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Synthesis and biotinylation of oligosaccharide fragments of mannosylated and 5-deoxy-5-methylthio-xylofuranosylated lipoarabinomannan from *Mycobacterium tuberculosis*

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ABSTRACT

The attachment of biotin to a molecule provides a powerful tool in biology. Here, we report an efficient synthesis and biotinylation of mannosylated and 5-deoxy-5-methylthio-xylofuranosylated Lipoarabinomannan from *Mycobacterium tuberculosis*. Preparation of the oligosaccharides involved the sequential addition of thioglycoside donors with arabinofuranosyl-containing acceptors. Methylthio group was introduced near the end of the synthesis.

1. Introduction

Tuberculosis (TB) continues to be world's deadliest communicable disease, with an estimated 9 million new cases and 1.5 million deaths in 2013.[1] The hallmark of *anti*-mycobacterial treatment is the use of multiple antibiotics over several months,[2] which has led to development of Multi-Drug-Resistant Tuberculosis (MDR-TB) and Extremely-Drug-Resistant Tuberculosis (XDR-TB).[1] The reason behind difficulty in treatment of mycobacterial infections is the unusual structure of its cell wall, which provides a daunting barrier for the passage of antibiotics.[3,4]

The cell wall of mycobacteria is composed of a complex mixture of polysaccharides, proteins, and lipids. Arabinogalactan (AG) and lipoarabinomannan (LAM) are the major structural polysaccharide components of the cell wall.[5] The polysaccharide component of LAM is composed of mannopyranosyl and arabinofuranosyl residues, linked via inositol moiety to the lipid portion of mycobacterial cell wall. LAM has shown the ability to inhibit protein kinases[6] and macrophage activation along with the ability to neutralize cytotoxic oxygen free radicals.[7] In some species the peripheral portion of LAM consisting of arabinofuranosyl residue is capped with short mannopyranosyl oligosaccharides, together being termed Mannosylated Lipoarabinomannan (ManLAM). It has been noted that adherence of ManLAM to human cells through its interactions with mannose binding proteins plays an important role in initial stages of infection.[8] Drugs inhibiting the enzymes that assemble these oligosaccharides can be useful in the prevention and treatment of mycobacterial disease, design of which would be greatly aided by the isolation and characterization of respective mannosyltransferases. In addition to this, there are reports involving binding of mycobacterium specific antibodies to oligosaccharide fragments of LAM, making them potential haptens for *anti*-tuberculosis vaccines.[9]

The detailed structural features of LAM demonstrated in 1990's[10,11] were later modified by the discovery of a novel natural thiosugar i.e. 5-deoxy-5-methylthio-pentose, being attached to LAM (Fig. 1).[12] The 5-deoxy-5-methylthio-xylofuranose (MTX) sugar has been identified in both, laboratory strains (H37Rv and H37Ra)[12] as well as clinical isolates (CSU20 and MT103)[12,13] of *Mycobacterium tuberculosis*. Studies on MTX have indicated its ability to inhibit cytokine response similar to ManLAM,[14] and their immuno-modulatory potential has been demonstrated by their ability to inhibit cytokine response induced by *Staphylococcus*

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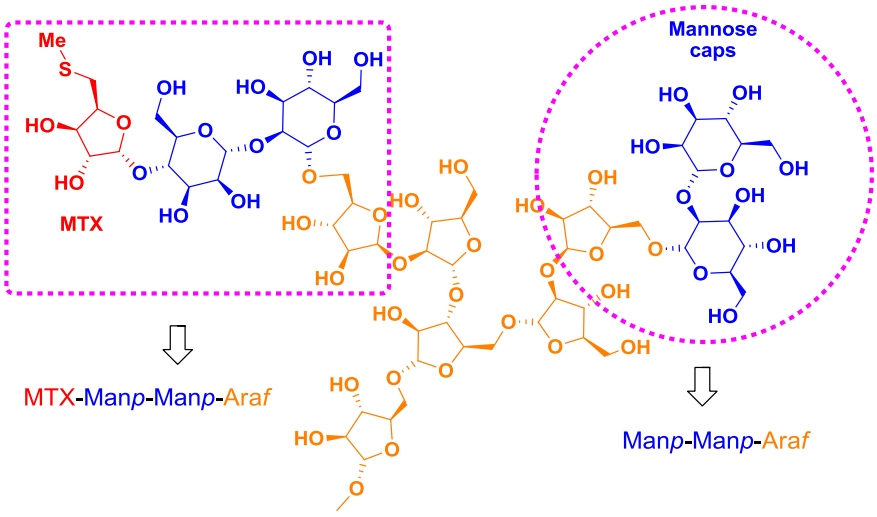


Fig. 1. Representative fragment structure for lipoarabinomannan (LAM) based on the typical carbohydrate composition of Mtb.

aureus Cowan strain (SAC) and Interferon- γ (IFN-γ) in human mono-cytic cell line (THP-1).[15]

MTX, like other furanose sugars, would be inherently less stable than the pyranose form. Mycobacteria host a number of furanosidic oligosaccharides, which are biosynthesized from UDP-galactopyranose with the help of specific mutase enzymes.[16,17] But in case of MTX, due to the presence of a thioether substituent at C-5, the pyranose ring form is not possible and thus mycobacterium would be investing a lot of effort in the biosynthesis of MTX. Thus the study of the biosynthetic pathway for MTX would be helpful in developing newer antibiotics and certain MTX specific antibodies, which would ease the process of quantitation of MTX substituents. The progress in this direction has been rather slow mainly due to the lack of synthetic substrates useful for fundamental biochemical studies leading to clarification of these biosynthetic pathways.

Venisse et al. have reported the utilization of biotinylated ManLAM for studying its antigenic properties and its ability to interact selectively with murine phagocytes.[18] Biotinylation involving a disulphide bond is useful for labelling and purifying proteins and carbohydrates on the cell surface, as it attaches the desired molecule on the surface, without permeating the cell membrane. To add to this, binding of biotinylated nucleotides to avidin-agarose affinity column, followed by subsequent elution by reduction of the disulphide bond using reducing agents such as Dithiothreitol (DTT),[19] provided us with further rationale for biotinylation of synthesized oligosaccharides.

With the aim of recognising antibodies generated against LAM, and to determine their specificity towards ManLAM, Gadikota et al.[20] had reported the synthesis of some oligosaccharide fragments of ManLAM, covalently conjugated to 8-aminooctyl aglycone, having the ability to form neoglycoconjugates. Adding to this; with the additional motive of providing synthetic substrates for biochemical studies, herein we describe an accessible synthesis of compounds **1** & **2** along with their biotinylated analogs **3** & **4** containing a cleavable disulfide bond in the 12-atom linker connecting them (Fig. 2).

