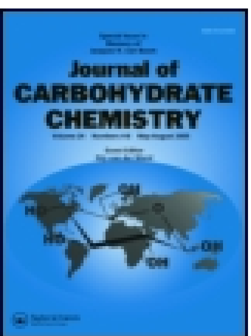

Journal of Carbohydrate Chemistry

ISSN: 0732-8303 (Print) 1532-2327 (Online) Journal homepage: http://www.tandfonline.com/loi/lcar20

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To cite this article: Andreja Jakas, Milica Perc, Josipa Suć, Maria C. Rodriguez, Mare Cudic & Predrag Cudic (2016): Synthesis of anthrose lipidic derivative as mimic of B. anthracis BclA glycoprotein for use in ELISA-like binding assays, Journal of Carbohydrate Chemistry

To link to this article: http://dx.doi.org/10.1080/07328303.2016.1139124

Published online: 17 Mar 2016.

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JOURNAL OF CARBOHYDRATE CHEMISTRY
2016, VOL. 0, NO. 0, 1–17
http://dx.doi.org/10.1080/07328303.2016.1139124

Synthesis of anthrose lipidic derivative as mimic of *B. anthracis* BclA glycoprotein for use in ELISA-like binding assays

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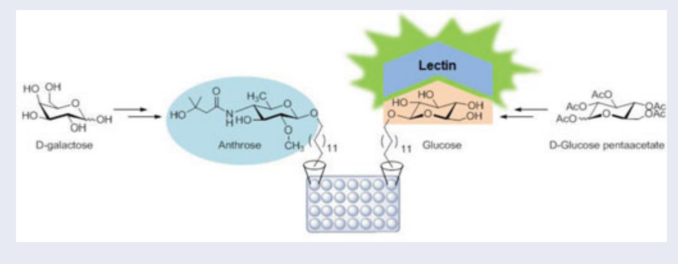
ABSTRACT

The surfaces of *Bacillus anthracis* endospores expose anthrose-containing oligosaccharides, which have been considered for use as a target for specific detection of the spores. In this direction, we have developed an efficient and straightforward synthetic strategy toward anthrose lipidic derivate tetradecyl 4,6-dideoxy-4-(3-hydroxy-3-methylbutanamido)-2-*O*-methyl-β-D-glucopyranoside **16** as a model target for *B. anthracis* spores. The ability of the prepared anthrose and glucose (for control purposes) lipidic derivatives to display on a multiwell plate was demonstrated by a colorimetric phenol-sulfuric acid assay and their potential utility in multiwell binding assays was assessed using fluorescein-labeled concanavalin A (ConA-FITC) and *Aleuria aurantia* (AAL-FITC).

GRAPHICAL ABSTRACT



ARTICLE HISTORY
Received 24 November 2015
Accepted 4 January 2016

KEYWORDS
Anthrax; anthrose; binding; glucose; synthesis

Introduction

Anthrax is an acute infectious disease caused by the spore-forming bacterium *Bacillus anthracis*. While predominantly a disease of animals, humans can become infected by contact as well. If not treated rapidly, it can be deadly. Therefore, the rapid detection of *B.anthracis* spores in the environment prior to infection is of utmost

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importance for both human and animal safety. Considering that the work with *B. anthracis* spores requires special training and a strictly controlled laboratory environment, not readily available to the majority of researchers, alternative options that can mimic the surface of *B. anthracis* spores are highly desirable.

It has been shown in literature that one of the components of *B. anthracis* exosporuim is a glycoprotein called BclA (*Bacillus* collagen-like protein of *anthracis*), whose carbohydrate portion is composed of the tetrasaccharide β-Ant(1→3)α-l-Rha*p*(1→3)α-l-Rha*p*(1→2)l-Rha*p*.[1] The upstream terminal residue of this tetrasaccharide is anthrose {4,6-dideoxy-4-[(3-hydroxy-3-methyl-1-oxobutyl)amino]-2-*O*-methyl-D-glucopyranose}, a highly specific monosaccharide for *B. anthracis* spores. P. H. Seeberger and co-workers demonstrated that anthrose-containing tetrasaccharides can be successfully used as a model of *B. anthracis* spores.[2,3] However, a cumbersome synthesis hampered its wide utilization as an initial target for the development of novel *B. anthracis* sensory systems. This is especially evident in the case of high-throughput screening (HTS) approaches in which larger quantities of this oligosaccharide are required. Alternatively, the structurally simple and synthetically more accessible anthrose monosaccharide may serve the same purpose. Synthesis of anthrose derivatives as building blocks for a more complex oligosaccharide has been described in literature.[4,5] However, these synthetic approaches require either use of a rather expensive D-fucose,[4,6] or use of D-mannose and 2-acetylfuran in rather lengthy syntheses.[5,7,8] In addition, the reported approaches require synthesis of thio derivatives of sugars in further modifications. A disadvantage of this procedure is the use of toxic and malodorous thiols and thiophenols.

Herein we report a simplified and straightforward synthetic route to the anthrose lipidic derivative **16** for application in screening assays to identify specific binders. In our strategy, use of the lipidic group has a dual function. It serves as a protecting group at *O*-1 position throughout the synthesis, whereas in the final product it serves as an anchoring moiety to the plastic surface of a multiwell plate for screening purposes.[9–12] The synthesis commences with relatively inexpensive D-galactose and includes attachment of the lipidic moiety from the beginning of the synthesis without a need for preparation of a thio derivative.[13–15] The lipidic derivative of glucose **19** was prepared for control purposes. The ability of the prepared lipidic anthrose and glucose derivatives to display on a multiwell plate were examined by colorimetric phenol-sulfuric acid assay.[16–18] Furthermore, their application in an Enzyme Linked Lectin Assay (ELLA) using a multiwell plate format was assessed by binding to fluorescein-labeled lectins concanavalin A (ConA-FITC) and *Aleuria aurantia* (AAL-FITC) lectin.

Results and discussion

Our synthetic strategy toward the desired anthrose lipidic derivative **16** is outlined in Scheme 1. In order to simplify synthesis of the lipidated anthrose, we have decided to incorporate the lipidic moiety in the first synthetic step. This moiety

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Scheme 1. Synthesis of anthrose lipidic derivative **16** from D-galactose. Reagents and conditions: (a) tetradecanol, $BF_3 \cdot OEt_2$, DMF, 0°C, 1 h, rt, overnight; (b) NaOMe/MeOH, 0°C→rt, 3 h; (c) benzaldehyde dimethyl acetal, p-toluene sulfonic acid, DMF, 60°C, 240 mBar, 5 h; (d) benzoylcyanide, $MeCN/CH_2Cl_2/Et_3N$, −25°C, 2 h; (e) pyridine/Ac_2O, rt, overnight; (f) AcOH/water (4:1), 50°C, 18 h; (g) PPh_3/imidazole/iodine, CH_2Cl_2, reflux, 12 h; (h) Pd/C (20%), $NaHCO_3$, H_2, DMF, rt, overnight; (i) Tf_2O, pyridine/CH_2Cl_2, −20°C, 2 h; (j) NaN_3, DMF, 80°C, 1 h; (k) AcCl/MeOH, CH_2Cl_2, 0°C, 15 min, rt, 24 h; (l) Ag_2O, MS 4Å, CH_2Cl_2, rt, 1 h, IMe, rt, 3 d; (m) $NaBH_4$, $NiCl_2 \cdot 6H_2O$, CH_2Cl_2, rt, 1 h; (n) $OH(CH_3)_2CCH_2COOH$, HATU, DIPEA, CH_2Cl_2, rt, 18 h; (o) NaOMe/MeOH, rt, 2 h.

not only serves as an *O*-1 protecting group throughout the synthesis but also facilitates adhesion of the final product to the plastic surface. Therefore, the key intermediate tetradecyl β-D-galactopyranoside **3** (Sch. 1) was obtained in two steps starting with commercially available β-D-galactose pentaacetate **1** by combining the procedures previously reported by Hui and co-workers[19] and Wong and co-workers using $BF_3 \cdot OEt_2$ as a promoter.[20] Treatment of **3** with benzaldehyde dimethyl acetal resulted in the 4,6-*O*-benzylidene derivative **4**.[14,21] In the next step, selective benzoylation was performed with benzoyl cyanide in the presence of triethylamine as a base at −25°C. Esterification occurred mainly at the *O*-3 position, as expected for a β-D-galactopyranoside, affording **5** in high yield (85%).[14] Further *O*-acetylation at *O*-2, followed by acid-catalyzed *O*-debenzylidenation, gave **7** in 81% overall yield. Transformation of the primary hydroxy group of diol **7** into a deoxy functionality was carried out by a two-step reaction sequence. The C-6 hydroxy group was first regioselectively iodinated using modified Garegg–Samuelsson's conditions[22] as previously reported by Bundle and co-workers for a

