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Total synthesis of Lewis^X using a late-stage crystalline intermediate

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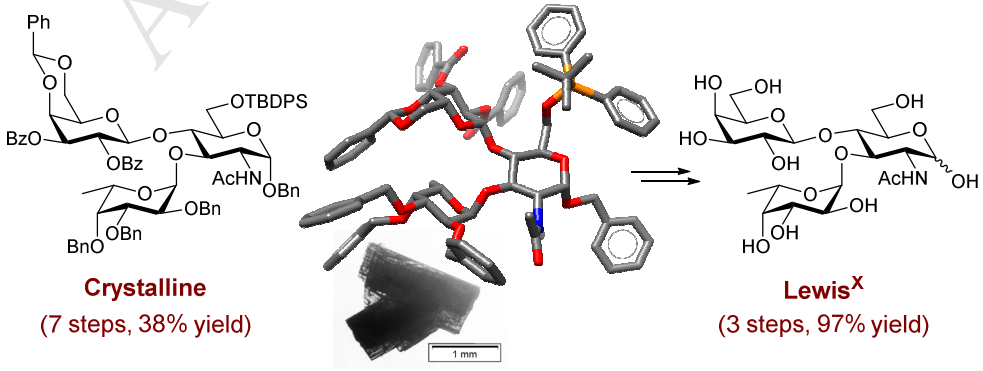
Abstract:

Herein, we report on a highly efficient synthesis of a crystalline protected Lewis^X trisaccharide that was converted to Lewis^X following global deprotection. The trisaccharide was prepared in a highly convergent synthesis (7 steps, longest linear sequence) and in a 38% overall yield using a strategy that involved the regioselective glycosylation of a GlcNAc acceptor with a galactose thioglycoside donor, followed by fucosylation of the remaining free GlcNAc hydroxyl as key steps. The core trisaccharide also has the potential to be converted to other members of the Type-2 Lewis family of antigens due to the orthogonal nature of the protecting groups employed.

Keywords

Lewis^X, Carbohydrate, Synthesis, Glycosylation.

Graphical Abstract



1. Introduction

The Lewis antigens (Le) are a related set of glycans characterised by the presence of α1→4 or α1→3 fucosylated GlcNAc residues, termed the Type-1 and Type-2 Lewis antigens, respectively.[1] The Type-1 antigens, also known as the Lewis blood group antigens (*e.g.* Le^A and Le^B, Figure 1), are found on erythrocytes, in blood plasma, and in various tissues, while Type-2 Lewis antigens, [*e.g.* Le^X and Le^Y, and their sialylated forms] are serological characters widely distributed in human and animal organisms.[2] Lewis glycans have important roles in biology, and indeed, the Lewis blood group antigens owe their name to the Lewis family who suffered from a red blood cell incompatibility.[3] Members of both subsets of Lewis antigens have been implicated in favourable and unfavourable selectin-dependent leukocyte and cell adhesion processes[2] with, for example, Sialyl Le^A and Sialyl Le^X (SLe^X) being upregulated during cancer metastasis,[4,5,6,7] while SLe^X also has an essential role in human fertilisation.[8] Targeting the dendritic cell (DC) C-type lectin, DC-SIGN, which binds Lewis antigens (*i.e.* Le^X, Le^Y, Le^B and Le^A), has been shown to increase antigen cross-presentation and enhance vaccine efficacy for cancer and intracellular pathogens.[9,10,11,12,13] Accordingly, there has been much interest in targeting this lectin using antigen-conjugated antibodies or antigen-conjugated Lewis glycans (*e.g.* Le^X), whereby the latter approach is thought to be advantageous due to reduced side-effects and the ease of synthesis.[14]

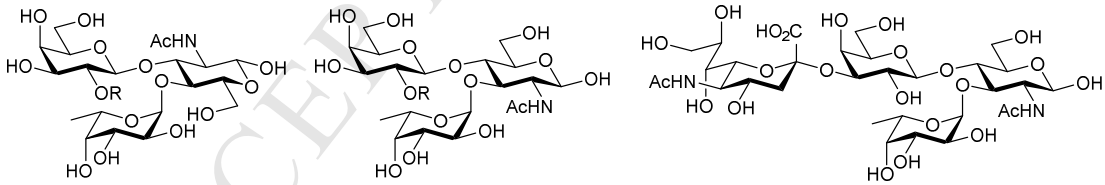
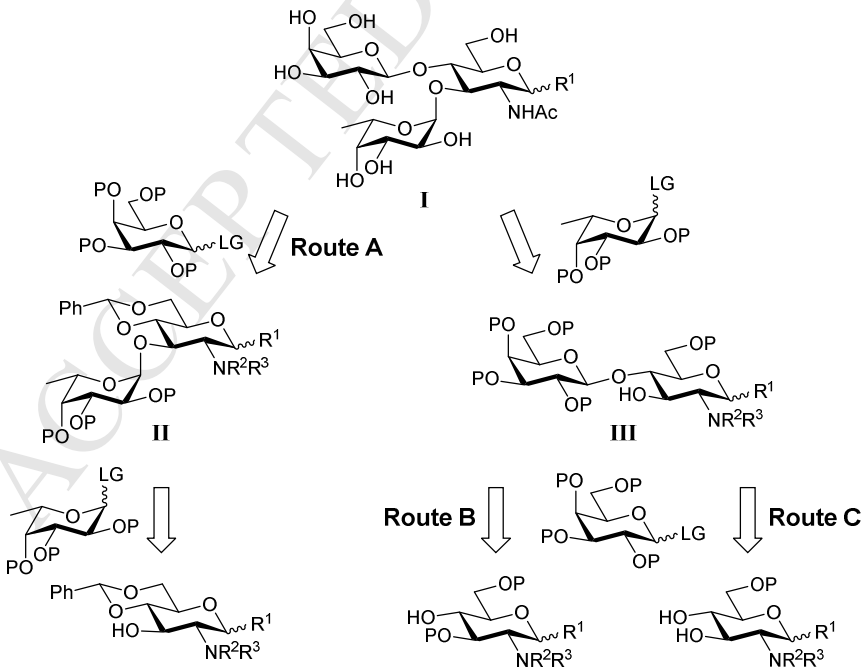


Figure 1. Representative Lewis antigens

Although various synthetic strategies can be envisaged for the synthesis of Type-2 Lewis glycans, the incorporation of an orthogonally protected trisaccharide $Lewis^X$ intermediate has particular merit, as this would allow access to a variety of Type-2 Lewis antigens, including sialylated and sulfated derivatives. There are three general retrosynthetic approaches that can be used for the construction of $Lewis^X$ derivatives

(**I**, Scheme 1). First, the target trisaccharide can be constructed through galactosylation of an α-L-Fuc-(1→3)-D-GlcNAc acceptor, which in turn is accessible via reductive ring opening of benzylidene protected dimer **II** (Route A).[15] Dimer **II** is conveniently constructed by 3-*O*-fucosylation of a benzylidene protected GlcNAc acceptor. A number of elegant syntheses of Lewis^X have been achieved in this manner, including the synthesis by Stahl *et al.* using an *N*-acetylglucosamine building block,[16] and the one-pot synthesis by Boons and co-workers using more advanced building blocks.[17] The lability of the fucose residue during the 2+1 coupling, however, can be troublesome,[18,19,20] and this has instigated the need for more complex fucose building blocks that contain electron-withdrawing protecting groups.[15,17,18,21,22,23] Alternatively, Le^X (**I**) can be constructed from the 2+1 coupling of a fucose donor with a β-D-Gal-(1→4)-D-GlcNAc acceptor **III**, which is derived from a protected GlcNAc precursor (Route B).[24] Again, some remarkable syntheses of the Type-2 Lewis glycans have been achieved using this approach, including the synthesis of SLe^X by Seeberger and co-workers.[25] The most efficient route to Le^X, however, involves a regioselective coupling at the 4-position of a glucosamine precursor (Route C), as elegantly shown by Roy and co-workers,[26] and later adapted

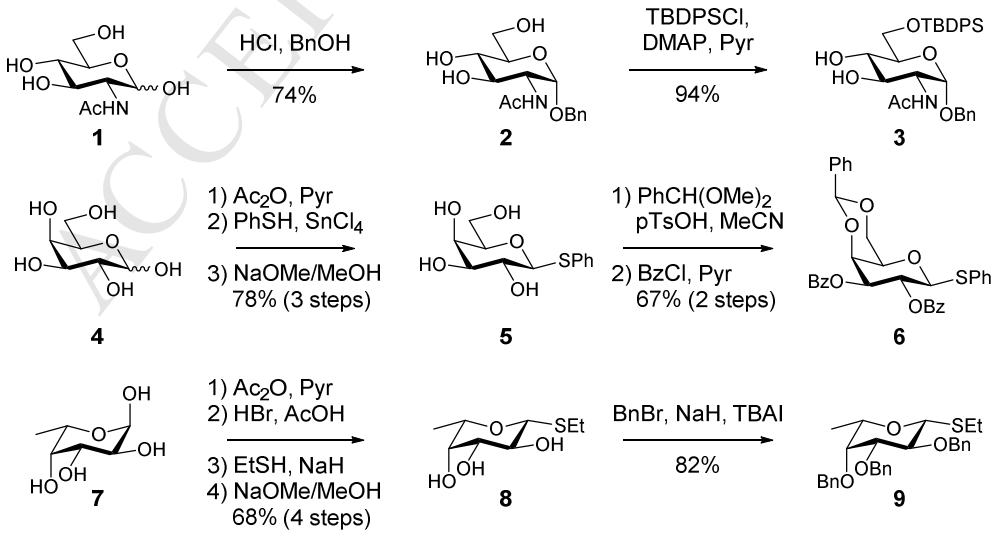


Scheme 1. Retrosynthetic approaches that have been used for the synthesis of Le^X.

by others.[27,28,29,30] Accordingly, we envisioned using this third regioselective glycosylation approach to develop a highly efficient synthesis suitable for the preparation of Le^X, as well as other Type-2 Lewis glycans. To this end, we sought to avoid the use of a glucosamine N-protecting group, such as Troc or Phth, as this would require a late stage N-deprotection and acetylation step. For the fucose donor, we opted for a perbenzylated thioglycoside, as the synthesis of a donor with electron-withdrawing groups would require a more complex building block synthesis and additional late-stage deprotection steps.