2. Result and discussion

2.1. Synthesis of monosaccharide *5*

The target molecules **1** & **2** were straightforwardly synthesized using a combination of sequential glycosylation with arabinofuranosyl-containing acceptors. The arabinofuranosyl-containing acceptor **5** was synthesized employing the process reported by Fletcher et al.[21], which involves chlorination of **10**[22] at the anomeric position, by bubbling hydrochloride gas through its solution in dry CH_2Cl_2 followed by simple concentration to give the labile chloride **11**.[22] This was reacted without purification with benzyl N-(3-hydroxypropyl)carbamate in dry CH_2Cl_2 in the absence of a promoter to give an intermediate β-glycoside product, contaminated with minor quantity (~5%) of the α isomer. This crude product was deacetylated under Zemplén conditions[23] and then purified by column chromatography to give desired compound **5** in 42% overall yield (Scheme 1).

2.2. Synthesis of trisaccharide *1*

With all the required building blocks in hand, arabinofuranosyl derivative **5** was glycosylated with thioglycoside derivative **6**[24] in the presence of N-iodosuccinimide and silver triflate[25] to provide the protected disaccharide derivative **12** in 82% yield with trace amounts of β isomer. The disaccharide **12** was then deacetylated under Zemplén conditions[23] to furnish the disaccharide alcohol **13** in 93% yield. The trisaccharide **15** was synthesized using a similar procedure involving initial glycosylation of disaccharide **13** with the thioglycoside donor **7**[26] under the same reaction conditions, to furnish the trisaccharide **14** in 86% yield.

Trisaccharide **14** was deacetylated using sodium methoxide to furnish the trisaccharide **15** in 90% yield. A portion of this product was completely deprotected by hydrogenation[27] providing **1** in 82% yield (Scheme 2).

2.3. Synthesis of tetrasaccharide *2*

To synthesize the tetrasaccharide **2** containing the D-enantiomer of MTX, an approach in which the methylthio substituent would be introduced at the final step was developed. The process required the synthesis of tetrasaccharide **2** having a leaving group at 5-position of the xylofuranosyl moiety. The penultimate tetrasaccharide **16** was synthesized by glycosylation between remaining trisaccharide **15** and monosaccharide **8**[15] using N-iodosuccinimide and silver triflate. A minor quantity of 1,2-*trans* isomer product was also formed under these reaction conditions. The crude product was purified by column chromatography to give **16** in 77% yield and the stereochemistry of compound **16** was confirmed from its spectroscopic analysis [signals at δ 5.24 (d, J=4.2 Hz, H-1_D), 5.18 (br

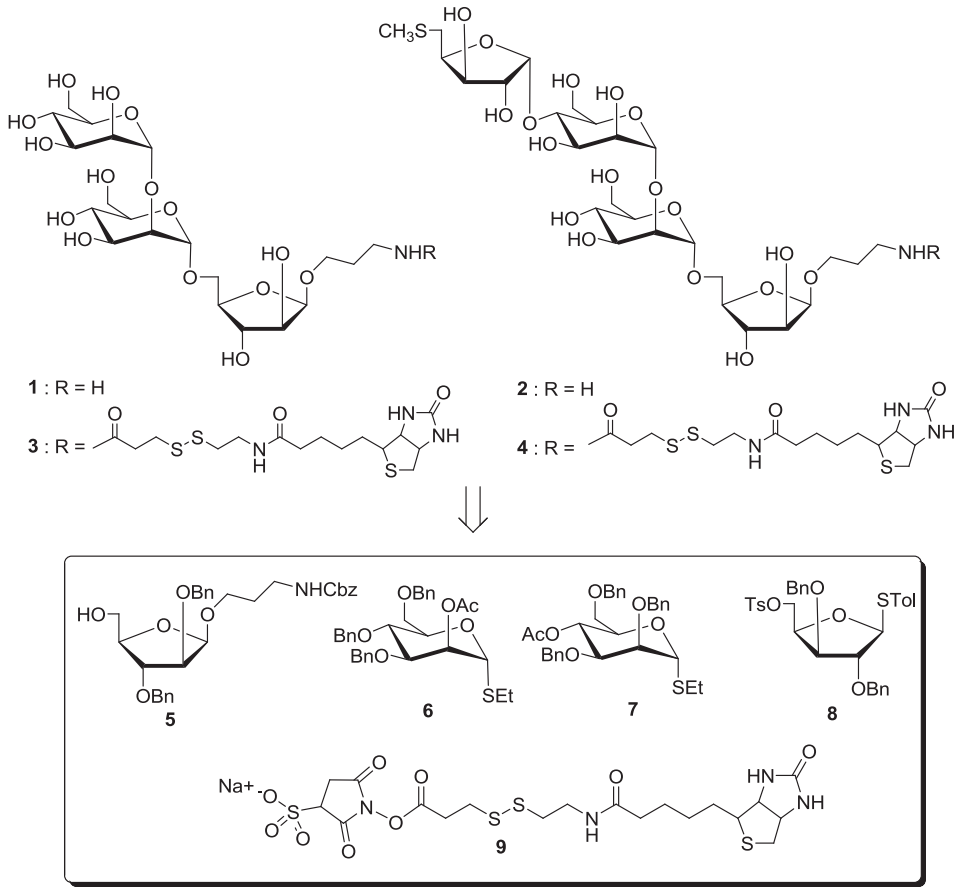


Fig. 2. Synthetic targets and their precursors.

s, H-1_C), 4.91 (br s, H-1_B), 4.79 (d, J=3.8 Hz, H-1_A) in the ^{1}H NMR and δ 100.8 (C-1_D), 100.7 (C-1_A), 99.4 (C-1_B), 99.2 (C-1_C) in the ^{13}C NMR spectra] (Scheme 2). Finally the methylthio group was introduced on the xylofuranose residue by refluxing tetrasaccharide **16** together with sodium thiomethoxide and 18-crown-6 in acetonitrile.[15] The desired benzylated tetrasaccharide was purified by passing through a short pad of SiO_2. With the required methylthio group in place, the benzyl ethers and benzyloxycarbonyl (CBz) groups were cleaved by Birch reduction employing sodium and ammonia at −78 °C in THF.[28] The final tetrasaccharide **2** was isolated after purification over Sephadex LH-20 column using CH_3OH–H_2O (4:1 v/v) as eluent in 72% overall yield (Scheme 2).

2.4. Biotinylation of compound *1* and *2*

Finally, compounds **1** & **2** were coupled with sulfosuccinimidyl 2-(biotinamido) ethyl-l,3-dithiopropionate (**9**) in methanol and the resulting solution was allowed to stir at room temperature for 8 h (Scheme 3). The resulting biotin labelled glycoconjugates **3** & **4** were isolated in 82% and 72% respectively.

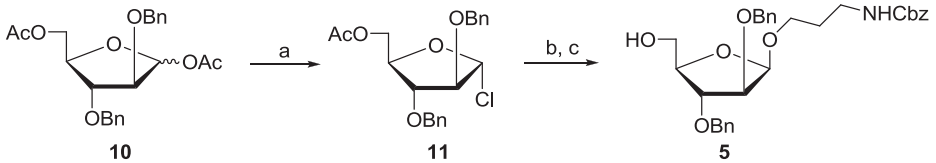
3. Conclusion

In summary, we carried out the synthesis of mannosyl-arabinoside and MTX containing mannosyl-arabinoside fragments of LAM from *M. tuberculosis* and their biotinylated analogs **3** & **4**, containing a disulfide bond in a 12-atom linker joining biotin. These biotinylated oligosaccharides can be used as probes to investigate the role of MTX and ManLAM in mycobacterial pathogenicity and will find application in studies involving development of vaccines against *M. tuberculosis*. A readily prepared *p*-tolyl 2,3-di-O-benzyl-5-O-toluenesulfonyl-1-thio-β-D-xylofuranoside (**8**) was used as final glycosyl donor having a leaving group at the 5-position. The methylthio group was introduced on xylofuranose at 5-position in the last step.

