a BCD segment closely resembling the selected ABC'D' acceptor. Besides benzyl ethers at O-4_C, O-3_B and O-4_B, it bears all protecting groups making the glucosyl acceptor **12** unique *per se*. It was synthesized from two easily accessible crystalline precursors, the rhamnoside diol **1**,⁴⁴ itself evolved into trichloroacetimidate **3**,¹⁶ and the glucosaminide acceptor **7**¹⁸ (Scheme 1). Regioselective chloroacetylation at the axial 2-OH of diol **1** by means of the acid-mediated opening of the orthoester formed by reaction with 2-chloro-1,1,1-trimethoxyethane was high yielding.⁴⁵ Glycosylation of acceptor **2** with rhamnosyl donor **3** gave disaccharide **4**, then converted to trichloroacetimidate **6** by means of hemiacetal **5**. Trimethylsilyl trifluoromethanesulfonate (TMSOTf)-mediated glycosylation of acceptor **7** with donor **6** gave the fully protected trisaccharide **8** together with orthoester **11** (27%) as evidenced from the signal typical of an orthoester quaternary carbon at 120.6 ppm in the ¹³C NMR spectrum of this side-product. The engagement of a chloroacetate masking position 2 of a glycosyl donor in the formation of intersaccharidic orthoesters is not without precedent,⁴⁶ and has occasionally been exploited to differentiate between ester protecting groups.⁴⁷ Such orthoester formation may be overcome by using harsher glycosylation conditions.⁴⁸ Irrespective of this outcome, trisaccharide **8** was delevulinoylated into the target alcohol **9**, which was then converted to monoacetate **10** upon Pd/C concomitant debenzoylation, allyl to propyl reduction and hydrodechlorination of the TCA and CA moieties into the corresponding acetamide and acetyl group, respectively.

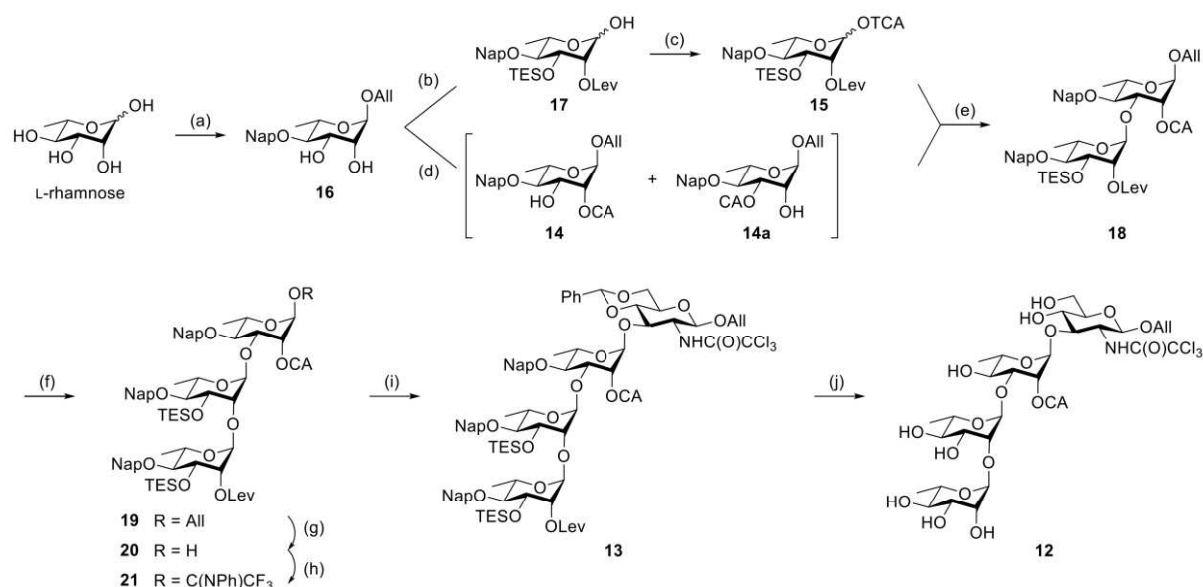
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Scheme 2. Retrosynthesis analysis of Tetrasaccharide 12 Showing Key Intermediate 13 and Monosaccharide Building Blocks. (site selective was removed)



Having achieved the proof of concept supporting CA as a suitable precursor to either a 2_C-O-acetyl or a 2_C-hydroxyl group (Scheme 2), we turned to the chemical synthesis of the targeted ABC'D'. A highly convergent strategy was established, exploiting the utility of crystalline diol **16** as precursor to rhamnosyls A, B and C and the iterative use of donor **15** for the introduction of rhamnosyls B and A. It also ensured a minimal number of deprotection steps, using hydroxyl masking groups orthogonal to allyl, TCA and CA. A strategy still barely adopted in oligosaccharide synthesis, although it has met some success for the synthesis of demanding glycolipids bearing unsaturated lipid chains⁴⁹⁻⁵¹ and is the subject of increasing interest,⁵² was favored. It involves a panel of protecting groups compatible with acid-mediated removal. Therein, the selected combination entails a 4_D,6_D-O-benzylidene acetal, a 2-naphthylmethyl (Nap) ether⁵² to mask OH-4 of the three rhamnose residues and a triethylsilyl (TES) ether⁵³ for its ease of introduction at O-3 of diol **16**. Recently developed tunable acid-sensitive ester protecting groups⁵² do not fulfill orthogonality criteria with the above selection. Emphasis was therefore on convergence and the remaining hydroxyl group in donor **15** was masked by means of a levulinoyl ester to ensure anchimeric assistance during glycosylation and subsequent selective cleavage in the presence of the 2_C-O-chloroacetyl moiety.⁵⁴ As for trisaccharide **10**, the synthesis of tetrasaccharide **12** employed rhamnosyl trichloroacetimidate donors. In contrast, a trirhamnosyl *N*-phenyltrifluoroacetimidate⁵⁵ was favored over the corresponding trichloroacetimidate in the case of the ABC donor.

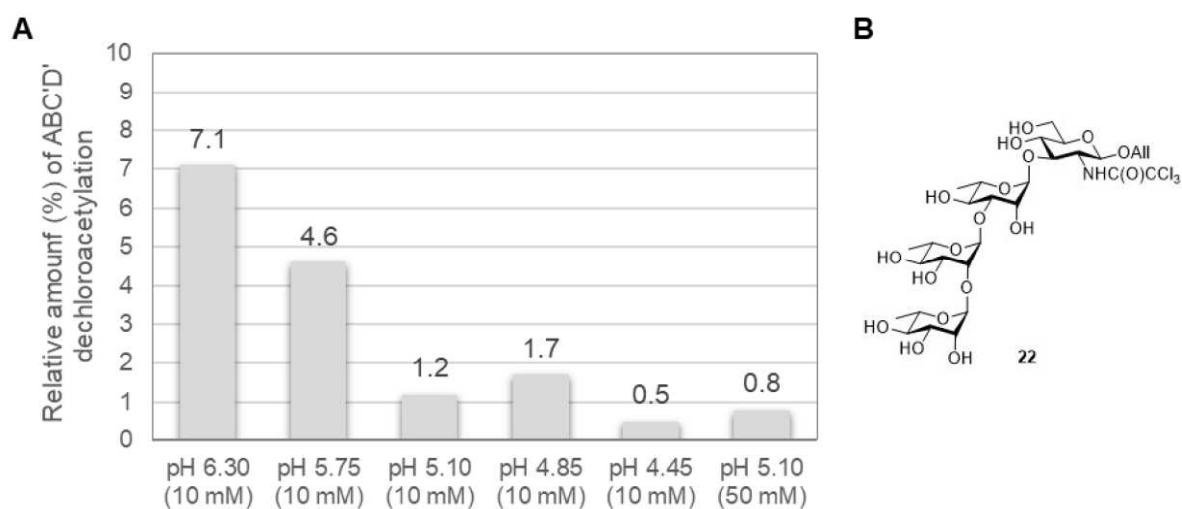
Scheme 3. Chemical Synthesis of Tetrasaccharide 12.



Reaction conditions: (a) (i) AcCl, AlOH, reflux, (ii) Me₂C(OMe)₂, PTSA, CH₃CN, (iii) NaH, NapBr, DMF, -5 °C to rt, (iv) 80% aq AcOH, 80 °C, 87%; (b) (i) TES-Cl, DIPEA, CH₃CN, (ii) DCC, DMAP, LevOH, DCM, (iii) [Ir], THF, (iv) I₂/NaHCO₃, THF/H₂O 5:1, 80% (α/β 4:1); (c) Cl₃CCN, DBU, DCE, 91% (α/β 19:1); (d) (i) (MeO)₃CCH₂Cl, PTSA, CH₃CN, (ii) 90% aq. TFA, quant. (14/14a: 19:1); (e) TBSOTf, PhMe, -40 °C, 82%; (f) (i) NH₂NH₂·H₂O, Pyridine/AcOH 3:2, (ii) 15, TBSOTf, PhMe, -20 °C, 83%; (g) (i) [Ir], THF, (ii) NIS, THF/H₂O 5:1, 80%; (h) PTFA-Cl, K₂CO₃, Acetone, 91%; (i) 7, TBSOTf, PhMe/DCM (3/1), -20 °C, 84%; (j) (i) NH₂NH₂·H₂O, Pyridine/AcOH 3:2; (ii) TFA/PhMe 9:1, 36%. DIPEA: diisopropylethylamine.

Crystalline diol **16** was obtained in four steps from L-rhamnose according to a protocol adapted from the well-established synthesis of diol **1** (Scheme 3). Regioselective silylation at OH-3 with triethylsilyl chloride in the presence of diisopropylethylamine and subsequent levulinoylation at OH-2 gave the fully protected allyl rhamnoside intermediate. Anomeric deallylation of the later was best performed by means of conventional metal-catalyzed allyl isomerization and iodine-mediated hydrolysis of the propen-1-yl intermediate in a buffered medium (Scheme S1).⁵⁶ To our satisfaction, by avoiding intermediate purification, this three-step process was achieved in a gratifying 80% overall yield to give hemiacetal **17** (Scheme 3), the key intermediate to the crystalline trichloroacetimidate **15**, then isolated with an excellent yield. The established optimized conditions enabled the synthesis of diol **16** on an 80 g scale and that of donor **15** on a 30 g scale. Alternatively, diol **16** was converted to the 2-*O*-chloroacetyl acceptor **14** as described to obtain the 4-*O*-benzyl analog **2**. The crude acceptor, isolated as a 95:5 mixture of the 2- and 3-chloroacetate regioisomers, was engaged in a TMSOTf-mediated glycosylation with donor **15** to give the crystalline disaccharide **18**. Hydrazine mediated-delevulinoylation in buffered medium¹⁶ unmasked OH-2_B to give the corresponding acceptor (Scheme S2), which was directly engaged in glycosylation with donor **15** to avoid TES loss and achieve trisaccharide **19** in 83% yield over two steps (Scheme 3). The two-step deallylation process optimized to obtain hemiacetal **17** was adapted to the later, albeit changing iodine for *N*-iodosuccinimide (NIS) to ensure mild hydrolysis conditions compatible with protecting group integrity.⁵⁷ The resulting hemiacetal **20** was evolved into

1 the *N*-phenyltrifluoroacetimidate donor **21**, which was set to react with an excess of the less demanding
 2 glucosaminide acceptor **7** under conditions similar to those used to obtain trisaccharide **8**. However, the
 3 glycosylation product **13** was isolated from a complex mixture in only 22% yield. Careful optimization
 4 of this crucial step (solvent, promoter, temperature, dilution) resulted in the identification of improved
 5 conditions. To our satisfaction, the glycosylation proceeded smoothly at low temperature and high
 6 dilution (17 mM) in anhyd. Toluene/DCM (3/1) containing catalytic *tert*-butyldimethylsilyl
 7 trifluoromethanesulfonate (TBSOTf), and no orthoester formation was observed. Tetrasaccharide **13**
 8 was isolated in a very good yield on a 5 g scale and engaged in a two-step controlled deprotection
 9 process. Conventional delevulinoylation unmasked OH-2_A providing an intermediate, which was not
 10 isolated. Instead, the obtained alcohol was treated in trifluoroacetic acid (TFA)/toluene (9:1, v/v), under
 11 conditions inspired from the work of Liu et al,⁵² to achieve the concomitant chemoselective removal of
 12 the TES, Bzl and Nap protecting groups, albeit in a modest 36% yield over two steps post extensive RP-
 13 HPLC purification. In agreement with previous reports,^{50, 52} all acid-sensitive protecting groups on
 14 residues A, B and D' were readily cleaved. In contrast, the isolated 4_C-Nap ether was found more
 15 resistant to these established conditions. Prolonged reaction time at room temperature were necessary
 16 to achieve full unmasking of OH-4_C, possibly at the extent of the glycosidic linkage integrity when
 17 working on a large scale. To our knowledge, this work exemplifies for the first time the applicability of
 18 TFA-mediated treatment for the removal of multiple protecting groups, including an isolated Nap ether,
 19 on a complex oligosaccharide featuring several acid-sensitive glycosidic linkages. As expected, the CA
 20 moiety survived these conditions. In contrast, it was found sensitive to the original reverse phase
 21 chromatography/lyophilization conditions (CH₃CN/H₂O), which resulted in the repeated formation of
 22 minute amounts of dechloroacetylated tetrasaccharide **22** (HRMS (ESI⁺) *m/z*: [M+N_a]⁺ Calcd for
 23 C₂₉H₄₆Cl₃NO₁₈Na 824.1678; found 824.1676), RP-LC *t_R* (215 nm) = 10.37 min, *t_R* (ELSD): 18.00 min
 24 vs *t_R* (215 nm) = 17.64 min, *t_R* (ELSD): 18.00 min for **12**).



57 **Figure 2.** A/ Stability of tetrasaccharide **12** at a concentration of 10 mM or 50 mM in an aqueous solution at
 58 different pH as estimated from the proportion of tetrasaccharide **22** relative to the total amount of tetrasaccharide
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1 ([22]/([22]+[12])), present at 24 h in the solution, based on RP-HPLC-UV_{210nm} analysis. **B/** Structure of
2 tetrasaccharide **22**.
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5 Since the CA ester had to comply with conditions most suitable for a transglycosylase-mediated
6 glucosylation, these observations prompted the evaluation of tetrasaccharide **12** integrity over time for
7 a range of pH (4.45 – 6.3). RP-HPLC analysis demonstrated ABC'D' stability at pH below 5 (Figures
8 2 and S1). At pH 6.3, the hydrolyzed product **22** was clearly visible at 6 h (1.8%), to reach 8% after a
9 day while steadily increasing over time (over 37% after seven days). As expected, reducing the pH was
10 found favorable. At pH 5.75, as used by default for enzymatic glucosylation mediated by the GH70 α -
11 transglycosylases of interest, hydrolysis remained visible to provide tetrasaccharide **22** in close to 5%
12 amount at 24 h. At pH 4.45, dechloroacetylation was hardly visible reaching 0.5% and 2% after one and
13 six days, respectively, while no other degradation product could be detected. Tetrasaccharide **12** was
14 considered to be stable at this pH. At pH 4.85, tetrasaccharide **22** was detected to a 1.8% and 3% extent
15 at one and six days, respectively, suggesting that the upper pH limit for long term stability was reached.
16 This tendency was confirmed at pH 5.1. Under these conditions, partial dechloroacetylation (0.7%) was
17 detected at 4 h to then slowly rise over time (1.25% after 24 h). Moreover, increasing the concentration
18 of ABC'D' by a factor five to reach 50 mM, had a positive impact and the extent of dechloroacetylation
19 (0.8% of **22**) observed during a 24 h timeframe, which exceeds that of the enzymatic glucosylation step
20 (16 h, see below), was considered to be acceptable (Figure 2).
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33 **Enzymatic glucosylation of tetrasaccharide 12 (ABC'D')**

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36 Branching sucrases are GH70 enzymes produced by some strains of lactic acid bacteria that use a cheap
37 and abundant substrate, sucrose, to glucosylate hydroxylated molecules.^{41, 58} Naturally, they branch
38 glucosyl moieties via α -1,2 or α -1,3-linkages onto linear chains of α -1,6 linked glucosyl units called
39 dextrans. But these enzymes have also demonstrated a remarkable promiscuity toward exogenous
40 acceptors such as carbohydrates, flavonoids or polyols for instance,^{41, 59} making them candidates of
41 choice to attempt the α -D-glucosylation of tetrasaccharide **12**. One of these enzymes, namely Δ N₁₂₃-
42 GBD-CD2 was used for the efficient glucosylation of the lightly protected CD' disaccharide acceptor
43 (Figure 1) to produce a *S. flexneri* 2a glucosylated intermediate, which was subsequently chemically
44 evolved into a ready-for-conjugation 15mer hapten of interest for vaccine development.³⁰ Furthermore,
45 the X-ray structure of this branching sucrose revealed an exposed active site⁶⁰ – much less buried than
46 that of *Neisseria polysaccharea* amylosucrase from GH13 family used in our prior studies^{40-41, 59} – and
47 thus more compatible with larger protected oligosaccharide accommodation. All these reasons prompted
48 us to test a set of six recently described sucrose-utilizing branching sucrases^{41, 58} for tetrasaccharide **12**
49 glucosylation. Three of them (BRS-A, Δ N₁₂₃-GBD-CD2, BRS-D) branch dextrans through α -1,2 osidic
50 linkages whereas the three others (BRS-B Δ 1, BRS-C, BRS-E) add glucosyl units only through α -1,3
51 linkages.
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1 After optimization of the reaction conditions and miniaturization of the assays, enzymes were screened
2 in 50 μ L reaction volume at pH 5.1 using 1 M of sucrose as glucosyl donor and 50 mM of tetrasaccharide
3 **12** as acceptor. This pH was chosen as a suitable compromise between enzyme activity and acceptor
4 stability in the buffer (Figures 2, S1, and S3). HPAEC-PAD analyses confirmed that sucrose depletion
5 was quasi-total in all reactions (data not shown) after 16 h of reaction and RP-HPLC-UV-MS analyses
6 revealed that all enzymes could transfer glucosyl units issued from sucrose onto acceptor **12** ($t_R = 22.5$
7 min). Two main products, called **P1** ($t_R = 21.65$ min) and **P2** ($t_R = 21.9$ min), were shown to have a
8 molecular mass m/z of 1057.24, corresponding to the NH_4^+ adducts of monoglucosylated forms of
9 tetrasaccharide **12** (Figure 3A). All enzymes also produced several polyglucosylated products
10 containing up to 6 glucosyl units as inferred from mass spectrometry analysis (Figures 3 and S4). HPLC-
11 UV detection further indicates that enzymes such as ΔN_{123} -GBD-CD2 and BRS-D are more prone to
12 synthesize longer oligosaccharides.

20 Tetrasaccharide conversion was determined from acceptor glucosylation (determined from the peak
21 areas of the glucosylated products detected by RP-HPLC-UV-MS) (Figure 3B, Table S1). Values
22 ranging from 2.5 to 22.8 % were obtained depending on the enzyme. BRS-E and BRS-B Δ 1 clearly
23 stand as the most efficient enzymes for the tetrasaccharide conversion, with conversion values 2.3 to 9.3
24 times higher than those obtained with the other branching sucrases. Moreover, these two enzymes
25 display a high specificity for the synthesis of the monoglucosylated product **P1**. Otherwise, the other
26 enzymes are more versatile and produce both **P1**, and **P2** as well as polyglucosylated compounds.