2. Results and discussion

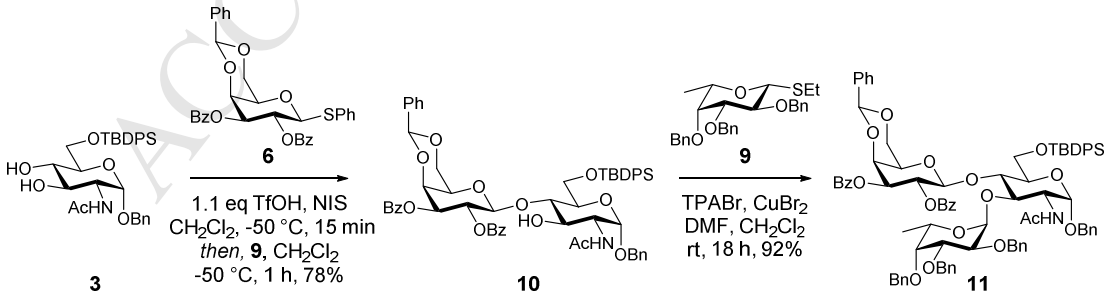
To begin the synthesis of Le^X, we selected three building blocks that could not only be readily synthesised, but moreover, would provide an orthogonally protected Le^X core that could be used for the construction of the full family of Type-2 Lewis antigens. The three building blocks, all of which can be synthesised in 2-5 steps, are depicted in Scheme 2. The simplest of these to prepare was the GlcNAc acceptor, which was synthesised on a multi gram scale in two-steps from GlcNAc (**1**). Here, Fisher glycosylation of lactol **1** with benzyl alcohol gave the crystalline benzyl glucoside **2**,[31] which was then selectively protected at the 6-position using *tert*-butyldiphenylsilyl (TBDPS) chloride to yield protected GlcNAc acceptor **3** in 70% over the two steps.[32] We observed the TBDPS-protection was sluggish at room temperature and that warming the reaction to 40 °C increased the solubility of the



Scheme 2. Synthesis of key building blocks

sugar and reaction yields significantly. Next, the galactose donor was prepared via a 5-step synthesis that commenced with the peracetylation of D-galactose (**4**), followed by installation of the anomeric thiophenol and deacetylation to give crystalline thiogalactoside **5** in 80% yield over the three steps.[33] Installation of the 4,6-benzylidine acetal protecting group,[33] followed by benzoylation of the remaining free hydroxyls then gave the required crystalline donor **6**,[34] equipped with a 2-*O*-acyl protecting group to allow for neighbouring group participation and β-selective glycosylation. Finally, the fucose acceptor was also prepared in five-steps. L-Fucose (**7**) was per-acetylated, converted to the anomeric bromide with subsequent conversion to the thioglycoside, and deacetylated to give triol **8**. Perbenzylation then afforded the crystalline target donor **9** in 56% overall yield.[35]

With the target building blocks in hand, the assembly of the Le^X trisaccharide began with the regioselective glycosylation of GlcNAc acceptor **3** and the galactose donor **6** (Scheme 2). It was observed that the slow addition of the donor (1.2 equivalents) to a mixture of the acceptor and activator at -50 °C resulted in a high yielding (74%) regioselective glycosylation, however, when the reaction was performed at higher temperatures (or allowed to warm to 0 °C) small amounts of the over-glycosylated trimer were formed. Given that the acceptor is obtained in fewer steps compared to the donor, the reaction was repeated using a slight excess of acceptor (1.1 equivalents of GlcNAc) at -50 °C for 1 hour, which, following purification by silica gel flash column chromatography, gave disaccharide **10** in 78%. NMR analysis was used to confirm that the resulting glycoside was the β-pyranoside, whereby ^{1}H NMR analysis



Scheme 3. Assembly of the Le^X core **11**

revealed a $J_{1'',2''}$ = 8.2 Hz, indicative of the β-anomer, while the regioselectivity of the glycosylation was confirmed using 2D NMR analysis in which HMBC correlations between both H-1´´/C-4´, and H-4´/C-1´´ were observed.

With the disaccharide acceptor in hand, the glycosylation between disaccharide **10** and fucose donor **6** was explored. Activation of the thioethyl donor **6** using Ogawa's conditions[36] resulted in the smooth formation of the desired α-fucosylated trisaccharide **11** in excellent yield (92%) and excellent α-selectivity ($^1J_{C,H}$ = 170 Hz[37]). Much to our delight, trisaccharide **11** could be purified via silica gel chromatography and/or readily crystallised from methanol (Figure 2),[38] which allowed for rapid purification. The obtained crystal structure also confirmed the regio- and stereo-selectivity of both glycosylation reactions. While one other crystalline Le^X intermediate has been reported (though no crystal structure was obtained),[39] the synthetic route employed was more complex and did not readily allow for the further derivatisation of the Le^X scaffold into other Type-II Lewis glycans. Moreover, our yield over the two glycosylation reactions (72%) is comparable to other reported strategies. While there are several notable syntheses of protected Le^X trisaccharides,[25,29,30,40] with for example an 80% yield over the two coupling steps,[30] these strategies employ N-protected glucosamine acceptors, thereby requiring more complex building block syntheses and late stage functional group interconversions. Comparing our route to the synthesis by Roy and co-workers who pioneered the regioselective glycosylation of an *N*-acetylglucosamine acceptor,[26] we obtained similar glycosylation yields, however, our crystalline derivative contains orthogonally protected 6´- and 6´´-positions, thereby allowing for the synthesis of 6-sulfo- or 6´-sulfo-Le^X derivatives.[41] In addition, deacetylation of **11** gives the 2´´,3´´-diol which in turn can be used *en route* towards dimeric Le^X,[42] 3´-sulfo-Le^X,[43] as well as SLe^X derivatives.[44] Taken as a whole, our short synthetic sequence (longest linear sequence of 7 steps) and high overall yield (38%) for the synthesis of orthogonally protected Le^X **11** provides a highly efficient and versatile route for the synthesis of Type-2 Lewis glycans.

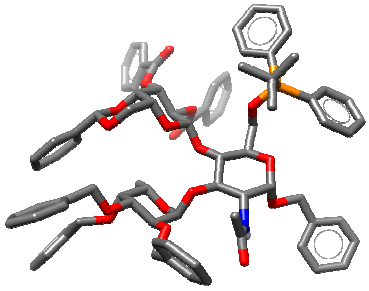
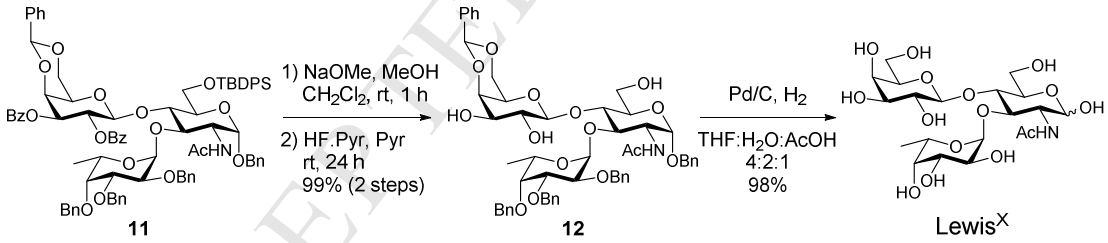


Figure 2. Crystal structure of trisaccharide **11**.[38]

To complete the synthesis of Le^X, trisaccharide **11** was globally deprotected (Scheme 4). Accordingly, the benzoyl and TBDPS groups were first removed using NaOMe/MeOH and HF.pyridine, respectively, to give triol **12**. The conditions of Zang and co-workers were then employed for the reductive removal of both the benzyl ether and benzylidene protecting groups.[45] Here, it should be noted that the solvent mixture THF:H_2O:AcOH (4:2:1, v/v/v) was essential in obtaining near quantitative yields of the target trisaccharide without concomitant de-fucosylation. With an overall yield of 37% (longest linear sequence of 10-steps), our synthesis of Le^X is the highest yielding to date and should facilitate in the preparation of complex glycoconjugates via the use of a variety of ligation strategies.[46]



Scheme 4. Global deprotection and total synthesis of Le^X

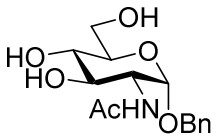
3. Conclusion

In conclusion, the synthesis of the Le^X trisaccharide was completed in excellent yield and in few linear steps. To achieve this, highly efficient syntheses of the core building blocks were accomplished (2-5 steps, 54-70% yield), and a regioselective glycosylation between the glucosamine acceptor and galactose donor was performed in 78% yield. An α-selective fucosylation then gave the resulting trisaccharide as crystalline material in excellent (92%) yield, with global deprotection affording Le^X in near quantitative yield. It is envisioned that this highly efficient route will find further

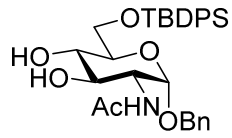
application, not only for the targeting of Le^X to receptors such as DC-SIGN, but also for the synthesis of other members of the Type-2 family of Lewis antigens.

4. Experimental

General procedure. Unless otherwise stated all reactions were performed under argon. Prior to use, THF (Pancreac) was distilled from sodium and benzophenone, pyridine was distilled and dried over 4Å molecular sieves (4Å MS), CH_2Cl_2 (Fisher) was distilled from P_2O_5, DMF was distilled from BaO, and H_2O was distilled. NaOMe was freshly prepared from sodium [Aldrich] and MeOH. *N*-Acetyl glucosamine (Fluka), BnOH (Ajax Chemicals), conc. HCl (Panreac), TBDPS-Cl (Aldrich), DMAP (Pierce), *N*-iodosuccinimide (Aldrich), TfOH (Aldrich), TPABr (Aldrich), $CuBr_2$ (Chempur), HF.Pyridine (Aldrich), $Ca(OAc)_2$ (Sigma), AcOH (Sigma-Aldrich), Pd/C (Aldrich), Et_3N (Sigma), Dowex H^+ (Sigma-Aldrich 50WX8), 4Å molecular sieves (ROTH), Toluene (Panreac), Et_2O (Pancreac), EtOAc (Pancreac), petroleum ether (Pure Science), MeOH (Pure Science), $CHCl_3$ (Pancreac), EtOH (absolute, Pure Science), $NaHCO_3$ (Pure Science), $Na_2S_2O_3$ (Merck) and NaCl (Pancreac) were used as received. Galactose donor **6**[33,34] and fucose donor **9**[35] were prepared according to literature procedures. All solvents were removed by evaporation under reduced pressure. Reactions were monitored by TLC-analysis on Macherey-Nagel silica gel coated plastic sheets (0.20 mm, with fluorescent indicator UV_{254}) with detection by UV-absorption (short wave UV – 254 nm; long wave UV – 366 nm), by dipping in 10% H_2SO_4 in EtOH followed by charring at ~150 °C, by dipping in I_2 in silica, or by dipping into a solution of ninhydrin in EtOH followed by charring at ~150 °C. Column chromatography was performed on Pure Science silica gel (40 – 63 micron). AccuBOND II ODS-C_{18} (Agilent) was used for reverse phase chromatography. Infrared spectra were recorded as thin films using a Bruker Tensor 27 FTIR spectrometer equipped with an Attenuated Total Reflectance (ATR) sampling accessory and are reported in wave numbers (cm^{-1}). Nuclear magnetic resonance spectra were recorded at 20 °C in D_2O, CD_3OD, $CDCl_3$, or pyridine-d_5 using either a Varian INOVA operating at 500 MHz or Varian VNMRS operating at 600 MHz. Chemical shifts are given in ppm (δ) relative to solvent residues. NMR peak assignments were made using COSY, HSQC and HMBC 2D experiments.