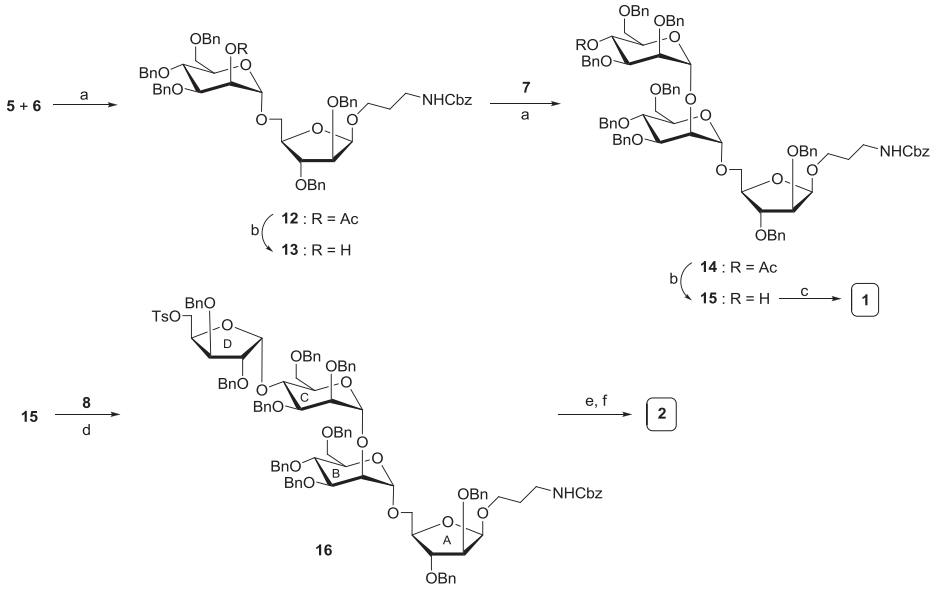
4. Experimental

4.1. General methods

All reactions were monitored by thin layer chromatography over silica gel-coated TLC plates. The spots on TLC were visualized by



Scheme 1. Reagents and conditions: (a) HCl, CH_2Cl_2, room temperature; (b) benzyl N-(3-hydroxypropyl)carbamate, CH_2Cl_2, room temperature; (c) 0.1 M CH_3ONa, CH_3OH, room temperature, 42% overall yield.

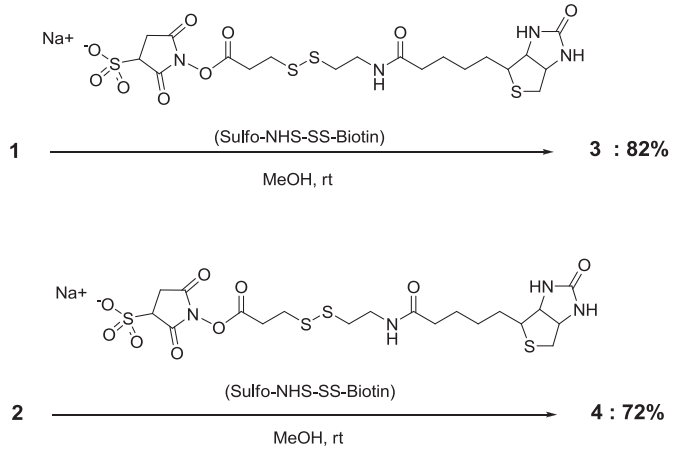


Scheme 2. Reagents and conditions: (a) N-iodosuccinimide (NIS), AgOTf, MS-4Å, CH_2Cl_2, −40 °C→0 °C, 82% for compound **12** and 86% for compound **14**; (b) 0.1 M CH_3ONa, CH_3OH, room temperature, 93% for compound **13** and 90% for compound **15**; (c) H_2, 20% $Pd(OH)_2$–C, CH_3OH, room temperature, 82%; (d) NIS, AgOTf, MS-4Å, CH_2Cl_2, 0 °C→room temperature, 77%; (e) $NaSCH_3$, 18-crown-6, CH_3CN, reflux; (f) Na, NH_3, THF, −78 °C, 72% overall yield.

warming ceric sulfate [2% $Ce(SO_4)_2$ in 5% H_2SO_4 in EtOH]-sprayed plates on a hot plate. Silica gel 230–400 mesh was used for column chromatography. ^1H and ^{13}C NMR, spectra were recorded on Brucker Avance 500 MHz and Bruker Avance 300 MHz instrument spectrometers using $CDCl_3$ and D_2O as solvents and TMS as internal reference unless stated otherwise. Chemical shift values are expressed in δ parts per million. ESI-MS were recorded on a JEOL spectrometer. Elementary analysis was carried out on Carlo ERBA analyzer. IR spectra were recorded on Shimadzu Spectrophotometers. Optical rotations were determined on Autopol III polarimeter. Commercially available grades of organic solvents of adequate purity are used in all reactions.

*4.2. 3-(Benzyloxycarbonylamino)propyl 2,3-di-O-benzyl-β-D-arabinofuranoside (**5**)*