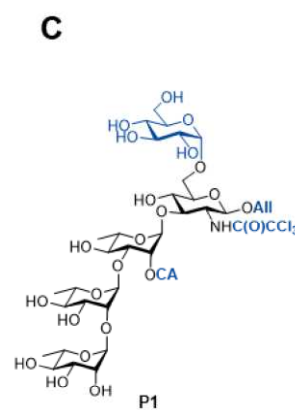
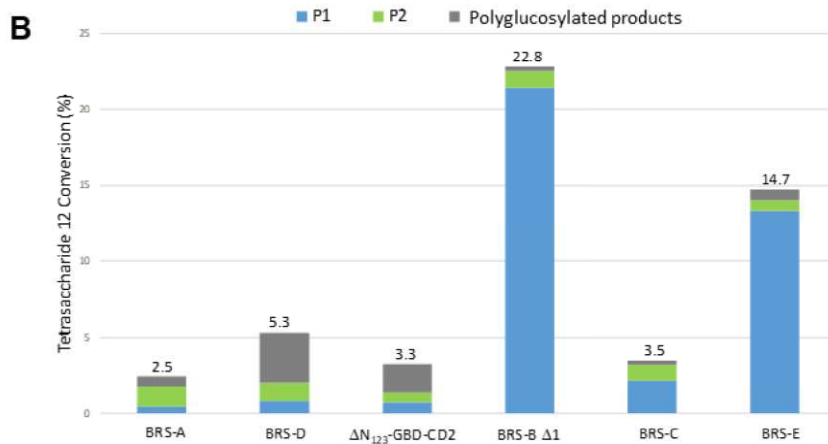
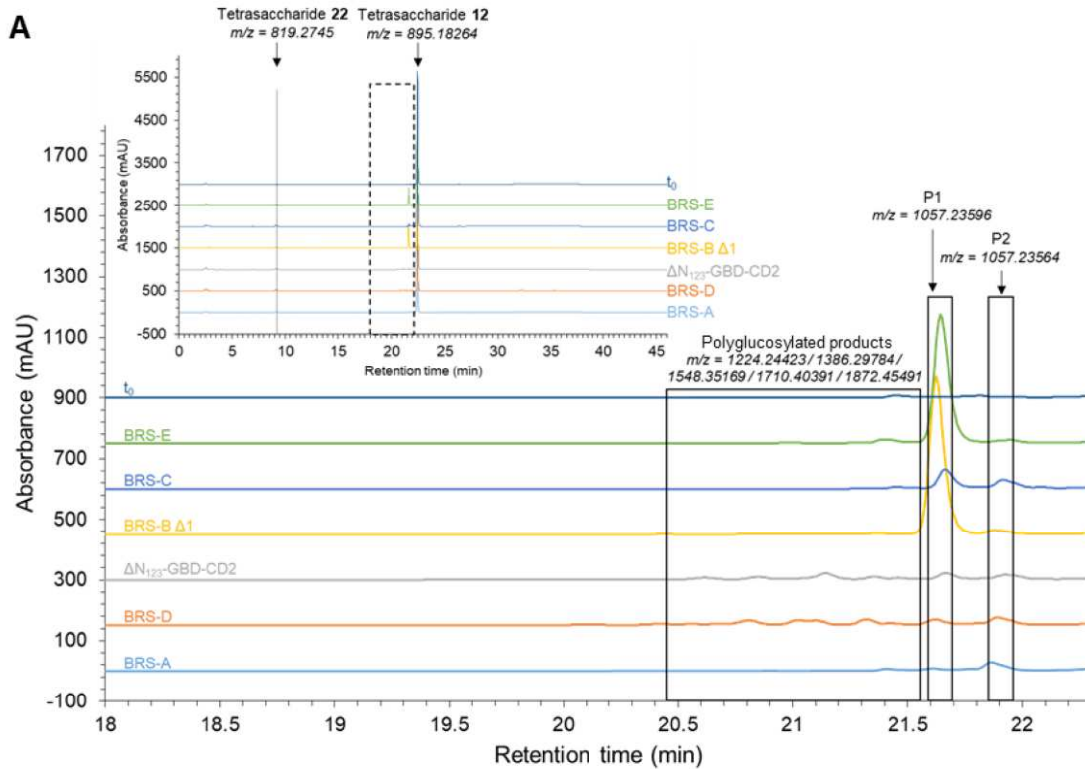


Figure 3: **A/** Chromatograms of the RP-HPLC-UV-MS analysis for the enzymatic glucosylation of tetrasaccharide **12** by six selected GH70 branching sucrases after 16 h of reaction in presence of sucrose (1 M) and tetrasaccharide **12** (50 mM) at pH 5.1. The region between 18.0 and 22.3 minutes corresponding to the frame in dash line has been enlarged for clarity purpose. The initial time (t_0) is indicated as reference. **P1** ($t_R = 21.65$ min), **P2** ($t_R = 21.9$ min) and polyglucosylated products ($t_R = 20.6 / 20.8 / 21.0 / 21.1 / 21.15 / 21.2 / 21.3 / 21.45$ min) are products of the enzymatic glucosylation. A shift in molecular mass by 162 Da, characteristic of monoglucosylation, was observed for **P1** and **P2**. For polyglucosylated products, incremental shifts of 162 Da were observed up to a shift of 1008 Da corresponding to 6 glucosyl units and reaching the sensitivity limit of the spectrometer. Tetrasaccharide **22** ($t_R = 22.5$ min), the dechloroacetylated form of **12**, was detected in the reaction mixture after 16 h of reaction, albeit only in non-interfering small amounts (< 2%); m/z are indicated below each product. **B/** Tetrasaccharide **12** conversion = $\frac{A_{P1}^{final} + A_{P2}^{final} + A_{Polyglucosylated}^{final}}{A_{12}^{initial}}$. The relative proportions of **P1**, **P2** and polyglucosylated products are represented in bar charts. **C/** Structure of **P1** characterized by NMR spectroscopy.

1 To produce **P1** at larger scale, a 1 mL reaction was performed using a preparation of purified BRS-B
 2 Δ 1 and an optimized acceptor **12**:sucrose ratio of 1:20 (carbohydrate total dry mass = 386 g/L). In these
 3 conditions, the conversion rate of tetrasaccharide **12** into pentasaccharide **P1** was 31% (Figure S5).
 4 NMR characterization of **P1** was based firstly on the comparison of the HSQC spectra (Figures 4A and
 5 B) of tetrasaccharide **12** and pentasaccharide **P1** as glucosylation was anticipated to result in shifted
 6 resonances at the glucosylated position and adjacent ones. Obviously, C-6D' was shifted toward higher
 7 frequency, from 61.78 ppm to 65.87 ppm, while the adjacent 5D' carbon was shifted to lower frequency,
 8 from 76.23 ppm to 74.51 ppm. In addition, the ^1H and ^{13}C resonances corresponding to the neighboring
 9 4D' position slightly shifted, by around 0.1 ppm each. All data converged, revealing that glucosylation
 10 occurred on the D' moiety of tetrasaccharide **12**, yielding the ABC'[$\alpha\text{E}(1\rightarrow6)$]D' pentasaccharide
 11 (Figures 3C, 4 and S6). Glucosylation at OH-6D' is representative of the repeating units corresponding
 12 to *S. flexneri* serotypes 4a and 4b O-Ags.¹⁹ This finding demonstrates the potential of the devised
 13 synthetic strategy, opening new routes for the glucodiversification of tetrasaccharide **12** to access
 14 additional pentasaccharides representative of *S. flexneri* serotype-specific O-Ag repeating units.
 15 Unfortunately, pentasaccharide **P2** was produced in trace amounts, thus limiting its further
 16 characterization. **P2** production was not improved for any of the wild-type branching sucrases at pH
 17 5.75 compared to results obtained at pH 5.1 (not shown). Nonetheless, this original contribution sets the
 18 groundwork for further engineering of sucrose-utilizing branching sucrases to improve their catalytic
 19 performances and product yields, in particular to achieve higher **P2** production, or expand their α -D-
 20 glucosylation regioselectivity, on the chemically synthesized ABC'D' tetrasaccharide core (**12**).
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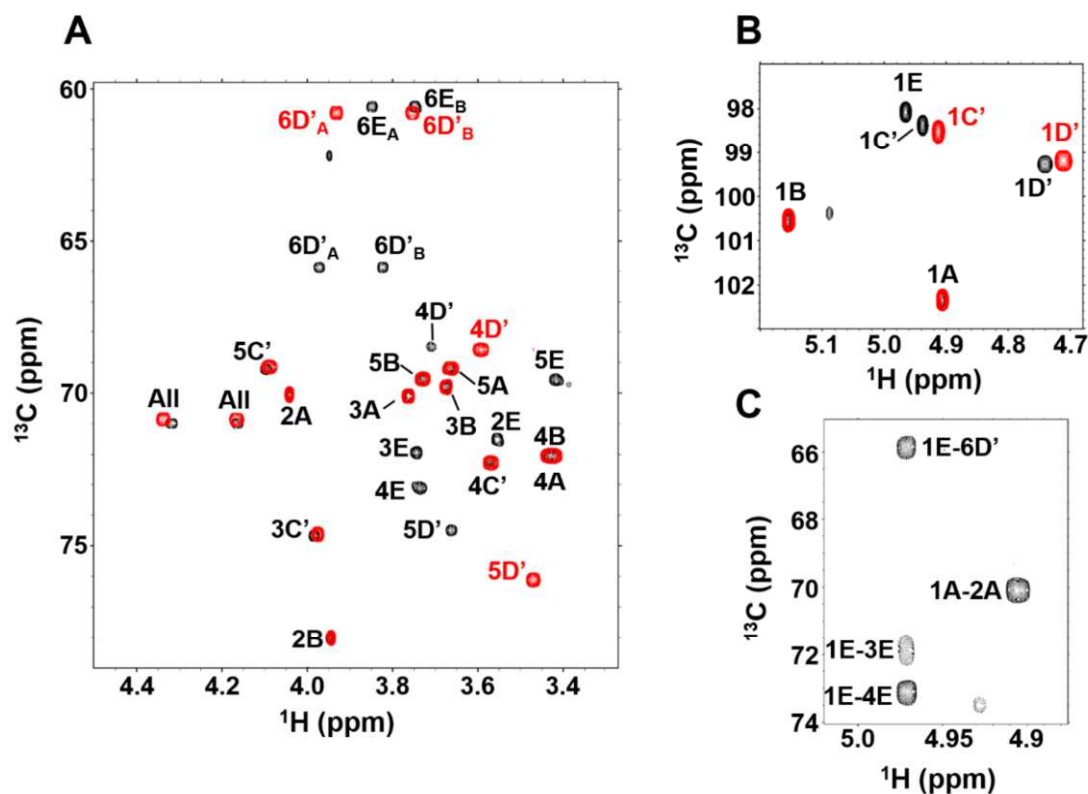


Figure 4: Superimposed ^1H - ^{13}C HSQC NMR spectra of the: A/ C2, C3, C4 and C5 resonances, and B/ anomeric region of pentasaccharide **P1** (black) and tetrasaccharide **12** (red). C/ The ^1H - ^{13}C HMBC illustrates the correlation

1 between the E and D' units of **P1**. Spectra were acquired at 950 MHz in 99.9% DCl-containing D₂O at pH 4.9
2 (pD 4.5).
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8 **CONCLUSION**

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10 The concept validation exemplified herein provides a novel access to a large diversity of *S. flexneri* type-
11 specific pentasaccharides from a simple non-natural lightly protected tetrasaccharide scaffold (**12**),
12 representative of the backbone repeating units of most *S. flexneri* O-Ags. Tetrasaccharide **12** was
13 designed for optimal manipulation compatible with chain elongation at both ends post-enzymatic
14 glycosylation. It was obtained in 13 chemical steps from two easily available crystalline
15 monosaccharides, each synthesized in four steps. Going beyond the synthesis of a panel of *S. flexneri*
16 O-Ag fragments representing multiple different *Shigella* serotypes, this attractive diverging
17 chemoenzymatic strategy offers new opportunities to provide libraries of complex microbial
18 oligosaccharides derived from a common core, albeit differing by their branching pattern, whether in
19 the form of glucosyl residues⁶¹ or other substitutions. Glycan diversity characterizing the cell surface of
20 numerous microbial species, many of which of vaccinal interest such as *Shigella*, calls for innovative
21 ways toward expanding our arsenal of structurally well-defined oligosaccharides to decipher critical
22 biological phenomena or for use in glycoarrays enabling improved diagnosis. Bacteria such as
23 *Salmonella enterica*⁶² or *Escherichia coli*⁶³ and fungi such as *Cryptococcus neoformans*,⁶⁴ are other
24 examples of families of pathogens featuring closely resembling polysaccharide antigens differentiating
25 groups and types within species. While the proof-of-concept is demonstrated herein for a small subset
26 of available α -transglucosylases, impressive developments in glycoenzyme engineering technologies,
27 that enable the tailoring on purpose of naturally available biocatalysts toward improved and/or novel
28 substrate specificity for the high yielding conversion of a non-natural substrate into the targeted
29 product,⁵⁹ add to the exciting potential foreseen for the proposed approach. In particular, ongoing
30 investigation aims at exploring further this promising convergent chemoenzymatic strategy in the
31 context of *S. flexneri* O-antigens of vaccinal interest.
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49 **EXPERIMENTAL**

50 *General procedures*

51 All reagents and solvents were of commercial grade and used as received. All moisture sensitive
52 reactions were performed under an argon (Ar) atmosphere. Reactions were monitored by thin layer
53 chromatography (TLC) analysis with UV detection (254 nm) and by spraying with orcinol (1 mg/mL)
54 in 10% aq. H₂SO₄ followed by charring. Flash chromatography was performed on silica gel (40-60 μ m)
55 with UV (254 nm) and ELSD detection. Reverse phase flash chromatography was performed on a C-18
56 column with UV (215 nm) and ELSD detection. Except spectra for compound **5**, which were recorded
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1 on a Thermo Scientific™ LTQ Orbitrap Velos™ hybrid FT mass spectrometer, all HRMS spectra were
2 recorded on a WATERS QTOF Micromass instrument in the positive-ion electrospray ionization (ESI⁺)
3 mode. Solutions were prepared using 1:1 CH₃CN/H₂O containing 0.1% formic acid or using 7:3
4 CH₃CN/aq. MeOH containing 10 mM CH₃CO₂NH₄.
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7 Analytical RP-HPLC was performed using

8 *Conditions A.* an Akzo Nobel Kromasil® column, C18, 4.6 x 150 mm, 3.5 μm, 100 Å, eluting at 1.0
9 mL.min⁻¹ with CH₃CN in 0.08% aq. TFA, 0 to 40% for 20 min, 15 °C. Detection was by UV at λ = 215,
10 230 or 254 nm, and by ELSD.
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12 *Conditions B.* a C18 RP Fusion analytical column, 250 x 4.6 mm, 4 μm, 80 Å. The flow rate was set to
13 1.0 mL.min⁻¹. Reaction media were diluted 5 times in H₂O/CH₃CN (70:30, v/v) + 0.08% TFA. Elution
14 started with 20% aq. CH₃CN for 15 min, then reaching 80% aq. CH₃CN over 15 min, which was held
15 for 5 min, 30 °C. Detection was by UV at λ = 220 nm.
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21 Nuclear magnetic resonance (NMR) spectra of the chemically synthesized compounds were recorded at
22 303 K on a Bruker Avance spectrometer at 400 MHz (¹H) and 100 MHz (¹³C) equipped with a BBO
23 probe. Spectra were recorded in deuterated chloroform (CDCl₃) or in deuterated water (D₂O).
24 Elucidations of chemical structures was based on ¹H, COSY, DEPT-135, HSQC, decoupled HSQC, ¹³C,
25 decoupled ¹³C and HMBC experiments. Signals are reported as m (multiplet), s (singlet), d (doublet), t
26 (triplet), dd (doublet of doublet), q (quadruplet), dt (doublet of triplet), dq (doublet of quadruplet), ddd
27 (doublet of doublet of doublet). Signals can also be described with a prefix br (broad), p (pseudo) or
28 with a subscript o (overlap), po (partially overlap). Coupling constants (*J*) are reported in hertz (Hz).
29 Chemical shifts (*δ*) are reported in ppm relative to the residual solvent peak. Sugar residues are lettered
30 according to the lettering of the repeating unit of the *S. flexneri* O-Ags (A, B, C, D, E) and identified by
31 a subscript in the listing of signal assignments.
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40 High field NMR spectra of tetrasaccharide **12** and of the product of glucosylation **P1** were recorded at
41 298 K on a Bruker Avance spectrometer equipped with a 5-mm gradient indirect cryoprobe (TGIR-
42 RMN-THC Fr3050 CNRS, Gif-sur-Yvette), operating at a proton frequency of 950 MHz and at a carbon
43 frequency of 238 MHz. Spectra or on a Bruker Avance spectrometer equipped with a 5-mm gradient
44 indirect cryoprobe (MetaToul, TBI INRA-CNRS-INSAT, Toulouse), operating at a proton frequency of
45 800 MHz and at a carbon frequency of 200 MHz. Spectra were processed and analyzed with Topspin
46 software (Bruker) and/or Sparky (T. D. Goddard and D. G. Kneller, SPARKY 3, University of
47 California, San Francisco). Samples were lyophilized three times and dissolved in 180 μL of 99.9%
48 DCl-containing D₂O at pH 4.9 (pD 4.5) or simply dissolved in 180 μL of D₂O for spectra recorded at
49 950/235 MHz and 800/200 MHz, respectively. 65536 Data points were acquired with 32 and 2048 scans
50 for the ¹H and DEPT-135 NMR experiments, respectively. ¹H-¹³C HSQC, HMBC and COSY spectra
51 were recorded as follows: (i) on the 950 MHz instrument: 512 increments of 2048 complex points are
52 acquired with an accumulation of 16 scans, spectral widths were 16025 Hz for the ¹H dimension and
53 44267 Hz for the ¹³C dimension; and (ii) on the 800 MHz instrument: 1024 increments of 2048 complex
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1 points are acquired with an accumulation of 8 scans, spectral widths were 16025 Hz for the ^1H dimension
2 and 48077 Hz for the ^{13}C dimension. Elucidation of the chemical structures was based on ^1H , COSY,
3 DEPT-135, HSQC, and HMBC experiments.
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6 **Chemical synthesis**