Scheme 2. Synthesis of glucose lipidic derivative **19** from peracetylated-β-D-glucose. Reagents and conditions: (a) pyridine/Ac_2O, rt, overnight; (b) tetradecanol, $BF_3 \cdot OEt_2$, DMF, 0°C, 1 h, rt, overnight; (c) NaOMe/MeOH, 0°C→rt.

mannopyranoside derivative.[23] However, under these conditions (triphenylphosphine/imidazole/iodine in toluene at reflux) compound **8** was obtained in trace amounts. A significant improvement in the reaction yield was achieved by replacing toluene with CH_2Cl_2 at room temperature (rt), affording compound **8** in 67% yield. This is consistent with previous reports indicating CH_2Cl_2 as the preferred solvent for iodination of a variety of alcohols using the triphenylphosphine/iodine system.[14,24,25] Catalytic hydrogenation with Pd/C of **8** afforded a quantitative yield of pure 6-deoxygalactopyranoside **9**. Subsequent reaction of **9** with triflic anhydride followed by the nucleophilic displacement of the resulting 4-*O*-triflate **10** with azide[26] gave compound **11** in 95% overall yield. Deacetylation at *O*-2 in compound **11** was performed by acid-catalyzed methanolysis (AcCl/MeOH) to afford **12** in 82% yield.[27] The methoxy group at *O*-2 of anthrose was then introduced following the procedure previously reported by Porter and co-workers.[28] Following this procedure, treatment of compound **12** with iodomethane in the presence of silver(I) oxide resulted in **13** in 88% yield. Reduction of the azide in **13** with sodium borohydride in the presence of nickel chloride afforded amine **14**.[14] Treatment of **14** with 3-hydroxy-3-methylbutyric acid in the presence of *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-(4,5-*b*)-pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) gave **15** in 80% yield over a two-step sequence. Finally, 3-*O* debenzoylation using base-catalyzed methanolysis conditions gave the target anthrose derivative **16** in a 67% yield.

Glucose derivative **19** was obtained over two steps starting with β-D-glucose pentaacetate (Sch. 2) in 47% yield. No change of stereochemistry at the C-1 position was observed under the applied experimental conditions.

In order to assess the suitability of the prepared lipidic anthrose **16** and glucose **19** derivatives for use in microtiter plate assays, we determined the ability of these lipidic monossacharides to adhere to the surface of a polystyrene 96-well plate.[9–12] Anthrose **16** and glucose **19** derivatives at concentrations ranging from 0.625 to 10 mM were added to the wells and left overnight to allow the solvent to completely evaporate, promoting the monosaccharides' adhesion to the well surface through hydrophobic interactions. The amount of bound monosaccharides in each well, before and after repeated washings with PBS (3 × and 5 ×, respectively), was quantified using a colorimetric phenol-sulfuric acid method.[10,16–18] As shown in Figure 1, both lipidated monosaccharides, **16** and **19**, adhered to the polystyrene surface of the well. The observed difference in the absortivity between anthrose and glucose derivatives upon treatment with phenol and sulfuric acid could be attributed to the differences in the monosaccharides' reactivity with the derivatization reagent.[29]

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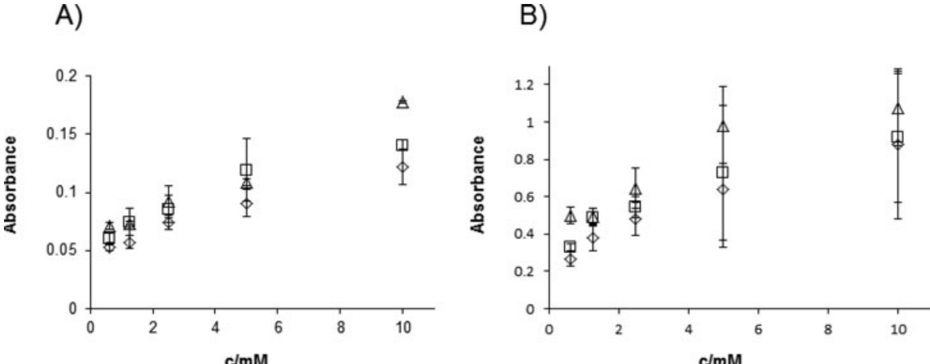

Figure 1. Phenol-sulfuric acid test suggesting a strong adhesion to the polystyrene well surface of (A) anthrose lipidic derivative **16** and (B) glucose lipidic derivative **19**. Washings with PBS : 0 × (◊), 3 × (□), 5 × (△).

Notably, amounts of bound monosaccharides in each well were not significantly altered by repeated washings with PBS (frequently used in binding assays), indicating not only a strong adhesion to the polystyrene surface, but also that adhesion is not influenced by the monosaccharide moiety's structure and physical properties. This also suggests that the lipidated anthrose **16** as well as glucose **19** derivatives can be used in ELLA screening assays in a multiwell-plate format for identification of specific binders.

To assess the applicability of this method in biological binding studies, a lectin-binding assay was performed (Fig. 2). For this purpose, two lectins were used: concanavalin A (ConA), a glucose and mannose binding lectin,[30,31] and *Aleuria aurantia* lectin (AAL), a fucose binding lectin (in this case, used as a negative control).[32–35] To the best of our knowledge, there is no commercially available lectin capable of binding anthrose. Upon immobilization of lipidated monosaccharides on the microtiter plate, as described above, all wells were incubated overnight with PBS containing 3% of bovine serum albumin (BSA) at rt to block nonspecific interactions. Wells without monosaccharides were treated with the same blocking solution and lectins to evaluate nonspecific binding of lectins to BSA. The blocking solution

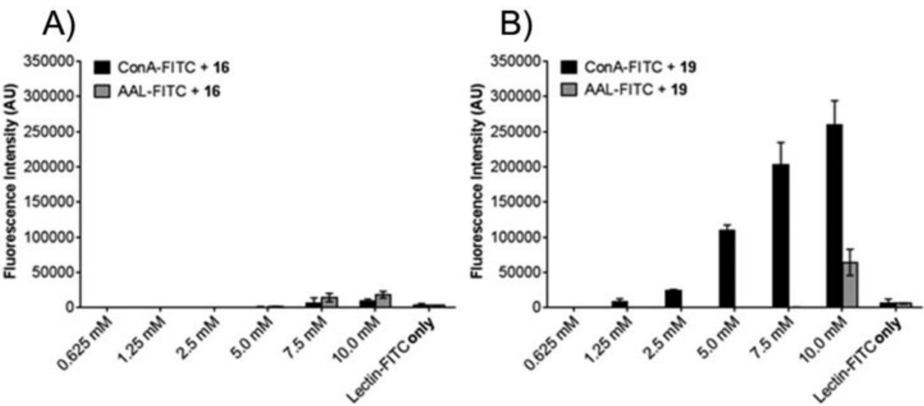

Figure 2. Binding of ConA-FITC and AAL-FITC to anthrose lipidic derivative **16** and glucose lipidic derivative **19** in a multiwell ELLA.

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was then removed and fluorescein-labeled ConA-FITC or AAL-FITC (10 μg/mL) was added to each well and allowed to incubate for 1 h. In the next step, wells were washed with PBS (3 $\times$) and fluorescence intensities were measured as an indication of lectin's binding to the monosaccharide substrate. As expected, no interaction with **16** (Fig. 2A) and strong interaction with **19** (Fig. 2B) were observed for ConA, indicating that under the applied experimental conditions, binders such as ConA can clearly distinguish one monosaccharide over another (i.e., in this case glucose over anthrose). The amount of the bounded ConA following the washing steps is proportional to the amount of **19** in each well (Fig. 2B), thus making it applicable in ELISA-type formats. The predicted absence of any appreciable interaction of AAL with **16** and **19** (Fig. 2), additionally supported the utility of this method for screening of monosaccharide specific binders. Nonspecific binding to BSA was not observed for either lectin, ConA, or AAL (Fig. 2).