Compound **10** (8 g, 19.32 mmol) was dissolved in dry dichloromethane (30 ml) and HCI gas was bubbled through the solution for 30 min. The reaction mixture was further stirred for an additional 10 min. After completion of reaction, the reaction mixture was concentrated to yield 5-O-acetyl-2,3-di-O-benzyl-α-D-arabinofuranosyl chloride **11** as a light yellow syrup. The concentrated crude product **11** was dissolved in dry CH_2Cl_2 (50 mL) without any purification and benzyl N-(3-hydroxypropyl)carbamate (6.1 g, 28.98 mmol) was added to it. After 6 h of stirring, the reaction mixture was diluted with CH_2C1_2 (80 mL) and washed with water (30 mL) and then saturated $NaHCO_3$ solution (2×50 mL) and water (50 mL). The organic layers were collected and dried over anhydrous Na_2SO_4, then filtered and concentrated as light yellow syrup. De-acetylation was done by adding few drops of 0.1 M NaOMe to a solution of crude product in methanol (90 mL). The reaction was stirred for 1 h. The reaction mixture was concentrated and the light yellow oil obtained was purified by chromatography (2:1 hexane: EtOAc) to give **5** (4.5 g, 42%) as a colorless oil. $[\alpha]_D^{25}$ −47.5 (*c* 1.5, $CHCl_3$); IR (neat): 2837, 1738, 1303, 1218, 1224, 1088, 1078, 986, 760, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.33–7.23 (m, 15H, Ar–H), 5.55–5.54 (m, 1H, N*H*Cbz), 5.05 (ABq, 2H, $COOCH_2Ph$), 4.80 (d, *J*=4.4 Hz, 1H, H-1_A), 4.64 (d, *J*=11.6 Hz, 1H, $PhCH_2$), 4.59–4.57 (m, 2H, $PhCH_2$), 4.54 (d, *J*=11.6 Hz, $PhCH_2$), 4.19 (dd, *J*=6.2, 6.7 Hz, 1H, H-3_A), 4.05 (dd, *J*=4.5, 7.0 Hz, 1H, H-4_A), 4.03–3.99 (m, 1H, H-2_A), 3.83–3.78 (m, 1H, OCH_2R), 3.67–3.65 (m, 1H, H-5_{aA}), 3.57–3.54 (m, 1H, H-5_{bA}), 3.40–3.38 (m, 1H, OCH_2R), 3.37–3.32 (m, 1H, NCH_2), 3.27–3.23 (m, 1H, NCH_2), 2.64 (dd, *J*=4.8, 7.1 Hz, 1H, H-5OH), 1.77–1.75 (m, 2H, $-CH_2-$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.6 ($COOCH_2Ph$), 138.3–126.1 (Ar–C), 100.8 (C-1_A), 84.0 (C-4_A), 82.0 (C-2_A), 81.2 (C-3_A), 72.5 ($PhCH_2$), 67.1 (OCH_2R), 66.5 ($COOCH_2Ph$), 63.9 (C-5_A), 38.9 (NCH_2), 29.5 ($-CH_2-$); ESI-MS: *m*/*z* 544.2 $[M+Na]^+$; Anal. Calcd for $C_{30}H_{35}NO_7$ (521.24): C, 69.08; H, 6.76%; found: C, 68.95; H, 6.87%.



Scheme 3. Biotinylation of compounds **1** & **2**.

*4.3. 3-(Benzyloxycarbonylamino)propyl 5-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-2,3-di-O-benzyl-β-D-arabinofuranoside (**12**)*

Thioglycoside **6** (4.9 g, 9.21 mmol) and alcohol **5** (4.0 g, 7.68 mmol) were placed in a round-bottom flask to which powdered MS-4Å (4.0 g) were added. The contents were dried in vacuo overnight. To this, dry CH_2C1_2 (80 mL) was added and the

mixture was stirred for 10 min at −40 °C. N-iodosuccinimide (2.49 g, 11.10 mmol) was added at −40 °C and the reaction was stirred for 20 min followed by addition of silver triflate (594 mg, 2.30 mmol). The reaction mixture was gradually warmed to 0 °C and then quenched by addition of triethylamine (0.5 mL). The yellow solution formed was filtered, diluted with CH_2Cl_2 (100 mL) and washed with 5% aq $Na_2S_2O_3$ and water. The organic layers were collected, dried (Na_2SO_4) and evaporated to light yellow syrup. The crude product was purified by column chromatography using hexane-ethylacetate (5:1) as eluent to furnish disaccharide **12** (6.2 g, 82%) as a colourless liquid; $[\alpha]_D^{25}$ +3.7 (*c* 1.5, $CHCl_3$); IR (neat): 2907, 1848, 1473, 1248, 1124, 1107, 1088, 986, 750, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.36–7.27 (m, 28H, Ar–H), 7.17–7.16 (m, 2H, Ar–H), 5.59–5.57 (m, 1H, NHCbz), 5.40 (dd, *J*=1.8, 3.0 Hz, 1H, H-2_B), 5.08 (br s, 2H, $COOCH_2Ph$), 4.87 (br s, 1H, H-1_B), 4.86 (d, *J*=10.8 Hz, 1H, $PhCH_2$), 4.84 (d, *J*=4.4 Hz, 1H, H-1_A), 4.68 (dd, *J*=11.6 Hz, 2H, $PhCH_2$), 4.62 (dd, *J*=11.6 Hz, 2H, $PhCH_2$), 4.57 (dd, *J*=11.6 Hz, 2H, $PhCH_2$), 4.49 (m, 3H, $PhCH_2$), 4.09 (dd, *J*=6.2, 6.7 Hz, 1H, H-3_A), 4.06–4.02 (m, 2H, H-2_A, H-4_A), 3.99 (dd, *J*=3.3, 9.4 Hz, 1H, H-3_B), 3.91 (t, *J*=9.5 Hz, 1H, H-4_B), 3.85–3.73 (m, 4H, H-5_{abA}, H-5_B, OCH_2), 3.69–3.67 (m, 1H, H-6_{aB}), 3.51 (dd, *J*=5.6, 10.1 Hz, 1H, H-6_{bB}), 3.44–3.33 (m, 2H, OCH_2, NCH_2), 3.24–3.17 (m, 1H, NCH_2), 2.16 (s, 3H, $COCH_3$), 1.77–1.75 (m, 2H, $-CH_2-$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6 ($COCH_3$), 156.6 ($COOCH_2Ph$), 138.5–127.8 (Ar–*C*), 101.2 (C-1_A), 98.5 (C-1_B), 84.1 (C-4_A), 83.2 (C-3_A), 80.0 (C-2_A), 78.4 (C-3_B), 75.5 ($PhCH_2$), 74.4 (C-4_B), 73.7 ($PhCH_2$), 72.7 (2C, $PhCH_2$), 72.1 ($PhCH_2$), 72.0 (C-5_B) 70.2 (C-6_B), 69.0 (OCH_2R), 68.9 (C-2_B), 67.2 (C-5_A), 66.7 ($COOCH_2Ph$), 39.6 (NCH_2), 29.6 ($-CH_2-$), 21.4 ($COCH_3$); ESI-MS: *m*/*z* 1018.4 $[M+Na]^+$; Anal. Calcd for $C_{59}H_{65}NO_{13}$ (995.44): C, 71.14; H, 6.58%; found: C, 71.03; H, 6.67%.

4.4. 3-(Benzyloxycarbonylamino)propyl 5-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-2,3-di-O-benzyl-β-D-arabinofuranoside (**13**)