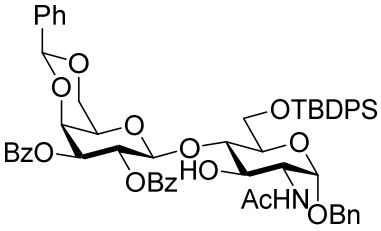


Benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside (2). To a solution of *N*-acetylglucosamine (6.40 g, 28.9 mmol) in benzyl alcohol (50 mL, 480 mmol), conc. aq. HCl (3.0 mL) was added and the reaction mixture was stirred at 90 °C for 3 h. The crude mixture was cooled down to rt, poured into Et_2O (500 mL) and left to crystallize at -4 °C for 18 h. The crystalline product was filtered and washed with petroleum ether. Purification by silica gel column chromatography (CH_2Cl_2:MeOH, 95:5 → 88:12, v/v) gave benzyl glycoside **2** (6.67 gram, 21.4 mmol, 74%) as a white foam. R_f = 0.25 (CH_2Cl_2:MeOH, 90:10, v/v); Mp 182 – 184 °C; $\alpha_D^{17.8}$ = +222 (c = 0.1, MeOH); IR (film) 3298, 3092, 2938, 2901, 2844, 1648, 1552, 1497, 1455, 1375, 1309, 1230, 1156, 1093, 1047, 778, 732, 695 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.40 – 7.25 (m, 5H, CH_{arom}), 4.85 (d, 1H, $J_{1´,2´}$ = 3.6 Hz, H-1´), 4.74 (d, 1H, $J_{1a,1b}$ = 12.0 Hz, H-1a), 4.49 (d, 1H, $J_{1a,1b}$ = 12.0 Hz, H-1b), 3.89 (dd, 1H, $J_{1´,2´}$ = 3.6 Hz, $J_{2´,3´}$ = 10.8 Hz H-2´), 3.83 (dd, 1H, $J_{5´,6a´}$ = 1.6 Hz, $J_{6a´,6b´}$ = 11.4 Hz, H-6a´), 3.73 – 3.64 (m, 3H, H-6b´, H-3´, H-4´), 3.36 (dd, 1H, $J_{4´,5´}$ = $J_{5´,6b´}$ = 9.6 Hz, H-5´), 1.95 (s, 3H, CH_3 Ac); ^{13}C NMR (125 MHz, CD_3OD) δ 173.6 (C=O Ac), 139.0 (Cq_{arom}), 129.4 (C-o_{arom}), 129.3 (C-m_{arom}), 128.8 (C-p_{arom}), 97.5 (C-1´), 74.1 (C-4´), 72.7 (C-3´), 72.5 (C-5´), 70.1 (C-1), 62.7 (C-6´), 55.4 (C-2´), 22.5 (CH_3 Ac); HRMS(ESI) *m/z* calcd. for $[C_{15}H_{22}NO_6]^+$: 312.1442, obsd.: 312.1446.



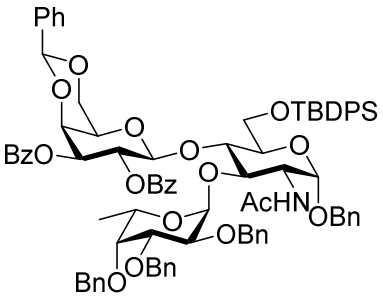
Benzyl 2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-α-D-glucopyranoside (3). To a solution of benzyl glycoside **2** (837 mg, 8.74 mmol) in pyridine (10 mL), TBDPS-Cl (2.75 mL, 33.9 mmol) and DMAP (200 mg, 1.64 mmol) were added and the reaction mixture was stirred at 40 °C for 18 h. The crude mixture was concentrated *in vacuo* and purified by silica gel column chromatography (CH_2Cl_2:MeOH, 100:0 → 95:5, v/v) to give diol **3** (1.39 gram, 94%) as a white solid. R_f = 0.50 (CH_2Cl_2:MeOH, 95:5, v/v); $\alpha_D^{17.8}$ = +58.4 (c = 0.5, $CHCl_3$); IR (film) 3324, 3071, 2930, 2857, 1739, 1655, 1544, 1472, 1456, 1428, 1376, 1217, 1112, 1048, 1025, 823, 775, 739, 701 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.73 – 7.68 (m, 4H, CH_{arom}), 7.46 – 7.27 (m, 11H, CH_{arom}), 5.84 (d, 1H, $J_{2´,NH}$ = 8.5 Hz, NH), 4.88 (d, 1H, $J_{1´,2´}$ = 3.0 Hz, H-1´), 4.72 (d, 1H, $J_{1a,1b}$ = 11.8 Hz, H-1a), 4.44 (d, 1H, $J_{1a,1b}$ = 11.8 Hz, H-1b), 4.14 – 4.06 (m, 1H, H-2´), 3.94 – 3.85 (m, 2H, H-6a´, H-6b´), 3.77 – 3.68 (m, 2H, H-3´, H-5´), 3.63 (dd, 1H, $J_{3´,4´}$ = $J_{4´,5´}$ = 9.1 Hz, H-4´), 3.24 (bs, 1H, OH), 2.86 (bs, 1H, OH), 2.00 (s, 3H, CH_3 Ac), 1.07 (s,

9H, CH_3 *t*Bu); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0 (C=O), 137.1, 135.81, 135.78, 133.3, 133.2, 130.0, 128.8, 128.3, 128.2, 127.9 (C_{arom}), 96.5 (C-1´), 74.5 (C-3´), 72.9 (C-5´), 71.5 (C-4´), 69.4 (C-1), 64.3 (C-6´), 53.8 (C-2´), 27.0 (CH_3 *t*Bu), 23.4 (CH_3 Ac), 19.4 (Cq *t*Bu); HRMS(ESI) *m/z* calcd. for $[C_{31}H_{40}NO_6Si]^+$: 550.2619, obsd.: 550.2620.



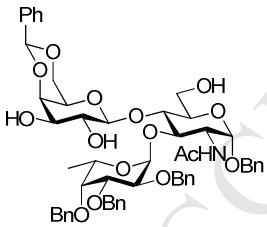
Benzyl 2-acetamido-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-α-D-glucopyranoside (10). Glycosyl acceptor **3** (680 mg, 1.24 mmol) was co-evaporated with toluene (3 × 3 mL) and dissolved in dry CH_2Cl_2 (5 mL). In a separate flask, glycosyl donor **6** (798 mg, 1.40 mmol)[33, 34] was co-evaporated with toluene (3 × 3 mL) and dissolved in dry CH_2Cl_2 (10 mL). Activated molsieves (4Å) were added to both the donor and the acceptor solutions, and both solutions were stirred at rt for 30 min. *N*-iodosuccinamide (631 mg, 2.81 mmol) was added to the mixture with acceptor **3** and the reaction mixture was cooled to -50 °C. Freshly distilled TfOH (124 µL) was added at -50 °C and the reaction mixture was stirred for 15 min before adding the solution of donor **6**. The crude reaction mixture was stirred for 1 h at -50 °C and when TLC analysis showed full conversion of the glycosyl donor, the reaction mixture was quenched by the addition of $NaHCO_3$ (830 mg). The crude reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with sat. aq. $Na_2S_2O_3$ (30 mL) and the water layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried with $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel flash column chromatography (PE:EtOAc 75:25 → 0:100, v/v) gave disaccharide **10** (909 mg, 73%) as a white foam. R_f = 0.56 (EtOAc); $\alpha_D^{17.8}$ = +91.1 (c = 1, $CHCl_3$); IR (film) 3422, 3382, 3069, 2931, 2889, 2857, 1722, 1668, 1602, 1531, 1452, 1428, 1369, 1315, 1273, 1219, 1177, 1112, 1070, 1040, 1027, 821, 773, 741, 708 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.97 (d, 2H, $J_{o\text{-}m}$ = 7.3 Hz, CH-*o* Bz), 7.76 – 7.66 (m, 6H, CH_{arom}), 7.55 – 7.30 (m, 16H, CH_{arom}), 7.28 – 7.14 (m, 8H, CH_{arom}), 5.88 (dd, 1H, $J_{1´´,2´´}$ = 8.2 Hz, $J_{2´´,3´´}$ = 10.6 Hz, H-2´´), 5.64 (d, 1H, $J_{2´,NH}$ = 8.8 Hz, NH) 5.52 (s, 1H, CHPh), 5.31 (dd, 1H, $J_{3´´,4´´}$ = 3.0 Hz, $J_{2´´,3´´}$ = 10.7 Hz, H-3´´), 4.98 (d, 1H, $J_{1´´,2´´}$ = 8.2 Hz, H-1´´), 4.88 (d, 1H, $J_{1´,2´}$ = 3.2 Hz, H-1´), 4.58 (d, 1H, $J_{3´´,4´´}$ = $J_{4´´,5´´}$ = 3.0 Hz, H-4´´), 4.50 (d, 1H, $J_{1a,1b}$ =

12.0 Hz, H-1a), 4.45 (d, 1H, $J_{6a',6b'}$ = 12.0 Hz, H-6a´), 4.34 (d, 1H, $J_{1a,1b}$ = 12.0 Hz, H-1b), 4.20 – 4.00 (m, 3H, H-2´, H-6b´, H-4´), 3.87 (dd, 1H, $J_{2',3'} = J_{3',4'}$ = 9.8 Hz, H-3´), 3.75 (d, 1H, $J_{6a'',6b''}$ = 11.6 Hz, H-6a´´), 3.64 (bs, 1H, H-5´´), 3.57 (d, 1H, $J_{6a'',6b''}$ = 11.6 Hz, H-6b´´), 3.45 (d, 1H, $J_{4',5'}$ = 9.9 Hz, H-5´), 1.99 (s, 3H, CH_3 Ac), 1.08 (s, 9H, CH_3 *t*Bu); ^{13}C NMR (125 MHz, D_2O) δ 170.3 (C=O Ac), 166.2 (C=O 3-*O*-Bz), 165.1 (C=O 2-*O*-Bz), 137. 6, 137.3, 136.1, 135.7, 134.0, 133.6, 133.3, 133.0, 130.1, 130.0, 129.8, 129.2, 129.1, 128.6, 128.5, 128.3, 128.1, 128.0, 127.8, 126.4 (C_{arom}), 100.93 (CPh), 100.91 (C-1´´), 96.8 (C-1´), 79.0 (C-4´), 73.5 (C-4´´), 72.7 (C-3´´), 70.7 (C-5´), 70.2 (C-3´), 69. 8 (C-1), 69.3 (C-2´´), 68.6 (C-6´), 67.0 (C-5´´), 61.7 (C-6´´), 53.4 (C-2´), 27.1 (CH_3 *t*Bu), 23.6 (CH_3 Ac), 19.7 (Cq *t*Bu); HRMS(ESI) *m/z* calcd. for $[C_{58}H_{62}NO_{13}Si]^+$: 1008.3985, obsd.: 1008.4009.