7 *Synthesis of trisaccharide 10 from rhamnoside 1 and glucosaminide 7*

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10 **Allyl 4-O-benzyl-2-O-chloroacetyl- α -L-rhamnopyranoside (2).** Diol **1** (1.07 g, 3.65 mmol) was
11 dissolved in anhyd. CH_3CN (750 mL) then 2-chloro-1,1,1-trimethoxyethane (1.0 mL, 7.42 mmol, 2.0
12 equiv.) and PTSA (170 mg, 0.89 mmol, 0.2 equiv.) were added. After stirring for 40 min at rt, a TLC
13 control (Toluene/EtOAc 8:2) showed the total conversion of the starting diol **1** (Rf 0.1) into a less polar
14 product (Rf 0.8). 80% aq. AcOH (3 mL) was then added and the solution was stirred for 25 min at rt. A
15 TLC control (Toluene/EtOAc 8:2) showed the appearance of a more polar product (Rf 0.5). The mixture
16 was diluted with H_2O (150 mL) then extracted with EtOAc (300 mL). The organic phase was washed
17 with H_2O (100 mL) and brine (150 mL) then dried over anhyd. Na_2SO_4 and concentrated to dryness to
18 give alcohol **2** as a colorless oil. Acceptor **2** had ^1H NMR (400 MHz, CDCl_3) δ 7.41-7.26 (m, 5H, H_{ArBn}),
19 5.89 (m, 1H, $\text{CH}=\text{CH}_{2\text{All}}$), 5.30 (m, 1H, $J_{\text{trans}} = 17.3$ Hz, $J_{\text{gem}} = 1.6$ Hz, $\text{CH}=\text{CH}_{2\text{All}}$), 5.24-5.21 (m, 2H,
20 H-2, $\text{CH}=\text{CH}_{2\text{All}}$), 4.82 (d, 1H, $J_{1,2} = 1.2$ Hz, H-1), 4.81 (d, 1H, H_{Bn}), 4.75 (d, 1H, $J = 11.0$ Hz, H_{Bn}),
21 4.20-4.15 (m, 4H, $\text{CH}_2\text{-CH}=\text{All}$, H-3, H_{CA}), 4.00 (m, 1H, $\text{CH}_2\text{-CH}=\text{All}$), 3.80 (dq, 1H, H-5), 3.37 (pt, 1H,
22 $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4), 2.05 (d, 1H, $J_{3,\text{OH}} = 4.4$ Hz, OH), 1.38 (d, 3H, $J_{5,6} = 6.3$ Hz, H-6). $^{13}\text{C}\{^1\text{H}\}$
23 NMR (100 MHz, CDCl_3) δ 167.1 (CO_2CA), 138.1 (C_{IVBn}), 133.3 ($\text{CH}=\text{CH}_{2\text{All}}$), 128.7-127.9 (5C, C_{ArBn}),
24 117.7 ($\text{CH}=\text{CH}_{2\text{All}}$), 96.1 (C-1, $^1J_{\text{C,H}} = 171.0$ Hz), 81.5 (C-4), 75.3 ($\text{CH}_{2\text{Bn}}$), 74.4 (C-2), 70.1 (C-3), 68.2
25 ($\text{CH}_2\text{-CH}=\text{All}$), 67.6 (C-5), 40.8 ($\text{CH}_{2\text{CA}}$), 18.0 (C-6). HRMS (ESI $^+$) m/z : $[\text{M}+\text{Na}]^+$ Calcd for
26 $\text{C}_{18}\text{H}_{23}\text{ClO}_6\text{Na}$ 393.1081; found 393.1069.
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41 **Allyl 3,4-di-O-benzyl-2-O-levulinoyl- α -L-rhamnopyranosyl-(1 \rightarrow 3)-4-O-benzyl-2-O-chloroacetyl- α -L-rhamnopyranoside (4).** The crude material from above was taken into anhyd. Et_2O (20 mL) and
42 donor **3** (2.64 g, 4.51 mmol, 1.3 equiv.) was added. The suspension was stirred at rt for 25 min then
43 cooled down to -15 $^\circ\text{C}$ and TMSOTf was added (40 μL , 0.22 mmol, 0.06 equiv.). After stirring for 25
44 min at this temperature, a TLC control (Toluene/EtOAc 8:2) showed the total conversion of the
45 intermediate alcohol **2** (Rf 0.4) and the presence of a less polar product (Rf 0.45). The mixture was
46 neutralized by addition of Et_3N , and the suspension was filtered over a pad of Celite $^\circledR$. Volatiles were
47 removed under reduced pressure and the residue was purified by flash chromatography
48 (Cyclohexane/Acetone 9:1 to 0:1) to give disaccharide **4** (2.63 g, 91%) as a colorless oil. The coupling
49 product had ^1H NMR (400 MHz, CDCl_3) δ 7.38-7.25 (m, 15H, H_{ArBn}), 5.88 (m, 1H, $\text{CH}=\text{CH}_{2\text{All}}$), 5.41
50 (dd, 1H, $J_{1,2} = 1.9$ Hz, $J_{2,3} = 3.3$ Hz, H-2 $_B$), 5.29 (m, 1H, $J_{\text{trans}} = 17.2$ Hz, $J_{\text{gem}} = 1.6$ Hz, $\text{CH}=\text{CH}_{2\text{All}}$),
51 5.23-5.20 (m, 2H, H-2 $_C$, $\text{CH}=\text{CH}_{2\text{All}}$), 5.04 (d, 1H, H-1 $_B$), 4.91 (d, 1H, $J = 11.3$ Hz, H_{Bn}), 4.81 (d, 1H, J
52 = 10.9 Hz, H_{Bn}), 4.80 (d $_{\text{po}}$, 1H, $J_{1,2} = 1.8$ Hz, H-1 $_C$), 4.65 (d, 1H, H_{Bn}), 4.61 (d $_{\text{po}}$, 1H, $J = 11.3$ Hz, H_{Bn}),
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1 4.59 (d_{po}, 1H, H_{Bn}), 4.46 (d, 1H, $J = 11.3$ Hz, H_{Bn}), 4.20 (dd, 1H, $J_{2,3} = 3.2$ Hz, H-3_C), 4.15 (m, 1H, CH₂-
2 CH=All), 4.11 (brs, 2H, H_{CA}), 3.99 (m, 1H, CH₂-CH=All), 3.87 (dd, 1H, H-3_B), 3.82-3.72 (m, 2H, H-5_B,
3 H-5_C), 3.45 (pt, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4_C), 3.41 (pt, $J_{3,4} = J_{4,5} = 9.2$ Hz, H-4_B), 2.75-2.63 (m, 4H, CH_{2Lev}),
4 2.17 (s, 3H, CH_{3Lev}), 1.30 (d, 3H, $J_{5,6} = 6.3$ Hz, H-6_B), 1.29 (d, 3H, $J_{5,6} = 6.4$ Hz, H-6_C). ¹³C{¹H} NMR
5 (100 MHz, CDCl₃) δ 206.0 (CO_{Lev}), 171.8 (CO_{2Lev}), 166.7 (CO_{2CA}), 138.5-137.9 (3C, C_{IVBn}), 133.3
6 (CH=CH_{2All}), 129.0-127.6 (15C, C_{ArBn}), 117.6 (CH=CH_{2All}), 99.6 (C-1_B, ¹J_{C,H} = 172.8 Hz), 97.6 (C-1_C,
7 ¹J_{C,H} = 171.0 Hz), 80.2 (C-4_B), 79.7 (C-4_C), 77.4 (C-3_B), 77.0 (C-3_C), 75.5-75.0 (2C, CH_{2Bn}), 74.0 (C-
8 2_C), 71.6 (CH_{2Bn}), 69.3 (C-2_B), 68.7 (C-5_B), 68.2 (CH₂-CH=All), 67.9 (C-5_C), 40.8 (CH_{2CA}), 38.0 (CH_{2Lev}),
9 29.8 (CH_{3Lev}), 28.2 (CH_{2Lev}), 17.9 (2C, C-6_B, C-6_C). HRMS (ESI⁺) m/z : [M+Na]⁺ Calcd for
10 C₄₃H₅₁ClO₁₂Na 817.2967; found 817.3044.

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18 **3,4-Di-O-benzyl-2-O-levulinoyl- α -L-rhamnopyranosyl-(1 \rightarrow 3)-4-O-benzyl-2-O-chloroacetyl- α/β -**
19 **L-rhamnopyranose (5).** 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)-iridium
20 hexafluorophosphate (51 mg, 59 μ mol, 0.02 equiv.) was dissolved in anhyd. THF (10 mL) and the
21 solution was degassed repeatedly then stirred under a satd. H₂ atmosphere for 25 min. The resulting
22 yellow solution was degassed repeatedly before being poured into a solution of disaccharide **4** (1.97 g,
23 2.48 mmol) in anhyd. THF (35 mL). The mixture was stirred under an Ar atmosphere at rt for 17 h. A
24 TLC control (Toluene/EtOAc 8:2) indicated the consumption of the starting allyl glycoside (Rf 0.6) and
25 the presence of a less polar intermediate (Rf 0.7). A solution of I₂ (1.25 g, 4.63 mmol, 2.0 equiv.) in
26 THF/H₂O (5:1, 36 mL) was added. After stirring at rt, for 2 h, a TLC control (Toluene/EtOAc 8:2)
27 revealed the absence of the intermediate compound and the presence of more polar products (Rf 0.35).
28 The reaction was quenched with 10% aq. NaHSO₃ (100 mL). The mixture was concentrated under
29 reduced pressure, and extracted with DCM (3 \times 250 mL). The combined organic phases were washed
30 with brine (150 mL), dried over anhyd. Na₂SO₄ and passed through a phase separator filter. The residue
31 was purified by flash chromatography (Toluene/EtOAc 8:2 to 5:5) to give hemiacetal **5** (1.56 g, 83%)
32 as a pale yellow oil (α/β 9:1). The α -anomer had ¹H NMR (400 MHz, CDCl₃) δ 7.39-7.25 (m, 15H,
33 H_{ArBn}), 5.41 (dd, 1H, $J_{1,2} = 1.8$ Hz, $J_{2,3} = 3.2$ Hz, H-2_B), 5.23 (dd, 1H, $J_{1,2} = 1.9$ Hz, $J_{2,3} = 3.2$ Hz, H-2_C),
34 5.18 (dd, 1H, $J_{1,OH} = 4.0$ Hz, H-1_C), 5.05 (d, 1H, H-1_B), 4.90 (d, 1H, $J = 11.2$ Hz, H_{Bn}), 4.81 (d, 1H, $J =$
35 10.9 Hz, H_{Bn}), 4.65 (d, 1H, H_{Bn}), 4.60 (d_{po}, 1H, H_{Bn}), 4.59 (d_{po}, 1H, H_{Bn}), 4.46 (d, 1H, $J = 11.4$ Hz, H_{Bn}),
36 4.24 (dd, 1H, H-3_C), 4.11 (brs, 2H, H_{CA}), 3.99 (dq, 1H, $J_{5,6} = 6.3$ Hz, H-5_C), 3.86 (dd, 1H, H-3_B), 3.76
37 (dq, 1H, $J_{5,6} = 6.1$ Hz, H-5_B), 3.46 (pt, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4_C), 3.41 (pt, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4_B),
38 2.75-2.63 (m, 5H, OH-1_C, CH_{2Lev}), 2.17 (s, 3H, CH_{3Lev}), 1.30-1.28 (m, 6H, H-6_B, H-6_C). ¹³C{¹H} NMR
39 (100 MHz, CDCl₃) δ 206.2 (CO_{Lev}), 171.8 (CO_{2Lev}), 166.8 (CO_{2CA}), 138.4-137.9 (3C, C_{IVBn}), 128.5-
40 127.6 (15C, C_{ArBn}), 99.4 (C-1_B, ¹J_{C,H} = 171.8 Hz), 91.3 (C-1_C, ¹J_{C,H} = 172.2 Hz), 80.3 (C-4_B), 79.7 (C-
41 4_C), 77.4 (C-3_B), 76.2 (C-3_C), 75.4, 75.1 (2C, CH_{2Bn}), 74.3 (C-2_C), 71.6 (CH_{2Bn}), 69.3 (C-2_B), 68.7 (C-
42 5_B), 67.8 (C-5_C), 40.8 (CH_{2CA}), 38.0 (CH_{2Lev}), 29.8 (CH_{3Lev}), 28.1 (CH_{2Lev}), 17.9 (2C, C-6_B, C-6_C).
43 HRMS (ESI⁺) m/z : [M+NH₄]⁺ Calcd for C₄₀H₅₁ClNO₁₂ 772.3094; found 772.3100.

1 **3,4-Di-O-benzyl-2-O-levulinoyl- α -L-rhamnopyranosyl-(1 \rightarrow 3)-4-O-benzyl-2-O-chloroacetyl- α / β -**
2 **L-rhamnopyranosyl trichloroacetimidate (6).** Hemiacetal **5** (1.39 g, 1.84 mmol) was dissolved in
3 anhyd. DCE (15 mL). CCl₃CN (0.55 mL, 5.49 mmol, 3.0 equiv.) and DBU (83 μ L, 0.56 mmol, 0.3
4 equiv.) were added at rt. After stirring for 1 h under Ar at rt. Follow up by TLC (Toluene/EtOAc 8:2 +
5 1% Et₃N) showed the absence of the starting hemiacetal **5** (Rf 0.3) and the presence of a less polar
6 product (Rf 0.65). The mixture was concentrated and directly purified by flash chromatography
7 (Toluene/EtOAc 95:5 to 0:1 + 1% Et₃N) to give donor **6** (1.59 g, 96 %, α / β 95:5) as a brownish oil.
8 Trichloroacetimidate **6** had ¹H NMR (400 MHz, CDCl₃) δ 8.73 (brs, 1H, NH), 7.40-7.26 (m, 15H, H_{ArBn}),
9 6.23 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1_C), 5.44 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.2 Hz, H-2_B), 5.38 (dd, 1H, $J_{2,3}$ =
10 3.3 Hz, H-2_C), 5.10 (d, 1H, H-1_B), 4.91 (d, 1H, J = 11.3 Hz, H_{Bn}), 4.82 (d, 1H, J = 10.9 Hz, H_{Bn}), 4.67-
11 4.60 (m, 3H, H_{Bn}), 4.51 (d, 1H, J = 11.3 Hz, H_{Bn}), 4.28 (dd, 1H, H-3_C), 4.05 (d, 1H, J = 15.1 Hz, H_{CA}),
12 4.01 (d, 1H, J = 15.1 Hz, H_{CA}), 3.97 (dq, 1H, H-5_C), 3.87 (dd, 1H, H-3_B), 3.79 (dq, 1H, H-5_B), 3.57 (pt,
13 $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4_C), 3.43 (pt, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4_B), 2.73-2.65 (m, 4H, CH_{2Lev}), 2.17 (s, 3H,
14 CH_{3Lev}), 1.34 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6_C), 1.27 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_B). ¹³C{¹H} NMR (100 MHz,
15 CDCl₃) δ 206.0 (CO_{Lev}), 171.8 (CO_{2Lev}), 166.3 (CO_{2CA}), 160.0 (CNH), 138.5-137.5 (3C, C_{IVBn}), 128.5-
16 127.6 (15C, C_{ArBn}), 99.4 (C-1_B), 94.1 (2C, C-1_C, CCl₃), 79.7 (C-4_B), 79.6 (C-4_C), 77.3 (C-3_B), 75.4
17 (CH_{2Bn}), 75.2 (C-3_C), 74.9 (CH_{2Bn}), 72.2 (C-2_C), 71.7 (CH_{2Bn}), 70.9 (C-5_C), 69.3 (C-2_B), 68.9 (C-5_B),
18 40.5 (CH_{2CA}), 38.0 (CH_{2Lev}), 29.8 (CH_{3Lev}), 28.1 (CH_{2Lev}), 17.9 (2C, C-6_B, C-6_C). HRMS (ESI⁺) m/z :
19 [M+NH₄]⁺ Calcd for C₄₂H₅₁Cl₄N₂O₁₂ 915.2196; found 915.2114.
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32 **Allyl 3,4-di-O-benzyl-2-O-levulinoyl- α -L-rhamnopyranosyl-(1 \rightarrow 3)-(4-O-benzyl-2-O-chloroacetyl-**
33 **α -L-rhamnopyranosyl)-(1 \rightarrow 3)-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido- β -D-**
34 **glucopyranoside (8) and 3,4-di-O-benzyl-2-O-levulinoyl- α -L-rhamnopyranosyl-(1 \rightarrow 3)-(4-O-**
35 **benzyl- β -L-rhamnopyranose)-1,2-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido- β -D-**
36 **glucopyranosid)-3-yl) orthochoacetate (11).** Alcohol **7** (630 mg, 1.39 mmol) and donor **6** (1.45 mg,
37 1.61 mmol, 1.2 equiv.) were dissolved in DCM (15 mL) and activated 4 Å MS (1.0 g) was added. The
38 suspension was stirred for 20 min at rt, then cooled to -15 °C, and TMSOTf (25 μ L, 138 μ mol, 0.1
39 equiv.) was added. After stirring for 45 min at -20 °C, a TLC control (Toluene/EtOAc 8:2) showed the
40 disappearance of acceptor **7** (Rf 0.25) and the presence of two less polar products (Rf 0.4 and 0.5). The
41 mixture was neutralized by addition of Et₃N, and the suspension was filtered over a pad of Celite®.
42 Volatiles were removed under reduced pressure and the crude material was purified by flash
43 chromatography (Toluene/EtOAc 95:5 to 0:100) to provide in order of elution first orthoester **11** (441
44 mg, 27%) then trisaccharide **8** (1.02 g, 62%) both as a white foam. The former had ¹H NMR (400 MHz,
45 CDCl₃) δ 7.64-7.61 (m, 2H, H_{ArBn}), 7.41-7.18 (m, 18H, H_{ArBn}, H_{ArBzl}), 7.07 (d, 1H, $J_{2,NH}$ = 7.2 Hz, NH),
46 5.89 (m, 1H, CH=CH_{2All}), 5.50 (s, 1H, H_{Bzl}), 5.39 (dd, 1H, $J_{1,2}$ = 1.4 Hz, $J_{2,3}$ = 3.3 Hz, H-2_B), 5.30 (m,
47 1H, J_{trans} = 17.2 Hz, J_{gem} = 1.5 Hz, CH=CH_{2All}), 5.23 (m, 1H, J_{cis} = 10.3 Hz, CH=CH_{2All}), 5.07 (d, 1H,
48 $J_{1,2}$ = 8.2 Hz, H-1_D), 4.93 (brs, 1H, H-1_B), 4.87 (d, 1H, J = 10.7 Hz, H_{Bn}), 4.84-4.79 (m, 4H, H-1_C, H-3_D,
49 H_{Bn}), 4.63 (d, 1H, J = 10.8 Hz, H_{Bn}), 4.61-4.58 (m, 2H, H-2_C, H_{Bn}), 4.33 (m, 1H, CH₂-CH=All), 4.16-
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4.04 (m, 5H, CH₂-CH=All, H-6a_D, H-5_B, 2H_{Bn}), 3.86 (dd, 1H, H-3_B), 3.72 (d, 1H, *J* = 12.6 Hz, CH₂Cl), 3.68 (d, 1H, *J* = 12.6 Hz, CH₂Cl), 3.68 (dd_{po}, 1H, H-6b_D), 3.63 (dd_{po}, 1H, *J*_{2,3} = 4.2 Hz, H-3_C), 3.58 (m, 1H, H-5_D), 3.51 (pt, 1H, *J*_{3,4} = *J*_{4,5} = 8.9 Hz, H-4_D), 3.45 (pt, 2H, *J*_{3,4} = *J*_{4,5} = 9.3 Hz, H-4_B, H-4_C), 3.39 (ddd, 1H, H-2_D), 3.11 (dq, 1H, H-5_C), 2.78-2.64 (m, 4H, CH_{2Lev}), 2.18 (s, 3H, CH_{3Lev}), 1.48 (d, 3H, *J*_{5,6} = 6.2 Hz, H-6_B), 1.24 (d, 3H, *J*_{5,6} = 6.2 Hz, H-6_C). ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 206.2 (CO_{Lev}), 171.8 (CO_{2Lev}), 162.0 (NHCO), 138.7-137.5 (4C, C_{IVBn}, C_{IVBzl}), 133.2 (CH=CH_{2All}), 129.4-126.7 (18C, C_{ArBn}, C_{IVBzl}), 120.6 (CO_{2CA}), 118.5 (CH=CH_{2All}), 101.6 (CH_{Bzl}), 100.2 (C-1_B, ¹*J*_{C,H} = 171.5 Hz), 98.4 (C-1_D, ¹*J*_{C,H} = 165.9 Hz), 97.3 (C-1_C, ¹*J*_{C,H} = 179.5 Hz), 92.4 (CCl₃), 81.3 (C-4_D), 79.9 (C-4_B), 79.2 (C-2_C), 78.6 (C-4_C), 78.5 (C-5_B), 77.8 (C-3_B), 75.7, 75.3, 71.2 (3C, CH_{2Bn}), 70.9 (CH₂-CH=All), 70.7 (C-5_C), 69.9 (C-3_D), 69.5 (C-2_B), 68.6 (2C, C-5_B, C-6_D), 65.8 (C-5_D), 58.8 (C-2_D), 46.6 (CH_{2CA}), 38.1 (CH_{2Lev}), 29.8 (CH_{3Lev}), 28.2 (CH_{2Lev}), 18.0 (C-6_B), 17.9 (C-6_C). HRMS (ESI⁺) *m/z*: [M+Na]⁺ Calcd for C₅₈H₆₅Cl₄NO₁₇Na 1212.2869; found 1212.2871.

Trisaccharide **8** had ¹H NMR (400 MHz, CDCl₃) δ 7.49-7.46 (m, 2H, H_{ArBn}), 7.38-7.23 (m, 18H, H_{ArBn}, H_{ArBzl}), 6.96 (d, 1H, *J*_{2,NH} = 7.4 Hz, NH), 5.88 (m, 1H, CH=CH_{2All}), 5.55 (s, 1H, H_{Bzl}), 5.37 (dd, 1H, *J*_{1,2} = 1.9 Hz, *J*_{2,3} = 3.1 Hz, H-2_B), 5.30 (m, 1H, *J*_{trans} = 17.3 Hz, *J*_{gem} = 1.6 Hz, CH=CH_{2All}), 5.24-5.21 (m, 2H, H-2_C, CH=CH_{2All}), 5.07 (d, 1H, *J*_{1,2} = 8.2 Hz, H-1_D), 5.02 (d, 1H, H-1_B), 4.89 (d, 1H, *J* = 11.2 Hz, H_{Bn}), 4.85 (d, 1H, *J*_{1,2} = 1.5 Hz, H-1_C), 4.70 (d, 1H, *J* = 11.1 Hz, H_{Bn}), 4.63 (d, 1H, H_{Bn}), 4.57 (d_{po}, 1H, *J* = 11.3 Hz, H_{Bn}), 4.54 (m, 1H, H-3_D), 4.47 (d, 1H, H_{Bn}), 4.43 (d_{po}, 1H, H_{Bn}), 4.39 (dd_o, 1H, H-6a_D), 4.35 (m, 1H, H_{All}), 4.14-4.07 (m, 2H, H-3_C, H_{All}), 4.02 (brs, 2H, H_{CA}), 3.98 (dq_{po}, 1H, H-5_C), 3.82 (dd_{po}, 1H, H-3_B), 3.80-3.71 (m, 2H, H-6b_D, H-5_B), 3.63-3.55 (m, 2H, H-4_D, H-5_D), 3.45 (ddd, 1H, H-2_D), 3.39 (pt, 1H, *J*_{3,4} = *J*_{4,5} = 9.4 Hz, H-4_B), 3.30 (pt, 1H, *J*_{3,4} = *J*_{4,5} = 9.6 Hz, H-4_C), 2.72-2.62 (m, 4H, CH_{2Lev}), 2.17 (s, 3H, CH_{3Lev}), 1.28 (d, 3H, *J*_{5,6} = 6.1 Hz, H-6_B), 0.74 (d, 3H, *J*_{5,6} = 6.1 Hz, H-6_C). ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 206.2 (CO_{Lev}), 171.8 (CO_{2Lev}), 166.3 (CO_{2CA}), 162.2 (NHCO), 138.6-136.9 (4C, C_{IVBn}, C_{IVBzl}), 133.2 (CH=CH_{2All}), 129.1-126.5 (18C, C_{ArBn}, C_{ArBzl}), 118.4 (CH=CH_{2All}), 102.1 (CH_{Bzl}), 99.3 (C-1_B, ¹*J*_{C,H} = 171.8 Hz), 98.4 (C-1_D, ¹*J*_{C,H} = 164.1 Hz), 97.2 (C-1_C, ¹*J*_{C,H} = 172.7 Hz), 92.2 (CCl₃), 80.2 (C-4_B), 80.1 (C-4_D), 79.7 (C-4_C), 77.4 (C-3_B), 76.3 (C-3_C), 75.1, 74.9 (2C, CH_{2Bn}), 74.2 (C-3_D), 73.2 (C-2_C), 71.5 (CH_{2Bn}), 70.8 (CH₂-CH=All), 69.3 (C-2_B), 68.7 (2C, C-5_B, C-6_D), 68.1 (C-5_C), 66.2 (C-5_D), 59.9 (C-2_D), 40.6 (CH_{2CA}), 38.0 (CH_{2Lev}), 29.8 (CH_{3Lev}), 28.1 (CH_{2Lev}), 18.0 (C-6_B), 17.1 (C-6_C). HRMS (ESI⁺) *m/z*: [M+Na]⁺ Calcd for C₅₈H₆₅Cl₄NO₁₇Na 1212.2869; found 1212.2820.