Conclusion

In conclusion, we have designed an efficient synthetic strategy that provides us with straightforward access to anthrose lipidic derivative **16** as a model target for *B. anthracis* spores, suitable for immobilization through hydrophobic interactions on the plastic surface of multiwell plates. The synthesis of **16** commences with affordable β-D-galactose pentaacetate and allows incorporation of a lipidic group at the beginning of the synthetic path without a need for preparation of a thio derivative, as previously described.[14] This lipidic tail serves as a protecting group throughout the synthesis and as an anchoring moiety in the final product, significantly reducing the complexity of the entire synthetic approach. Prepared glucose derivate **19** served as a control to ensure that the monosaccharide's structure and physical properties do not affect adhesion to the plastic surface and to demonstrate the utility of this approach to detect selective binders. Suitability of the prepared lipidated monosaccharides **16** and **19** for use in multiwell format binding assays was demonstrated by their resistance to washing steps after adhesion to the polystyrene surface and in multiwell binding assays using fluorescently labeled ConA and AAL lectins. Predictably, ConA exhibits selectivity for glucose over anthrose, and as expected, AAL did not show appreciable affinity toward these two monosaccharides. The reported binding study clearly demonstrated that this multiwell-plate assay is functional for use in biological screenings and that it is therefore applicable in ELISA-type formats. A similar synthetic strategy can be applied for the synthesis of various complex lipidated monosaccharides, which can be further successfully used in ELLA screening assays to identify selective binders.

Experimental section

General methods

Melting points were determined on a Tottoli (Büchi) apparatus and are uncorrected. Optical rotations were measured at 25°C using an Optical Activity LTD

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automatic AA-10 polarimeter. NMR spectra were recorded on a Bruker AV 600 spectrometer, operating at 150.91 MHz for ^{13}C and 600.13 MHz for ^{1}H nuclei. The spectra were measured in DMSO-d_6 at 25°C. Chemical shifts in parts per million were referenced to tetrametylsilane (TMS) and expressed in ppm. Spectra were assigned based on one-dimensional ^{1}H, ^{13}C, and APT (Attached Proton Test) and two-dimensional homonuclear COSY (Correlation Spectroscopy) and heteronuclear HMQC (Heteronuclear Multiple-Quantum Correlation) and HMBC (Heteronuclear Multiple Bond Correlation) experiments. Mass spectra were recorded on a triple quadrupole Agilent 6410 spectrometer operating in Electrospray ionization (ESI) mode. Analytical TLC was carried out on silica gel 60 with detection by charring with 10% H_2SO_4. Column chromatography was performed on silica gel (Merck, 0.040–0.063 mm). Penta-*O*-acetyl-*β*-D-galactopyranonoside, penta-*O*-acetyl-*β*-D-glucopyranonoside, tetradecanol, benzaldehyde dimethyl acetal, *p*-toluene sulfonic acid, $BF_3 \cdot OEt_2$, 3-hydroxy-3-methylbutanoic acid, and HATU were purchased from Sigma-Aldrich. Concentrated sulphuric acid, phenol, and phosphate buffered saline (PBS) powder were purchased from Fisher Scientific. Colorimetric assays were performed in Nunc™ 96-well polystyrene microplates (Thermo Scientific, Cat. No.: 8404). Lectins binding studies were performed in OptiPlate-96 Black, Black Opaque 96-well Microplate (PerkinElmer, Cat. No: 6005270). All measurements were done using a BioTek Synergy H4 microplate reader.

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Synthesis of anthrose lipidic derivative

Tetradecyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (2a) tetradecyl 3,4,6-tri-O-acetyl-β-D-galactopyranoside (2b)

Tetradecanol (14.47 g; 67.5 mmol, 2 equiv.) was added to a solution of compound **1** (13.18 g; 33.76 mmol) in dry DMF (210 mL). The reaction mixture was cooled to 0°C under nitrogen atmosphere, and $BF_3 \cdot OEt_2$ (7.6 mL) was added. The reaction mixture was stirred at 0°C for 1 h and at rt overnight. The reaction progress was monitored by TLC (toluene:EtOAc 2:1). After almost all starting material was consumed, the reaction was quenched by addition of saturated $NaHCO_3$ aqueous solution. The organic layer was extracted with saturated aqueous solution of $NaHCO_3$ (3×) and brine (1×), dried over Na_2SO_4, and evaporated. The resulting oil was purified by silica gel flash column chromatography (toluene:EtOAc 2:1) to give compound **2a** (1.85 g; 3.39 mmol; 10.1%) and compound **2b** (6.69 g; 13.31 mmol; 39.5%) as a yellow oil. R*f* (**2a**) = 0.70; R*f* (**2b**) = 0.49 (toluene:EtOAc 2:1).

HRMS for compound **2a** Calcd. for $C_{28}H_{48}O_{10}Na$ ($M+Na^+$) 567.3145, Found 567.3148.

Tetradecyl β-D-galactopyranoside (3)

Compound **2b** (2.9 g; 5.77 mmol) was added to 113 mL of dry MeOH. The suspension was cooled in an ice bath, and 4.5 mL of 0.1 M solution of NaOMe in MeOH was added. The reaction was allowed to warm to rt and stirred for 3 h. Amberlite IR 120 (H^+) was added to neutralize the mixture, then diluted with

methanol, and the exchange resin was filtered off. The resin was washed thoroughly, and the filtrate was concentrated to give compound **3** (1.33 g; 3.53 mmol; 61.2%) as a white powder. Tetradecyl β-D-galactofuranoside (0.66 g; 30.4%) and tetradecyl α-D-galactopyranoside (0.007 g; 0.3%) were also isolated.

mp(β-*p*, **3**) = 165–168°C; $[\alpha]_D$ (β-*p*, **3**) = −11° (c 1, MeOH); R*f* (β-*p*, **3**) = 0.25 (EtOAc:AcOH:H_2O 70:2:2); ^{13}C NMR (600.13 MHz, $CDCl_3$): δ 103.4 (C-1), 75.1 (C-5), 73.5 (C-3), 70.5 (C-2), 68.4 (CH_2-O, TD), 68.1 (C-4), 60.4 (C-6), 31.3–22.1 (CH_2, TD), 13.9 (CH_3, TD).

mp(β-*f*) = 160–162°C; $[\alpha]_{D(\beta\text{-}f)}$ = −68° (c 1, MeOH); R*f* (β-*f*) = 0.42 (EtOAc:AcOH:H_2O 70:2:2); ^{13}C NMR (600.13 MHz, $CDCl_3$): δ 107.7 (C-1), 82.0 (C-2, C-4), 76.6 (C-3), 70.5 (C-5), 66.8 (CH_2-O, TD), 62.9 (C-6), 31.3–22.1 (CH_2, TD), 13.9 (CH_3, TD).

R*f* (α-*p*) = 0.31 (EtOAc:AcOH:H_2O 70:2:2); ^{13}C NMR (600.13 MHz, $CDCl_3$): δ 98.8 (C-1), 71.2 (C-4), 69.6 (C-2), 68.8 (C-5), 68.4 (C-3), 66.9 (CH_2-O, TD), 60.6 (C-6), 31.3–22.1 (CH_2, TD), 13.9 (CH_3, TD). HRMS Calcd. for $C_{20}H_{40}O_6Na$ ($M+Na^+$) 399.2723, Found 399.2725.

Tetradecyl 4,6-O-benzylidene-β-D-galactopyranoside (4)

Compound **3** (1.997 g, 5.3 mmol) was dissolved in dry DMF (20 mL) and benzaldehyde dimethyl acetal (0.972 mL, 10.6 mmol) and *p*-toluene sulfonic acid (∼200 mg; to adjust pH ∼3) was added. The mixture was stirred at 60°C and reduced pressure (250 mbar) for ∼5 h. The solvents were removed under diminished pressure at 70°C in water bath. The residue was dissolved in CH_2Cl_2 (200 mL) and washed with a saturated $NaHCO_3$ aqueous solution (3 × 60 mL) and brine (2 × 60 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under vacuum. Crude oily residue was purified by silica gel flash column chromatography (toluene:EtOAc 2:1), to give pure compound **4** (1.364 g, 2.93 mmol, 55.5%) as a white powder.

mp = 133–139°C; $[\alpha]_D$ = –17° (c 1, MeOH); R*f* = 0.18 (toluene:EtOAc 2:1); ^1H NMR (150 MHz, DMSO-d_6): δ 7.49–7.38 (m, 5H, Ph), 5.58 (s, 1H, H-7), 4.87 (dd, 1H, *J* = 1 Hz, H-6b), 4.22 (d, 1H, *J* = 8 Hz, H-1), 4.09 (d, 1H, *J* = 3.3 Hz, H-4), 4.07 (d, 1H, *J* = 1.2 Hz, H-6a), 3.78 (1H, CH_{2b}-O (TD)), 3.50 (m, 1H, H-2), 3.48 (m, 1H, H-3), 3.47 (1H, CH_{2a}-O (TD)), 3.37 (m, 1H, H-5), 1.57–1.30 (24H, CH_2 (TD)), 0.88 (3H, CH_3 (TD)); ^{13}C NMR (600.13 MHz, DMSO-d_6): δ 138.6–126.2 (Ph), 103.11 (C-1), 99.7 (C-7), 76.0 (C-4), 72.0 (C-3), 70.0 (C-5), 68.6 (CH_2-O (TD)), 68.6 (C-6), 65.9 (C-2), 32.2–22.0 (CH_2 (TD)), 13.9 (CH_3 (TD)).