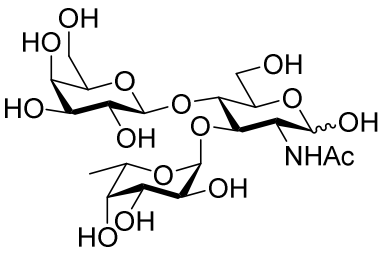
Benzyl 2-acetamido-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranoside)-2-deoxy-6-*O*-*tert*-butyldiphenylsilyl-α-D-glucopyranoside (11). A mixture of glycosyl acceptor **10** (0.90 g, 0.89 mmol) and glycosyl donor **9** (512 mg, 1.07 mmol)[35] were co-evaporated with dry DMF (3 × 3 mL) and dissolved in dry DMF (5 mL) and dry CH_2Cl_2 (10 mL). Activated molsieves (4Å) were added and the reaction mixture was stirred at rt for 30 min. TPABr (570 mg, 2.14 mmol) and $CuBr_2$ (480 mg, 2.14 mmol) were added and the reaction mixture was stirred ar rt for 15 h. When TLC analysis showed full conversion of the glycosyl acceptor, the crude reaction mixture was quenched by the addition of sat. aq. $Na_2S_2O_3$ (30 mL). The water layer was extracted with CH_2Cl_2 (3 × 10 mL), and the combined organic layers were washed with brine, dried with $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (PE:EtOAc, 9:1 → 2:1, v/v) and/or crystallized from hot methanol (200 mL) to afford trisaccharide **11** (1.05 g, 0.74 mmol, 92%) as white crystals. R_f = 0.23 (PE:EtOAc, 2:1, v/v); $\alpha_D^{24.4}$ = +110 (c = 0.5, $CHCl_3$); IR (film) 3088, 3064, 3032, 3007, 2932, 2894, 2859, 1735, 1671, 1497, 1453, 1428, 1367, 1315, 1273, 1250, 1250, 1219, 1165, 1143, 1098, 1061, 1046, 1027, 1002, 772, 708, 699 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (d, 2H, $J_{o,m}$ = 7.7 Hz, CH-*o* Bz), 7.87 – 7.81 (m, 4H, CH_{arom}), 7.64 – 7.07 (m, 39H,

CH_{arom}), 5.88 (dd, 1H, $J_{2'',3''}$ = 9.0 Hz, C-2´´), 5.60 (s, 1H, CHPh), 5.49 (d, 1H, $J_{2',NH}$ = 9.9 Hz, NH), 5.28 – 5.20 (m, 3H, H-1´´, H-1´´´, H-3´´), 4.95 (q, 1H, $J_{5''',6'''}$ = 6.7 Hz, H-5´´´), 4.84 – 4.77 (m, 2H, H-1´, CH_2-a 2´´´-*O*-Bn), 4.73 (d, 1H, $J_{a,b}$ = 11.7 Hz, CH_2-a 3´´´-*O*-Bn), 4.68 (d, 1H, $J_{a,b}$ = 11.7 Hz, CH_2-b 3´´´-*O*-Bn), 4.58 – 4.48 (m, 4H, H-4´´, CH_2-b 3´´´-*O*-Bn, H-2´, H-6a´), 4.41 – 4.31 (m, 3H, H-1a, H-1b, H-4´), 4.20 (d, 1H, $J_{a,b}$ = 11.4 Hz, CH_2-a 4´´´-*O*-Bn), 4.12 (d, 1H, $J_{6a',6b'}$ = 12.1 Hz, H-6b´), 4.06 – 4.01 (m, 1H, H-3´´´), 3.99 (dd, 1H, $J_{2',3'}$ = $J_{3',4'}$ = 9.7 Hz, H-3´), 3.96 – 3.89 (m, 2H, H-2´´´, H-6a´´), 3.54 – 3.47 (m, 3H, H-6b´´, H-5´´, CH_2-b 4´´´-*O*-Bn), 3.29 (d, 1H, $J_{4',5'}$ = 9.8 Hz, C-5´), 3.25 (bs, 1H, H-4´´´), 1.81 (s, 3H, CH_3 Ac), 1.33 (d, 3H, $J_{5''',6'''}$ = 6.2 Hz C-6´´´), 1.14 (s, 9H, CH_3 *t*Bu); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.7 (C=O Ac), 166.0 (C=O 3-*O*Bz), 164.8 (C=O 2-*O*Bz), 139.9, 139.8, 138.6, 137.8, 137.1, 136.2, 135.6, 134.0, 133.6, 133.2, 132.4, 130.3, 130.1, 130.0, 129.7, 129.2, 129.1, 129.0, 128.7, 128.64, 128.55, 128.52, 128.48, 128.45, 128.35, 128.3, 128.23, 128.20, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4, 127.1, 126.9, 125.9 (C_{arom}), 99.9 (C-1´´), 99.8 (CHPh), 97.9 (C-1´´´), 96.8 (C-1´), 79.4 (C-4´´´), 78.8 (C-3´´´), 75.3 (C-2´´´), 75.0 (CH_2 4´´´-*O*-Bn), 73.9 (C-4´), 73.6 (C-4´´), 72.9 (C-3´´, CH_2 2´´´-*O*-Bn), 72.7 (C-3´), 72.0 (CH_2 3´´´-*O*-Bn), 71.3 (C-5´), 70.1 (CH_2 1-*O*-Bn), 69.2 (C-2´´), 69.1 (C-6´), 66.6 (C-5´´, C-5´´´), 61.3 (C-6´´), 54.1 (C-2´), 27.1 (CH_3 *t*Bu), 23.7 (CH_3 Ac), 19.7 (Cq *t*Bu), 16.4 (C-6´´´); HRMS(ESI) *m/z* calcd. for $[C_{85}H_{89}NO_{17}SiNa]^+$: 1446.5792, obsd.: 1446.5797.



Benzyl 2-acetamido-4-*O*-(4,6-O-benzylidene-β-D-galactopyranosyl)-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2-deoxy-α-D-glucopyranoside (12). To a solution of trisaccharide **11** (201 mg, 141 µmol) in methanol (3.0 mL) and CH_2Cl_2 (3.0 mL), 1M methanolic NaOMe (2.0 mL) was added and the reaction mixture was stirred at rt for 4 h. The reaction mixture was quenched by the addition of Dowex H^+ and the reaction mixture was filtered, washed with MeOH and concentrated *in vacuo*. The crude product was used in the next step without further purification. R_f = 0.57 (PE:EtOAc, 50:50, v/v); HRMS(ESI) *m/z* calcd. for $[C_{71}H_{81}NO_{15}SiNa]^+$: 1238.5268, obsd.: 1238.5276.; The crude trisaccharide was dissolved in pyridine (5 mL), HF.pyridine (0.5 mL) was added at 0 °C and the reaction mixture was stirred ar rt for 18 h. After TLC analysis showed

complete conversion, the mixture was diluted with CH_2Cl_2 (25 mL) and washed with 1M aq. $Ca(OAc)_2$ (50 mL). The aqeaous layer was extracted with CH_2Cl_2 (2 × 25 mL) and the combined organic extracts were washed with brine, dried with $MgSO_4$, filtered and concentrated *in vacuo*. The residue was co-evaporated with toluene (3 × 5 mL) in order to remove traces of pyridine, and purified by silica gel flash column chromatography (EtOAc:MeOH, 100:0 → 95:5, v/v) to afford triol **12** (137 mg, 140 µmol, 99%) as a white foam. R_f = 0.45 (EtOAc:MeOH, 8:1, v/v), $\alpha_D^{24.6}$ = -24.2 (c = 0.5, $CHCl_3$); IR (film) 3428, 3334, 3063, 3031, 2974, 2929, 2906, 2872, 1658, 1543, 1497, 1454, 1397, 1363, 1338, 1246, 1213, 1165, 1136, 1093, 1047, 966, 909, 858, 734, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.48 – 7.42 (m, 2H, CH_{arom}), 7.31 – 7.14 (m, 22H, CH_{arom}), 7.11 (t, 1H, *J* = 7.6 Hz, CH_{arom}), 7.00 (t, 2H, *J* = 7.6 Hz, CH_{arom}), 6.79 (d, 1H, $J_{NH,2'}$ = 7.2 Hz, NH), 5.47 (s, 1H, CHPh), 5.38 (d, 1H, $J_{1'',2''}$ = 3.2 Hz, H-1´´), 4.98 (d, 1H, $J_{1''',2'''}$ = 3.4 Hz, H-1´´´), 4.80 (d, 1H, $J_{1',2'}$ = 7.6 Hz, H-1´), 4.70 – 4.62 (m, 3H, CH_2a 2-*O*-Bn, CH_2a 3-*O*-Bn, CH_2a 4-*O*-Bn), 4.60 (d, 1H, $J_{a,b}$ = 10.4 Hz, CH_2b 2-*O*-Bn), 4.55 (d, 1H, $J_{1a,1b}$ = 12.0 Hz, CH_2a-1-*O*-Bn), 4.52 (d, 1H, $J_{a,b}$ = 11.7 Hz, CH_2b 3-*O*-Bn), 4.35 (d, 1H, $J_{1a,1b}$ = 12.0 Hz, CH_2b-1-*O*-Bn), 4.31 – 4.20 (m, 2H, H-6a´´, H-5´´´), 4.16 (dd, $J_{2',3'}$ = 9.1 Hz, $J_{3',4'}$ = 10.2 Hz, H-3´), 4.10 – 4.03 (m, 2H, H-4´, H-4´´), 4.00 (bd, $J_{6a',6b'}$ = 12.2 Hz, H-6a´), 3.98 – 3.90 (m, 3H, H-2´, H-2´´, H-6b´´), 3.84 (dd, 1H, $J_{3''',4'''}$ = 2.5 Hz, $J_{2''',3'''}$ = 10.0 Hz, H-3´´´), 3.74 – 3.64 (m, 3H, H-2´´, H-5´, H-6b´), 3.59 – 3.51 (m, 2H, H-4´´´, H-5´´), 3.51 – 3.44 (m, 2H, H-3´´, OH), 2.85 (bs, 1H, OH), 1.70 (bs, 2H, 2 × OH), 1.45 (s, 3H, CH_3 Ac), 1.04 (d, 3H, $J_{5''',6'''}$ = 6.6 Hz, H-6´´´); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3 (C=O, Ac), 138.9, 137.8, 137.5, 137.5 (CH_{arom}), 129.2, 128.8, 128.6, 128.51, 128.50, 128.33, 128.28, 128.10, 128.06, 128.0, 127.7, 127.3, 126.5 (CH_{arom}), 102.3 (C-1´´), 101.1 (CHPh), 98.6 (C-1´´´), 96.3 (C-1´), 79.4 (C-3´´´), 77.7 (C-4´´´), 77.5 (C-2´´´), 76. 7 (C-3´), 75.6 (C-4´), 75.3 (C-4´´), 75.1 (CH_2 4-OBn), 74.7 (CH_2 2-OBn), 73.2 (C-2´´), 72.8 (C-5´´), 72.2 (CH_2 3-OBn), 71.1 (C-5´), 69.9 (CH_2-1), 69.5 (C-6´´), 67.5 (C-5´´´), 67.0 (C-3´´), 61.0 (C-6´), 54.1 (C-2´), 22.8 (CH_3 Ac), 17.1 (C-6´´´); HRMS(ESI) *m/z* calcd. for $[C_{55}H_{64}NO_{15}]^+$: 978.4270, obsd.: 978.4274.