Allyl 3,4-di-O-benzyl-α-L-rhamnopyranosyl-(1→3)-(4-O-benzyl-2-O-chloroacetyl-α-L-rhamnopyranosyl)-(1→3)-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside (9). To a solution of trisaccharide **8** (430 mg, 0.36 mmol) in Pyridine (6 mL) stirred at rt under an Ar atmosphere were added AcOH (4 mL) and NH₂NH₂·H₂O (100 μL, 2.06 mmol, 5.7 equiv.). After stirring for 35 min at rt, a TLC control (Toluene/Acetone 9:1) showed the absence of the starting material (R_f 0.35) and the formation of a more polar product (R_f 0.2). Following addition of H₂O (100 mL) and DCM (50 mL), the two layers were separated and the aq. one was extracted with DCM (2 x 50 mL). The combined organic extracts were washed with brine (100 mL), dried over anhyd. Na₂SO₄ and

1 concentrated to dryness. The residue was purified by flash chromatography (Toluene/EtOAc 85:15 to
2 0:1) to give alcohol **9** (342 g, 86%) as a white solid. The later had ^1H NMR (400 MHz, CDCl_3) δ 7.50-
3 7.47 (m, 2H, H_{ArBn}), 7.39-7.23 (m, 18H, H_{ArBn} , H_{ArBzl}), 7.04 (d, 1H, $J_{2,\text{NH}} = 7.4$ Hz, NH), 5.88 (m, 1H,
4 $\text{CH}=\text{CH}_{2\text{All}}$), 5.55 (s, 1H, H_{Bzl}), 5.30 (m, 1H, $J_{\text{trans}} = 17.2$ Hz, $J_{\text{gem}} = 1.5$ Hz, $\text{CH}=\text{CH}_{2\text{All}}$), 5.24-5.21 (m,
5 2H, H-2_C, $\text{CH}=\text{CH}_{2\text{All}}$), 5.07 (d_o, 1H, H-1_B), 5.06 (d_{po}, 1H, $J_{1,2} = 7.9$ Hz, H-1_D), 4.86 (d, 1H, $J_{1,2} = 1.6$
6 Hz, H-1_C), 4.86 (d, 1H, $J = 11.2$ Hz, H_{Bn}), 4.66 (d, 1H, H_{Bn}), 4.64 (d_{po}, 1H, $J = 11.8$ Hz, H_{Bn}), 4.60 (brs,
7 2H, H_{Bn}), 4.58 (d_{po}, 1H, H_{Bn}), 4.54 (m, 1H, H-3_D), 4.49 (d, 1H, $J = 11.0$ Hz, H_{Bn}), 4.39 (dd_{po}, 1H, $J_{5,6a} =$
8 4.6 Hz, H-6_{aD}), 4.36 (m, 1H, $\text{CH}_2\text{-CH}=\text{All}$), 4.13-4.08 (m, 2H, H-3_C, $\text{CH}_2\text{-CH}=\text{All}$), 4.05 (d, $J = 14.7$ Hz,
9 H_{CA}), 4.02 (d, $J = 14.7$ Hz, H_{CA}), 3.99 (dq_{po}, 1H, H-5_C), 3.91 (dd, 1H, $J_{1,2} = 1.7$ Hz, $J_{2,3} = 3.1$ Hz, H-2_B),
10 3.81 (pt, 1H, $J_{6b,5} = 9.6$ Hz, H-6_{bD}), 3.75-3.69 (m, 2H, H-3_B, H-5_B), 3.64-3.55 (m, 2H, H-4_D, H-5_D), 3.48
11 (ddd_{po}, 1H, H-2_D), 3.44 (pt, 1H, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4_B), 3.30 (pt, 1H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4_C), 2.36
12 (brs, 1H, OH), 1.28 (d, 3H, $J_{5,6} = 6.3$ Hz, H-6_B), 0.77 (d, 3H, $J_{5,6} = 6.2$ Hz, H-6_C). ^{13}C $\{^1\text{H}\}$ NMR (100
13 MHz, CDCl_3) δ 166.3 ($\text{CO}_{2\text{CA}}$), 162.2 (NHCO), 138.5-137.0 (4C, C_{IVBn} , C_{IVBzl}), 133.2 ($\text{CH}=\text{CH}_{2\text{All}}$),
14 129.1-126.4 (18C, C_{ArBn} , C_{IVBzl}), 118.4 ($\text{CH}=\text{CH}_{2\text{All}}$), 102.1 (CH_{Bzl}), 101.2 (C-1_B, $^1J_{\text{C,H}} = 170.8$ Hz), 98.3
15 (C-1_D, $^1J_{\text{C,H}} = 165.5$ Hz), 97.2 (C-1_C, $^1J_{\text{C,H}} = 172.1$ Hz), 92.2 (CCl_3), 80.4 (C-4_C), 80.1 (C-4_D), 79.7 (C-
16 4_B), 79.4 (C-3_B), 76.4 (C-3_C), 75.1, 75.0 (2C, $\text{CH}_{2\text{Bn}}$), 74.1 (C-3_D), 73.7 (C-2_C), 72.1 ($\text{CH}_{2\text{Bn}}$), 70.8 ($\text{CH}_2\text{-}$
17 $\text{CH}=\text{All}$), 69.0 (C-2_B), 68.7 (C-6_D), 68.4 (C-5_B), 68.1 (C-5_C), 66.2 (C-5_D), 60.0 (C-2_D), 40.6 ($\text{CH}_{2\text{CA}}$), 17.9
18 (C-6_B), 17.1 (C-6_C). HRMS (ESI⁺) m/z : $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{53}\text{H}_{59}\text{Cl}_4\text{NO}_{15}\text{Na}$ 1112.2537; found
19 1112.2651.

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32 **Propyl α -L-rhamnopyranosyl-(1 \rightarrow 3)-2-O-acetyl- α -L-rhamnopyranosyl-(1 \rightarrow 3)-2-acetamido-2-**
33 **deoxy- β -D-glucopyranoside (**10**).** To a stirred solution of trisaccharide **9** (76.9 mg, 70 μmol) in 96%
34 aq. EtOH (6 mL) and DCE (150 μL), were added Pd/C (75.6 mg). The suspension was stirred under H_2
35 atmosphere at rt for 24 h. After this time, a solution of Na_2HPO_4 (0.5 M, 500 μL) was added and the
36 suspension was stirred for another 3 d at rt. MS analysis of the reaction mixture revealed a molecular
37 weight of corresponding to that of the target trisaccharide. The reaction mixture was passed through a
38 0.2 μm filter. After freeze-drying, purification of the residue by RP-LC (215 nm, 0-40% linear gradient
39 of $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ over 27 CV at a flow rate of 30 $\text{mL}\cdot\text{min}^{-1}$), gave trisaccharide **10** (28.7 mg, 47%) as a
40 fluffy solid after repeated freeze-drying. Propyl glycoside **10** had ^1H NMR (400 MHz, D_2O) δ 5.00-4.96
41 (m, 2H, H-2_C, H-1_B), 4.86 (brs, 1H, H-1_C), 4.50 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1_D), 4.08 (dq, 1H, H-5_C), 4.01
42 (brd, 1H, H-2_B), 3.96-3.89 (m, 2H, H-6_{aD}, H-3_C), 3.85-3.69 (m, 3H, $\text{OCH}_{2\text{Pr}}$, H-2_D, H-6_{bD}), 3.65 (dd,
43 1H, $J_{2,3} = 2.7$ Hz, $J_{3,4} = 9.4$ Hz, H-3_B), 3.60-3.40 (m, 7H, H-4_C, $\text{OCH}_{2\text{Pr}}$, H-3_D, H-4_D, H-5_B, H-4_B), 2.16
44 (s, 3H, $\text{CH}_{3\text{Ac}}$), 2.05 (s, 3H, $\text{CH}_{3\text{NHAc}}$), 1.54 (psex, 2H, $J = 6.9$ Hz, $\text{CH}_{2\text{Pr}}$), 1.25-1.22 (m, 6H, H-6_B, H-
45 6_C), 0.86 (t, 3H, $\text{CH}_{3\text{Pr}}$). ^{13}C $\{^1\text{H}\}$ NMR (100 MHz, D_2O) δ 177.1 (NHCO), 175.8 (CO_{Ac}), 105.4 (C-1_B,
46 $^1J_{\text{C,H}} = 171.3$ Hz), 103.3 (C-1_D, $^1J_{\text{C,H}} = 163.5$ Hz), 101.1 (C-1_C, $^1J_{\text{C,H}} = 173.4$ Hz), 85.1 (C-3_D), 79.8 (C-
47 3_C), 78.7 (C-5_D), 75.1 (C-2_C), 74.9 ($\text{OCH}_{2\text{Pr}}$), 74.4 (C-4_B), 73.9 (C-4_C), 72.8 (C-3_B), 72.7 (C-2_B), 72.0
48 (C-5_B), 71.6 (C-5_C), 71.1 (C-4_D), 63.5 (C-6_D), 57.9 (C-2_D), 25.0 ($\text{CH}_{3\text{NHAc}}$), 24.7 ($\text{CH}_{2\text{Pr}}$), 22.9 ($\text{CH}_{3\text{Ac}}$),
49 19.2, 19.0 (2C, C-6_C, C-6_B), 12.3 ($\text{CH}_{3\text{Pr}}$). HRMS (ESI⁺) m/z : $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{25}\text{H}_{43}\text{NO}_{15}\text{Na}$
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620.2531; found 620.2496; [M+H]⁺ Calcd for C₂₅H₄₄NO₁₅ 598.2711; found 598.2674. RP-HPLC (Conditions A) t_R (215 nm): 9.82 min.

Synthesis of tetrasaccharide 22 from L-rhamnose 1 and glucosaminide 7

Allyl 4-O-(2-naphthylmethyl)- α -L-rhamnopyranoside (16). Acetyl chloride (50 mL, 693 mmol, 2.5 equiv.) was added dropwise to allylic alcohol (610 mL) at 0 °C, the solution was stirred for 25 min, and then L-rhamnose monohydrate (50 g, 277 mmol) was added. The mixture was heated for 2.5 h at 70 °C then for 15 h at 40 °C. Follow up by TLC (DCM/MeOH 8:2) indicated the conversion of the starting hemiacetal (R_f 0.2) into a less polar product (R_f 0.7). The bath temperature was cooled to 0 °C and the solution was neutralized by addition of NaHCO_{3(s)} (103 g). The suspension was filtered over a pad of Celite® and solvents were evaporated and co-evaporated three times with Toluene. The crude material was dissolved in anhyd. acetone (300 mL) then 2,2-dimethoxypropane (100 mL, 0.81 mol, 3.0 equiv.) and PTSA (3.04 g, 16 mmol, 0.05 equiv.) were successively added. After stirring at rt for 3 h, TLC (DCM/MeOH 9:1) indicated the conversion of the intermediate allyl glycoside (R_f 0.3) into a less polar product (R_f 0.6). The solution was neutralized by adding Et₃N (4.0 mL) and solvents were evaporated under reduced pressure. The residue was dissolved in DCM (600 mL) and washed with H₂O (3 x 300 mL) and brine (200 mL). The organic layer was dried by passing through phase separator filter and concentrated to dryness. The crude residue was dissolved in DMF (800 mL) under an Ar atmosphere, the bath temperature was cooled to -5 °C and NaH (60% oil dispersion, 29.1 g, 0.73 mol, 2.4 equiv.) was added portion wise to this suspension. The mixture was stirred at rt for 2 h under Ar and then 2-(bromomethyl)naphthalene (73.5 g, 0.33 mol, 1.2 equiv.) was added portion wise at -5 °C. After stirring the reaction mixture for 2 h under Ar at rt, a TLC control (Cyclohexane/EtOAc 7:3) indicated the absence of the intermediate alcohol (R_f 0.3) and the presence of a less polar product (R_f 0.7). The reaction was quenched at 0 °C by addition of MeOH (50 mL). Solvents were eliminated under reduced pressure and volatiles were co-evaporated with Toluene. The residue was taken in EtOAc (400 mL) and washed with H₂O (3 x 300 mL) and brine (150 mL). The organic phase was dried over anhyd. Na₂SO₄, filtered, and concentrated to dryness. The crude residue was dissolved in 80% aq. AcOH (500 mL) and the solution was stirred for 20 h at 80 °C. A TLC control (Cyclohexane/EtOAc 5:5) indicated that the intermediate (R_f 1.0) had been converted into a more polar product (R_f 0.2). Solvents were removed under vacuum and traces of AcOH were eliminated by co-evaporation with Toluene (3 x 400 mL). The residue was taken in CH₃CN (500 mL) and washed with cold Cyclohexane (2 x 300 mL). The CH₃CN layer was evaporated to dryness and recrystallization in hot Cyclohexane was performed to afford the expected diol **16** (82.5 g, 87%) as a white to off-white crystal. The later had mp = 74.8 °C (Cyclohexane). ¹H NMR (400 MHz, CDCl₃) δ 7.89-7.81 (m, 4H, H_{ArNap}), 7.55-7.46 (m, 3H, H_{ArNap}), 5.89 (dddd, 1H, *J* = 17.2, 10.4, 6.0, 5.2 Hz, CH=CH_{2All}), 5.30 (ddd, 1H, *J* = 17.2, 3.2, 1.5 Hz, CH=CH_{2All}), 5.21 (ddd, 1H, *J* = 10.4, 2.8, 1.5 Hz, CH=CH_{2All}), 4.93 (s, 2H, H_{Nap}), 4.84 (d, *J*_{1,2} = 0.95 Hz, 1H, H-1), 4.19 (ddpt, 1H, *J* = 12.9, 5.1, 1.5 Hz, CH₂-CH=All), 4.04-3.95 (m, 3H, CH₂-CH=All, H-2, H-3), 3.81 (dq, 1H, *J*_{5,4} = 9.2 Hz, *J*_{5,6} = 6.3 Hz, H-5), 3.43 (pt, 1H, *J*_{3,4} = 9.2 Hz, H-4), 2.38 (brs, 2H, OH-2, OH-3), 1.40 (d, 3H, *J*_{6,5} = 6.3 Hz, H-6). ¹³C {¹H} NMR (100 MHz, CDCl₃) δ 133.7 (CH=CH_{2All}), 128.5 (C_{IVAr}), 127.9 (C_{IVAr}), 127.7

(C_{IVAr}), 126.7-125.8 (7C, C_{ArNap}), 117.4 (CH=CH_{2All}), 98.5 (C-1, ¹J_{C,H} = 170.1 Hz), 81.8 (C-4), 75.1 (CH_{2Nap}), 71.6 (C-3), 71.3 (C-2), 68.0 (CH₂-CH=All), 67.3 (C-5), 18.1 (C-6). HRMS (ESI⁺) *m/z*: [M+NH₄]⁺ Calcd for C₁₆H₂₆NO₅ 362.1967; found 362.1985; [M+Na]⁺ Calcd for C₁₆H₂₂O₅Na 367.1521; found 367.1576.

Allyl 4-*O*-(2-naphthylmethyl)-3-*O*-triethylsilyl- α -L-rhamnopyranoside (S1). To a solution of diol **16** (20.0 g, 58.1 mmol) in anhyd. CH₃CN (800 mL) stirred at 0 °C under an Ar atmosphere were successively added dropwise TES-Cl (11.70 mL, 69.7 mmol, 1.2 equiv.) and DIPEA (15.2 mL, 87.1 mmol, 1.5 equiv.). After stirring at rt for 2 h under Ar, TLC (Cyclohexane/EtOAc 8:2) showed complete consumption of the starting material (R_f 0.15) and the presence of a main product (R_f 0.7). MeOH (1.2 mL, 29.0 mmol, 0.5 equiv.) was added, and after 15 min, volatiles were evaporated under reduced pressure. Toluene was added and a precipitate appeared. The suspension was filtered and the filtrate was concentrated to dryness. The residue was purified by flash chromatography (Toluene/EtOAc 95:5 to 9:1) to give alcohol **S1** (19.7 g, 74%) as a yellow oil. The later had ¹H NMR (400 MHz, CDCl₃) δ 7.90-7.79 (m, 4H, H_{ArNap}), 7.55-7.45 (m, 3H, H_{ArNap}), 5.9 (dddd, 1H, *J* = 17.2, 10.4, 6.0, 5.2 Hz, CH=CH_{2All}), 5.30 (ddd, 1H, *J* = 17.2, 3.2, 1.5 Hz, CH=CH_{2All}), 5.22 (ddd, 1H, *J* = 10.4, 2.9, 1.5 Hz, CH=CH_{2All}), 5.06 (d, 1H, *J* = 11.5 Hz, H_{Nap}), 4.89 (d, 1H, *J*_{1,2} = 1.5 Hz, H-1), 4.81 (d, 1H, *J* = 11.5 Hz, H_{Nap}), 4.25-4.15 (ddpt, 1H, *J* = 13.1, 5.0, 1.5 Hz, CH₂-CH=All), 4.14 (dd, 1H, *J*_{3,4} = 9.2 Hz, *J*_{3,2} = 3.5 Hz, H-3), 4.06-3.97 (m, 1H, CH₂-CH=All), 3.88 (dd, 1H, *J*_{2,3} = 3.5 Hz, *J*_{2,1} = 1.5 Hz, H-2), 3.81 (dq, 1H, *J*_{5,4} = 9.2 Hz, *J*_{5,6} = 6.3 Hz, H-5), 3.46 (pt, 1H, *J*_{4,3} = *J*_{4,5} = 9.2 Hz, H-4), 2.74 (brs, 1H, OH), 1.33 (d, 3H, *J*_{6,5} = 6.3 Hz, H-6), 1.00 (t, 9H, *J* = 8.0 Hz, CH_{3TES}), 0.74 (q, 6H, *J* = 8.0 Hz, CH_{2TES}). ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 136.0 (C_{IVNap}), 134.0 (CH=CH_{2All}), 133.3 (C_{IVNap}), 133.0 (C_{IVNap}), 128.0-125.8 (7C, C_{ArNap}), 117.0 (CH=CH_{2All}), 98.0 (C-1, ¹J_{C,H} = 169.0 Hz), 81.3 (C-4), 75.5 (CH_{2Nap}), 73.3 (C-3), 72.2 (C-2), 67.8 (CH_{2All}), 67.4 (C-5), 17.9 (C-6), 6.8 (CH_{3TES}), 5.1 (CH_{2TES}). HRMS (ESI⁺) *m/z*: [M+Na]⁺ Calcd for C₂₆H₃₈O₅SiNa 481.2394; found 481.2386.