Tetradecyl 3-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside (5)

Compound **4** (1.364 g, 2.93 mmol) was dissolved in a mixture of dry MeCN (9.6 mL), CH_2Cl_2 (9.6 mL), and Et_3N (4.8 mL). The mixture was cooled to –25°C in refrigerator and a solution of benzoyl cyanide (424 mg, 3.52 mmol) in dry CH_2Cl_2 (7.3 mL) was added dropwise under nitrogen atmosphere. The reaction progress was monitored by TLC (toluene:EtOAc 2:1). After 2 h, the mixture was washed with a saturated aqueous solution of $NaHCO_3$ (120 mL). The aqueous layer was extracted with

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CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtrated, and concentrated under vacuum. The residue was purified by flash column chromatography (silica gel, toluene:EtOAc 10:1) to give **5** (1.422 g; 2.5 mmol; 85%) as a white powder.

mp = 85–90°C; $[\alpha]_D = +67°$ (c 1, MeOH); R*f* = 0.31 (toluene:EtOAc 10:1); ^{1}H NMR (150 MHz, DMSO-d_6): δ 7.34–7.98 (m, 10H, Ph), 5.59 (s, 1H, H-7), 5.40 (d, 1H, J = 5.8 Hz, OH-4), 5.05 (m, 1H, H-3), 4.45 (d, 1H, J = 7.8 Hz, H-1), 4.44 (d, 1H, J = 3.9 Hz, H-4), 4.10 (d, 1H, H-6a), 3.79 (1H, CH_{2b}-O (TD)), 3.72 (2H, H-5, H-2), 3.51 (1H, CH_{2a}-O (TD)), 1.57–1.33 (24H, CH_2 (TD)), 0.85 (3H, CH_3 (TD)); ^{13}C NMR (600.13 MHz, DMSO-d_6): δ 165.5 (OCO-Ph), 138.6–126.2 (Ph), 102.6 (C-1), 99.3 (C-7), 74.9 (C-4), 73.4 (C-3), 68.7 (CH_2-O (TD)), 68.4 (C-6), 67.5 (C-5), 65.5 (C-2), 31.3–22.1 (CH_2 (TD)), 13.9 (CH_3 (TD)); HRMS Calcd. for $C_{34}H_{48}O_7Na$ ($M+Na^+$) 591.3298, Found 591.3288.

Tetradecyl 2-O-acetyl-3-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside (6)

Acetic anhydride (5 mL) was added to a solution of **5** (1.422 g, 2.5 mmol) in pyridine (3.8 mL). The reaction mixture was stirrred at rt overnight, quenched by the addition of MeOH (20 mL) at 0°C, and then concentrated under vacuum. The residue was dissolved in CH_2Cl_2 (20 mL). The organic layer was washed with a saturated $KHSO_4$ aqueous solution (1 × 10 mL) and water (1 × 10 mL), dried over Na_2SO_4, filtered, and concentrated to give **6** (1.525 g; 2.5 mmol) as a white powder that was used in the next step without further purification.

R*f* = 0.52 (toluene:EtOAc 10:1); mp = 85–90°C; MS-ESI(*m/z*) Calcd. for $C_{36}H_{50}O_8Na$ ($M+Na^+$) 633.3, Found 633.0; HRMS Calcd. for $C_{36}H_{50}O_8Na$ ($M+Na^+$) 633.3404, Found 633.3394.

Tetradecyl 2-O-acetyl-3-O-benzoyl-β-D-galactopyranoside (7)

Compound **6** (1.525 g, 2.5 mmol) was dissolved in AcOH:water (4:1, 16 mL) and heated at 50°C. After 18 h of stirring, the mixture was extracted with CH_2Cl_2 (5 × 40 mL) and the organic layers were combined and washed with a saturated $NaHCO_3$ aqueous solution (6 × 50 mL), brine (2 × 50 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by flash column chromatography (silica gel, toluene:EtOAc 2:1) to give **7** (1.052 g; 2.0 mmol; 80.5%) as a white powder.

mp = 110–115°C; $[\alpha]_D = +40°$ (c 1, MeOH); R*f* = 0.21 (toluen:EtOAc 2:1); ^{1}H NMR (150 MHz, DMSO-d_6): δ 7.93–7.51 (m, 10H, Ph), 5.21 (t, 1H, $J_{2,1} = J_{2,3}$ = 10 Hz, H-2) 4.99 (dd, 1H, $J_{3,4}$ = 3 Hz, H-3), 4.67 (1H, OH-4), 4.59 (d, 1H, J = 8 Hz, H-1), 4.09 (d, 1H, $J_{4,5}$ = 3.5 Hz, H-4), 3.75 (1H, CH_{2b}-O (TD)), 3.64 (t, 1H, $J_{5,6a}$ = $J_{5,6b}$ = 5.5 Hz, H-5) 3.57, 3.52 (m, 2H, H-6), 3.44 (1H, CH_{2a}-O (TD)), 1.92 (CH_3CO), 1.49–1.26 (24H, CH_2 (TD)), 0.85 (3H, CH_3 (TD)); ^{13}C NMR (600.13 MHz, DMSO-d_6): δ 169.2 (CH_3*C*O), (165.2 (OCO-Ph), 133.4–128.6 (Ph), 100.1 (C-1), 75.0 (C-5) 74.9 (C-3), 69.3 (C-2), 68.5 (CH_2-O (TD)), 65.3 (C-4), 59.7 (C-6), 31.2–22.0 (CH_2 (TD)), 20.4 (CH_3CO), 13.9 (CH_3 (TD)); MS-ESI (*m/z*) Calcd. for $C_{29}H_{46}O_8Na$

(M+Na^+) 545.3, Found 545.3; HRMS Calcd. for $C_{29}H_{46}O_8Na$ (M+Na^+) 545.3091, Found 545.3093.

Tetradecyl 2-O-acetyl-3-O-benzoyl-6-deoxy-6-iodo-β-D-galactopyranoside (8)

Triphenylphosphine (2.11 g; 8.05 mmol), imidazole (676 mg; 8.05 mmol) and iodine (766 mg; 6.04 mmol) were added at rt to a solution of compound **7** (1.052 mg; 2.01 mmol) in CH_2Cl_2 (146 mL). The reaction mixture was stirred at reflux for 12 h. The reaction mixture was cooled, saturated $NaHCO_3$ aqueous solution was added (866 mg/10.4 mL water), and the mixture was stirred for an additional 5 min. Iodine was added in portions (approximately 300 mg each). Once the CH_2Cl_2 supernatant remained iodine-colored, the reaction mixture was stirred for an additonal 10 min. Excess of iodine was removed by the addition of saturated $Na_2S_2O_3$aqueous solution (20 mL). The mixture, diluted with CH_2Cl_2 (30 mL), was extracted with water (2 × 40 mL). The organic layer was concentrated and then diluted with cold diethyl ether to precipitate triphenylphosphine oxide. The precipitate was filtrated, and the filtrate further evaporated. The residue was purified by flash column chromatography (silica gel, toluene:EtOAc 10:1) to give compound **8** (851 mg; 1.35 mmol; 67%) as a white powder.

mp = 64–68°C; $[\alpha]_D$ = +38° (c 1, MeOH); R*f* = 0.5 (toluene : EtOAc 10 : 1); ^{1}H NMR (150 MHz, DMSO-d_6): δ 7.93–7.52 (m, 5H, Ph), 5.52 (1H, OH-4), 5.20 (t, 1H, $J_{2,1}$ = $J_{2,3}$ = 10 Hz, H-2) 5.05 (m, 1H, H-3), 4.65 (d, 1H, *J* = 6 Hz, H-1), 4.20 (d, 1H, $J_{4,5}$ = 3.5 Hz, H-4), 3.91 (t, 1H, H-5), 3.77 (1H, CH_{2b}-O (TD)), 3.48 (1H, CH_{2a}-O (TD)), 3.41, 3.26 (m, 2H, H-6), 1.92 (s, 3H, CH_3CO), 1.51–1.25 (24H, CH_2 (TD)), 0.84 (3H, CH_3 (TD)); ^{13}C NMR (600.13 MHz, DMSO-d_6): δ 169.2 (CH_3CO), 165.2 (OCO-Ph), 139.5–128.7 (Ph), 99.7 (C-1), 74.4 (C-3), 74.3 (C-5), 68.8 (C-2), 68.8 (CH_2-O (TD)), 66.4 (C-4), 31.2–22.0 (CH_2 (TD)), 20.4 (CH_3CO), 13.9 (CH_3 (TD)), 4.4 (C-6); MS-ESI (*m/z*) Calcd. for $C_{29}H_{45}IO_7Na$ (M+Na^+) 655.2 found 655.2; HRMS Calcd. for $C_{29}H_4$ IO_7Na (M+Na^+) 655.2108, Found 655.2108.