2-Acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-(β-D-galactopyranosyl))-D-glucopyranoside (Le^x). To trisaccharide **12** (137 mg, 140 µmol) in

distilled THF (4 mL), H_2O (2 mL), AcOH (1.0 mL) and Pd/C (100 mg) were added and hydrogen gas was bubbled though the reaction mixture at rt for 3 h. The crude mixture was diluted with water (20 mL), filtered over paper (Whatman 42) and lyophilised. Purification by size exclusion chromatography (BioGel P-2, 1200 × 10 mm) gave Le^X (72.5 mg 137 µmol, 98%) as a white foam. R_f = 0.12 (*n*-butanol:AcOH:H_2O, 4:1:1, v/v/v); IR (film) 3313, 2971, 2938, 1640, 1555, 1427, 1378, 1162, 1118, 1071, 1034, 1021, 968, 811 cm^{-1}; ^1H NMR (300 MHz, D_2O) *α-anomer:* δ 5.11 – 5.08 (m, 2H, H-1´, H-1), 4.84 (q, 1H, $J_{5´´,6´´}$ = 6.7 Hz, H-5´´), 4.46 (d, 1H, $J_{1´,2´}$ = 7.8 Hz, H-1´ α), 4.15 (dd, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.4 Hz, H-2 α), 4.03 – 3.92 (m, 4H, H-3, H-4, H-5, H-6a), 3.92 – 3.81 (m, 8H, H-6b, H-3´´, H-4´), 3.79 (d, 1H, $J_{3´´,4´´}$ = $J_{4´´,5´´}$ = 3.0 Hz, H-4´´), 3.77 – 3.62 (m, 4H, H-6´a, H-6´b, H-2´´, H-3´), 3.62 – 3.57 (m, 1H, H-5´), 3.53 – 3.46 (m, 1H, H-2´), 2.03 (s, 3H, CH_3 Ac), 1.19 – 1.15 (m, 3H, H-6´´); *β-anomer:* δ 5.11 – 5.08 (m, 1H, H-1´), 4.84 (q, 1H, $J_{5´´,6´´}$ = 6.7 Hz, H-5´´), 4.72 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1 β), 4.45 (d, 1H, $J_{1´,2´}$ = 7.8 Hz, H-1´ β), 4.03 – 3.92 (m, 2H, H-4, H-6a), 3.92 – 3.81 (m, 5H, H-6b, H-3´´, H-4´, H-2, H-3), 3.79 (d, 1H, $J_{3´´,4´´}$ = $J_{4´´,5´´}$ = 3.0 Hz, H-4´´), 3.77 – 3.62 (m, 4H, H-6´a, H-6´b, H-2´´, H-3´), 3.62 – 3.57 (m, 2H, H-5, H-5´), 3.53 – 3.46 (m, 1H, H-2´), 2.03 (s, 6H, CH_3 Ac), 1.19 – 1.15 (m, 6H, H-6´´); ^{13}C NMR (125 MHz, D_2O) δ 174.4 (C=O β), 174.2 (C=O β), 101.76 (C-1´ β), 101.74 (C-1´ α), 98.6 (C-1´´ β), 98.5 (C-1´´ α), 94.7 (C-1 β), 91.0 (C-1 α), 75.4 (C-5 β), 74.91 (C-3 β), 74.85 (C-5´ α + β), 73.2 (C-4 α+β), 72.8 (C-3 α), 72.4 (C-3´ α+β), 71.8 (C-4´´ α+β), 71.3 (C-5 α), 71.0 (C-2´ α+β), 69.20 (C-3´´ α), 69.15 (C-3´´ β), 68.3 (C-4´ α+β), 67.6 (C-2´´ α+β), 66.6 (C-5´´ α+β), 61.5 (C-6´ α+β), 59.7 (C-6 β), 59.6 (C-6 α), 56.9 (C-2 β), 54.0 (C-2 α), 22.2 (CH_3 Ac β), 21.9 (CH_3 Ac α), 15.2 (C-6´´ α+β); HRMS(ESI) *m/z* calcd. for $[C_{20}H_{36}NO_{15}]^+$: 530.2079, obsd.: 530.2093.

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Highlights

A crystalline orthogonally protected LewisX trisaccharide was synthesised

Sequential regioselective glycosylation of a GlcNAc acceptor with galactose and fucose donors provided the trisaccharide

The efficient total synthesis of Lewis^{X} was completed

Supporting Information

Total synthesis of Lewis^X using a late-stage crystalline intermediate

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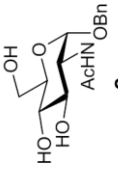
^{1}H-NMR (500 MHz, CD_3OD)



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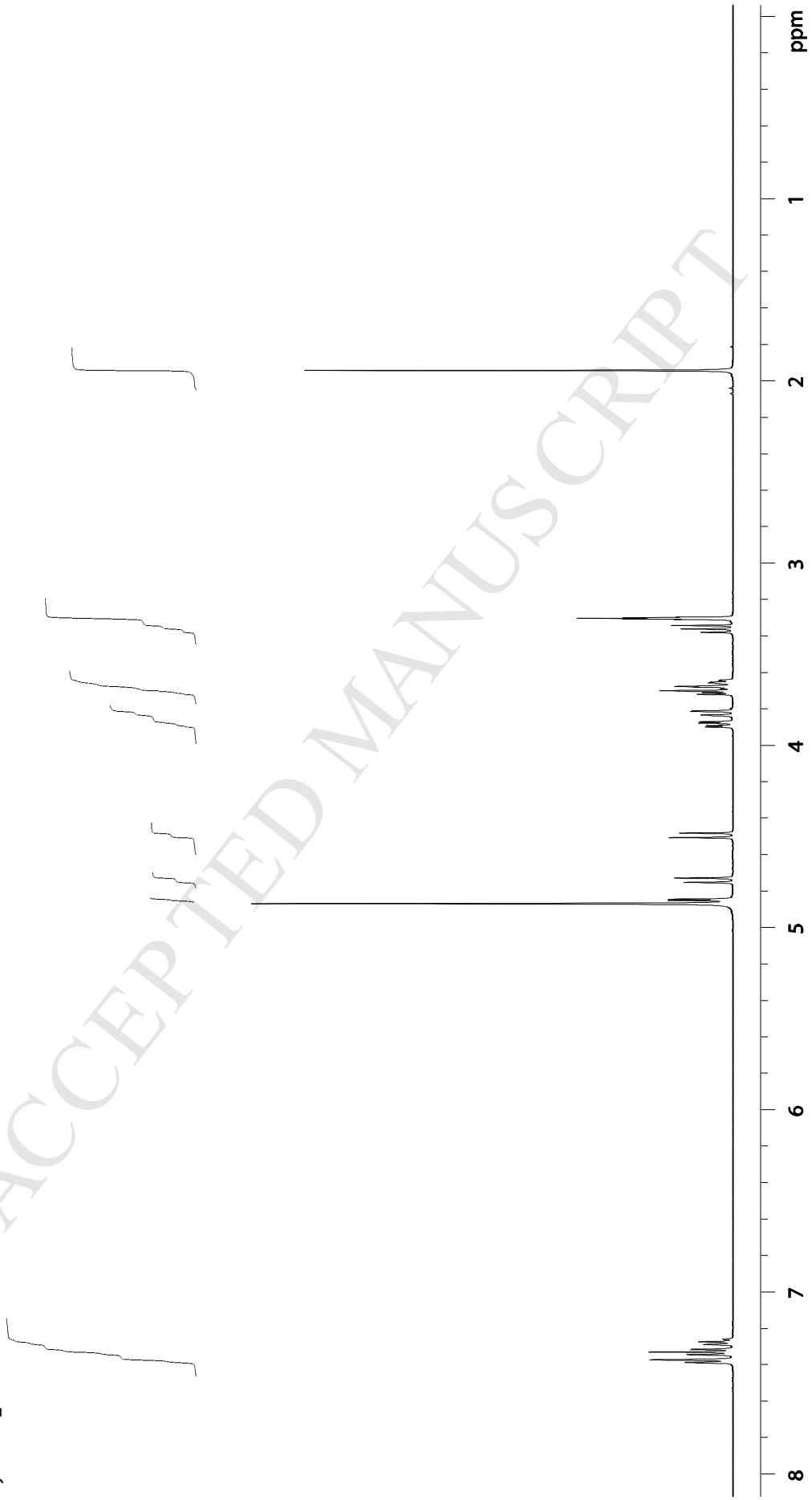

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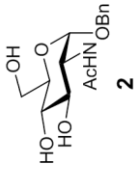

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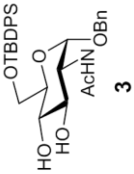