Allyl 2-*O*-levulinoyl-4-*O*-(2-naphthylmethyl)-3-*O*-triethylsilyl- α -L-rhamnopyranoside (S2). Alcohol **S1** (19.7 g, 43.0 mmol) was dissolved in anhyd. DCM (145 mL) and stirred at rt. DCC (17.7 g, 85.9 mmol, 2.0 equiv.), DMAP (4.2 g, 34.4 mmol, 0.8 equiv.) and levulinic acid (13.2 mL, 128.9 mmol, 3.0 equiv.) were successively added. After stirring at rt for 2 h, a TLC control (Cyclohexane/EtOAc 7:3) showed complete consumption of the starting material (R_f 0.6) and the presence of a more polar product (R_f 0.55). The reaction mixture was concentrated under reduced pressure. The crude material was taken in EtOAc (100 mL) and the resulting suspension was filtered on a pad of Celite®. H₂O (30 mL) was added to the filtrate and the organic layer was washed successively with 10% aq. CuSO₄ (30 mL), H₂O (30 mL), satd aq. NaHCO₃ (30 mL) and brine (30 mL). The organic layer was dried by stirring over anhyd. Na₂SO₄, filtered and concentrated under vacuum. The residue was purified by flash chromatography (Toluene/EtOAc 8:2) to give the protected monosaccharide **S2** (17.7 g, 74%) as a yellow to brown oil. The later had ¹H NMR (400 MHz, CDCl₃) δ 7.90-7.79 (m, 4H, H_{ArNap}), 7.54-7.45 (m, 3H, H_{ArNap}), 5.91 (dddd, 1H, *J* = 17.2, 10.4, 6.0, 5.2 Hz, CH=CH_{2All}), 5.30 (ddd, 1H, *J* = 17.2, 3.2,

1 1.5 Hz, CH=CH_{2All}), 5.22 (ddd, 1H, *J* = 10.4, 2.9, 1.5 Hz, CH=CH_{2All}), 5.13-5.08 (m, 2H, H_{Nap}, H-2),
2 4.82 (d, 1H, *J* = 11.5 Hz, H_{Nap}), 4.72 (d, 1H, *J*_{1,2} = 1.6 Hz, H-1), 4.25 (dd, 1H, *J*_{3,2} = 3.4 Hz, *J*_{3,4} = 9.4 Hz,
3 H-3), 4.17 (ddpt, 1H, *J* = 13.1, 5.0, 1.4 Hz, CH₂-CH=All), 3.98 (ddpt, 1H, *J* = 13.1, 5.8, 1.4 Hz, CH₂-
4 CH=All), 3.80 (dq, 1H, *J*_{5,4} = 9.4 Hz, *J*_{5,6} = 6.2 Hz, H-5), 3.44 (pt, 1H, *J*_{4,5} = *J*_{4,3} = 9.4 Hz, H-4), 2.82-2.70
5 (m, 4H, CH_{2Lev}), 2.20 (s, 3H, CH_{3Lev}), 1.32 (d, 3H, *J*_{6,5} = 6.2 Hz, H-6), 1.00 (t, 9H, *J* = 8.1 Hz, CH_{3TES}),
6 0.74 (q, 6H, *J* = 8.1 Hz, CH_{2TES}). ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 206.0 (CO_{Lev}), 172.1 (CO_{2Lev}),
7 136.1 (C_{IVNap}), 133.8 (CH=CH_{2All}), 133.3 (C_{IVNap}), 133.0 (C_{IVNap}), 128.0-125.8 (7C, C_{ArNap}), 117.0
8 (CH=CH_{2All}), 96.6 (C-1, ¹*J*_{C,H} = 170.6 Hz), 81.4 (C-4), 75.5 (CH_{2Nap}), 73.5 (C-2), 71.1 (C-3), 68.0
9 (CH_{2All}), 67.9 (C-5), 38.0 (CH_{2Lev}), 29.8 (CH_{3Lev}), 28.2 (CH_{2Lev}), 18.0 (C-6), 6.8 (CH_{3TES}), 4.9 (CH_{2TES}).
10 HRMS (ESI⁺) *m/z*: [M+Na]⁺ Calcd for C₃₁H₄₄O₇SiNa 479.2791; found 479.2754.

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18 **2-O-Levulinoyl-4-O-(2-naphthylmethyl)-3-O-triethylsilyl-α-L-rhamnopyranose (17).** *Route 1.* 1,5-
19 Cyclooctadiene-bis(methyldiphenylphosphine)-iridium hexafluorophosphate (358 mg, 0.636 mmol,
20 0.02 equiv.) was dissolved in anhyd. THF (30 mL) and the solution was degassed repeatedly then stirred
21 under a satd. H₂ atmosphere for 30 min. The resulting yellow solution was degassed repeatedly before
22 being poured into a solution of rhamnoside **S2** (17.7 g, 31.8 mmol) in anhyd. THF (320 mL). The
23 mixture was stirred under an Ar atmosphere at rt for 2 h. A TLC control (Cyclohexane/EtOAc 7:3)
24 showed complete conversion of the starting material (Rf 0.5) into a less polar intermediate (Rf 0.55). A
25 solution of I₂ (16.1 g, 63.6 mmol, 2.0 equiv.) and NaHCO₃ (21.4 g, 254.4 mmol, 8.0 equiv.) in 1:5
26 H₂O/THF (389 mL) was then poured at 0 °C. After stirring for 2 h at this temperature, a TLC control
27 (Cyclohexane/EtOAc 7:3) showed complete conversion of the intermediate into a more polar product
28 (Rf 0.3). 10% aq. Na₂S₂O₅ (100 mL) was added. THF was evaporated under reduced pressure and DCM
29 (300 mL) was added. The aq. layer was extracted with DCM (2 x 200 mL) and the combined organic
30 phases were washed with satd aq. NaHCO₃ (100 mL) and brine (100 mL). The organic layer was dried
31 over anhyd. Na₂SO₄, filtered and concentrated under vacuum. The residue was purified by flash
32 chromatography (Cyclohexane/EtOAc 8:2 to 6:4 + 1% Et₃N) to give hemiacetal **17** (14.6 g, 89%, α/β
33 4:1) as an off-white to yellow oil.

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43 *Route 2.* To a solution of diol **16** (20.0 g, 58.07 mmol) in anhyd. CH₃CN (580 mL) stirred at rt were
44 successively added dropwise TES-Cl (10.23 mL, 61 mmol, 1.05 equiv.) and DIPEA (15.17 mL, 87
45 mmol, 1.5 equiv.). After stirring the RM at rt for 2 h, a TLC control (Cyclohexane/EtOAc 8:2) showed
46 complete consumption of the starting material (Rf 0.15) and the presence of a main product (Rf 0.7).
47 MeOH (2.35 mL, 58.07 mmol, 1 equiv.) was added, the mixture was stirred for 15 min, and volatiles
48 were evaporated under reduced pressure. Toluene was added and a precipitate appeared. The suspension
49 was filtered and the filtrate was concentrated to dryness. The crude residue was dissolved in anhyd.
50 DCM (194 mL) and stirred at rt. Then were successively added DCC (23.96 g, 116.14 mmol, 2.0 equiv.),
51 DMAP (5.68 g, 46.46 mmol, 0.8 equiv.) and levulinic acid (17.84 mL, 174.21 mmol, 3.0 equiv.). After
52 stirring at rt for 15 h, TLC (Cyclohexane/EtOAc 7:3) showed complete consumption of the starting
53 material (Rf 0.6) and the presence of a more polar compound (Rf 0.55). The reaction mixture was
54 concentrated under reduced pressure. The crude material was taken in EtOAc (200 mL) and the resulting
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suspension was filtered on a pad of Celite®. H₂O (150 mL) was added to the filtrate and the organic layer was washed successively with 10% aq. CuSO₄ (2 x 150 mL), H₂O (2 x 150 mL), satd aq. NaHCO₃ (150 mL) and brine (150 mL). The organic layer was dried over anhyd. Na₂SO₄, filtered and concentrated under vacuum. 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)-iridium hexafluorophosphate (982 mg, 1.16 mmol, 0.02 equiv.) was dissolved in anhyd. THF (50 mL) and the solution was degassed repeatedly then stirred under a satd. H₂ atmosphere for 1 h. The resulting yellow solution was degassed repeatedly before being poured into a solution of the crude residue in anhyd. THF (580 mL). The mixture was stirred under an Ar atmosphere at rt for 3 h. A TLC control (Cyclohexane/EtOAc 7:3) showed complete conversion of the starting material (Rf 0.5) into a less polar product (Rf 0.55). A mixture of I₂ (29.5 g, 116.1 mmol, 2 equiv.) and NaHCO₃ (39.0 g, 464.6 mmol, 8 equiv.) in 1:5 H₂O/THF (710 mL) was added to the mixture stirred at 0 °C. After stirring for 2 h at this temperature, a TLC control (Cyclohexane/EtOAc 7:3) showed complete conversion of the intermediate and the presence of a more polar product (Rf 0.3). Satd aq. Na₂S₂O₅ (500 mL) was added. THF was evaporated under reduced pressure and DCM (500 mL) was added. The aq. layer was extracted with DCM (2 x 250 mL) and the combined organic phases were washed with satd aq. NaHCO₃ (500 mL) and brine (500 mL). The organic layer was dried over anhyd. Na₂SO₄, filtered and concentrated under vacuum. The residue was purified by flash chromatography (Cyclohexane/EtOAc 8:2 to 6:4 + 1% Et₃N) to give hemiacetal **17** (24.0 g, 80%, α/β 3:1) as an off-white to yellow oil. The later had ¹H NMR (400 MHz, CDCl₃) δ 7.89-7.78 (m, 4H, H_{ArNap}), 7.53-7.44 (m, 3H, H_{ArNap}), 5.30 (dd, 0.25H, $J_{2,1} = 1.1$ Hz, $J_{2,3} = 3.4$ Hz, H-2 β), 5.13-5.03 (m, 2.5H, H-1 α , H-2 α , H_{Nap}), 4.85-4.77 (m, 1.25H, H-1 β , H_{Nap}), 4.28 (dd, 0.75H, $J_{3,2} = 2.7$ Hz, $J_{3,4} = 9.3$ Hz, H-3 α), 4.00 (dq, 0.75H, $J_{5,4} = 9.3$ Hz, $J_{5,6} = 6.2$ Hz, H-5 α), 3.92 (dd, 0.25H, $J_{3,2} = 3.3$ Hz, $J_{3,4} = 9.0$ Hz, H-3 β), 3.50-3.43 (m, 0.25H, H-5 β), 3.43 (pt, 0.75H, $J_{4,5} = J_{4,3} = 9.3$ Hz, H-4 α), 3.37 (pt, 0.25H, $J_{4,5} = J_{4,3} = 9.0$ Hz, H-4 β), 2.93-2.61 (m, 4H, CH_{2Lev}), 2.22 (s, 2.25H, CH_{3Lev} α), 2.21 (s, 0.75H, CH_{3Lev} β), 1.36 (d, 0.75H, $J_{6,5} = 6.2$ Hz, H-6 β), 1.30 (d, 2.25H, $J_{6,5} = 6.2$ Hz, H-6 α), 0.98 (t, 6.75H, $J = 8.1$ Hz, CH_{3TES} α), 0.97 (t, 2.25H, $J = 8.1$ Hz, CH_{3TES} β), 0.68 (q, 6H, $J = 8.1$ Hz, CH_{2TES}). ¹³C {¹H} NMR (100 MHz, CDCl₃) δ 206.2 (CO_{Lev}), 172.2 (CO_{2Lev}), 136.0 (C_{IVNap}), 133.3 (C_{IVNap}), 133.0 (C_{IVNap}), 128.0-125.8 (C_{ArNap}), 93.1 (C-1 β , $^1J_{C,H} = 158.5$ Hz), 92.2 (C-1 α , $^1J_{C,H} = 171.3$ Hz), 81.3 (C-4 α), 80.9 (C-4 β), 75.3 (CH_{2Nap}), 74.2 (C-2 β), 73.8 (C-2 α), 73.6 (C-3 β), 71.7 (C-5 β), 70.6 (C-3 α), 68.0 (C-5 α), 38.0 (CH_{2Lev}), 29.8 (CH_{3Lev}), 28.2 (CH_{2Lev}), 18.2 (C-6 β), 18.1 (C-6 α), 6.8 (CH_{3TES}), 4.9 (CH_{2TES}). HRMS (ESI⁺) m/z : [M+Na]⁺ Calcd for C₂₈H₄₀O₇SiNa 539.2451; found 539.2441.

2-O-Levulinoyl-4-O-(2-naphthylmethyl)-3-O-triethylsilyl- α/β -L-rhamnopyranosyl

trichloroacetimidate (15). To a solution of hemiacetal **17** (24.0 g, 46.5 mmol) in anhyd. DCE (210 mL) stirred at rt under an Ar atmosphere were successively added CCl₃CN (14 mL, 139.6 mmol, 3.0 equiv.) and DBU (3.47 mL, 23.2 mmol, 0.5 equiv.). After stirring the RM for 3 h under Ar at rt, a TLC control (Cyclohexane/EtOAc 7:3) showed complete consumption of the starting material (Rf 0.3) and the presence of a less polar product (Rf 0.55). Volatiles were evaporated under reduced pressure. The residue was purified by flash chromatography (Cyclohexane/EtOAc 9:1 to 5:5 + 1% Et₃N) to give donor

1 **15** (28.0 g, 91%, α/β 95:5) as a whitish crystalline solid. Trichloroacetimidate **15** had mp = 65.7 °C. ¹H
2 NMR (400 MHz, CDCl₃) δ 8.67 (s, 0.95H, NH α), 8.46 (s, 0.05H, NH β), 7.89-7.79 (m, 4H, H_{ArNap}), 7.54-
3 7.47 (m, 3H, H_{ArNap}), 6.15 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1 α), 5.56 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1 β), 5.26 (dd, 0.95H,
4 $J_{2,3}$ = 3.2 Hz, $J_{2,1}$ = 2.1 Hz, H-2 α), 5.26 (dd, 0.05H, $J_{2,3}$ = 3.2 Hz, $J_{2,1}$ = 2.0 Hz, H-2 β), 5.10 (d, 1H, J =
5 11.5 Hz, H_{Nap}), 4.82 (d, 1H, J = 11.5 Hz, H_{Nap}), 4.32 (dd, 1H, $J_{3,2}$ = 3.2 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 3.95 (dq,
6 0.95H, $J_{5,4}$ = 9.3 Hz, $J_{5,6}$ = 6.2 Hz, H-5 α), 3.86-3.77 (m, 0.05H, H-5 β), 3.52 (pt, 1H, $J_{4,3}$ = $J_{4,5}$ = 9.3 Hz,
7 H-4), 2.84-2.74 (m, 4H, CH_{2Lev}), 2.23 (s, 2.85H, CH<sub>3Lev α), 2.22 (s, 0.15H, CH_{3Lev} β), 1.33 (d, 2.85H, $J_{6,5}$
8 = 6.2 Hz, H-6 α), 1.30 (d, 0.15H, $J_{6,5}$ = 6.3 Hz, H-6 β), 0.99 (t, 9H, J = 8.0 Hz, CH_{3TES}), 0.68 (q, 6H, J =
9 8.0 Hz, CH_{2TES}). ¹³C{¹H} (100 MHz, CDCl₃) δ 205.9 (CO_{Lev}), 171.3 (CO_{2Lev}), 160.1 (CNH) 135.7
10 (C_{IVNap}), 133.3 (C_{IVNap}), 133.0 (C_{IVNap}), 128.1-125.9 (7C, C_{ArNap}), 95.4 (C-1 β , ¹ $J_{C,H}$ = 175.3 Hz), 95.0 (C-
11 1 α , ¹ $J_{C,H}$ = 179.7 Hz), 91.0 (CCl₃), 80.5 (C-4), 75.7 (CH_{2Nap}), 71.9 (C-2), 71.0, 70.9 (2C, C-3, C-5), 38.0
12 (CH_{2Lev}), 29.8 (CH_{3Lev}), 28.1 (CH_{2Lev}), 18.0 (C-6), 6.8 (CH_{3TES}), 4.8 (CH_{2TES}). HRMS (ESI⁺) m/z :
13 [M+Na]⁺ Calcd for C₃₀H₄₀Cl₃NO₇SiNa 682.1526; found 682.1537.</sub>

23 **Allyl 2-O-chloroacetyl-4-O-(2-naphthylmethyl)- α -L-rhamnopyranoside (14)**. To a solution of diol **16**
24 (15.3 g, 44.5 mmol) in anhyd. CH₃CN (306 mL) stirred at rt was added 2-chloro-1,1,1-trimethoxyethane
25 (18.9 mL, 133.4 mmol, 3.0 equiv.) and PTSA·H₂O (0.85 g, 4.45 mmol, 0.1 equiv.). After stirring at rt
26 for 1 h, TLC (Cyclohexane/EtOAc 7:3) showed complete consumption of the starting material (Rf 0.13)
27 into a mix of two less polar products (Rf 0.75, 0.8). Then, 90% aq. TFA (215 mL) was added to the
28 reaction medium at 0 °C. After stirring for 30 min at rt, a TLC control (Cyclohexane/EtOAc 7:3)
29 indicated the complete consumption of the intermediate and the presence of a major more polar product
30 (Rf 0.5). H₂O was added until the mixture became completely cloudy (100 mL). The product was
31 extracted with EtOAc (2 x 200 mL). The organic phase was washed with satd aq. NaHCO₃ (2 x 200 mL)
32 and brine (200 mL). The organic layer was dried over anhyd. Na₂SO₄, filtered and concentrated under
33 vacuum to obtain a 95:5 mixture of alcohol **14** and its regioisomer **14a**. Acceptor **14** had ¹H NMR (400
34 MHz, CDCl₃) δ 7.92-7.81 (m, 4H, H_{ArNap}), 7.55-7.48 (m, 3H, H_{ArNap}), 5.89 (dddd, 1H, J = 17.0, 10.5,
35 5.9, 5.2 Hz, CH=CH_{2All}), 5.51 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{2,1}$ = 1.5 Hz, H-2), 5.31 (ddd, 1H, J = 17.2, 3.2, 1.5
36 Hz, CH=CH_{2All}), 5.24 (ddd, 1H, J = 10.5, 2.9, 1.5 Hz, CH=CH_{2All}), 4.97 (d, 1H, J = 11.3 Hz, H_{Nap}), 4.93
37 (d, 1H, J = 11.3 Hz, H_{Nap}), 4.86 (brd, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 4.17 (ddpt, 1H, J = 12.8, 5.2, 1.5 Hz, CH₂-
38 CH=All), 4.17 (d, 1H, J = 2.9 Hz, H_{CA}), 4.16 (d, 1H, J = 2.9 Hz, H_{CA}), 3.98 (ddpt, 1H, J = 12.8, 5.9, 1.5
39 Hz, CH₂-CH=All), 3.85 (dd, 1H, $J_{3,2}$ = 3.3 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 3.76 (dq, 1H, $J_{5,4}$ = 9.6 Hz, $J_{5,6}$ = 6.3
40 Hz, H-5), 3.45 (pt, 1H, $J_{4,3}$ = $J_{4,5}$ = 9.6 Hz, H-4), 2.07 (s, 1H, OH), 1.35 (d, 3H, $J_{6,5}$ = 6.3 Hz, H-6).
41 ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 167.1 (CO_{2CA}), 135.6 (C_{IVNap}), 133.4 (CH=CH_{2All}), 133.2 (C_{IVNap}),
42 133.1 (C_{IVNap}), 129.0-125.9 (C_{ArNap}), 117.7 (CH=CH_{2All}), 96.2 (C-1, ¹ $J_{C,H}$ = 170.4 Hz), 81.5 (C-4), 75.3
43 (CH_{2Nap}), 74.6 (C-2), 70.2 (C-3), 68.2 (CH_{2All}), 67.7 (C-5), 40.9 (CH_{2CA}), 18.0 (C-6). HRMS (ESI⁺) m/z :
44 [M+Na]⁺ Calcd for C₂₂H₂₅ClO₆Na 443.1232; found 443.1284.

