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Design, modeling and synthesis of functionalized paromamine analogs

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Abstract—Based on the published NMR structure of the antibiotic paromomycin complexed with the A-site of *Escherichia coli* 16S ribosomal RNA, a set of C-5 functionalized analogs of paromamine was designed and synthesized, incorporating cyclic and acyclic appendages and terminating with one or more ammonium groups.

1. Introduction

Aminoglycoside antibiotics are a group of clinically important antibacterial drugs which exert their function through specific binding to prokaryotic ribosomal RNA, thus affecting protein biosynthesis.[1] However, their widespread use over the last decades has been significantly compromised by oto- and nephrotoxicity,[2] and the rapid emergence of bacterial resistance.[3] A major breakthrough in this area of research was the discovery that the pseudo-trisaccharide butyrosin,[4] in which N-1 of the deoxystreptamine moiety was acylated with 4-amino-2(*S*)-hydroxyaminobutyric acid, was less susceptible to inactivating enzymes, while maintaining potency. Armed with this information the chemical modification of the existing aminoglycosides has resulted in the development of clinically useful antibiotics such as amikacin.[5]

The chemistry of aminoglycoside antibiotics remained in a state of hiatus for a number of years, in part due to the emergence of other classes of potent antibacterial agents such as carbapenems[6] and quinolones.[7] In spite of the polyfunctional nature of aminoglycosides, a great deal of chemical know-how has been developed over the years in order to allow selective functionalizations. The recent achievements in understanding the interactions of aminoglycosides with RNA[8] have rekindled interest in the design of mimetics and analogs that may simulate the biological activity of these compounds.[9–11] Efforts have been made to design analogs to overcome bacterial resistance to aminoglycosides.[12]

Past experience in our laboratory has led to the synthesis of a number of aminoglycoside analogs to probe functional and structural requirements for antibacterial activity.[13] We now report on the synthesis of a set of branched paromamines, the pseudo-disaccharide corresponding to paromomycin but lacking the D-ribose and the 2,6-deoxy-2,6-diamino-L-idose units.[14]

2. Library design

The A-site of the prokaryotic 16S ribosomal RNA has been localized as the binding site for the group of 4,5-disubstituted deoxystreptamines, including the antibiotics neomycin and paromomycin (Fig. 1).[8]

Moreover, the solution structure of the A-site of *E. coli* 16S ribosomal RNA complexed with paromomycin has been determined by NMR spectroscopy.[15] According to this elegant analysis, paromomycin binds in the major groove of the A-site within the internal loop (Fig. 2a). Rings I and II of paromomycin fit into the pocket created by an asymmetrical bulge, and make specific contacts with the nucleic acid bases and phosphate residues. Rings III and IV, on the other hand, extend along the major groove, and their contribution to the binding event is less important as no specific contacts with RNA are observed. This is also corroborated by the observation that ribostamycin, a pseudo-trisaccharide related to neomycin but lacking ring IV, and neamine itself

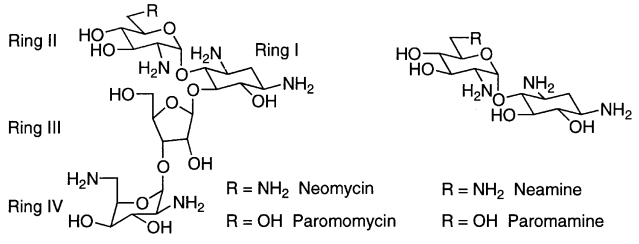

Figure 1.

Keywords: aminoglycoside; paromomycin; RNA; library; modeling.