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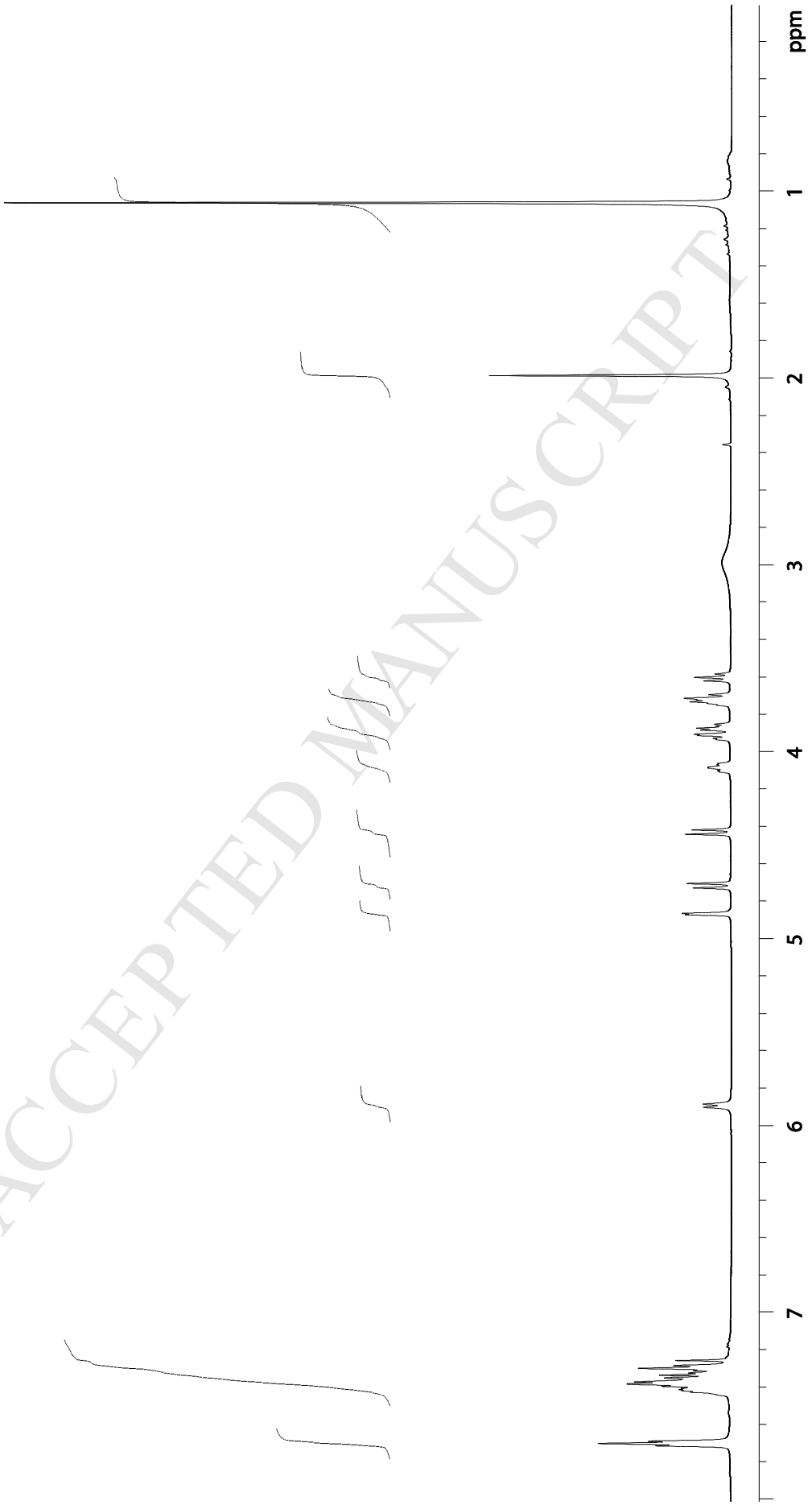

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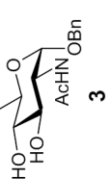

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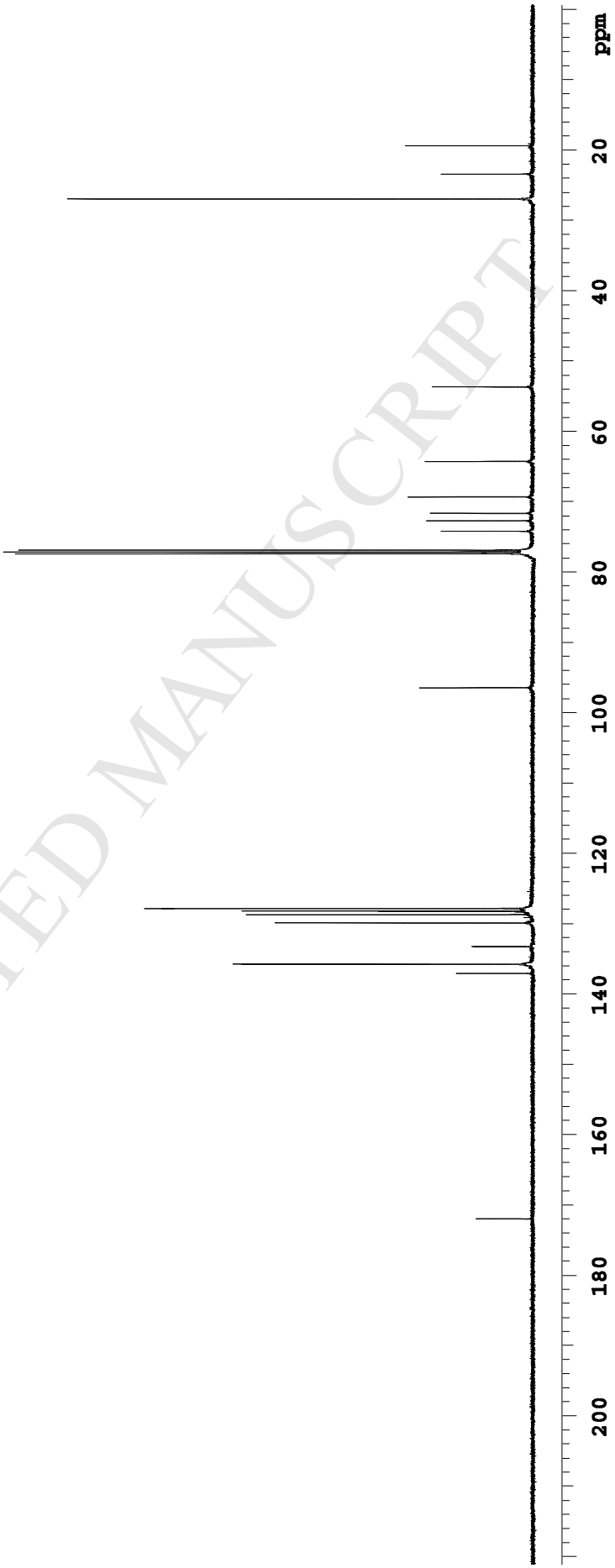