Tetradecyl 2-O-acetyl-3-O-benzoyl-6-deoxy-β-D-fucopyranoside (9)

20% palladium catalyst on charcoal (416 mg; 8.07 mmol) and $NaHCO_3$ (227 mg, 2.7 mmol) were added to a solution of compound **8** (851 mg; 1.35 mmol) in dry DMF (30 mL). The reaction mixture was stirred overnight in a hydrogen atmosphere. The catalyst was filtered on Celite 521 and the filtrate was concentrated under vacuum. The residue was purified by flash column chromatography (silica gel, toluene:EtOAc 4:1) to give compound **9** (640 mg; 1.26 mmol; 93.3%) as a white powder.

mp = 59–64°C; $[\alpha]_D$ = +43° (c 1, MeOH); R*f* = 0.6 (toluene:EtOAc 4:1); ^{1}H NMR (150 MHz, DMSO-d_6): δ 7.93–7.51 (m, 10H, Ph), 5.25 (d, 1H, *J* = 6.5 Hz, OH-4), 5.19 (dd, 1H, $J_{2,1}$ = $J_{2,3}$ = 10 Hz, H-2) 5.01 (dd, 1H, *J* = 3 Hz, H-3), 4.47 (d, 1H, *J* = 8 Hz, H-1), 3.83 (d, 1H, $J_{4,5}$ = 3 Hz, H-4), 3.81 (q, 1H, *J* = 6.5 Hz, H-5), 3.72 (1H, CH_{2b}-O (TD)), 3.43 (1H, CH_{2a}-O (TD)), 1.91 (s, 3H, CH_3CO), 1.48–1.24 (24H, CH_2 (TD)), 1.18 (d, 3H, *J* = 6.5 Hz, H-6), 0.85 (3H, CH_3 (TD)); ^{13}C NMR (600.13 MHz, DMSO-d_6): δ 169.2 (CH_3CO), 165.2 (OCO-Ph), 133.4–128.7 (Ph), 99.9 (C-1), 74.9

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(C-3), 69.8 (C-5), 69.1 (C-4), 68.3 (C-2), 68.4 (*CH_2*-O (TD)), 31.2–22.0 (CH_2 (TD)), 20.5 (*CH_3*CO), 16.2 (C-6), 13.9 (CH_3 (TD)); MS-ESI (*m/z*) Calcd. for $C_{29}H_{46}O_7Na$ (M+Na^+) 529.3, Found 529.4; HRMS Calcd. for $C_{29}H_{46}O_7Na$ (M+Na^+) 529.3142, Found 529.3132.

Tetradecyl 2-O-acetyl-4-azido-3-O-benzoyl-6-deoxy-β-D-glucopyranoside (11)

A solution of trufluoromethaneslufonic anhydride (314 μL, 1.89 mmol) in dry CH_2Cl_2 (3.5 mL) was added dropwise at –20°C under argon to a solution of compound **9** (640 mg; 1.26 mmol) in dry CH_2Cl_2 (14 mL) containing pyridine (273 μL). The reaction mixture was stirred at –20°C for 2 h. The mixture was then diluted in CH_2Cl_2 (10 mL) and extracted with water (15 mL). The organic layer was washed with saturated $KHSO_4$ aqueous solution (15 mL), saturated $NaHCO_3$ aqueous solution (10 mL), and brine (20 mL), dried over Na_2SO_4, filtered, and concentrated. Triflate derivative **10** (800 mg; 99%) was obtained as a white powder and was used in the next step without further purification.

R*f* = 0.45 (toluene:petroleum ether:EtOAc 2:6:1)

Sodium azide (333 mg; 5.01 mmol) was added to a solution of **10** (800 mg; 1.25 mmol) in dry DMF (18.2 mL). The reaction mixture was stirred at 80°C for 1 h, then concentrated under high vacuum. The residue was dissolved in EtOAc (50 mL). The organic layer was washed with water (20 mL) and brine (20 mL), dried over Na_2SO_4, filtrated, and concentrated under vacuum. The residue was purified by flash column chromatography (silica gel, toluene:petroleum ether:EtOAc 2:6:1) to give compound **11** (637 mg; 1.20 mml; 95% yield over two steps) as a colorless oil.

R*f* = 0.56 (toluene:petroleum ether:EtOAc 2:6:1); ^{1}H NMR (150 MHz, DMSO-*d_6*): δ 7.97–7.54 (m, 5H, Ph), 5.48 (dd, 1H, $J = 9.6$ Hz, H-3), 4.89 (dd, 1H, $J_{2,1} = J_{2,3} = 9.7$ Hz, H-2) 4.74 (d, 1H, $J = 8$ Hz, H-1), 3.79 (t, 1H, $J = 9.6$ Hz, H-5), 3.65 (d, 1H, $J_{4,5} = 3$ Hz, H-4), 3.72 (1H, *CH_{2b}*-O (TD)), 3.43 (1H, *CH_{2a}*-O (TD)), 1.89 (s, 3H, *CH_3*CO), 1.46–1.23 (24H, CH_2 (TD)), 1.30 (d, 3H, $J = 6$ Hz, H-6), 0.85 (3H, CH_3 (TD)); ^{13}C NMR (600.13 MHz, DMSO-*d_6*): δ 168.9 (CH_3CO), 165.0 (OCO-Ph), 133.8–128.7 (Ph), 99.1 (C-1), 73.8 (C-3), 71.6 (C-2), 69.3 (C-4), 68.8 (*CH_2*-O (TD)), 64.6 (C-5), 31.2–22.0 (CH_2 (TD)), 20.2 (*CH_3*CO), 17.8 (C-6), 13.9 (CH_3 (TD)); MS-ESI (*m/z*) Calcd. for $C_{29}H_{45}N_3O_6Na$ (M+Na^+) 554.3, Found 554.3; HRMS Calcd. for $C_{29}H_{45}N_3O_6Na$ (M+Na^+) 554.3206, Found 554.3199.

Tetradecyl 4-azido-3-O-benzoyl-4,6-dideoxy-β-D-glucopyranoside (12)

A solution of acetyl chloride (2.5 mL, 36 mmol) in MeOH (66 mL) was added dropwise to a solution of **11** (637 mg; 1.2 mmol) in dry CH_2Cl_2 (40 mL). The reaction mixture was stirred at 0°C for 15 min, then at rt for 24 h. The organic layer was washed with saturated $NaHCO_3$aqueous solution, filtered, and concentrated. The residue was purified by flash column chromatography (silica gel, toluene:petroleum ether:EtOAc 2:6:1) to give compound **12** (480 mg; 0.98 mmol; 82%) as a colorless oil.