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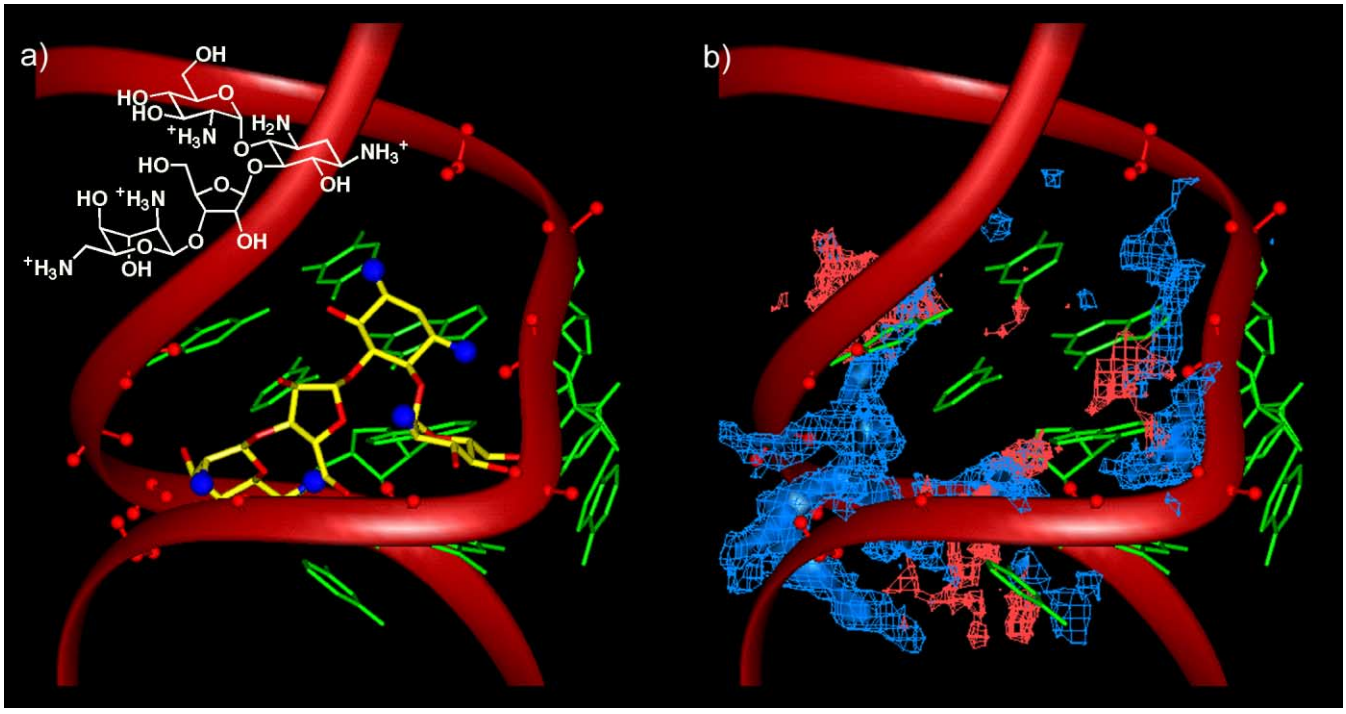


Figure 2. (a) NMR determined structure of paromomycin–RNA complex according to Puglisi.[15] (b) GRID mapping of the groove; energetically favored spaces for ammonium groups (blue) and for hydroxyl groups (red).

form similar complexes with the A-site RNA.[8] Modeling studies of RNA–aminoglycoside complexes have been reported by Cedergren[16]and Westhof.[17] Very recently, the crystal structure of the 30S subunit of ribosomal RNA from *Thermus thermophilus* complexed with paromomycin has been described, providing interesting insights into the binding of each residue.[18]

When we used the GRID program[19] to map the binding site of RNA, using the structures already established by Puglisi and coworkers,[15] we found that ammonium groups would occupy energetically favorable sites on the region normally occupied by ring IV of paromomycin, and surrounded by numerous phosphate groups. We reasoned that by replacing rings III and IV of paromomycin with appendages carrying terminal ammonium groups, we could perhaps mimic their functional and spacial requirements vis-à-vis the A-site RNA. This might also lead to simpler structures possibly possessing promising antibacterial activities. Simple aminols that bind RNA as surrogates for aminoglycoside antibiotics have been reported by Tok and Rando.[20]

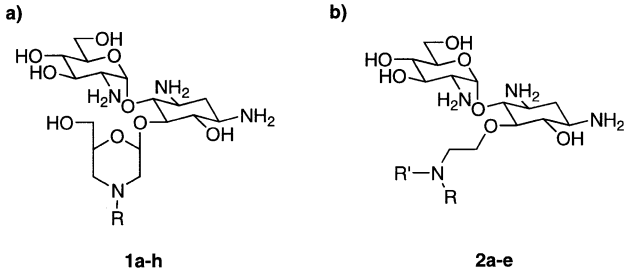


Figure 3.