To a solution of compound **12** (5.5 g, 5.52 mmol) in MeOH (80 mL), few drops of 0.1 M NaOMe were added and the reaction was stirred at room temperature for 3 h. The reaction was then neutralised with Amberlite-IR 120 (H^+) resin, filtered and evaporated to give the crude product, which was purified by column chromatography using hexane-EtOAc (2:1) as eluent, to give **13** (4.9 g, 93%); $[\alpha]_D^{25}$ +3.5 (*c* 1.5, $CHCl_3$); IR (neat): 2987, 1848, 1273, 1218, 1140, 1107, 1048, 986, 750, cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.23–7.17 (m, 28H, Ar–H), 7.09–7.06 (m, 2H, Ar–H), 5.49–5.46 (m, 1H, NHCbz), 4.97 (br s, 2H, $COOCH_2Ph$), 4.84 (d, *J*=1.4 Hz, 1H, H-1_B), 4.72 (d, *J*=10.7 Hz, 1H, $PhCH_2$), 4.71 (br s, 1H, H-1_A), 4.56–4.51 (m, 5H, $PhCH_2$), 4.47 (d, *J*=12.5 Hz, 1H, $PhCH_2$), 4.42 (dd, *J*=11.9 Hz, 3H, $PhCH_2$), 4.01 (dd, *J*=6.2, 6.8 Hz, 1H, H-3_A), 3.98–3.90 (m, 3H, H-2_A, H-2_B, H-4_A), 3.77–3.56 (m, 7H, H-3B, H-4B, H-5_{abA}, H-5_B, H-6_{aB}, OCH_2), 3.43 (dd, *J*=5.6, 10.1 Hz, 1H, H-6_{bB}), 3.34–3.22 (m, 2H, NCH_2, OCH_2), 3.13–3.07 (m, 1H, NCH_2), 2.48 (br s, 1H, O*H*), 1.68–1.65 (m, 2H, $-CH_2-$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.7 ($COOCH_2Ph$), 138.4–127.7 (Ar–C), 101.0 (C-1_A), 99.8 (C-1_B), 84.1 (C-3_A), 83.2 (C-4_A), 80.5 (C-2_A), 80.1 (C-3_B), 75.5 ($PhCH_2$), 74.5 (C-4_B), 73.8 ($PhCH_2$), 72.9 ($PhCH_2$), 72.8 ($PhCH_2$), 72.3 ($PhCH_2$), 71.6 (C-5_B), 69.7 (C-6_B), 69.1 (OCH_2R), 68.5 (C-2_B), 67.3 (C-5_A), 66.8 ($COOCH_2Ph$), 39.6 (NCH_2), 29.5 ($-CH_2-$); ESI-MS: *m*/*z* 976.4 $[M+Na]^+$; Anal. Calcd for $C_{57}H_{63}NO_{12}$ (953.43): C, 71.75; H, 6.66%; found: C, 71.64; H, 6.78%.

4.5. 3-(Benzyloxycarbonylamino)propyl 2,3-di-O-benzyl-5-O-[2-O-(4-O-acetyl-2,3,6-tri-O-benzyl-α-D-mannopyranosyl)-3,4,6-tri-O-benzy-α-D-mannopyranosyl]-β-D-arabinofuranoside (**14**)

MS-4Å (3.00 g) were added to a solution of compound **13** (4.5 g, 4.72 mmol) and thioglycoside **7** (3.0 g, 5.66 mmol) in anhydrous CH_2Cl_2 (60 mL). The reaction mixture was stirred for 1 h under N_2 atmosphere at room temperature. The reaction mixture was then cooled to −40 °C and stirred for 10 min. To this, N-iodosuccinimide (1.53 g, 6.79 mmol) was added and the reaction was allowed to stir for 20 min before adding silver triflate (365 mg, 1.42 mmol). The reaction mixture was then allowed to warm gradually to 0 °C followed by quenching using triethylamine (0.5 mL). The yellow solution was filtered and diluted with CH_2C1_2 (90 mL). This diluted solution was washed with saturated $Na_2S_2O_3$ solution (2×30 mL), followed by brine (50 mL) and water (40 mL). The organic layers were collected, dried (Na_2SO_4), filtered and concentrated over vacuum to light yellow syrup. The crude product was purified by column chromatography (4:1 hexane:EtOAc) to give **14** (5.8 g, 86%) as a colourless syrup; $[\alpha]_D^{25}$ +4.5 (*c* 1.5, $CHCl_3$); IR (neat): 2911, 1818, 1303, 1278, 1204, 1137, 1038, 1048, 976, 750, 679 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.24–7.05 (m, 45H, Ar–H), 5.44–5.42 (m, 1H, NHCbz), 5.25 (dd, *J*=9.7, 9.3 Hz, 1H, H-4_C), 5.05 (br s, 1H, H-1_C), 4.96 (br s, 2H, $COOCH_2Ph$), 4.91 (br s, 1H, H-1_B), 4.75 (d, *J*=10.7 Hz, 1H, $PhCH_2$), 4.69 (d, *J*=4.2 Hz, 1H, H-1_A), 4.55 (d, *J*=12.2 Hz, 1H, $PhCH_2$), 4.53–4.37 (m, 12H, $PhCH_2$), 4.35 (d, *J*=12.2 Hz, 1H, $PhCH_2$), 4.27 (d, *J*=12.2 Hz, $PhCH_2$), 3.99–3.97 (m, 1H, H-2_A), 3.95–3.88 (m, 4H, H-2_B, H-2_C, H-3_A, H-4_A), 3.85–3.83 (m, 1H, H-4_B), 3.75–3.54 (m, 8H, H-3_B, H-3_C, H-5_{aA}, H-5_B, H-6_{aB}, H-6_{abC}, OCH_2), 3.49 (dd, *J*=6.3, 6.70 Hz, 1H, H-5_C), 3.41 (dd, *J*=3.3, 11.2 Hz, 1H, H-5_{bA}), 3.35 (dd, *J*=5.6, 10.0 Hz, 1H, H-6_{bB}), 3.29–3.19 (m, 2H, NCH_2, OCH_2), 3.08–3.03 (m, 1H, NCH_2), 1.78 (s, 3H, $COCH_3$), 1.62–1.59 (m, 2H, $-CH_2-$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3 ($COCH_3$), 156.8 ($COOCH_2Ph$), 138.7–127.8 (Ar–C), 101.1 (C-1_A), 100.2 (C-1_C), 99.4 (C-1_B), 84.1 (C-4_A), 83.4 (C-2_A), 80.3 (C-3_A), 80.1 (C-3_B), 77.4 (C-2_B), 75.6 ($PhCH_2$), 75.1 (C-4_B), 75.0 (C-3_C), 74.8 (C-2_C), 73.7 ($PhCH_2$), 73.6 (2C, $PhCH_2$), 72.8 ($PhCH_2$), 72.7 (2C, $PhCH_2$), 72.3 ($PhCH_2$), 72.0 (C-5_C), 71.2 (C-5_B), 70.4 (C-6_C), 70.1 (OCH_2R), 69.4 (C-6_B), 69.2 (C-4_C), 67.3 (C-5_A), 66.8 ($COOCH_2Ph$), 39.8 (NCH_2), 29.7 ($-CH_2-$), 21.3 ($COCH_3$); ESI-MS: *m*/*z* 1450.5 $[M+Na]^+$; Anal. Calcd for $C_{86}H_{93}NO_{18}$ (1427.63): C, 72.30; H, 6.56%; found: C, 72.18; H, 6.67%.