1 **Allyl 2-O-levulinoyl-4-O-(2-naphthylmethyl)-3-O-triethylsilyl- α -L-rhamnopyranosyl-(1 \rightarrow 3)-2-O-**
2 **chloroacetyl-4-O-(2-naphthylmethyl)- α -L-rhamnopyranoside (18).** To a solution of crude acceptor **14**
3 (44.5 mmol, 1.1 equiv.) in anhyd. Toluene (424 mL), stirred at rt under an Ar atmosphere, were
4 successively added donor **15** (28.0 g, 42.4 mmol) and activated 4 Å MS (5.6 g). The suspension was
5 stirred for 15 min at rt, then cooled to -60 °C. After 15 min of stirring at this temperature, TBSOTf (781
6 μ L, 3.4 mmol, 0.08 equiv.) was slowly added. After stirring for 1.5 h under Ar while the temperature
7 wise rising from -60 to -15 °C, a TLC control (Cyclohexane/EtOAc 7:3) showed the complete
8 consumption of donor **15** (Rf 0.6) and the formation of a less polar product (Rf 0.7). The mixture was
9 cooled down to -60 °C and Et₃N (700 μ L, 5.0 mmol, 0.12 equiv.) was added for neutralization. The
10 reaction mixture was stirred for 15 min at -60 °C and then filtered on a pad of Celite®. The filtrate was
11 concentrated under reduced pressure. The residue was purified by flash chromatography
12 (Toluene/EtOAc 95:5) to give disaccharide **18** (32.1 g, 82%) as a white to off-white crystalline solid.
13 The glycosylation product had mp = 87.4 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.95-7.80 (m, 8H, H_{ArNap}),
14 7.56-7.45 (m, 6H, H_{ArNap}), 5.91 (dddd, 1H, J = 17.0, 10.6, 5.9, 5.3 Hz, CH=CH_{2All}), 5.32 (ddd, 1H, J =
15 17.2, 3.2, 1.5 Hz, CH=CH_{2All}), 5.30 (dd, 1H, $J_{2,1}$ = 1.4 Hz, $J_{2,3}$ = 3.3 Hz, H-2_C), 5.25 (dd, 1H, $J_{2,1}$ = 1.3
16 Hz, $J_{2,3}$ = 3.3 Hz, H-2_B), 5.24 (ddd, 1H, J = 10.5, 2.6, 1.5 Hz, CH=CH_{2All}), 5.11 (d, 1H, $J_{1,2}$ = 1.3 Hz, H-
17 1_B), 5.10 (d, 2H, J = 11.5 Hz, H_{Nap}), 4.85 (d, 1H, $J_{1,2}$ = 1.4 Hz, H-1_C), 4.84 (d, 1H, J = 11.5 Hz, H_{Nap}),
18 4.83 (d, 1H, J = 11.5 Hz, H_{Nap}), 4.31 (dd, 1H, $J_{3,2}$ = 3.3 Hz, $J_{3,4}$ = 9.4 Hz, H-3_C), 4.24 (dd, 1H, $J_{3,2}$ = 3.3
19 Hz, $J_{3,4}$ = 9.4 Hz, H-3_B), 4.21 (d, 1H, J = 3.3 Hz, H_{CA}), 4.20 (d, 1H, J = 3.3 Hz, H_{CA}), 4.19 (ddpt, 1H, J
20 = 12.9, 5.9, 1.4 Hz, CH₂-CH=All), 4.01 (ddpt, 1H, J = 12.9, 5.9, 1.5 Hz, CH₂-CH=All), 3.84 (dq, 2H, $J_{5,4}$
21 = 9.4 Hz, $J_{5,6}$ = 6.4 Hz, H-5_C, H-5_B), 3.60 (pt, 1H, $J_{4,5}$ = $J_{4,3}$ = 9.4 Hz, H-4_C), 3.45 (pt, 1H, $J_{4,5}$ = $J_{4,3}$ = 9.4
22 Hz, H-4_B), 2.72-2.56 (m, 4H, CH_{2Lev}), 2.14 (s, 3H, CH_{3Lev}), 1.36 (d, 3H, $J_{6,5}$ = 6.4 Hz, H-6_C), 1.27 (d,
23 3H, $J_{6,5}$ = 6.4 Hz, H-6_B), 0.92 (t, 9H, J = 8.0 Hz, CH_{3TES}), 0.62 (q, 6H, J = 8.0 Hz, CH_{2TES}). ¹³C {¹H}
24 NMR (100 MHz, CDCl₃) δ 206.0 (CO_{Lev}), 171.9 (CO_{2Lev}), 166.7 (CO_{2CA}), 136.1 (C_{IVNap}), 135.4 (C_{IVNap}),
25 133.4 (2C, CH=CH_{2All}, C_{IVNap}), 133.3 (C_{IVNap}), 133.1 (C_{IVNap}), 133.0 (C_{IVNap}), 128.4-125.7 (14C, C_{ArNap}),
26 117.7 (CH=CH_{2All}), 99.6 (C-1_B, ¹J_{C,H} = 173.2 Hz), 95.8 (C-1_C, ¹J_{C,H} = 172.0 Hz), 80.5 (C-4_B), 80.3 (C-
27 4_C), 76.7 (C-3_C), 75.5 (CH_{2Nap}), 75.1 (CH_{2Nap}), 74.2 (C-2_C), 73.5 (C-2_B), 70.9 (C-3_B), 68.9 (C-5_B), 68.3
28 (CH_{2All}), 67.9 (C-5_C), 40.8 (CH_{2CA}), 37.9 (CH_{2Lev}), 29.7 (CH_{3Lev}), 28.1 (CH_{2Lev}), 18.0 (C-6_C), 17.9 (C-
29 6_B), 6.8 (CH_{3TES}), 4.7 (CH_{2TES}). HRMS (ESI⁺) m/z : [M+Na]⁺ Calcd for C₅₀H₆₃ClO₁₂SiNa 941.3787;
30 found 941.3675.

31 **Allyl 4-O-(2-naphthylmethyl)-3-O-triethylsilyl- α -L-rhamnopyranosyl-(1 \rightarrow 3)-2-O-chloroacetyl-4-**
32 **O-(2-naphthylmethyl)- α -L-rhamnopyranoside (S3).** To a solution of disaccharide **18** (3.33 g, 3.62
33 mmol) in a mix of 3:2 Pyridine/AcOH (45 mL), stirred at rt, was added H₂NNH₂·H₂O (652 μ L, 7.24
34 mmol, 2.0 equiv.). After stirring at rt for 2 h, TLC (Cyclohexane/EtOAc 7:3) showed complete
35 consumption of the starting material (Rf 0.75) and the presence of a less polar product (Rf 0.8). H₂O (40
36 mL) and DCM (100 mL) were added. The aq. layer was extracted with DCM (2 x 50 mL) and the
37 combined organic phases were washed times with 10% aq. CuSO₄ (40 mL), H₂O (40 mL), satd aq.
38 NaHCO₃ (50 mL) and brine (40 mL). The organic layer was dried over anhyd. Na₂SO₄, filtered and
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1 concentrated under vacuum. The residue was purified by flash chromatography (Cyclohexane/EtOAc
2 7:3) to give alcohol **S3** (1.96 g, 66%) as a white foam. Disaccharide **S3** had ^1H NMR (400 MHz, CDCl_3)
3 δ 7.92-7.78 (m, 8H, H_{ArNap}), 7.55-7.43 (m, 6H, H_{ArNap}), 5.89 (dddd, 1H, $J = 17.2, 10.6, 6.0, 5.3$ Hz,
4 $\text{CH}=\text{CH}_{2\text{All}}$), 5.31 (ddd, 1H, $J = 17.2, 3.1, 1.5$ Hz, $\text{CH}=\text{CH}_{2\text{All}}$), 5.28 (dd, 1H, $J_{2,1} = 1.7$ Hz, $J_{2,3} = 3.3$ Hz,
5 H-2C), 5.23 (ddd, 1H, $J = 10.6, 2.7, 1.2$ Hz, $\text{CH}=\text{CH}_{2\text{All}}$), 5.18 (d, 1H, $J_{1,2} = 1.0$ Hz, H-1B), 5.01 (d, 1H,
6 $J = 11.5$ Hz, H_{Nap}), 5.00 (d, 1H, $J = 11.2$ Hz, H_{Nap}), 4.84 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1C), 4.83 (d, 1H, $J =$
7 11.2 Hz, H_{Nap}), 4.79 (d, 1H, $J = 11.5$ Hz, H_{Nap}), 4.27 (dd, 1H, $J_{3,2} = 3.3$ Hz, $J_{3,4} = 9.5$ Hz, H-3C), 4.19
8 (ddpt, 1H, $J = 12.7, 5.3, 1.5$ Hz, $\text{CH}_2-\text{CH}=\text{All}$), 4.18 (bpd, 2H, H_{CA}), 4.07 (dd, 1H, $J_{3,2} = 3.3$ Hz, $J_{3,4} = 9.1$
9 Hz, H-3B), 4.01 (ddpt, 1H, $J = 12.7, 6.0, 1.2$ Hz, $\text{CH}_2-\text{CH}=\text{All}$), 3.90-3.81 (m, 2H, H-2B, H-5C), 3.78 (dq,
10 1H, $J_{5,4} = 9.1$ Hz, $J_{5,6} = 6.3$ Hz, H-5B), 3.56 (pt, 1H, $J_{4,3} = J_{4,5} = 9.5$ Hz, H-4C), 3.43 (pt, 1H, $J_{4,3} = J_{4,5} =$
11 9.1 Hz, H-4B), 2.59 (brs, 1H, OH-2B), 1.35 (d, 3H, $J_{6,5} = 6.2$ Hz, H-6C), 1.28 (d, 3H, $J_{6,5} = 6.3$ Hz, H-6B),
12 0.94 (t, 9H, $J = 7.8$ Hz, CH_3TES), 0.63 (q, 6H, $J = 7.8$ Hz, CH_2TES). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ
13 166.7 (CO_2CA), 136.0 (C_{IVNap}), 135.3 (C_{IVNap}), 133.3 ($\text{CH}=\text{CH}_{2\text{All}}$), 133.1-128.2 (4C, C_{IVNap}), 127.9-125.7
14 (14C, C_{ArNap}), 117.7 ($\text{CH}=\text{CH}_{2\text{All}}$), 101.4 (C-1B, $^1J_{\text{C,H}} = 172.9$ Hz), 95.9 (C-1C, $^1J_{\text{C,H}} = 171.4$ Hz), 80.8
15 (C-4B), 80.3 (C-4C), 77.5 (C-3C), 75.5 ($\text{CH}_{2\text{Nap}}$), 75.2 ($\text{CH}_{2\text{Nap}}$), 74.4 (C-2C), 73.0 (C-3B), 72.5 (C-2B),
16 68.4 (C-5B), 68.3 ($\text{CH}_{2\text{All}}$), 67.9 (C-5C), 40.8 (CH_2CA), 18.0 (C-6C), 17.9 (C-6B), 6.7 (CH_3TES), 5.0
17 (CH_2TES). HRMS (ESI $^+$) m/z : $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{45}\text{H}_{57}\text{ClO}_{10}\text{SiNa}$ 838.3743; found 838.3753.

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29 **Allyl 2-O-levulinoyl-4-O-(2-naphthylmethyl)-3-O-triethylsilyl- α -L-rhamnopyranosyl-(1 \rightarrow 2)-(4-O-**
30 **(2-naphthylmethyl)-3-O-triethylsilyl- α -L-rhamnopyranosyl)-(1 \rightarrow 3)-2-O-chloroacetyl-4-O-(2-**
31 **naphthylmethyl)- α -L-rhamnopyranoside (**19**). *Route 1.* To a solution of disaccharide **18** (15.0 g, 16.3
32 mmol) in 3:2 Pyridine/AcOH (204 mL) stirred at rt was added $\text{H}_2\text{NNH}_2\cdot\text{H}_2\text{O}$ (1.58 mL, 32.6 mmol, 2.0
33 equiv.). After stirring at rt for 1.5 h, TLC (Cyclohexane/EtOAc 7:3) showed complete consumption of
34 the starting material (Rf 0.75) and the presence of a less polar product (Rf 0.8). H_2O (50 mL) and EtOAc
35 (200 mL) were added. The aq. layer was extracted with EtOAc (2 x 100 mL) and the combined organic
36 phases were washed times with 10% aq. CuSO_4 (3 x 50 mL), H_2O (2 x 50 mL), satd aq. NaHCO_3 (50
37 mL) and brine (2 x 50 mL). The organic layer was dried over anhyd. Na_2SO_4 , filtered and concentrated
38 under vacuum. The crude residue was dissolved in anhyd. Toluene (326 mL), stirred at rt under an Ar
39 atmosphere, and donor **15** (13.7 g, 21.2 mmol, 1.3 equiv.) and activated 4 Å MS (3.4 g) were
40 successively added. The suspension was stirred for 15 min at rt, then cooled to -40 °C. After 15 min of
41 stirring at this temperature, TBSOTf (215 μL , 0.815 mmol, 0.05 equiv.) was slowly added. After stirring
42 for 2 h under Ar from -40 to -10 °C, a TLC control (Cyclohexane/EtOAc 7:3) showed complete
43 consumption of the disaccharide **18** (Rf 0.55) and the presence of a less polar product (Rf 0.75). The
44 mixture was cooled down to -60 °C and Et_3N (204 μL , 1.47 mmol, 0.09 equiv.) was added. The reaction
45 mixture was stirred for 15 minutes at -60 °C and then filtered on a pad of Celite®. The filtrate was
46 concentrated under reduced pressure. The residue was purified by flash chromatography
47 (Cyclohexane/EtOAc 9:1 to 8:2) to give trisaccharide **19** (17.9 g, 83 % over two steps) as a colorless
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1 *Route 2.* Donor **15** (1.04 g, 1.46 mmol, 1.24 equiv.) and activated 4 Å MS (1.4 g) were successively
2 added to a solution of alcohol **S3** (1.0 g, 1.22 mmol) in anhyd. Toluene (30 mL), stirred at rt under an
3 Ar atmosphere. The suspension was stirred for 15 min at rt, then cooled to -20 °C. After 15 min of
4 stirring at this temperature, TMSOTf (11 µL, 61 µmol, 0.05 equiv.) was slowly added. After stirring for
5 2 h under Ar from -20 °C to rt, a TLC control (Cyclohexane/EtOAc 7:3) showed complete consumption
6 of the disaccharide **S3** (Rf 0.55) into a less polar product (Rf 0.75). The mixture was cooled down to -
7 40 °C and Et₃N (15 µL, 110 µmol, 0.09 equiv.) was added. The reaction mixture was stirred for 15
8 minutes at -40 °C and then filtered on a pad of Celite®. The filtrate was concentrated under reduced
9 pressure. The residue was purified by flash chromatography (Cyclohexane/EtOAc 9:1 to 8:2) to give
10 trisaccharide **19** (1.36 g, 85%) as a colorless oil. Trisaccharide **19** had ¹H NMR (400 MHz, CDCl₃) δ
11 7.91-7.77 (m, 12H, H_{ArNap}), 7.54-7.43 (m, 9H, H_{ArNap}), 5.90 (dddd, 1H, *J* = 17.1, 10.3, 6.0, 5.3 Hz,
12 CH=CH_{2All}), 5.30 (ddd, 1H, *J* = 17.1, 3.0, 1.4 Hz, CH=CH_{2All}), 5.28 (dd, 1H, *J*_{2,1} = 1.7 Hz, *J*_{2,3} = 3.3 Hz,
13 H-2_A), 5.25 (dd, 1H, *J*_{2,1} = 1.7 Hz, *J*_{2,3} = 3.3 Hz, H-2_C), 5.21 (ddd, 1H, *J* = 10.3, 2.7, 1.4 Hz, CH=CH_{2All}),
14 5.10 (d, 1H, *J*_{1,2} = 1.7 Hz, H-1_B), 5.09-5.02 (m, 4H, H_{Nap}, H-1_A), 4.82 (d, 1H, *J*_{1,2} = 1.7 Hz, H-1_C), 4.78
15 (d, 1H, *J* = 12.0 Hz, H_{Nap}), 4.77 (d, 1H, *J* = 11.1 Hz, H_{Nap}), 4.75 (d, 3H, *J* = 11.5 Hz, H_{Nap}), 4.28 (dd,
16 1H, *J*_{3,2} = 3.3 Hz, *J*_{3,4} = 9.3 Hz, H-3_A), 4.23 (dd, 1H, *J*_{3,2} = 3.3 Hz, *J*_{3,4} = 9.6 Hz, H-3_C), 4.20-4.13 (m, 4H,
17 H-3_B, CH_{2All}, H_{CA}), 4.00 (ddpt, 1H, *J* = 12.9, 6.0, 1.4 Hz, CH₂-CH=All), 3.94 (dd, 1H, *J*_{2,1} = 1.7 Hz, *J*_{2,3}
18 = 2.3 Hz, H-2_B), 3.85 (dq, 1H, *J*_{5,4} = 9.3 Hz, *J*_{5,6} = 6.2 Hz, H-5_A), 3.81 (dq, 1H, *J*_{5,4} = 9.6 Hz, *J*_{5,6} = 6.0
19 Hz, H-5_C), 3.68 (dq, 1H, *J*_{5,4} = 9.4 Hz, *J*_{5,6} = 6.0 Hz, H-5_B), 3.54 (pt, 1H, *J*_{4,3} = *J*_{4,5} = 9.6 Hz, H-4_C), 3.45
20 (pt, 1H, *J*_{4,3} = *J*_{4,5} = 9.4 Hz, H-4_B), 3.40 (pt, 1H, *J*_{4,3} = *J*_{4,5} = 9.3 Hz, H-4_A), 2.81-2.70 (m, 4H, CH_{2Lev}),
21 2.21 (s, 3H, CH_{3Lev}), 1.26 (d, 3H, *J*_{6,5} = 6.0 Hz, H-6_C), 1.24 (d, 3H, *J*_{6,5} = 6.0 Hz, H-6_B), 1.15 (d, 3H, *J*_{6,5}
22 = 6.2 Hz, H-6_A), 0.99 (t, 9H, *J* = 8.0 Hz, CH_{3TES}), 0.93 (t, 9H, *J* = 7.9 Hz, CH_{3TES}), 0.69 (q, 6H, *J* = 7.9
23 Hz, CH_{2TES}), 0.62 (q, 6H, *J* = 8.0 Hz, CH_{2TES}). ¹³C {¹H} NMR (100 MHz, CDCl₃) δ 206.0 (CO_{Lev}), 171.6
24 (CO_{2Lev}), 166.7 (CO_{2CA}), 136.3 (C_{IVNap}), 136.1 (C_{IVNap}), 135.3 (C_{IVNap}), 133.4 (CH=CH_{2All}), 133.4
25 (C_{IVNap}), 133.3 (C_{IVNap}), 133.3 (C_{IVNap}), 133.1 (C_{IVNap}), 132.9 (2C, C_{IVNap}), 128.3-125.7 (21C, C_{ArNap}),
26 117.7 (CH=CH_{2All}), 101.5 (C-1_B, ¹*J*_{C,H} = 171.6 Hz), 99.1 (C-1_A, ¹*J*_{C,H} = 173.0 Hz), 95.7 (C-1_C, ¹*J*_{C,H} =
27 171.9 Hz), 81.2 (2C, C-4_A, C-4_B), 79.7 (C-4_C), 78.7 (C-2_B), 78.1 (C-3_C), 75.5 (CH_{2Nap}), 75.3 (CH_{2Nap}),
28 75.2 (CH_{2Nap}), 74.5 (C-2_C), 73.3 (C-2_A), 72.7 (C-3_B), 71.1 (C-3_A), 69.2 (C-5_B), 68.6 (C-5_A), 68.3 (CH_{2All}),
29 67.8 (C-5_C), 40.7 (CH_{2CA}), 38.1 (CH_{2Lev}), 29.8 (CH_{3Lev}), 28.2 (CH_{2Lev}), 18.0 (3C, C-6_A, C-6_B, C-6_C), 17.9
30 (C-6_B), 6.9 (CH_{3TES}), 6.8 (CH_{3TES}), 5.0 (CH_{2TES}), 4.9 (CH_{2TES}). HRMS (ESI⁺) *m/z*: [M+NH₄]⁺ Calcd for
31 C₇₃H₉₉ClNO₁₆Si₂ 1336.6224; found 1336.6191.