We chose to synthesize a series of C-5 substituted paromamines which carried cyclic and acyclic appendages containing amino groups (Fig. 3a, b) which could make electrostatic contacts with the phosphate groups of the A-site nucleic acid backbone. To this end, we considered morpholines as conformationally constrained mimics of the ribosyl ring, and linear primary amines branched at C-5 as acyclic equivalents. Morpholino analogs of dibekacin have been reported by Tsuchiya and coworkers[21].

The proposed interactions of a 5-(guanidinoethylmorpholino)glycoside, and a 5-(guanidinoethylamino)ethoxy ether of paromamine with the A-site nucleic acid backbone based on the original Puglisi model[15] are shown in Fig. 4.

3. Results and discussion

Ring IV of paromomycin was removed via a sequence of reactions involving a β-elimination as a key step following our published work of some years ago (Scheme 1).[22] The periodic acid cleavage of the diol in **3** gave dialdehyde **4**, which was not purified due to its instability, but directly subjected to a reductive coupling with a variety of primary amines (Scheme 2). The yields of cyclic amines were somewhat modest due to the formation of open chain diamines and the reactions required carefully selected conditions. By adjusting the pH to 6, employing high dilution conditions and slow addition of an amine to a solution of **4** in MeOH/DMF we were able to obtain the desired morpholines **5a**–**h**. The monoprotected amines were synthesized by conventional methods[23] and the protected guanidinoethylamine was prepared using Goodman's methodology.[24] The β-(2-aminoethyl)glucoside precursor to **5f** was synthesized by using a glycosylation method developed in our group.[25a]

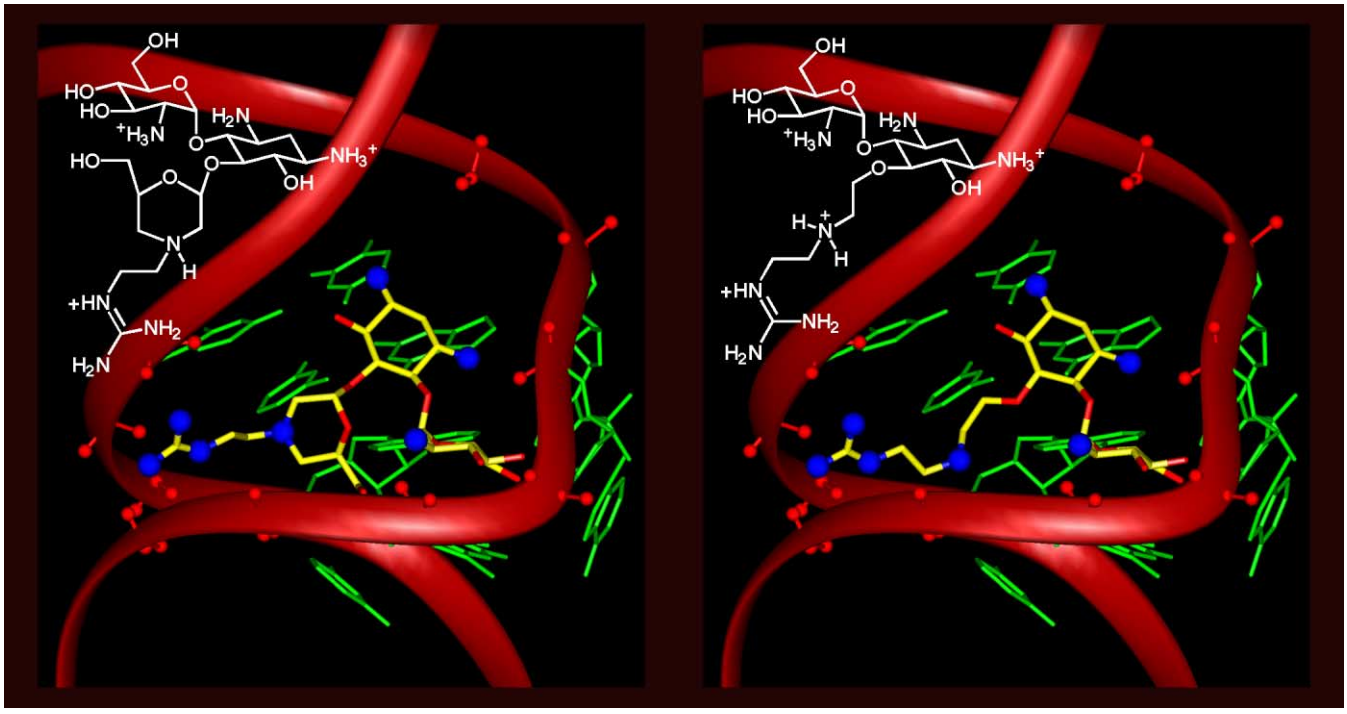
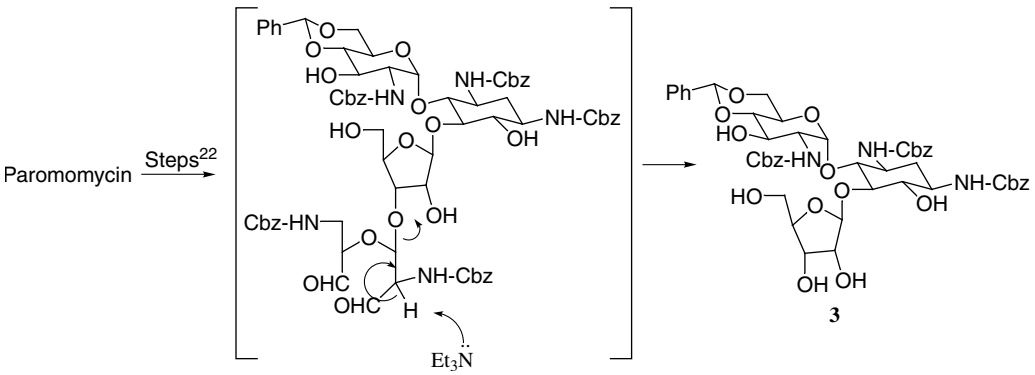