4.6. 3-(Benzyloxycarbonylamino)propyl 2,3-di-O-benzyl-5-O-[2-O-(2,3,6-tri-O-benzyl-α-D-mannopyranosyl)-3,4,6-tri-O-benzy-α-D-mannopyranosyl]-β-D-arabinofuranoside (**15**)

To a solution of **14** (4.2 g, 2.94 mmol) in MeOH (80 mL), few drops of 0.1 M MeONa solution were added. The reaction mixture was stirred for 5 h at room temperature and then concentrated to yield a syrup. The crude product was purified by chromatography (4:1 hexane:EtOAc) to give **15** (3.7 g, 90%) as a syrup; $[\alpha]_D^{25}$ +4.9 (*c* 1.5, $CHCl_3$); IR (neat): 2997, 1858, 1373, 1238, 1204, 1137, 1088, 1048, 976, 760 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.25–7.09 (m, 45H, $PhCH_2$), 5.46–5.44 (m, 1H, NHCbz), 5.10 (br s, 1H, H-1_C), 4.96 (br s, 2H, $COOCH_2Ph$), 4.86 (d, *J*=1.4 Hz, 1H, H-1_B), 4.77 (d, *J*=10.8 Hz, 1H, $PhCH_2$), 4.70 (d, *J*=3.6 Hz, 1H, H-1_A), 4.57 (d, *J*=12.2 Hz, 1H, $PhCH_2$), 4.55–4.42 (m, 11H, $PhCH_2$), 4.37 (d, *J*=12.2 Hz, 1H, $PhCH_2$), 4.34 (d, *J*=12.2 Hz, 1H, $PhCH_2$), 4.27 (d, *J*=12.2 Hz, $PhCH_2$), 4.00–3.92 (m, 5H, H-2_A, H-2_B, H-2_C, H-3_A, H-4_A), 3.86–3.78 (m, 2H, H-4_B, H-4_C), 3.75–3.57 (m, 10H, H-3_B, H-3_C, H-5_{abA}, H-5_B, H-5_C, H-6_{aB}, H-6_{abC}, OCH_2), 3.46 (dd, *J*=5.5, 10.0 Hz, 1H, H-6_{bB}), 3.31–3.21 (m, 2H, NCH_2, OCH_2), 3.10–3.04 (m, 1H, NCH_2), 1.63–1.59 (m, 2H, $-CH_2-$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.8 ($COOCH_2Ph$), 138.8–127.9 (Ar–C), 101.1 (C-1_A), 99.9 (C-1_C), 99.5 (C-1_B), 84.1 (C-4_A), 83.5 (C-2_A), 80.4 (C-3_A), 80.1 (C-3_B), 79.6 (C-2_B),75.5 ($PhCH_2$), 75.1 (C-4_B), 74.8 (C-2_C), 74.2 (C-3_C), 73.8 (2C, $PhCH_2$), 72.9 (3C, $PhCH_2$), 72.8 ($PhCH_2$), 72.4 (C-5_B), 72.1 ($PhCH_2$), 72.0 (C-5_C), 70.6 (C-6_C),70.4 (OCH_2R), 69.5 (C-6_B), 67.9 (C-4_C), 67.2 (C-5_A), 66.8 ($COOCH_2Ph$), 39.8 (NCH_2), 29.7 ($-CH_2-$); ESI-MS: *m*/*z* 1408.5 $[M+Na]^+$; Anal. Calcd for $C_{84}H_{91}NO_{17}$ (1385.62): C, 72.76; H, 6.61%; found: C, 72.62; H, 6.74%.

*4.7. 3-(Benzyloxycarbonylamino)propyl 5-O-[3,4,6-tri-O-benzy-2-O-[2,3,6-tri-O-benzyl-4-O-(2,3-di-O-benzyl-5-O-toluenesulfonyl-α-D-xylofuranosyl)-α-D-mannopyranosyl]-α-D-mannopyranosyl]-2,3-di-O-benzyl-β-D-arabinofuranoside (**16**)*

After drying thioglycoside **8** (1.75 g, 3.03 mmol) and alcohol **15** (3.5 g, 2.52 mmol) for 6 h over vacuum, both were dissolved in CH_2Cl_2 (40 mL), and the resulting solution was cooled to 0 °C. To this solution, powdered 4 Å molecular sieves (2.5 g) were added, and the suspension was stirred for 20 min at 0 °C. N-iodosuccinimide (819 mg, 3.63 mmol) was added, followed by silver triflate (196 mg, 0.76 mmol). The reaction mixture was stirred for 15 min and then neutralized with Et_3N. The reaction mixture was further diluted with CH_2Cl_2 (90 mL) and filtered through Celite® bed. The filtrate was washed successively with saturated aqueous $Na_2S_2O_3$ solution (50 mL) followed by water (60 mL). The organic layers were collected, dried over Na_2SO_4 and concentrated to give a syrup that was purified by column chromatography (4:1 hexane:EtOAc) to afford **16** (2.8 g, 77%), as a syrup; $[\alpha]_D^{25}$ +6.2 (*c* 1.5, $CHCl_3$); IR (neat): 2637, 1708, 1333, 1278, 1214, 1137, 1048, 986, 770, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.59–7.57 (m, 2H, Ar–H), 7.24–7.05 (m, 55H, Ar–H), 6.90–6.88 (m, 2H, Ar–H), 5.24 (d, *J*=4.2 Hz, 1H, H-1_D), 5.18 (br s, 1H, H-1_C), 5.10 (br s, 2H, COOCH_2Ph), 4.91 (br s, 1H, H-1_B), 4.79 (d, *J*=3.8 Hz, 1H, H-1_A), 4.76 (d, *J*=11.5 Hz, 1H, PhCH_2), 4.59–4.47 (m, 8H, PhCH_2), 4.45–4.41 (m, 5H, PhCH_2), 4.40–4.31 (m, 6H, H-2_A, H-2_C, H-4_A, PhCH_2), 4.24–4.09 (m, 6H, H-2_B, H-3_A, H-4_D PhCH_2), 4.04–3.87 (m, 6H, H-2_D, H-3_D, H-4_B, H-4_C, H-6_{abC}), 3.85–3.56 (m, 9H, H-3_B, H-3_C, H-5_{abA}, H-5_B, H-5_C, H-5_{abD}, H-6_{aB}), 3.51–3.47 (m, 2H, H-6_{bB}, OCH_2-), 3.44–3.41 (m, 2H, OCH_2-, NCH_2), 3.27–3.24 (m, 1H, NCH_2), 2.27 (s, 3H, CH_3), 1.86–1.78 (m, 2H, –CH_2–); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.1 (NHCOOCH_2Ph), 144.8–125.0 (Ar–C), 100.8 (C-1_D), 100.7 (C-1_A), 99.4 (C-1_B), 99.2 (C-1_C), 84.5 (C-4_D), 83.7 (C-2_C), 82.3 (C-4_A), 80.9 (C-4_C), 80.6 (C-5_C), 79.9 (C-2_D), 79.7 (C-3_D), 75.4 (PhCH_2), 75.2 (C-3_C), 74.4 (C-2_A), 74.3 (C-3_B), 74.0 (C-3_A), 73.8 (PhCH_2), 73.4 (PhCH_2), 73.1 (PhCH_2), 72.8 (PhCH_2), 72.6 (PhCH_2), 72.4 (2C, C-2_A, C-3_B), 72.3 (PhCH_2), 72.1 (PhCH_2), 71.7 (C-3_A), 70.8 (PhCH_2), 70.5 (PhCH_2), 69.9 (OCH_2-), 69.6(C-6_B), 69.1 (2C, C-6_C, COOCH_2Ph), 68.5 (C-5_D), 65.2 (C-5_A), 51.6 (NCH_2), 29.1 (–CH_2–), 22.1 (CH_3); ESI-MS: *m*/*z* 1874.7 $[M+Na]^+$; Anal. Calcd for $C_{110}H_{117}NO_{23}S$ (1851.77): C, 71.29; H, 6.36%; found: C, 71.18; H, 6.45%.