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51 **2-O-Levulinoyl-4-O-(2-naphthylmethyl)-3-O-triethylsilyl-α-L-rhamnopyranosyl-(1→2)-(4-O-(2-**
52 **naphthylmethyl)-3-O-triethylsilyl-α-L-rhamnopyranosyl)-(1→3)-2-O-chloroacetyl-4-O-(2-**
53 **naphthylmethyl)-α/β-L-rhamnopyranose (20).** 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)-
54 iridium hexafluorophosphate (103 mg, 0.12 mmol, 0.02 equiv.) was dissolved in anhyd. THF (10 mL)
55 and the solution was degassed repeatedly then stirred under a satd. H₂ atmosphere for 30 min. The
56 resulting yellow solution was degassed repeatedly before being poured into a solution of trisaccharide
57 **19** in anhyd. THF (67 mL). The mixture was stirred under an Ar atmosphere at rt for 4 h. A TLC control
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(Cyclohexane/EtOAc 7:3) showed complete conversion of the starting material (Rf 0.6) into a less polar product (Rf 0.65). A solution of NIS (1.64 g, 7.27 mmol, 1.2 equiv.) in 1:5 H₂O/THF (34 mL) and then H₂O (84 mL) were poured into the mixture. After stirring at rt for 4 h, a TLC control (Cyclohexane/EtOAc 7:3) showed complete conversion of the intermediate into a more polar product (Rf 0.35). Satd aq. Na₂S₂O₅ (200 mL) and EtOAc (500 mL) were added. The aq. layer was extracted with EtOAc (2 x 250 mL) and the combined organic phases were washed with satd aq. NaHCO₃ (200 mL) and brine (200 mL). The organic layer was dried over anhyd. Na₂SO₄, filtered and concentrated under vacuum. The residue was purified by flash chromatography (Cyclohexane/EtOAc 8:2 to 6:4) to give hemiacetal **20** (6.2 g, 80%, α/β 8:2) as a yellow oil. The α anomer had ¹H NMR (400 MHz, CDCl₃) δ 7.91-7.75 (m, 12H, H_{ArNap}), 7.54-7.41 (m, 9H, H_{ArNap}), 5.26 (dd, 2H, $J_{2,1} = 1.9$ Hz, $J_{2,3} = 3.0$ Hz, H-2_A, H-2_C), 5.20 (dd, 1H, $J_{1,2} = 1.9$ Hz, $J_{1,OH} = 3.8$ Hz, H-1_A), 5.12 (d, 1H, $J_{1,2} = 1.6$ Hz, H-1_B), 5.09-5.02 (m, 4H, H_{Nap}, H-1_A), 5.05 (d, 3H, $J = 11.9$ Hz, H_{Nap}), 4.04 (s_o, 1H, H-1_C), 4.77 (d, 2H, $J = 11.7$ Hz, H_{Nap}), 4.75 (d, 1H, $J = 11.3$ Hz, H_{Nap}), 4.27 (dd, 2H, $J_{3,2} = 3.0$ Hz, $J_{3,4} = 9.3$ Hz, H-3_A, H-3_C), 4.18 (d, 1H, $J = 15.5$ Hz, H_{CA}), 4.16-4.12 (m, 2H, H_{CA}, H-3_B), 4.02 (dq, 1H, $J_{5,4} = 9.3$ Hz, $J_{5,6} = 6.2$ Hz, H-5_C), 3.93 (dd, 1H, $J_{2,1} = 1.6$ Hz, $J_{2,3} = 2.4$ Hz, H-2_B), 3.85 (dq, 1H, $J_{5,4} = 9.3$ Hz, $J_{5,6} = 6.3$ Hz, H-5_A), 3.68 (dq, 1H, $J_{5,4} = 9.3$ Hz, $J_{5,6} = 6.2$ Hz, H-5_B), 3.54 (pt, 1H, $J_{4,3} = J_{4,5} = 9.3$ Hz, H-4_C), 3.45 (pt, 1H, $J_{4,3} = J_{4,5} = 9.3$ Hz, H-4_B), 3.39 (pt, 1H, $J_{4,3} = J_{4,5} = 9.3$ Hz, H-4_A), 2.80-2.69 (m, 4H, CH_{2Lev}), 2.67 (d, 1H, $J_{OH,1} = 3.8$ Hz, OH-1_A), 2.21 (s, 3H, CH_{3Lev}), 1.23 (d, 6H, $J_{6,5} = 6.2$ Hz, H-6_B, H-6_C), 1.15 (d, 3H, $J_{6,5} = 6.3$ Hz, H-6_A), 0.99 (t, 9H, $J = 8.0$ Hz, CH_{3TES}), 0.92 (t, 9H, $J = 8.0$ Hz, CH_{3TES}), 0.69 (q, 6H, $J = 8.0$ Hz, CH_{2TES}), 0.61 (q, 6H, $J = 8.0$ Hz, CH_{2TES}). ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 206.0 (CO_{Lev}), 171.6 (CO_{2Lev}), 166.7 (CO_{2CA}), 136.3 (C_{IVNap}), 136.1 (C_{IVNap}), 135.3 (C_{IVNap}), 133.4 (C_{IVNap}), 133.3 (C_{IVNap}), 133.3 (C_{IVNap}), 133.1 (C_{IVNap}), 132.9 (C_{IVNap}), 132.9 (C_{IVNap}), 128.3-125.7 (C_{ArNap}), 101.4 (C-1_B, $^1J_{C,H} = 171.6$ Hz), 99.1 (C-1_C, $^1J_{C,H} = 172.5$ Hz), 91.4 (C-1_A, $^1J_{C,H} = 172.8$ Hz), 81.2 (C-4_A, C-4_C), 79.6 (C-4_B), 78.8 (C-2_B), 77.2 (C-3_C), 75.4 (CH_{2Nap}), 75.3 (CH_{2Nap}), 75.2 (CH_{2Nap}), 74.7 (C-2_C), 73.3 (C-2_A), 72.7 (C-3_B), 71.1 (C-3_A), 69.3 (C-5_B), 68.7 (C-5_A), 67.9 (CH_{2All}), 67.8 (C-5_C), 40.7 (CH_{2CA}), 38.1 (CH_{2Lev}), 29.8 (CH_{3Lev}), 28.2 (CH_{2Lev}), 18.0 (C-6_A, C-6_B, C-6_C), 17.9 (C-6_B), 6.9 (CH_{3TES}), 6.8 (CH_{3TES}), 5.0 (CH_{2TES}), 4.9 (CH_{2TES}). HRMS (ESI⁺) m/z : [M+NH₄]⁺ Calcd for C₇₀H₉₅ClNO₁₆Si₂ 1296.5891; found 1296.5878.

2-O-Levulinoyl-4-O-(2-naphthylmethyl)-3-O-triethylsilyl- α -L-rhamnopyranosyl-(1 \rightarrow 2)-(4-O-(2-naphthylmethyl)-3-O-triethylsilyl- α -L-rhamnopyranosyl)-(1 \rightarrow 3)-2-O-chloroacetyl-4-O-(2-naphthylmethyl)- α/β -L-rhamnopyranosyl *N*-(phenyl)trifluoroacetimidate (21**).** To a solution of hemiacetal **20** (5.74 g, 4.48 mmol) in anhyd. acetone (90 mL), stirred at rt, were successively added PTFA-Cl (1.07 mL, 6.73 mmol, 1.5 equiv.) and K₂CO₃ (1.24 g, 8.97 mmol, 2.0 equiv.). After stirring at rt for 3 h, TLC (Cyclohexane/EtOAc 7:3) showed complete consumption of hemiacetal **20** (Rf 0.35) into a less polar product (Rf 0.65). The mixture was filtered on a pad of Celite® and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (Toluene/EtOAc 8:2 to 5:5) to give donor **21** (6.0 g, 91%, α/β 8:2) as a colorless oil. Imidate **21** had ¹H NMR (400 MHz, CDCl₃) δ 7.91-7.76 (m, 12H, H_{ArNap}), 7.30 (d, 2H, $J = 8.0$ Hz, H_{ArPh}), 7.54-7.39 (m, 9H, H_{ArNap}), 7.30 (t, 2H, $J = 8.0$ Hz, H_{ArPh}), 7.12 (t, 1H, $J = 7.4$ Hz, H_{ArPh}), 6.90-6.80 (m, 2H, H_{ArPh}), 6.17

1 (brs, 0.8H, H-1_Cα), 5.77 (brs, 0.2H, H-1_Cβ), 5.41 (t, 0.8H, $J_{2,3} = 2.3$ Hz, H-2_Cα), 5.41 (brs, 0.2H, H-2_Cβ),
2 5.29 (dd, 0.8H, $J_{2,1} = 0.9$ Hz, $J_{2,3} = 3.0$ Hz, H-2_Aα), 5.26 (dd, 0.2H, $J_{2,1} = 0.9$ Hz, $J_{2,3} = 2.8$ Hz, H-2_Aβ),
3 5.16 (d, 0.8H, $J_{1,2} = 0.9$ Hz, H-1_Bα), 5.16 (brs, 0.2H, H-1_Bβ), 5.10-5.01 (m, 4H, H_{Nap}, H-1_A), 4.78 (d,
4 1H, $J = 12.2$ Hz, H_{Nap}), 4.77 (d, 2H, $J = 11.5$ Hz, H_{Nap}), 4.32-4.23 (d, 2H, H-3_A, H-3_C), 4.20-4.10 (m,
5 3H, H_{CA}, H-3_B), 3.95 (dd, 1H, $J_{2,1} = 0.9$ Hz, $J_{2,3} = 2.8$ Hz, H-2_B), 3.91 (dq, 1H, $J_{5,4} = 9.3$ Hz, $J_{5,6} = 6.2$
6 Hz, H-5_C), 3.85 (dq, 1H, $J_{5,4} = 9.4$ Hz, $J_{5,6} = 6.2$ Hz, H-5_A), 3.70 (dq, 1H, $J_{5,4} = 9.2$ Hz, $J_{5,6} = 6.2$ Hz, H-
7 5_B), 3.63 (pt, 1H, $J_{4,3} = J_{4,5} = 9.3$ Hz, H-4_C), 3.48 (pt, 1H, $J_{4,3} = J_{4,5} = 9.2$ Hz, H-4_B), 3.41 (pt, 1H, $J_{4,3} =$
8 $J_{4,5} = 9.4$ Hz, H-4_A), 2.82-2.69 (m, 4H, CH_{2Lev}), 2.21 (s, 2.4H, CH_{3Lev}α), 2.20 (s, 0.6H, CH_{3Lev}β), 1.28
9 (d, 3H, $J_{6,5} = 6.2$ Hz, H-6_C), 1.25 (d, 3H, $J_{6,5} = 6.2$ Hz, H-6_B), 1.15 (d, 2.4H, $J_{6,5} = 6.2$ Hz, H-6_Aα), 1.11
10 (d, 0.6H, $J_{6,5} = 6.2$ Hz, H-6_Aβ), 1.04-0.90 (m, 18H, CH_{3TES}), 0.75-0.58 (m, 12H, CH_{2TES}). ¹³C{¹H} NMR
11 (100 MHz, CDCl₃) δ 206.0 (CO_{Lev}), 171.6 (CO_{2Lev}), 166.4 (CO_{2CA}), 142.9 (CN), 136.2 (C_{IVNap}), 136.0
12 (C_{IVNap}), 134.9 (C_{IVNap}), 133.4 (C_{IVNap}), 133.3 (2C, C_{IVNap}), 133.1 (C_{IVNap}), 132.9 (2C, C_{IVNap}), 129.4
13 (C_{IVPh}), 128.8-125.7 (25C, C_{ArNap}, C_{ArPh}), 119.4 (C_{ArPh}), 101.4 (C-1_B, $J_{C,H} = 171.6$ Hz), 99.3 (C_{IVCF3}),
14 99.1 (C-1_C, $J_{C,H} = 172.5$ Hz), 91.4 (C-1_A, $J_{C,H} = 172.8$ Hz), 81.2 (2C, C-4_A, C-4_C), 79.6 (C-4_B), 78.8
15 (C-2_B), 77.2 (C-3_C), 75.4 (CH_{2Nap}), 75.3 (CH_{2Nap}), 75.2 (CH_{2Nap}), 74.7 (C-2_C), 73.3 (C-2_A), 72.7 (C-3_B),
16 71.1 (C-3_A), 69.3 (C-5_B), 68.7 (C-5_A), 67.9 (CH_{2All}), 67.8 (C-5_C), 40.7 (CH_{2CA}), 38.1 (CH_{2Lev}), 29.8
17 (CH_{3Lev}), 28.2 (CH_{2Lev}), 18.0 (3C, C-6_A, C-6_B, C-6_C), 17.9 (C-6_B), 6.9 (CH_{3TES}), 6.8 (CH_{3TES}), 5.0
18 (CH_{2TES}), 4.9 (CH_{2TES}). HRMS (ESI⁺) m/z : [M+NH₄]⁺ Calcd for C₇₈H₉₉ClF₃N₂O₁₆Si₂ 1467.6160; found
19 1467.6174.

20 **Allyl 2-O-levulinoyl-4-O-(2-naphtylmethyl)-3-O-triethylsilyl-α-L-rhamnopyranosyl-(1→2)-(4-O-**
21 **(2-naphtylmethyl)-3-O-triethylsilyl-α-L-rhamnopyranosyl)-(1→3)-(2-O-chloroacetyl-4-O-(2-**
22 **naphtylmethyl)-α-L-rhamnopyranosyl)-(1→3)-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-**
23 **β-D-glucopyranoside (13).** To a solution of donor **21** (5.7 g, 3.93 mmol) in anhyd. 3:1 Toluene/DCM
24 (223 mL), stirred at rt under an Ar atmosphere, were successively added acceptor **7** (3.56 g, 7.86 mmol,
25 2.0 equiv.) and activated 4 Å MS (11 g). The suspension was stirred for 15 min at rt, then cooled to -20
26 °C. After 15 min of stirring at this temperature, TBSOTf (135 μL, 590 μmol, 0.15 equiv.) was slowly
27 added. After stirring for 1 h under Ar from -20 to 0 °C, TLC (Cyclohexane/EtOAc 7:3) showed complete
28 consumption of donor **21** (Rf 0.65) into a more polar product (Rf 0.5). The mixture was cooled down to
29 -40 °C and Et₃N (103 μL, 0.74 mmol, 0.19 equiv.) was added for neutralization. The suspension was
30 stirred for 15 minutes at -40 °C and then filtered on a pad of Celite®. The filtrate was concentrated under
31 reduced pressure. The residue was purified by flash chromatography (Toluene/EtOAc 95:5 to 5:5) to
32 give tetrasaccharide **13** (5.62 g, 84 %) as a colorless to yellow oil. The fully protected **13** had ¹H NMR
33 (400 MHz, CDCl₃) δ 7.90-7.77 (m, 12H, H_{ArNap}), 7.72 (s, 1H, H_{ArBzl}), 7.56-7.43 (m, 10H, H_{ArNap}, H_{ArBzl}),
34 7.38 (dd, 1H, $J = 8.4, 1.5$ Hz, H_{ArBzl}), 7.23-7.17 (m, 3H, H_{ArBzl}), 7.02 (d, 1H, $J = 7.3$ Hz, NH), 5.87 (dddd,
35 1H, $J = 17.2, 10.4, 6.4, 5.3$ Hz, CH=CH_{2All}), 5.56 (s, 1H, H_{Bzl}), 5.31 (ddd, 1H, $J = 17.2, 3.0, 1.2$ Hz,
36 CH=CH_{2All}), 5.28 (dd, 1H, $J_{2,1} = 1.3$ Hz, $J_{2,3} = 3.0$ Hz, H-2_C), 5.26 (dd, 1H, $J_{2,1} = 1.3$ Hz, $J_{2,3} = 3.0$ Hz,
37 H-2_A), 5.22 (ddd, 1H, $J = 10.4, 2.6, 1.2$ Hz, CH=CH_{2All}), 5.12-5.03 (m, 5H, H-1_A, H-1_B, H-1_D, H_{Nap}),
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4.97 (d, 1H, $J = 11.5$ Hz, H_{Nap}), 4.89 (d, 1H, $J_{1,2} = 1.3$ Hz, H-1_C), 4.79 (d, 1H, $J = 10.6$ Hz, H_{Nap}), 4.76 (d, 1H, $J = 11.1$ Hz, H_{Nap}), 4.65 (d, 1H, $J = 11.5$ Hz, H_{Nap}), 4.58 (dd, 1H, $J_{3,2} = 8.4$ Hz, $J_{3,4} = 9.9$ Hz, H-3_D), 4.40 (dd, 1H, $J_{6a,5} = 4.8$ Hz, $J_{6a,6b} = 10.9$ Hz, H-6a_D), 4.36 (ddpt, 1H, $J = 12.9, 5.3, 1.2$ Hz, CH₂-CH=All), 4.28 (dd, 1H, $J_{3,2} = 3.0$ Hz, $J_{3,4} = 9.4$ Hz, H-3_A), 4.18 (dd, 1H, $J_{3,2} = 3.0$ Hz, $J_{3,4} = 9.4$ Hz, H-3_C), 4.15-4.06 (m, 4H, CH₂-CH=All, H_{CA}, H-3_B), 3.78 (dq, 1H, $J_{5,4} = 9.4$ Hz, $J_{5,6} = 6.3$ Hz, H-5_C), 3.91 (t, 1H, $J_{2,3} = 2.1$ Hz, H-2_B), 3.87-3.76 (m, 2H, H-5_B, H-6b_D), 3.69 (dq, 1H, $J_{5,4} = 9.4$ Hz, $J_{5,6} = 6.3$ Hz, H-5_A), 3.65-3.55 (m, 2H, H-4_D, H-5_D), 3.53-3.36 (m, 4H, H-2_D, H-4_B, H-4_C, H-4_A), 2.83-2.70 (m, 4H, CH_{2Lev}), 2.22 (s, 3H, CH_{3Lev}), 1.24 (d, 3H, $J_{6,5} = 6.3$ Hz, H-6_B), 1.09 (d, 3H, $J_{6,5} = 6.3$ Hz, H-6_A), 1.01 (t, 9H, $J = 7.9$ Hz, CH_{3TES}), 0.94 (t, 9H, $J = 7.9$ Hz, CH_{3TES}), 0.76-0.68 (m, 9H, CH_{2TES}, H-6_C), 0.62 (q, 6H, $J = 7.9$ Hz, CH_{2TES}). ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 206.0 (CO_{Lev}), 171.6 (CO_{2Lev}), 166.2 (CO_{2CA}), 162.2 (CO_{NH}), 136.9 (C_{IVNap}), 136.3 (C_{IVNap}), 136.0 (C_{IVBzl}), 135.5 (C_{IVNap}), 133.4 (C_{IVNap}), 133.3 (2C, C_{IVNap}, CH=CH_{2All}), 133.2 (C_{IVNap}), 133.0 (2C, C_{IVNap}), 132.9 (C_{IVNap}), 129.1 (C_{ArBzl}), 129.0 (C_{ArBzl}), 128.3-127.6 (10C, C_{ArNap}), 126.5-125.6 (16C, C_{ArNap}, C_{ArBzl}), 118.4 (CH=CH_{2All}), 102.0 (CH_{Bzl}), 101.4 (C-1_B, ¹J_{C,H} = 172.7 Hz), 98.9 (C-1_A, ¹J_{C,H} = 173.4 Hz), 98.2 (C-1_D, ¹J_{C,H} = 165.0 Hz), 97.2 (C-1_C, ¹J_{C,H} = 172.8 Hz), 92.1 (C_{IVCCl3}), 81.3 (C-4_A), 81.1 (C-4_B), 80.1 (C-5_D), 79.8 (C-4_C), 78.3 (C-2_B), 77.2 (C-3_A), 75.5 (CH_{2Nap}), 75.2 (CH_{2Nap}), 75.1 (CH_{2Nap}), 74.1 (C-3_D), 74.0 (C-2_C), 73.2 (C-2_A), 72.7 (C-3_B), 71.1 (C-3_C), 70.8 (CH_{2All}) 69.3 (C-5_A), 68.7 (C-6_D), 68.6 (C-5_B), 68.2 (C-5_C), 66.2 (C-4_D), 60.2 (C-2_D), 40.6 (CH_{2CA}), 38.1 (CH_{2Lev}), 29.8 (CH_{3Lev}), 28.2 (CH_{2Lev}), 18.1 (C-6_B), 18.0 (C-6_A), 17.2 (C-6_C), 6.9 (CH_{3TES}), 6.8 (CH_{3TES}), 5.0 (3C, CH_{2TES}, C-6_D). HRMS (ESI⁺) m/z : [M+NH₄]⁺ Calcd for C₈₉H₁₁₃Cl₄N₂O₂₁Si₂ 1729.6145; found 1729.6128.

Allyl α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-rhamnopyranosyl-(1 \rightarrow 3)-(2-O-chloroacetyl- α -L-rhamnopyranosyl)-(1 \rightarrow 3)-2-deoxy-2-trichloroacetamido- β -D-glucopyranoside (12). To a solution of tetrasaccharide **13** (3.02 g, 1.76 mmol) in 3:2 Pyridine/AcOH (35 mL) stirred at rt, was added NH₂NH₂·H₂O (0.34 mL, 7.05 mmol, 4.0 equiv.). After stirring at rt for 1 h, a TLC control (Cyclohexane/EtOAc 8:2) showed complete consumption of the starting material (R_f 0.2) into a less polar product (R_f 0.45). Satd aq. NaHCO₃ (200 mL) and DCM (250 mL) were added. The aq. layer was extracted with DCM (150 mL) and the combined organic phases were washed with brine (150 mL). The organic layer was dried over anhyd. Na₂SO₄, filtered, co-evaporated five times with Toluene and concentrated under vacuum. The residue was then diluted in Toluene (21 mL) and the mixture was cooled down to 0 °C. TFA (189 mL) was slowly added and the reaction mixture was stirred at rt for 20 h under an Ar atmosphere. A TLC control (DCM/MeOH 8:2) showed complete consumption of the starting material (R_f 0.9) into a more polar product (R_f 0.15). Solvents were evaporated under reduced pressure and co-evaporations with Toluene (3 x 100 mL) then MeOH (3 x 100 mL) were performed. The residue was purified by reverse phase chromatography eluting with 20.5% aq. CH₃CN to give minutes amounts of the dechloroacetylated tetrasaccharide **22** (HRMS (ESI⁺) m/z : [M+N_a]⁺ Calcd for C₂₉H₄₆Cl₃NO₁₈Na 824.1678; found 824.1676). RP-LC t_R (215 nm): 13.46 min, t_R (ELSD): 13.85 min) and fraction containing the targeted ABC'D' **12** (HRMS (ESI⁺) m/z : [M+NH₄]⁺ Calcd for C₃₁H₅₁Cl₄N₂O₁₉ 895.1840; found 895.1860). RP-LC t_R (215 nm): 17.64 min, t_R (ELSD): 18.00 min).