Figure 4. Proposed docked structures for two representative paromamine derivatives, as tetra trifluoroacetate salts.

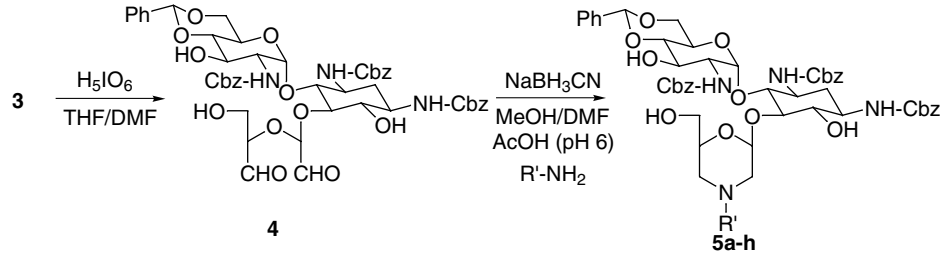
Thus, compound **6** was benzoylated, and the product **7** was treated with *N*-Cbz ethanolamine in the presence of $HBF_4{\cdot}Et_2O$ to afford **8** as a single isomer in a good yield (Scheme 3).[25b] The benzoates were removed with cat. NaOMe in MeOH to give **9** and the Cbz group was hydrogenolyzed to afford **10**, which was used for the reductive amination with **4**.

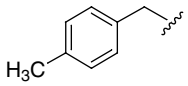
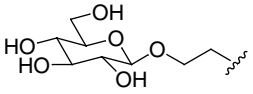
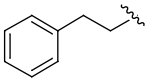
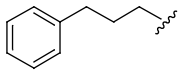
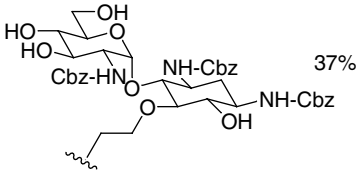
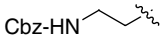
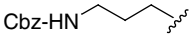
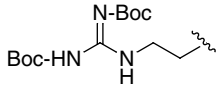
Deprotection of **5a**–**h** involved the removal of the benzylidene group with hot aqueous acetic acid followed by hydrogenolysis over 20% palladium hydroxide on carbon to afford the extended paromamine analogs **1a**–**h** representing a cross-section of appendages, some of which were guided by our modeling studies (Scheme 4).