*4.8. 3-Aminopropyl 5-O-[2-O-(α-D-mannopyranosyl)-α-D-mannopyranosyl]-β-D-arabinofuranoside (**1**)*

To a solution of compound **15** (500 mg, 0.36 mmol) in CH_3OH, 20% $Pd(OH)_2$–C (500 mg) was added. The reaction mixture was then stirred at room temperature for 7 h under positive pressure of hydrogen. The reaction mixture was filtered through Celite® bed and then evaporated to dryness. The crude product was purified over Sephadex-LH-20 column using CH_3OH-H_2O (8:1 v/v) as eluant, to give pure compound **1** (100 mg, 82%); $[\alpha]_D^{25}$ +5.5 (*c* 1.5, H_2O); IR (neat): 3200, 1948, 1393, 1348, 1224, 1088, 1048, 986 cm^{-1}; ^1H NMR (500 MHz, D_2O): δ 5.15 (d, *J*=1.6 Hz, 1H, H-1_C), 5.02 (br s. 2H, H-1_A, H-1_B), 4.10–4.06 (m, 3H, H-2_A, H-2_C, H-3_A), 4.03–3.98 (m, 2H, H-2_B, H-4_A), 3.94–3.82 (m, 5H, H-3_B, H-3_C, H-6_{abB}, H-6_{aC}), 3.79–3.65 (m, 6H, H-4_B, H-5_{aA}, H-5_B, H-5_C, H-6_{bC}, OCH_2R), 3.62–3.58 (m, 3H, H-4_C, H-5_{bA}, OCH_2R), 3.14–3.11 (m, 2H, NCH_2), 1.99–1.95 (m, 2H, –CH_2–); ^{13}C NMR (125 MHz, D_2O): δ 104.9 (C-1_A), 101.6 (C-1_B), 98.8 (C-1_C), 82.9 (C-4_A), 81.2 (C-2_B), 79.1 (C-3_A), 76.8 (C-2_A), 73.8 (C-5_C), 73.5 (C-5_B), 70.8 (C-3_C), 70.7 (C-3_B), 70.5 (C-2_C), 67.4 (OCH_2R), 67.3 (C-5_A), 66.9 (C-4_B), 66.3 (C-4_C), 61.7 (C-6_B), 61.4 (C-6_C), 38.4 (NCH2), 27.1 (–CH_2–); ESI-MS: *m*/*z* 532.2 $[M+H]^+$. Anal. Calcd for $C_{20}H_{37}NO_{15}$ (531.22): C, 45.20; H, 7.02%; found: C, 45.07; H, 7.15%.

*4.9. 3-Aminopropyl 5-O-{2-O-[4-O-(5-deoxy-5-methylthio-α-D-xylofuranosyl)-α-D-mannopyranosyl]-α-D-mannopyranosyl}-β-D-arabinofuranoside (**2**)*

To a solution of **16** (367 mg, 0.20 mmol) in acetonitrile, 18-crown-6 (40 mg) was added followed by sodium thiomethoxide (48 mg, 0.69 mmol). This mixture was then refluxed for 12 h and after completion of reaction, cooled to room temperature. It was then diluted with acetonitrile and filtered through Celite® bed. The filtrate after concentrating to a syrup was purified by passing through a short pad SiO_2. The resulting intermediate was dissolved in THF and cooled to −78 °C. Then using a dry ice trap, ammonia was condensed into the flask followed by addition of sodium metal in three portions till a deep blue colour persisted. After stirring the solution for 1.5 h at −78 °C, methanol was added and the flask was left open to atmosphere overnight to allow NH_3 to evaporate. Then the remaining solution was concentrated, dissolved in methanol and neutralized with glacial acetic acid. The mixture was reconcentrated and purified over Sephadex® LH-20 column using CH_3OH–H_2O (4:1 v/v) as eluant to give pure compound **2** (99 mg, 72%); $[\alpha]_D^{25}$ +6.5 (*c* 1.5, $CHCl_3$); IR (neat): 3307, 1948, 1873, 1248, 1224, 1137, 1088, 1048 cm^{-1}; ^1H NMR (500 MHz, D_2O): δ 5.39 (d, *J*=4.4 Hz, 1H, H-1_D), 5.12 (d, *J*=1.8 Hz, 1H, H-1_C), 5.02 (br s, 1H, H-1_B), 5.01 (d, *J*=4.5 Hz, 1H, H-1_A), 4.37 (ddd, *J*=5.0, 4.8, 8.4 Hz, 1H, H-4_D), 4.24 (dd, *J*=4.2, 5.0 Hz, 1H, H-3_D), 4.22–4.16 (m, 2H, H-2_D, H-3_A), 4.14–4.05 (m, 4H, H-2_A, H-2_B, H-2_C, H-4_A), 4.00–3.92 (m, 5H, H-3_B, H-3_C, H-6_{aB}, H-6_{abC}), 3.89–3.81 (m, 5H, H-4_B, H-4_C, H-5_{aA}, H-5_C, H-6_{bB}), 3.77–3.57 (m, 4H, H-5_{bA}, H-5_B, OCH_2), 3.04–2.96 (m, 2H, NCH_2), 2.78 (dd, *J*=4.8, 13.8 Hz, 1H, H-5_{aD}), 2.67 (dd, *J*=8.4, 13.8 Hz, 1H, H-5_{bD}), 2.16 (s, 3H, SCH_3), 1.90–1.88 (m, 2H, –CH_2–); ^{13}C NMR (125 MHz, D_2O): δ 104.3 (C-1_D), 104.1 (C-1_B), 102.9 (C-1_A), 100.0 (C-1_C), 81.5 (C-4_A), 80.5 (C-2_B), 79.6 (C-4_D), 78.4 (C-2_D), 77.8 (C-3_D), 77.4 (C-3_A), 76.1 (C-2_A), 76.0 (C-5_C), 74.9 (C-5_B), 73.7 (C-3_C), 72.3 (C-3_B), 72.1 (C-2_C), 72.0 (OCH_2), 70.2 (C-4_B), 68.8 (C-4_C), 67.9 (C-5_A), 63.1 (C-6_B), 62.8 (C-6_C), 38.8 (NCH_2), 34.8 (C-5_D), 29.8 (–CH_2–), 16.8 (SCH_3); ESI-MS: *m*/*z* 694.2 $[M+H]^+$; Anal. Calcd for $C_{26}H_{47}NO_{18}S$ (693.25): C, 45.02; H, 6.83%; found: C, 44.93; H, 6.91%.