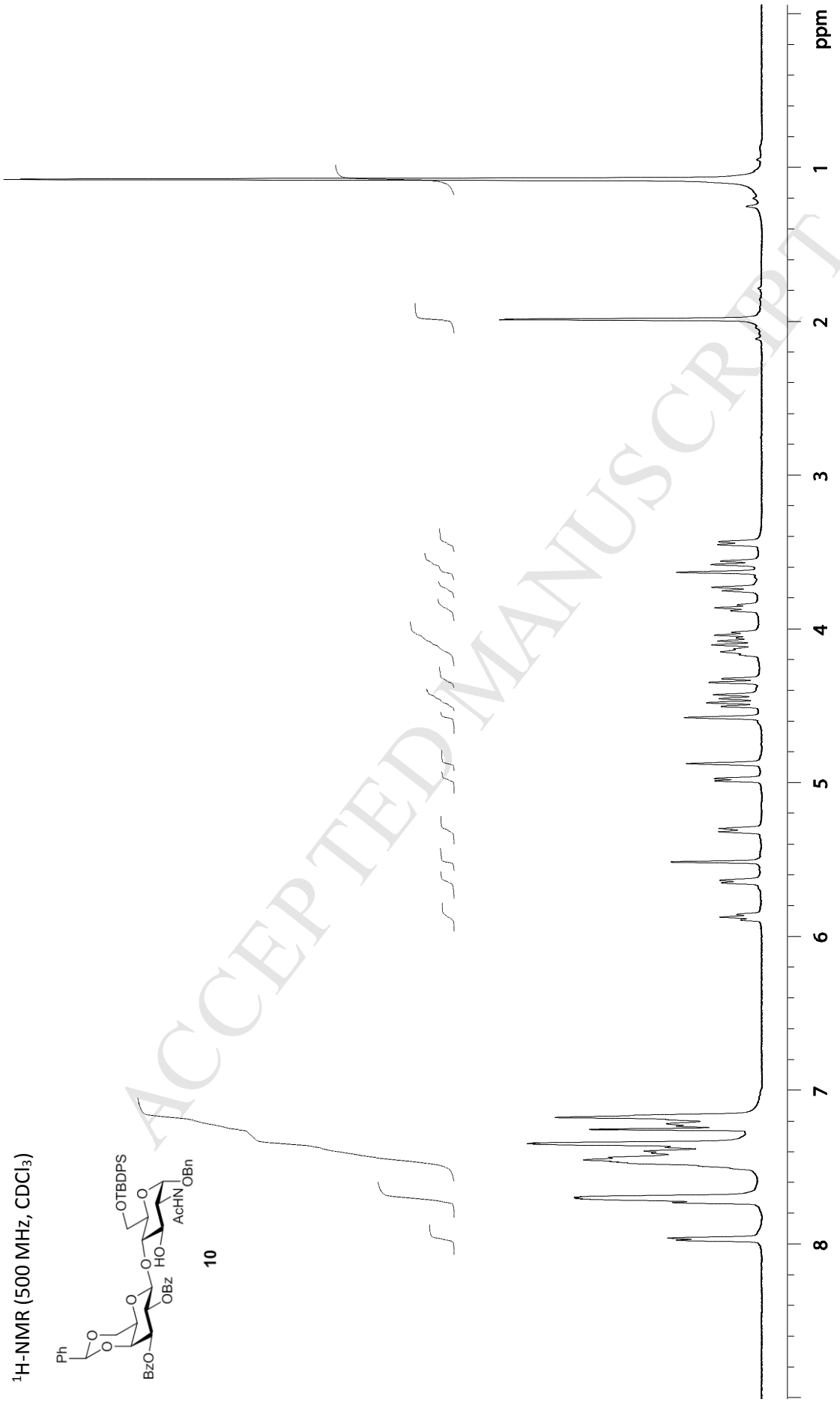

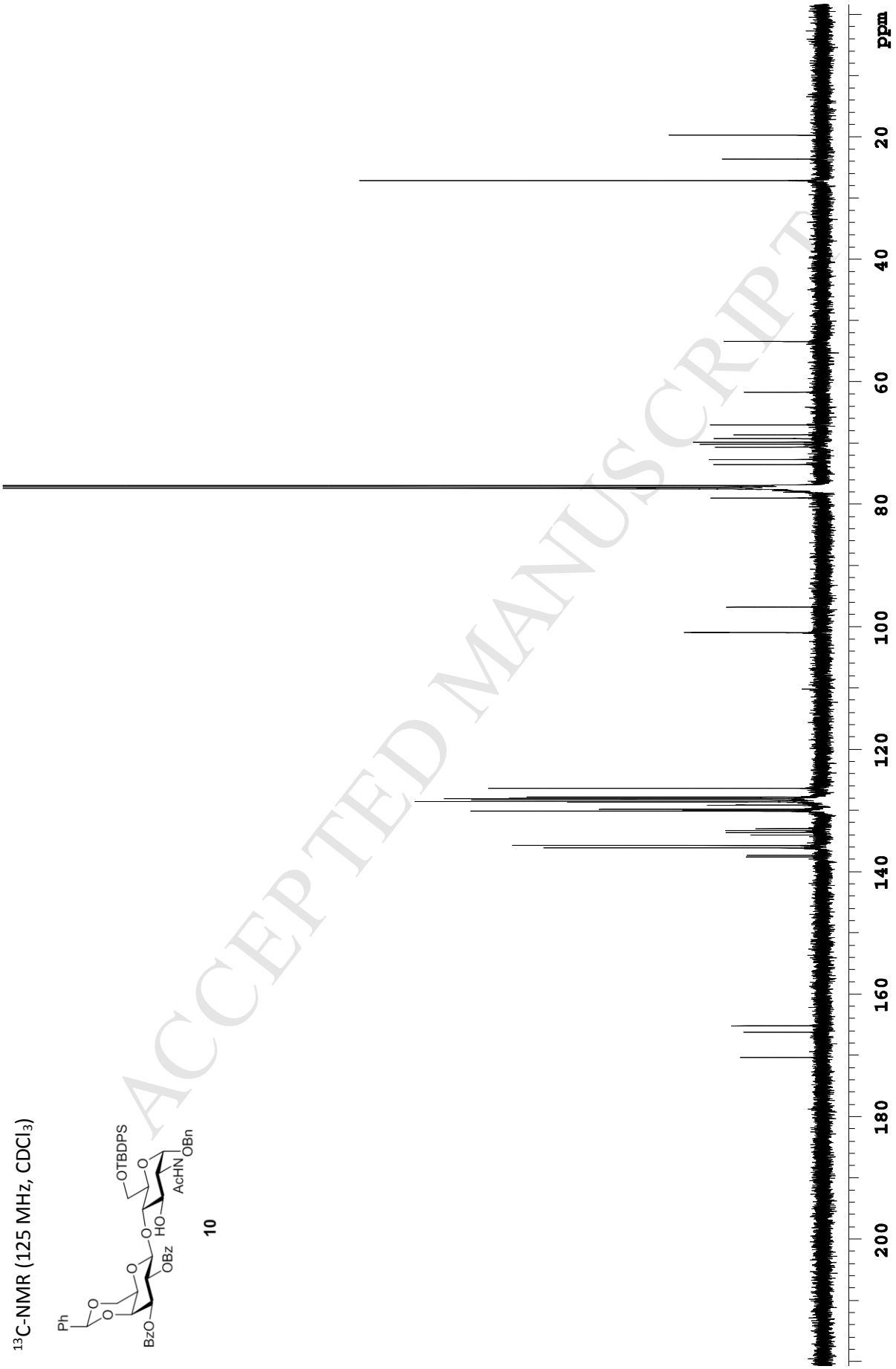

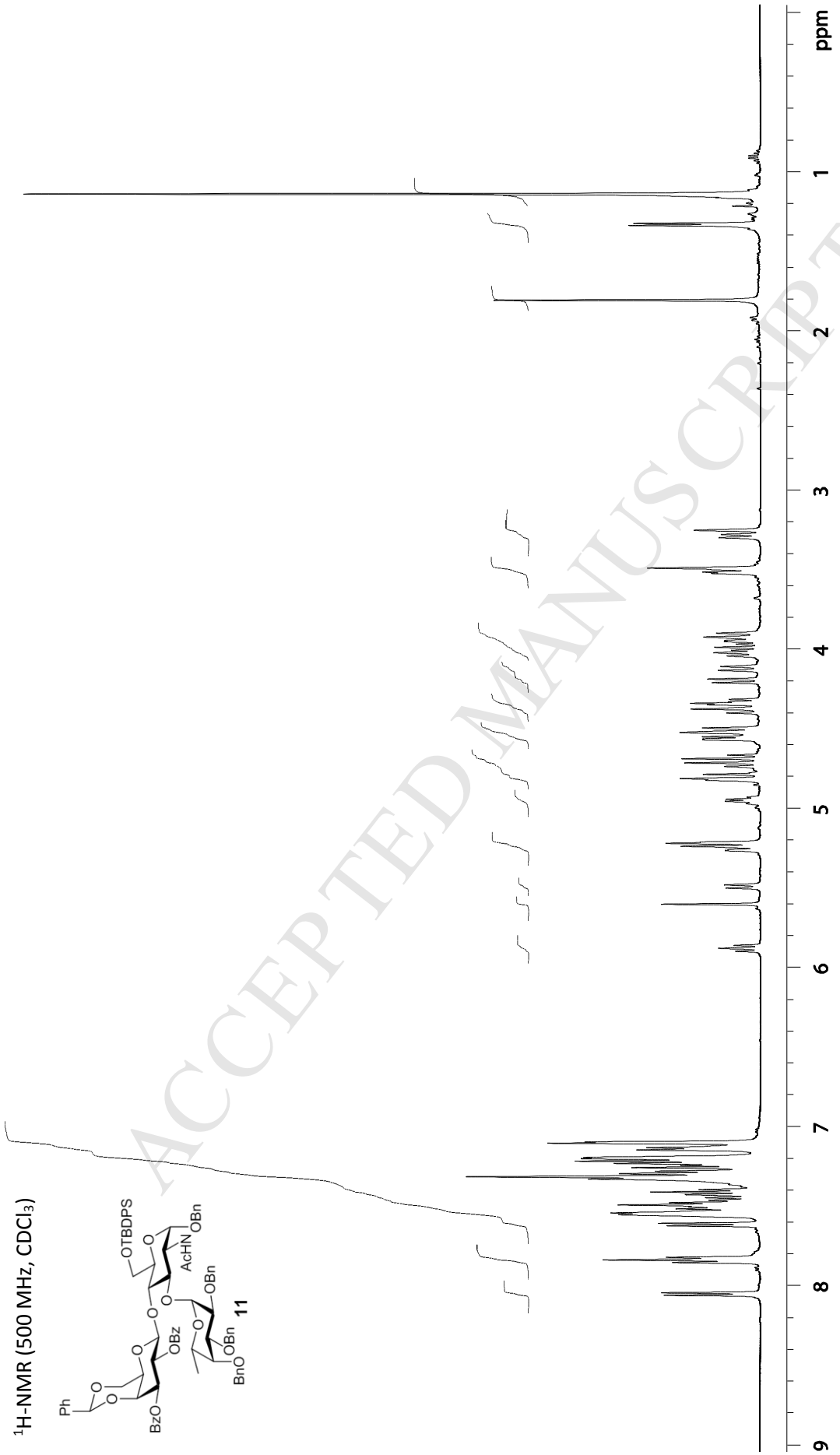

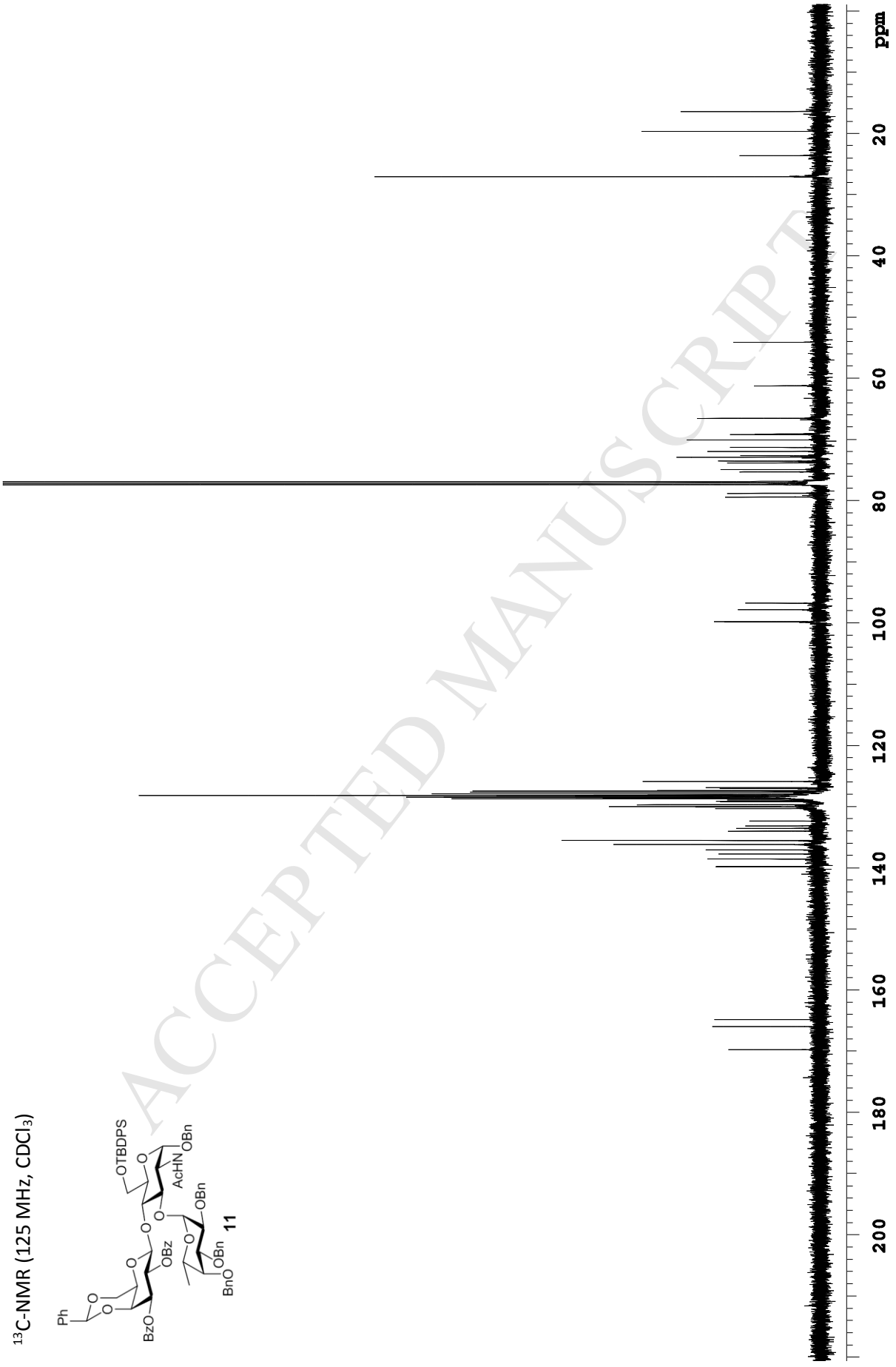

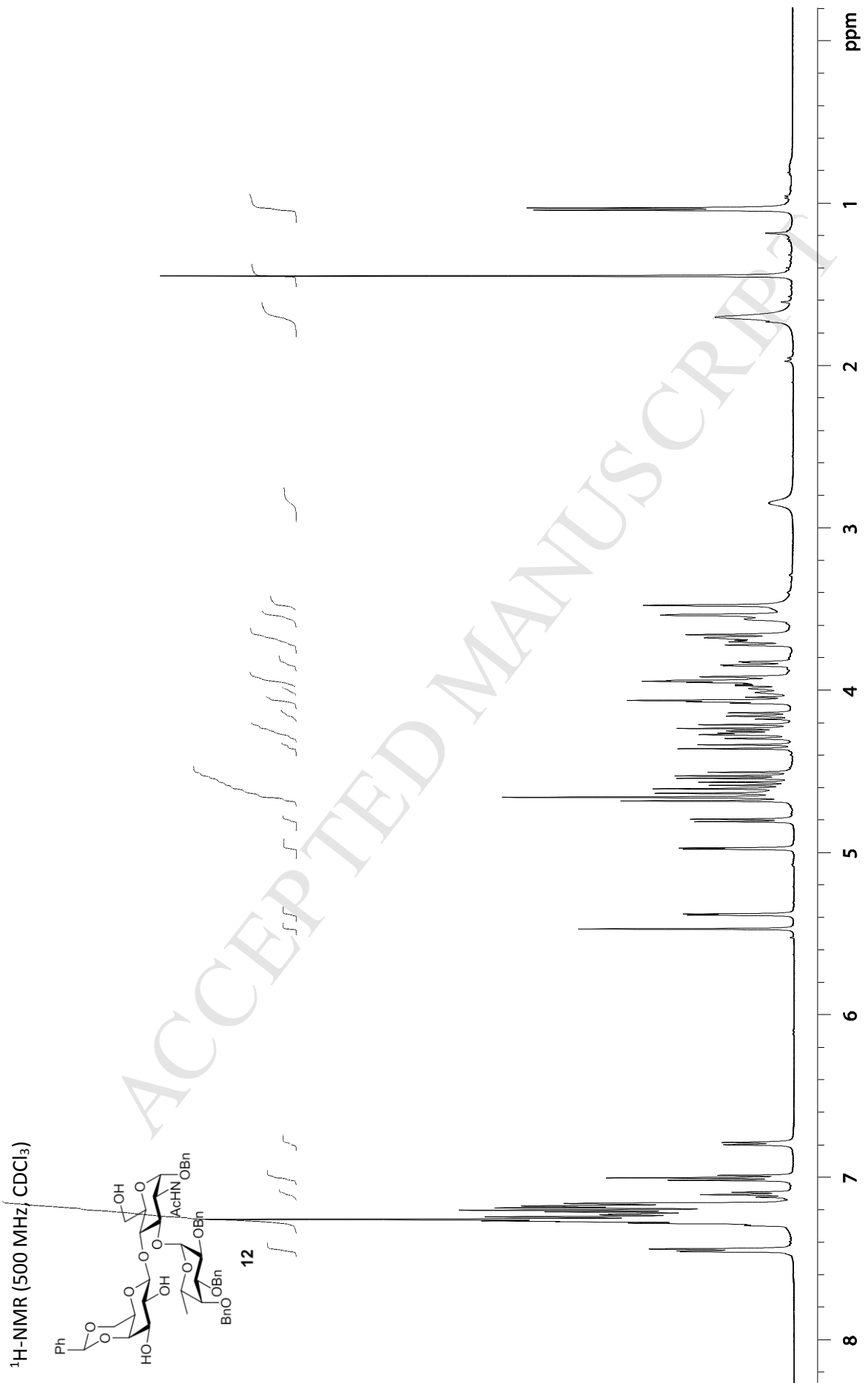