Fractions containing tetrasaccharide **12** were combined and lyophilized. Contaminated fractions were further purified by semi-preparative RP-HPLC (215 nm, 20.5% aq. CH₃CN) to give, after freeze-drying, the lightly protected ABC'D' **12** (559 mg, 36%) as a white powder. Tetrasaccharide **12** had ¹³C{¹H} NMR (200 MHz, D₂O), δ 168.6 (CO_{2CA}), 164.7 (CO_{NH}), 133.0 (CH=CH_{2All}), 118.7 (CH=CH_{2All}), 102.3 (C-1_A), 100.5 (C-1_B), 99.1 (C-1_D), 98.5 (C-1_C), 91.6 (C_{IVCCIB}), 81.7 (C-3_D), 78.0 (C-2_B), 76.1 (C-5_D), 74.5 (C-3_C), 73.4 (C-2_C), 72.2 (C-4_C), 72.0 (2C, C-4_B, C-4_A), 70.8 (CH₂-CH=All), 70.0 (2C, C-3_A, C-2_A), 69.9 (C-3_B), 69.5 (C-5_B), 69.2 (C-5_C), 69.1 (C-5_A), 68.5 (C-4_D), 60.7 (C-6_D), 57.1 (C-2_D), 40.7 (CH_{2CA}), 16.7 (C-6_B), 16.6 (C-6_A), 16.2 (C-6_C). ¹H NMR (950 MHz, DCl), δ 5.89 (dddd, 1H, J = 17.0, 10.4, 5.2 Hz, CH=CH_{2All}), 5.30 (ddd, 1H, J = 17.0, 1.5, 0.9 Hz, CH=CH_{2All}), 5.23 (dpt, 1H, J = 10.4, 1.5 Hz, CH=CH_{2All}), 5.18 (dd, 1H, $J_{2,3}$ = 2.5 Hz, $J_{2,1}$ = 1.6 Hz, H-2_C), 5.15 (brs, 1H, H-1_B), 4.91 (brd, 1H, $J_{1,2}$ = 1.6 Hz, H-1_C), 4.90 (brs, 1H, H-1_A), 4.70 (do, 1H, H-1_D), 4.34 (ddt_{po}, 1H, J = 13.0, 5.5, 1.5 Hz, CH₂-CH=All), 4.32 (d_{po}, 1H, J_{gem} = 15.3 Hz, H_{CA}), 4.28 (d, 1H, J_{gem} = 15.3 Hz, H_{CA}), 4.33 (ddq, 1H, J = 13.0, 6.3, 1.3 Hz, CH₂-CH=All), 4.09 (dq, 1H, $J_{5,4}$ = 9.7 Hz, $J_{5,6}$ = 6.3 Hz, H-5_C), 4.04 (dd, 1H, $J_{2,3}$ = 3.0 Hz, $J_{2,1}$ = 1.8 Hz, H-2_A), 3.98 (dd, 1H, $J_{3,4}$ = 9.7 Hz, $J_{3,2}$ = 3.4 Hz, H-3_C), 3.95 (dd_{po}, 1H, $J_{2,3}$ = 3.0 Hz, $J_{2,1}$ = 1.8 Hz, H-2_B), 3.93 (dd_{po}, 1H, $J_{6b,5}$ = 2.0 Hz, $J_{6b,6a}$ = 12.4 Hz, H-6_{bD}), 3.90 (pt, 1H, $J_{2,1}$ = $J_{2,3}$ = 9.7 Hz, H-2_D), 3.78 (pt_{po}, 1H, $J_{3,2}$ = $J_{3,4}$ = 9.7 Hz, H-3_D), 3.77 (dd_{po}, 1H, $J_{3,4}$ = 9.8 Hz, $J_{3,2}$ = 3.0 Hz, H-3_A), 3.75 (dd_{po}, 1H, $J_{6a,5}$ = 5.8 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6_{aD}), 3.73 (dq, 1H, $J_{5,4}$ = 9.5 Hz, $J_{5,6}$ = 6.3 Hz, H-5_B), 3.68 (dd_{po}, 1H, $J_{3,4}$ = 9.5 Hz, $J_{3,2}$ = 3.0 Hz, H-3_B), 3.66 (dq_{po}, 1H, $J_{5,4}$ = 9.8 Hz, $J_{5,6}$ = 6.2 Hz, H-5_A), 3.59 (pt, 1H, $J_{4,5}$ = $J_{4,3}$ = 9.7 Hz, H-4_D), 3.57 (pt, 1H, $J_{4,5}$ = $J_{4,3}$ = 9.7 Hz, H-4_C), 3.47 (dddd, 1H, $J_{5,4}$ = 9.7 Hz, $J_{5,6a}$ = 5.8 Hz, $J_{5,6b}$ = 2.0 Hz, H-5_D), 3.43 (pt_{po}, 1H, $J_{4,5}$ = $J_{4,3}$ = 9.5 Hz, H-4_B), 3.37 (pt_{po}, 1H, $J_{4,5}$ = $J_{4,3}$ = 9.8 Hz, H-4_A), 1.30 (d, 3H, $J_{6,5}$ = 6.3 Hz, H-6_B), 1.25 (d_{po}, 3H, $J_{6,5}$ = 6.2 Hz, H-6_A), 1.24 (d_{po}, 3H, $J_{6,5}$ = 6.3 Hz, H-6_C). HRMS (ESI⁺) m/z : [M+NH₄]⁺ Calcd for C₃₁H₅₁Cl₄N₂O₁₉ 895.1840; found 895.1860. RP-HPLC (Conditions A) t_R (215 nm): 17.64 min, t_R (ELSD): 18.00 min; (Conditions B) t_R (220 nm): 22.50 min.

Allyl α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-rhamnopyranosyl-(1 \rightarrow 3)-[α -D-glucopyranosyl-(1 \rightarrow 6)]-2-O-chloroacetyl- α -L-rhamnopyranosyl-(1 \rightarrow 3)-2-deoxy-2-trichloroacetamido- β -D-glucopyranoside (P1**).** A mixture of acceptor **12** (44 mg, 50 μ mol) and sucrose (343 mg, 20 equiv.) in sodium acetate buffer pH 5.1 (1 mL) was stirred for 16 h in the presence of purified BRS-B Δ 1 (1 U.mL⁻¹). Product **P1** was purified by automated fractionation on an Agilent 1260 Infinity HPLC using a C18-RP Fusion column by means of a 20 min run eluting at a flow of 1 mL.min⁻¹ with H₂O/CH₃CN 70:30 for 4 min then with a linear gradient of H₂O/CH₃CN 70:30 to 40:60 from 4 to 11 min, followed by washing and re-equilibration steps. Fractions containing products and the remaining acceptor as detected by UV were collected and re-analyzed by HPLC, using the same analytical conditions. Fractions containing a single peak product were pooled, concentrated to dryness using a SpeedVac to give pentasaccharide **P1** (1.0 mg, 2.3%) and tetrasaccharide **12** (20 mg). The glucosylation product had ¹H NMR (950 MHz, DCl), δ 5.89 (dddd, 1H, J = 17.0, 10.4, 5.2 Hz, CH=CH_{2All}), 5.30 (ddd, 1H, J = 17.0, 1.5, 0.9 Hz, CH=CH_{2All}), 5.23 (dpt, 1H, J = 10.4, 1.5 Hz, CH=CH_{2All}), 5.19 (dd, 1H, $J_{2,3}$ = 2.5 Hz, $J_{2,1}$ = 1.6 Hz, H-2_C), 5.16 (brs, 1H, H-1_B), 4.97 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1_E), 4.94 (brd, 1H, $J_{1,2}$ = 1.1 Hz, H-1_C), 4.91 (brd,

1 1H, $J_{1,2} = 0.8$ Hz, H-1_A), 4.74 (d, 1H, $J_{1,2} = 8.6$ Hz, H-1_D), 4.32 (d_{po}, 1H, $J_{gem} = 15.5$ Hz, H_{CA}), 4.32 (dd_o,
2 1H, $J = 12.8, 5.9$ Hz, CH₂-CH=All), 4.28 (d, 1H, $J_{gem} = 15.5$ Hz, H_{CA}), 4.16 (dd, 1H, $J = 12.8, 6.3$ Hz,
3 CH₂-CH=All), 4.10 (dq, 1H, $J_{5,4} = 9.7$ Hz, $J_{5,6} = 6.3$ Hz, H-5_C), 4.04 (dd, 1H, $J_{2,3} = 3.0$ Hz, $J_{2,1} = 1.8$ Hz,
4 H-2_A), 3.98 (dd_{po}, 1H, $J_{3,4} = 9.7$ Hz, $J_{3,2} = 3.4$ Hz, H-3_C), 3.97 (dd_{po}, 1H, $J_{6b,5} = 5.1$ Hz, $J_{6b,6a} = 11.6$ Hz,
5 H-6_B), 3.95 (dd, 1H, $J_{2,3} = 3.0$ Hz, $J_{2,1} = 1.8$ Hz, H-2_B), 3.90 (pt, 1H, $J_{2,1} = J_{2,3} = 9.7$ Hz, H-2_D), 3.82
6 (d_o, 1H, $J_{6a,6b} = 10.4$ Hz, H-6_a), 3.83 (dd_{po}, 1H, $J_{6b,5} = 3.7$ Hz, $J_{6b,6a} = 10.4$ Hz, H-6_b), 3.82 (pt_{po}, 1H,
7 $J_{3,2} = J_{3,4} = 9.5$ Hz, H-3_D), 3.77 (dd_{po}, 1H, $J_{3,4} = 9.8$ Hz, $J_{3,2} = 3.0$ Hz, H-3_A), 3.76 (dd_{po}, 1H, $J_{3,4} = 9.9$
8 Hz, $J_{3,2} = 3.6$ Hz, H-3_E), 3.75-3.73 (m_{po}, 2H, H-5_B, H-4_E), 3.72 (dd_{po}, 1H, $J_{4,5} = 9.6$ Hz, $J_{4,3} = 9.5$ Hz, H-
9 4_D), 3.71 (dd_{po}, 1H, $J_{6a,5} = 5.5$ Hz, $J_{6a,6b} = 10.4$ Hz, H-6_a), 3.68 (dd_o, $J_{3,4} = 9.5$ Hz, $J_{3,2} = 3.0$ Hz, H-3_B),
10 3.68- 3.65 (m_{po}, 2H, H-5_A, H-5_D), 3.57 (pt, 1H, $J_{4,5} = J_{4,3} = 9.7$ Hz, H-4_C), 3.55 (dd, 1H, $J_{2,3} = 10.0$ Hz,
11 $J_{2,1} = 3.6$ Hz, H-2_E), 3.43 (pt_{po}, 1H, $J_{4,5} = J_{4,3} = 9.5$ Hz, H-4_B), 3.42 (pt_o, 1H, $J_{4,5} = J_{4,3} = 9.8$ Hz, H-4_A),
12 3.42 (ddd_o, 1H, $J_{5,4} = 9.5$ Hz, H-5_E), 1.30 (d, 3H, $J_{6,5} = 6.3$ Hz, H-6_B), 1.25 (d, 3H, $J_{6,5} = 6.2$ Hz, H-6_A),
13 1.24 (d, 3H, $J_{6,5} = 6.3$ Hz, H-6_C). ¹³C{¹H} NMR (239 MHz, DCl), δ 168.6 (CO_{2CA}), 164.7 (CO_{NH}), 133.1
14 (CH=CH_{2All}), 118.8 (CH=CH_{2All}), 102.4 (C-1_A), 100.6 (C-1_B), 99.2 (C-1_D), 98.4 (C-1_C), 98.1 (C-1_E),
15 91.7 (C_{IVCCl3}), 81.4 (C-3_D), 78.0 (C-2_B), 74.6 (C-3_C), 74.5 (C-5_D), 73.4 (C-2_C), 73.1 (C-4_E), 72.2 (C-4_C),
16 72.1 (2C, C-4_A, C-4_B), 71.9 (C-3_E), 71.5 (C-2_E), 70.9 (CH₂-CH=All), 70.1 (2C, C-3_A, C-2_A), 70.0 (C-3_B),
17 69.6 (2C, C-5_B, C-5_E), 69.3 (C-5_C), 69.2 (C-5_A), 68.5 (C-4_D), 65.9 (C-6_D), 60.6 (C-6_E), 57.2 (C-2_D), 40.8
18 (CH_{2CA}), 16.8 (C-6_B), 16.6 (C-6_A), 16.3 (C-6_C). HRMS (ESI⁺) m/z : [M+NH₄]⁺ Calcd for
19 C₃₇H₅₇Cl₄NaNO₂₄ 1062.1922; found 1062.1947. RP-HPLC (Conditions A) t_R (215 nm): 16.84 min, t_R
20 (ELSD): 16.89 min; (Conditions B) t_R (220 nm): 21.65 min.

Enzymatic synthesis

Production of the native GH70 branching sucrases and purification of BRS-B Δ 1

21 The six branching sucrases were recombinantly expressed in *E. coli* as previously reported.^{58, 65} Briefly,
22 *E. coli* BL21 StarTM DE3 and *E. coli* BL21 AI competent cells (Life Technologies) were transformed
23 by pET53-55 plasmids containing the genes brsB Δ 1, brsC, brsD, brsE, Δ N₁₂₃-GBD-CD2; and pBAD49
24 plasmid containing the gene brsA, respectively. Twenty milliliters of LB-Miller medium, supplemented
25 with ampicillin (100 μ g.mL⁻¹), were inoculated with 100 μ L of transformation mix and incubated
26 overnight at 37 °C under agitation (200 rpm).

27 Enzyme productions were performed in Erlenmeyer flasks with modified ZYM-5052 medium (Studier
28 2005) that contains i) 0% lactose, 0% glucose, 0.5% glycerol and 0.01% L-arabinose for BRS-A
29 production, ii) 0.1% lactose, 0% glucose and 1% glycerol for BRS-B Δ 1, BRS-C, BRS-D, BRS-E
30 production or iii) 0.75% lactose, 0.05% glucose and 1.5% glycerol for Δ N₁₂₃-GBD-CD2 production.⁶⁵
31 All culture media were supplemented with ampicillin (100 μ g mL⁻¹) and inoculated with the
32 corresponding starter culture at an OD_{600nm} of 0.05. Cultures were incubated at 21 °C for 26 h (BRS-A,
33 BRS-B Δ 1, BRS-C, BRS-D, BRS-E) or 23 °C for 32 h (Δ N₁₂₃-GBD-CD2) under agitation (150 rpm).
34 Cells were then harvested by centrifugation, dispersed in 50 mM sodium acetate buffer (pH 5.75) at a

1 final OD_{600nm} of 80 for BRS-A, BRS-B Δ1, BRS-C, BRS-D, BRS-E, and an OD_{600nm} of 30 for ΔN₁₂₃-
2 GBD-CD2. Cells were disrupted by sonication and recombinant enzymes were recovered in the soluble
3 fraction after a cycle of centrifugation (15,500 g, 30 min, 8 °C) of the crude cell extract.

4 The BRS-B Δ1 enzyme was purified by affinity chromatography onto nickel resins.⁴¹ Briefly, cells were
5 centrifuged and re-suspended in the binding buffer (20 mM phosphate sodium buffer, pH 7.4, 500 mM
6 NaCl, 20 mM imidazole) at a final OD_{600nm} of 200. After disruption by sonication, centrifugation (18,000
7 g, 30 min, 4 °C) and filtration through a 0.22 μm Ministart® syringe filter (Sartorius), lysates were
8 applied onto a 1 mL HisTrap FF® column (GE Healthcare Life Sciences) that had been equilibrated
9 with the binding buffer, using an AKTAXpress system (GE Healthcare Life Sciences) kept at 10 °C.
10 Proteins were eluted by a 400 mM imidazole step and immediately stored in a 5 mL loop for online
11 automated re-injection into a Superose12 resin (GE Healthcare Life Sciences). Elution was performed
12 during 3 h 30 with 50 mM sodium acetate buffer at pH 5.75 supplemented with 100 mM NaCl at a flow
13 of 1 mL.min⁻¹. Protein fractions were automatically collected and purity was controlled by sodium
14 dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) using Mini-PROTEAN® TGX
15 Stain-Free™ precast gels (BioRad).
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25 **Measurement of enzyme activity on sucrose**

26 One unit of branching sucrase is defined as the amount of enzyme which catalyzes the production of
27 one micromole of fructose per min from 292 mM sucrose, at 30 °C, in 50 mM sodium acetate buffer at
28 pH 5.1 or pH 5.75. The enzyme activities were determined by measuring the amount of reducing sugars
29 using the dinitrosalicylic acid (DNS) method,⁶⁶ corresponding to the initial velocity of released fructose.
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35 **Enzyme screening for tetrasaccharide 12 glucosylation**

36 Transglucosylation assays were performed at 30 °C in 50 mM sodium acetate buffer containing 0.34
37 mM CaCl₂, pH 5.1, supplemented with 0.4 U.mL⁻¹ of enzyme, 1 M sucrose, and 50 mM tetrasaccharide
38 **12** corresponding to an acceptor:sucrose molar ratio of 1:20. Reactions were incubated in glass tubes for
39 16 h.
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45 **Analytical methods for the detection of the glucosylated products**

46 Glucose, fructose, leucrose and sucrose present in reaction medium were analyzed by HPAEC-PAD
47 (high performance anion exchange chromatography with pulsed amperometric detection) using a
48 CarboPac™ PA100 analytical column (4 mm x 250 mm) coupled with a CarboPac™ PA100 guard
49 (4 mm x 50 mm). Product separation was performed using a sodium acetate gradient (6–500 mM) in
50 150 mM NaOH over 45 min (1 mL/min).
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54 Tetrasaccharide **12** and related glucosylated products were analyzed on a LC-MS instrument composed
55 of a Dionex/Thermo Scientific™ HPLC UltiMate 3000 system with a VW Detector, coupled to a
56 Thermo Scientific™ LTQ Orbitrap Velos™ hybrid FT mass spectrometer (Thermo Fisher Scientific,
57 San Jose, CA, USA). Reaction media were diluted 5 times in H₂O/CH₃CN (70:30, v/v) + 0.08% TFA.
58 The presence of residual tetrasaccharide **12** and glucosylated products (pentasaccharides **P1** and **P2** and
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1 poly-glucosylated products) was determined by eluting through a C18 RP Fusion (4 μm , 80 \AA , 250 x
2 4.6 mm) analytical column placed in an oven at 30 $^{\circ}\text{C}$. The flow rate was set to 1 $\text{mL}\cdot\text{min}^{-1}$ and the
3 solvent system consisted of H_2O and CH_3CN . Elution started with 20% aq. CH_3CN for 15 min, then
4 reached 80% aq. CH_3CN within 15 min. The later was held for 5 min, followed by a drop to 20% aq.
5 CH_3CN within 0.1 min, which was held for 11 min to allow column re-equilibration. The injected sample
6 amount was 10 μL and samples were kept at 4 $^{\circ}\text{C}$. UV absorption spectra were monitored at 220 nm.
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8 Mass detection was carried out in a positive heated electrospray ionization (ESI) mode. Mass
9 spectrometer settings were as follows: the spray voltage was 4.0 kV, the capillary and desolvation
10 temperature were 400 and 450 $^{\circ}\text{C}$, respectively, and the maximum injection time was 50 ms in Full Scan
11 mode (500 - 1900 m/z). Nitrogen was used as sheath gas (pressure 75 units) and auxiliary gas (pressure
12 20 units). The automatic gain control (AGC) was set at $1\text{e}6$ for full scan mode with a mass resolution of
13 60,000 (at 400 m/z). Data acquisition was performed using the Thermo Scientific Xcalibur software.
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23 ASSOCIATED CONTENT

24 25 26 **Supporting Information**

27 The supporting Information is available free of charge on the ACS Publications website at DOI:
28 Schemes S1 and S2, figures S1-S6, and table S1. ^1H and ^{13}C NMR spectra of all novel compounds,
29 except for compounds **12** and **P1**. For those two compounds, ^1H , DEPT-135, HSQC and HMBC NMR
30 spectra.
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Notes

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14 **TOC: Graphical abstract**

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