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mp = 50–52°C; $[\alpha]_D = 0°$ (c 1, MeOH); R*f* = 0.5 (toluene:petroleum ether:EtOAc 2:6:1); ^{1}H NMR (150 MHz, DMSO-d_6): δ 8.01–7.54 (m, 10H, Ph), 5.16 (dd, 1H, J = 9 Hz, H-3), 4.40 (d, 1H, J = 8 Hz, H-1), 3.71 (t, 1H, J = 10 Hz, CH_{2b}-O (TD)), 3.55 (1H, H-4), 3.52 (1H, H-2) 3.38 (1H, H-5), 3.48 (t, 1H, J = 10 Hz, CH_{2a}-O (TD)), 1.53–1.26 (24H, CH_2 (TD)), 1.27 (d, 3H, J = 6 Hz, H-6), 0.85 (3H, CH_3 (TD)); ^{13}C NMR (600.13 MHz, DMSO-d_6): δ 165.2 (OCO-Ph), 133.5–128.7 (Ph), 102.06 (C-1), 76.2 (C-3), 71.4 (C-5), 69.2 (C-2), 68.8 (CH_2-O (TD)), 65.0 (C-4), 31.2–22.0 (CH_2 (TD)), 18.1 (C-6), 13.9 (CH_3 (TD)); MS-ESI (*m/z*) Calcd. for $C_{27}H_{43}N_3O_5Na$ (M+Na$^+$) 512.3, Found 512.3; HRMS Calcd. for $C_{27}H_{43}N_3O_5Na$ (M+Na$^+$) 512.3101, Found 512.3110.

Tetradecyl 4-azido-3-O-benzoyl-4,6-deoxy-2-O-methyl-β-D-glucopyranoside (13)

Silver(I) oxide (1.1 g, 5.88 mmol) and molecular sieves were added to a solution of compound **12** (480 mg; 0.98 mmol) in dry CH_2Cl_2(37 mL). The reaction mixture was stirred at rt for 1 h. Iodomethane (611 μL, 9.8 mmol) was then added dropwise and the reaction mixture was continued to stir in the dark at rt for 3 d. Silver(I) oxide was filtered over Celite 521 and the filtrate was concentrated under vacuum. The residue was purified by flash column chromatography (silica gel, toluene:EtOAc 20:1) to give compound **13** (433 mg; 0.86 mmol; 88%) as a white powder.

mp = 47–49°C; $[\alpha]_D = +4°$ (c 1, MeOH); R*f* = 0.6 (toluene : EtOAc 20 : 1); ^{1}H NMR (150 MHz, DMSO-d_6): δ 8.03–7.55 (m, 5H, Ph), 5.24 (dd, 1H, J = 9.7 Hz, H-3), 4.53 (d, 1H, J = 7.9 Hz, H-1), 3.76 (t, 1H, J = 10 Hz, CH_{2b}-O (TD)), 3.56 (1H, H-4), 3.48 (t, 1H, J = 9.6, H-5), 3.34 (3H, CH_3-O), 3.32 (1H, CH_{2a}-O (TD)), 3.20 (dd, 1H, J = 7.7 H-2), 1.53–1.23 (24H, CH_2 (TD)), 1.26 (d, 3H, J = 6 Hz, H-6), 0.85 (3H, CH_3 (TD)); ^{13}C NMR (600.13 MHz, DMSO-d_6): δ 165.1 (OCO-Ph), 133.7–128.8 (Ph), 101.7 (C-1), 81.0 (C-2), 74.7 (C-3), 69.0 (C-5), 68.8 (CH_2-O (TD)), 64.9 (C-4), 59.7 (CH_3-O), 31.2–22.0 (CH_2 (TD)), 18.0 (C-6), 13.9 (CH_3 (TD)); MS-ESI (*m/z*) Calcd. for $C_{28}H_{45}N_3O_5Na$ (M+Na$^+$) 526.3, Found 526.3; HRMS Calcd. for $C_{28}H_{45}N_3O_5Na$ (M+Na$^+$) 526.3257, Found 526.3261.

Tetradecyl 3-O-benzoyl-4,6-dideoxy-4-(3-hydroxy-3-methylbutanamido)-2-O-methyl-β-D-glucopyranoside (15)

$NaBH_4$ (65 mg; 1.7 mmol) and a catalytic amount of $NiCl_2 \times 6H_2O$ were added to a solution of compound **13** (433 mg; 0.86 mmol) in a mixture of dry CH_2Cl_2 (12.4 mL) and EtOH (60 mL). The reaction mixture was stirred at rt for 1 h and concentrated under vacuum. The residue was dissolved in CH_2Cl_2 (50 mL). The organic layer was washed with water (20 mL) and brine (20 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum. Compound **14** (398 mg; 97%) was used in the next step without further purification. R*f* = 0.45 (toluene:EtOAc 1:1)

3-Hydroxy-3-methylbutanoic acid (157 μL, 1.24 mmol), HATU (475 mg, 1.24 mmol), and DIPEA (207 μL, 1.24 mmol) were added to a solution of compound **14** (398 mg, 0.83 mmol) in dry CH_2Cl_2 (19.5 mL). The reaction mixture was stirred at rt under argon for 18 h. The reaction mixture was then diluted with CH_2Cl_2 (20 mL). The organic layer was washed with a saturated $NaHCO_3$ aqueous

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solution of (2 ×10 mL) water (10 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by flash column chromatography (silica gel, toluene:EtOAc 1:3) to give compound **15** (395 mg; 0.684 mmol; 79.5% yield over two steps) as a colorless oil.

R*f* = 0.7 (toluene:EtOAc 1:3); ^{1}H NMR (150 MHz, DMSO-d_6): δ 7.98–7.54 (m, 5H, Ph), 5.17 (dd, 1H, $J_{3,4}$ = 10 Hz, H-3), 4.60 (s, 1H, -$(CH_3)_2$-*OH*), 4.46 (d, 1H, J = 7.8 Hz, H-1), 3.83 (1H, CH_{2b}-O (TD)), 3.83 (1H, H-4), 3.59 (1H, H-5), 3.58 (3H, CH_3-O), 3.53 (1H, CH_{2a}-O (TD)), 3.21 (dd, 1H, J = 7.7 Hz, $J_{2,3}$ = 17 Hz, H-2), 2.12 (NHCOCH_2-), 1.58–1.36 (24H, CH_2 (TD)), 1.14 (d, 3H, J = 6.3 Hz, H-6), 0.95 (s, 6H, -$(CH_3)_2$-OH), 0.88 (3H, CH_3 (TD)); ^{13}C NMR (600.13 MHz, DMSO-d_6): δ 171.0 (NH-*CO*-CH_2), 165.7 (OCO-Ph), 133.3–128.6 (Ph), 102.1 (C-1), 81.5 (C-2), 74.5 (C-3), 69.8 (C-5), 68.7 (CH_2-O (TD)), 68.3 (-CH_2-*C*-$(CH_3)_2$-), 59.6 (CH_3-O), 54.0 (C-4), 48.53 (NHCOCH_2-), 31.2–22.0 (CH_2 (TD)), 28.8 (-$(CH_3)_2$-OH), 17.6 (C-6), 13.9 (CH_3 (TD)); MS-ESI (*m/z*) Calcd. for $C_{33}H_{55}NO_7Na$ ($M+Na^+$) 600.4, Found 600.4; HRMS Calcd. for $C_{33}H_{55}NO_7Na$ ($M+Na^+$) 600.3877, Found 600.3881.

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Tetradecyl 4,6-dideoxy-4-(3-hydroxy-3-methylbutanamido)-2-O-methyl-β-D-glucopyranoside (16)

A solution of NaOMe (2.8 mL, 1 M in MeOH) was added dropwise at 0°C to a solution of **15** (395 mg; 0.684 mmol) in MeOH (38 mL). The reaction mixture was stirred at rt for 2 h. An acidic resin (Amberlite IR 120 H^+) was added to neutralize NaOMe. The resin was filtrated and the solvent was removed. The residue was then purified by flash column chromatography (silica gel, EtOAc:AcOH:water 70:2:2) to give **16** (217 mg; 0.458; 67%) as a white powder.

mp = 59–63°C; $[\alpha]_D$ = −22° (c 1, MeOH); R*f* = 0.5 (EtOAc:AcOH:water 70:2:2); ^{1}H NMR (150 MHz, DMSO-d_6): δ 7.69 (*NH*COCH$_2$-), 4.95 (-$CH_3)_2$-*OH*) 4.82 (s, 1H, -$(CH_3)_2$-*OH*), 4.17 (d, 1H, J = 7.7 Hz, H-1), 3.73 (1H, CH_{2b}-O (TD)), 3.44 (3H, CH_3-O), 3.42 (1H, CH_{2a}-O (TD)), 3.37 (d, 1H, J = 0.63 Hz, H-4), 3.27 (1H, H-3), 3.26 (1H, H-5), 2.74 (dd, 1H, $J_{1,2}$ = $J_{2,3}$ = 8.6 Hz, H-2), 2.21 (s, 2H, NHCOCH_2-), 1.52–1.22 (24H, CH_2 (TD)), 1.16 (d, 6H, J = 7.1 Hz, -$(CH_3)_2$-OH), 1.06 (d, 3H, J = 6.2 Hz, H-6), 0.86 (3H, CH_3 (TD)); ^{13}C NMR (600.13 MHz, DMSO-d_6): δ 171.4 (NH-*CO*-CH_2), 102.5 (C-1), 84.1 (C-2), 72. (C-3), 70.3 (C-5), 68.6 (-CH_2-*C*-$(CH_3)_2$-), 68.5 (CH_2-O (TD)), 59.8 (CH_3-O), 56.5 (C-4), 48.6 (NHCOCH_2-) 31.2–22.0 (CH_2 (TD)), 29.5, 29.4 (-$(CH_3)_2$-OH), 17.9 (C-6), 13.9 (CH_3 (TD)); MS-ESI (*m/z*) Calcd. for $C_{26}H_{51}NO_6$ ($M+H^+$) 474.4, Found 474.4; HRMS Calcd. for $C_{26}H_{51}NO_6Na$ ($M+Na^+$) 496.3608, Found 496.3600.