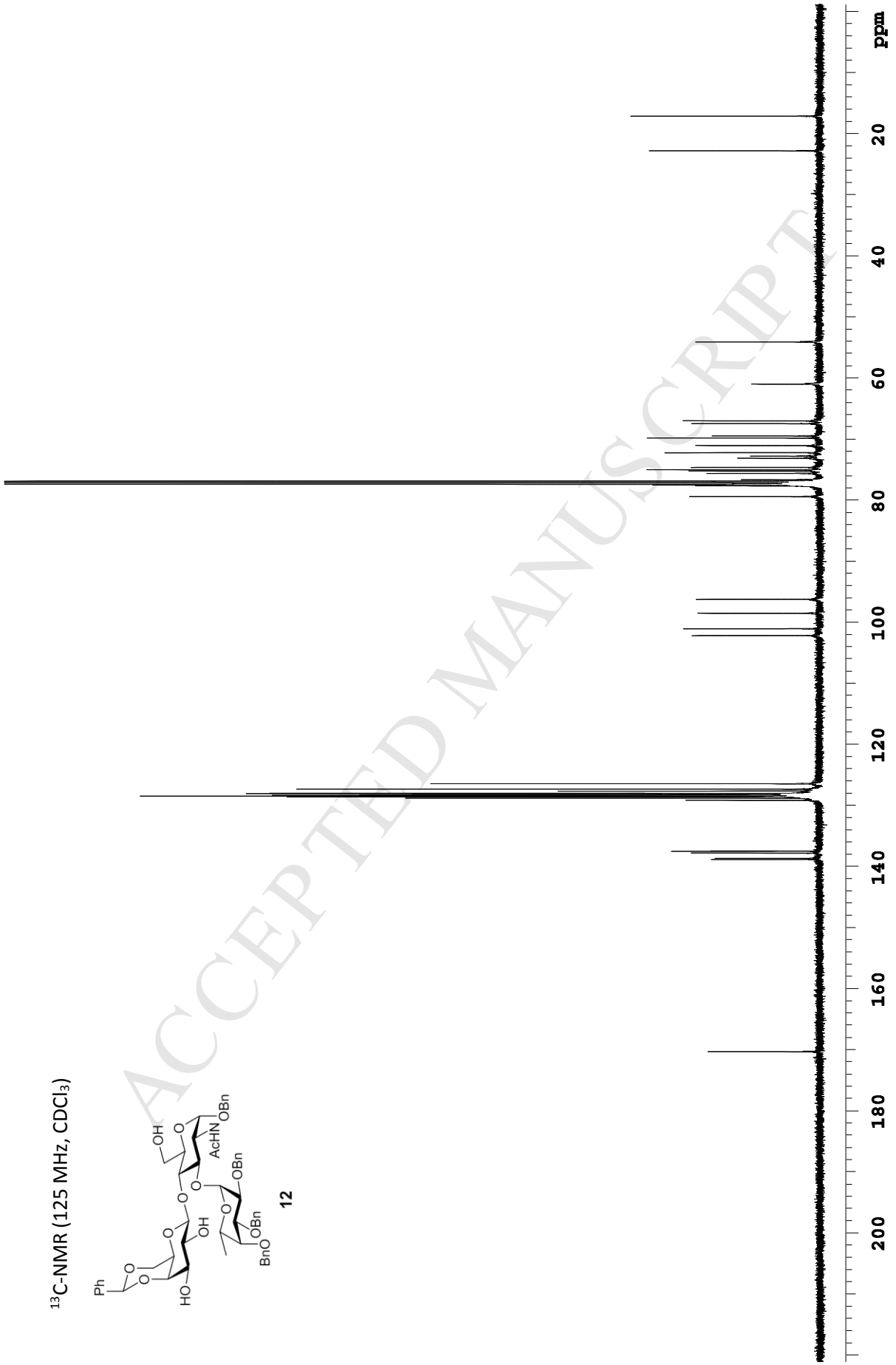

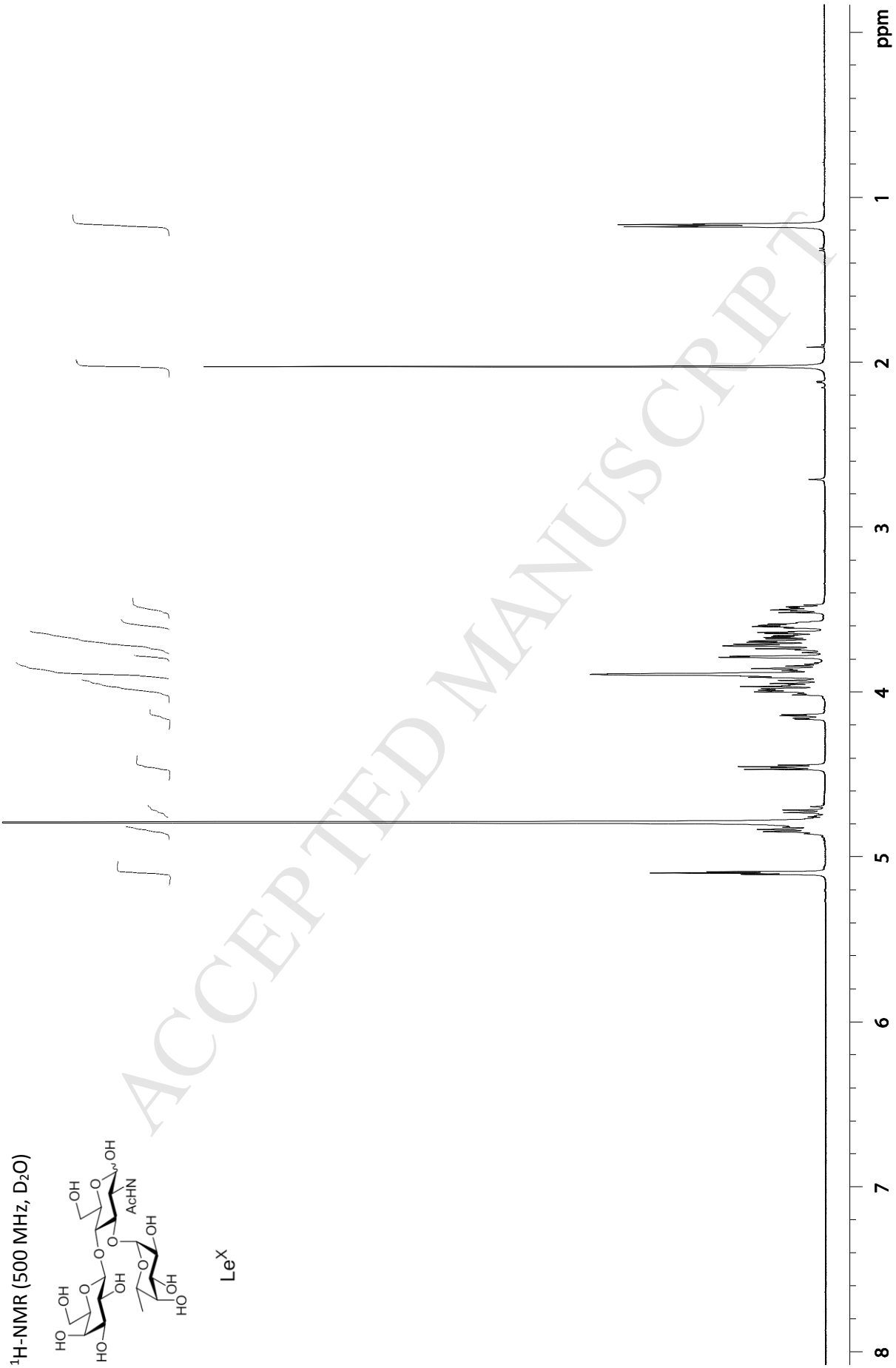

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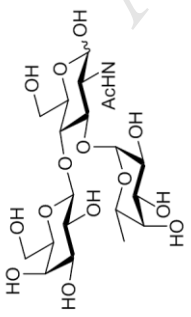

Le^X