*4.10. Biotinylation of compound **1***

To a solution of **1** (20 mg, 0.04 mmol) in methanol, sulfosuccinimidyl 2-(biotinamido) ethyl-l,3-dithiopropionate (**9**) (27.4 mg, 0.05 mmol) was added and the resulting solution was allowed to stir at room temperature for 8 h. After completion, the reaction was concentrated to a syrup, which was purified by Sephadex® LH-20 column using CH_3OH–H_2O (10:1 v/v) as eluant, to afford **3** (28.4 mg, 82%); $[\alpha]_D^{25}$ +4.9 (*c* 1.5, H_2O); IR (KBr): 3317, 1948, 1873, 1558, 1224, 1137, 1088, 1008 cm^{-1}; ^1H NMR (500 MHz, D_2O): δ 5.20 (d, *J*=1.6 Hz, 1H, H-1_C), 5.08 (br s. 2H, H-1_B, H-1_A),4.65–4.61 (m, 1H, H-3_A), 4.48–4.45 (m, 1H, NHC*H*-), 4.39–4.36 (m, 1H, NHC*H*-), 4.16–4.10 (m, 3H,, H-2_A, H-2_C, H-4_A), 4.08–3.98 (m, 2H, H-2_B, H-3_B), 3.96–3.85 (m, 4H, H-3_C, H-6_{abB}, H-6_{aC}), 3.83–3.74 (m, 6H, H-4_B,H-5_{aA}, H-5_B, H-5_C, H-6_{bC}, OCH_2R), 3.73–3.53 (m, 5H, H-4_C, H-5_{bA}, SCH_2-, OCH_2R), 3.29–3.23 (m, 2H, NCH_2-), 3.19–3.02 (m, 3H, –CH_2–, SCH_2-), 2.95–2.91 (m, 2H, –CH_2–), 2.84–2.78 (m, 2H, –CH_2–), 2.70–2.68 (m, 2H, –CH_2–), 2.34–2.30 (m, 2H, –CH_2–), 2.04–1.99 (m 2H, –CH_2–),1.77–1.62 (m, 2H, –CH_2–), 1.50–1.44 (m, 2H, –CH_2–), 1.23–1.20 (m, 2H, –CH_2–); ^{13}C NMR (125 MHz, D_2O): δ 174.2 (NHCO-), 172.7 (NHCO-), 164.1 (NHCONH), 104.9(C-1_A), 101.9(C-1_B), 99.2(C-1_C), 82.4(C-4_A), 81.3(C-2_B), 79.4(C-3_A), 76.4(C-2_A), 73.9(C-5_C), 73.5(C-5_B), 70.9(C-3_C), 70.8(C-3_B), 70.6(C-2_C), 67.5 (2C, H-4_B, OCH_2R), 66.4 (C-4_C), 62.7(C-5_A), 61.8(C-6_B), 61.5(C-6_C), 60.9 (NHCHCH-), 57.0 (NHCHCH-), 56.0 (SCH_2CH-), 40.3 (–CH_2–), 38.4(NCH_2), 37.3 (NCH_2-), 36.5 (2C, –CH_2–), 33.4 (–CH_2–), 30.2 (2C, –CH_2–), 28.4 (–CH_2–), 28.3 (–CH_2–), 25.7 (–CH_2–); ESI-MS: *m*/*z*

943.3 $[M+Na]^+$; Anal. Calcd for $C_{35}H_{60}N_4O_{18}S_3$ (920.31): C, 45.64; H, 6.57%; found: C, 45.51; H, 6.68%.

4.11. Biotinylation of compound **2**

To a solution of **2** (10 mg, 0.01 mmol) in methanol, sulfosuccinimidyl 2-(biotinamido) ethyl-l,3-dithiopropionate (**9**) (10.5 mg, 0.02 mmol) was added and the resulting solution was allowed to stir at room temperature for 8 h. It was then concentrated to a syrup that was purified by Sephadex® LH-20 column using CH_3OH-H_2O (6:1 v/v) as eluant, to afford **4** (11.2 mg, 72%); $[\alpha]_D^{25}$ +3.5 (*c* 1.5, H_2O); IR (KBr): 3337, 2248, 1873, 1548, 1424, 1137, 1088, 1048, 986 cm^{-1}; ^1H NMR (500 MHz, D_2O): δ 5.45 (d, *J*=4.5 Hz, 1H, H-1_D), 5.18 (d, *J*=1.8 Hz, 1H, H-1_C), 5.08 (d, *J*=1.6 Hz, 1H, H-1_B), 5.04 (d, *J*=4.5 Hz, 1H, H-1_A), 4.67–4.64 (m, 2H, C*H*NHCO-), 4.47 (ddd, *J*=5.0, 4.8, 8.4 Hz, 1H, H-4_D), 4.46–4.42 (m, 1H, H-3_D), 4.31–4.23 (m, 2H, H-2_D, H-3_A), 4.17–4.10 (m, 4H, H-2_A, H-2_B, H-2_C,H-4_A), 4.04–3.96 (m, 5H, H-3_B, H-3_C, H-6_{aB}, H-6_{abC}), 3.93–3.82 (m, 5H, H-4_B, H-4_C, H-5_{aA}, H-5_C, H-6_{bB}), 3.67–3.63 (m, 2H, SCH_2-), 3.61–3.53 (m, 4H, H-5_{bA}, H-5_B, OCH_2), 3.39–3.31 (m, 2H, NCH_2-), 3.06–2.90 (m, 5H, H-5_{aD}, $-CH_2-$, SCH_2-, SCH_2-), 2.85–2.69 (m, 6H, H-5_{bD}, $-CH_2-$), 2.34–2.30 (m, 2H, $-CH_2-$), 2.22 (s, 3H, SCH_3), 1.90–1.64 (m 4H, $-CH_2-$),1.50–1.44 (m, 2H, $-CH_2-$), 1.19–1.17 (m, 2H, $-CH_2-$); ^{13}C NMR (125 MHz, D_2O): δ 175.4 (NH*C*O-), 174.7 (NH*C*O-), 164.5 (NH*C*ONH), 104.1 (C-1_D), 104.0 (C-1_B), 102.6 (C-1_A), 99.7 (C-1_C), 80.2 (C-4_A), 79.3 (C-2_B), 78.3 (C-4_D), 77.1 (C-2_D), 76.7 (C-3_D), 76.1 (C-2_A), 75.0 (C-2_A), 74.8 (C-5_C), 73.5 (C-5_B), 72.4 (C-3_C), 70.9 (C-3_B), 70.8 (2C, C-2_C, C-4_C), 68.9 (O*C*H_2-), 67.4 (C-4_B), 66.5 (C-5_A), 62.7 (NH*C*HCH-), 61.8 (C-6_B), 61.4 (C-6_C), 60.8 (NH*C*HCH_2-), 55.9 (S*C*HCH_2-), 40.3 (S*C*H_2-), 38.4 (N*C*H_2), 37.4 (N*C*H_2), 37.2 ($-CH_2-$), 36.0 (S*C*H_2-), 35.6 (C-5D) 33.9 ($-CH_2-$), 33.5 ($-CH_2-$), 28.9 ($-CH_2-$), 28.4 ($-CH_2-$), 28.2 ($-CH_2-$), 25.7 ($-CH_2-$), 15.6 (S*C*H_3); ESI-MS: *m*/*z* 1105.3 $[M+Na]^+$; Anal. Calcd for $C_{411}H_{70}N_4O_{21}S_4$ (1082.34): C, 45.46; H, 6.51%; found: C, 45.33; H, 6.63%.

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Supplementary data

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