Synthesis of glucose lipidic derivative

Tetradecyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (17) and tetradecyl 3,4,6-tri-O-acetyl-β-D-glucopyranoside (18)

Tetradecanol (428 mg; 2 mmol) was added to a solution of penta-*O*-acetyl-β-D-glucopyranonoside (195 mg; 0.5 mmol) in dry CH_2Cl_2 (4 mL). Reaction mixture

was cooled to 0°C, and $BF_3 \cdot Et_2O$ (0.1 mL; 0.75 mmol) was added. The reaction mixture was stirred for 1 h at 0°C, and overnight at rt under nitrogen atmosphere. Additional amount of $BF_3 \cdot Et_2O$ (50 μL) was added, and the reaction mixture was stirred at rt over an additional overnight. The reaction was quenched with the addition of a saturated $NaHCO_3$ aqueous solution. The organic layer was extracted with brine, dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography (silica gel, toluene:hexane:EtOAc 10:10:1, and toluene:EtOAc 2:1) to give compound **17** (58.5 mg; 0.11 mmol; 22%) and compound **18** (72 mg; 1.43 mmol; 29%) as a colorless oil.

R*f* (**17**) = 0.56 (toluene:EtOAc 2:1); MS-ESI (*m/z*) Calcd. for $C_{28}H_{48}O_{10}Na$ (M+Na$^+$) 567.3, Found 567.3. R*f* (**18**) = 0.44 (toluene:EtOAc 2:1); MS-ESI (*m/z*) Calcd. for $C_{26}H_{46}NO_9Na$ (M+Na$^+$) 525.3, Found 525.3.

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Tetradecyl β-D-glucopyranoside (19)

Compound **17** (58.5 mg; 0.11 mmol) was dissolved in dry MeOH (2.7 mL) cooled to 0°C, and 0.1 M NaOMe/MeOH (75 μL) was added. Reaction mixture was stirred at rt 3 h. Amberlite IR-120 (H^+) was added to neutralize the reaction and stirred for an additional 30 min. The ion-exchange resin was removed by filtration and the solvent was evaporated. The residue was purified by flash column chromatography (silica gel, EtOAc:AcOH:H_2O 70:2:2) to give compound **19** (37 mg; 0.1 mmol; 92%) as a white powder. The same procedure was repeated for compound **18** (72 mg; 0.14 mmol) to obtain **19** (50.5 mg; 0.13; 94%)[36]. Overall yield was 47%.

R*f* (α) = 0.43, R*f* (β) = 0.45 (EtOAc:AcOH:H_2O 70:2:2); $[\alpha]_D$ = +28° (c 1, MeOH); mp = 118–120°C; ^1H NMR (150 MHz, $CDCl_3$): δ 4.29 (d, 1H, J = 7.5 Hz, H-1), 3.87 (2H, H-6), 3.84 (1H, CH_{2b}-O (TD)), 3.62 (1H, H-4), 3.55 (1H, H-3), 3.52 (1H, CH_{2a}-O (TD)), 3.38 (t, 1H, $J_{2,3}$ = 7.9 Hz, H-2), 3.29 (d, 1H, J = 9.1 Hz, H-5), 1.62–1.26 (24H, CH_2 (TD)), 0.88 (3H, CH_3 (TD)); ^{13}C NMR (600.13 MHz, $CDCl_3$): δ 102.8 (C-1), 76.3 (C-3), 75.5 (C-5), 73.4 (C-2), 70.5 (CH_2-O (TD)), 69.3 (C-4), 61.4 (C-6), 31.9–22.7 (CH_2 (TD)), 14.1 (CH_3 (TD)); MS-ESI (*m/z*) Calcd. for $C_{20}H_{40}O_6Na$ (M+Na$^+$) 399.3, Found 399.5; HRMS Calcd. for $C_{20}H_{40}O_6Na$ (M+Na$^+$) 399.2717, Found 399.2707.

Phenol-sulfuric acid assay

The colorimetric phenol-sulfuric acid assay was performed in a 96-well UV microplate as described previously with slight modifications.[13,16–18] In brief, solutions of sugars **16** and **19** at different concentrations (0.625, 0.125, 2.5, 5, and 10 mM) were prepared in MeOH:water (2:1) mixture, and 50 μL aliquots were added into each well of 96-well microplate. Plates were left overnight in a fume-hood at rt to allow the solvent to completely evaporate. Dry wells containing sugars were washed with 10 mM PBS (100 μL, 0 ×, 3 ×, 5 ×), followed by the addition of 30 μL of 5% phenol in water and 150 μL of concentrated sulfuric acid. The plates were left for

30 min at rt. A mixture of 30 µL of 5% phenol and 150 µL of concentrated sulfuric acid was used as a reference. A_{490} was measured using a microplate reader. All experiments were performed in triplicates, and the results were presented as a mean ± standard deviation.

Lectin-binding assay

The 96-well microplates with the immobilized monosaccharides were prepared as described above. The wells with and without monossacharides (to assess nonspecific binding) were treated with 3% BSA in PBS (100 μL) blocking solution overnight at rt. The blocking solution was then removed and fluorescein-labeled ConA or AAL (100 μL, 10 μg/mL) were incubated in the wells for 1 h in the dark at rt. Wells were then washed with PBS buffer (3 × 100 μL) and fluorescence intensities were measured ($\lambda_{ex} = 485$ nm and $\lambda_{em} = 535$ nm). All experiments were performed in triplicate, and the results were presented as a mean ± standard deviation.

Acknowledgments

The authors thank Ms. Karen Gottwald for editing the text.

Funding

We gratefully acknowledge the financial support of the NATO Public Diplomacy Division, Science for Peace and Security Programme (SfP 983154), to P. Cudic and A. Jakas, and Ministry of Science, Education and Sport of the Republic of Croatia (098-0982933-2936) and NIH grant NCI R21 CA178754 to M. Cudic.

References

1. Daubenspeck, J.M.; Zeng, H.; Chen, P.; Dong, S.; Steichen, C.T.; Krishna, N.R.; Pritchard, D.G.; Turnbough, C.L., Jr. Novel oligosaccharide side chains of the collagen-like region of BclA, the major glycoprotein of the *Bacillus anthracis* exosporium. *J. Biol. Chem.* **2004**, 279, 30945–30953.
2. Tamborrini, M.; Werz, D.B.; Frey, J.; Pluschke, G.; Seeberger, P.H. Anti-carbohydrate antibodies for the detection of anthrax spores. *Angew. Chem. Int. Ed.* **2006**, 45, 6581–6582.
3. Tamborrini, M.; Oberli, M.A.; Werz, D.B.; Schürch, N.; Frey, J.; Seeberger, P.H.; Pluschke, G. Immuno-detection of anthrose containing tetrasaccharide in the exosporium of *Bacillus anthracis* and *Bacillus cereus* strains. *J. Appl. Microbiol.* **2009**, 106, 1618–1628.
4. Werz, D.B.; Seeberger, P.H. Total synthesis of antigen *Bacillus anthracis* tetrasaccharide–creation of an anthrax vaccine candidate. *Angew. Chem. Int. Ed.* **2005**, 44, 6315–6318.
5. Saksena, R.; Adamo, R.; Kováč, P. Studies toward a conjugate vaccine for anthrax. Synthesis and characterization of anthrose [4,6-dideoxy-4-(3-hydroxy-3-methylbutanamido)-2-O-methyl-D-glucopyranose] and its methyl glycosides. *Carbohydr. Res.* **2005**, 340, 1591–1600.
6. Mehta, A.S.; Saile, E.; Zhong, W.; Buskas, T.; Carlson, R.; Kannenberg, E.; Reed, Y.; Quinn, C.P.; Boons, G.-J. Synthesis and antigenic analysis of the BclA glycoprotein oligosaccharide from the *Bacillus anthracis* exosporium. *Chem. Eur. J.* **2006**, 12, 9136–9149.

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7. Adamo, R.; Saksena, R.; Kováč, P. Synthesis of the beta anomer of the spacer-equipped tetrasaccharide side chain of the major glycoprotein of the *Bacillus anthracis* exosporium. *Carbohydr. Res.* **2005**, 340, 2579–2582.
8. Guo, H.; O'Doherty, G.A. De novo asymmetric synthesis of anthrax tetrasaccharide and related tetrasaccharide. *J. Org. Chem.* **2008**, 73, 5211–5220.
9. Bryan, M.C.; Plettenburg, O.; Sears, P.; Rabuka, D.; Wacowich-Sgarbi, S.; Wong C.-H. Saccharide display on microtiter plates. *Chem. Biol.* **2002**, 9, 713–720.
10. Saha, S.K.; Brewer, C.F. Determination of the concentrations of oligosaccharides, complex type carbohydrates, and glycoproteins using the phenol-sulfuric acid method. *Carbohydr. Res.* **1994**, 254, 157–167.
11. Wang, D.; Carroll, G.T.; Turro, N.J.; Koberstein, J.T.; Kováč, P.; Saksena, R.; Adamo, R.; Herzenberg, L.A.; Herzenberg, L.A.; Steinman, L. Photogenerated glycan arrays identify immunogenic sugar moieties of *Bacillus anthracis* exosporium. *Proteomics* **2007**, 7, 180–184.
12. Parthasarathy, N.; Saksena, R.; Kováč, P.; DeShazer, D.; Peacock, S.J.; Wuthiekanun,V.; Heine, H.S.; Friedlander, A.M.; Cote, C.K.; Welkos, S.L.; Adamovicz, J.J.; Bavari, S.; Waag, D.M. Application of carbohydrate microarray technology for the detection of Burkholderia pseudomallei, *Bacillus anthracis* and *Francisella tularensis* antibodies. *Carbohydr. Res.* **2008**, 343, 2783–2788.
13. Crich, D.; Vinogradova, O. Synthesis and glycosylation of a series of 6-mono-, di-, and trifluoro S-phenyl 2,3,4-tri-O-benzyl-thiorhamnopyranosides. Effect of the fluorine substituents on glycosylation stereoselectivity. *J. Org. Chem.* **2007**, 72, 6513–6520.
14. Dhénin, S.G.Y.; Moreau, V.; Morel, N.; Nevers, M.-C.; Volland, H.; Créminon, C.; Djedaïni-Pilard, F. Synthesis of an anthrose derivative and production of polyclonal antibodies for the detection of anthrax spores. *Carbohydr. Res.* **2008**, 343, 2101–2110.
15. Hou, S.; Kovač, P. A convenient synthesis of furanose-free D-fucose per-O-acetates and a precursor for anthrose. *Eur. J. Org. Chem.* **2008**, 1947–1952.
16. DuBois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, 28, 350–356.
17. Masuko, T.; Minami, A.; Iwasaki, N.; Majima, T.; Nishimura, S.-I.; Lee, Y.C. Carbohydrate analysis by a phenol–sulfuric acid method in microplate format. *Anal. Biochem.* **2005**, 339, 69–72.
18. Fazio, F.; Bryan, C.M.; Blixt, O.; Paulson, C.J.; Wong, C.-H. Synthesis of sugar arrays in microtiter plate. *J. Am. Chem. Soc.* **2002**, 124, 14397–14402.
19. Liu, M.-Z.; Fan, H.-N.; Guo, Z.-W.; Hui, Y.-Z. One-step glycosylation and selective deprotection of peracetylated monosaccharides for facile syntheses of allyl glycosides with a free C-2 hydroxyl group. *Carbohydr. Res.* **1996**, 290, 233–237.
20. Plettenburg, O.; Bodmer-Narkevitch, V.; Wong, C.-H. Synthesis of alpha-galactosyl ceramide, a potent immunostimulatory agent. *J. Org. Chem.* **2002**, 67, 4559–4564.
21. Zhang, Z.; Ollmann, I.R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. Programmable one-pot oligosaccharide synthesis. *J. Am. Chem. Soc.* **1999**, 121, 734–753.
22. Garegg, P.J.; Samuelsson, B. Novel reagent system for converting a hydroxy-group into an iodo-group in carbohydrates with inversion of configuration. *Part 2. J. Chem. Soc. Perkin Trans.* **1980**, 1, 2866–2869.
23. Bundle, D.R.; Gerken, M.; Peters, T. Synthesis of antigenic determinants of the Brucella A antigen, utilizing methyl 4-azido-4,6-dideoxy-α-d-mannopyranoside efficiently derived from d-mannose. *Carbohydr. Res.* **1988**, 174, 239–251.
24. Rokhum, L.; Vanlalveni, C.; Lalfakzuala, R. A simple, mild and selective iodination of alcohols. *IJETCAS* **2013**, 4, 370–376.
25. Alvarez-Manzaneda, E.J.; Chahboun, R.; Cabrera Torres, E.; Alvarez, E.; Alvarez-Manzaneda, R.; Haidour, A.; Ramos Lopez, J.M. Reaction of allylic and benzylic alcohols and esters

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with PPh3/I2: one-pot synthesis of β,γ-unsaturated compounds. *Tetrahedron Lett*. **2005**, 46, 3755–3759.
26. David, S.; Fernandez-Mayoralas, A. Nouvelle voie d'accès à la configuration β-d-mannopyranoside protégée temporairement en positions 3 et 6. *Carbohydr. Res.* **1987**, 165, C11–C13.
27. Yeom, C.-E.; Lee, S.Y.; Kim, Y.J.; Kim, B.M. Mild and chemoselective deacetylation method using a catalytic amount of acetyl chloride in methanol. *SYNLETT* **2005**, 10, 1527–1530.
28. Breen, E.P.; Gouin, S.G.; Murphy, A.F.; Haines, L.R.; Jackson, A.M.; Pearson, T.W.; Murphy, P.V.; Porter, R.K. On the mechanism of mitochondrial uncoupling protein 1 function. *J. Biol. Chem.* **2006**, 281, 2114–2119.
29. Rao, P.; Pattabiraman, T.N. Further studies on the mechanism of phenol-sulfuric acid reaction with furaldehyde derivatives. *Anal. Biochem.* **1990**, 189, 178–181.
30. Mandal, D.K.; Bhattacharyya, L.; Koenig, S.H.; Brown III, R.D.; Oscarson, S.; Brewer, C.F. Studies of the binding specificity of concanavalin A. Nature of the extended binding site for asparagine-linked carbohydrates. *Biochemistry* **1994**, 33, 1157–1162.
31. Baenziger, J.U.; Fiete, D. Structural determinants of concanavalin A specificity for oligosaccharides. *J. Biol. Chem.* **1979**, 254, 2400–2407.
32. Fujihashi, M.; Peapus, D.H.; Kamiya, N.; Nagata, Y.; Miki, K. Crystal structure of fucose-specific lectin from *Aleuria aurantia* binding ligands at three of its five sugar recognition sites. *Biochemistry* **2003**, 42, 11093–11099.
33. Kochibe, N.; Furukawa, K. Purification and properties of a novel fucose-specific hemagglutinin of *Aleuria aurantia*. *Biochemistry* **1980**, 19, 2841–2846.
34. Yamashita, K.; Kochibe, N.; Ohkura, T.; Ueda, I.; Kobata, A. Fractionation of l-fucose-containing oligosaccharides on immobilized Aleuria aurantia lectin. *J. Biol. Chem.* **1985**, 260, 4688–4693.
35. Fukumori, F.; Takeuchi, N.; Hagiwara, T.; Ohbayashi, H.; Endo, T.; Kochibe, N.; Nagata, Y.; Kobata, A. Primary structure of a fucose-specific lectin obtained from a mushroom, *Aleuria aurantia*. *J. Biochem.* **1990**, 107, 190–196.
36. Tickle, D.; George, A.; Jennings, K.; Camilleri, P.; Kirby, A.J. A study of the structure and chiral selectivity of micelles of two isomeric D-glucopyranoside-based surfactants. *J. Chem. Soc. Perkin Trans.* **1998**, 2, 467–474.

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