For the synthesis of C-5 alkylamino ether analogs of paromamine, rings III and IV of paromomycin were chemically removed, and the three amino groups of the resulting pseudo-disaccharide were protected as benzyl carbamates to afford **11**, following an earlier report (Scheme 5).[26] We then took advantage of our previous observation that the C-5 hydroxyl was resistant towards acetylation in a series of similarly protected paromamines.[13b] Gratifyingly, acetylation of **11** led to the same high level of regioselectivity, affording the tetraacetate **12** in acceptable yield. The regiochemistry was confirmed by COSY analysis after hydrogenolysis. *O*-Allylation[14a] of **12** afforded **13** without ester migration as ascertained by COSY analysis of a derivative. Ozonolysis of **13** gave **14**, which upon reductive coupling with a variety of *N*-protected primary alkylamines, secondary amines, and alkylguanidines provided fully protected disaccharides **17a**–**e** in good yields (Scheme 6). Compound **13** was also subjected to ozonolysis followed by reduction of the resulting aldehyde with sodium borohydride (Scheme 5). The primary alcohol was mesylated and replaced by azide to give **15** in good overall yield.



Scheme 1.



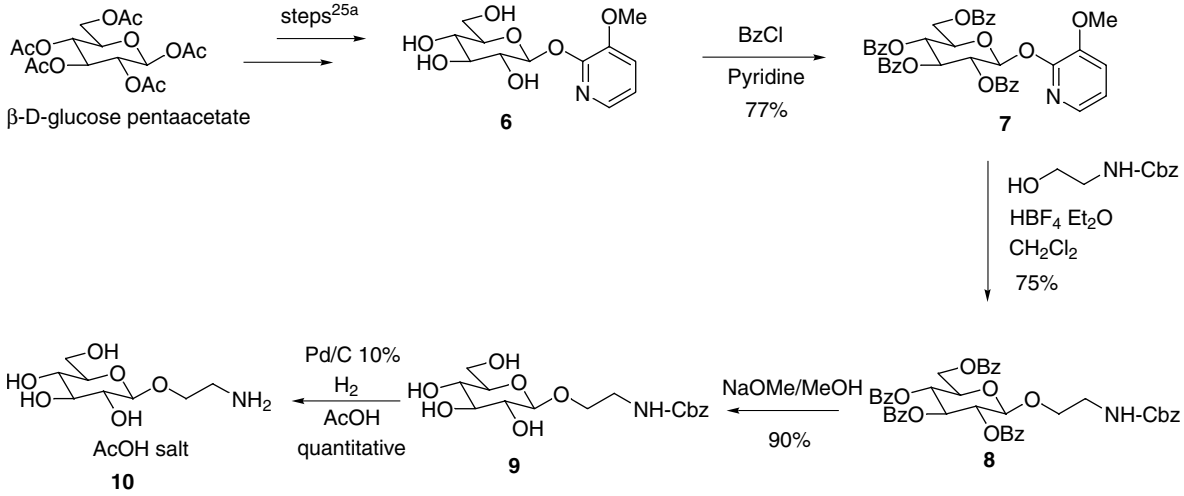
Product	R'	Yield	Product	R'	Yield
5a		26%	**5f**		42%
5b		20%			
5c		25%	**5g**		37%
5d		30%			
5e		41%	**5h**		47%

Scheme 2.

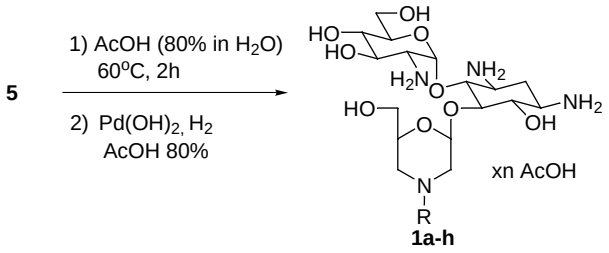
Finally, the acetates were removed with cat. NaOMe in MeOH and the azide was reduced with PPh_3/H_2O to give **16** that was used to provide **5g** (Scheme 2).

Deprotection of compounds **17a–e** with catalytic NaOMe in MeOH followed by hydrogenolysis led to the analogs **2a–e** (Scheme 7).

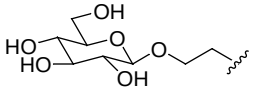
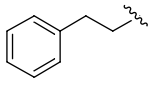
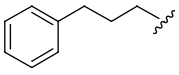
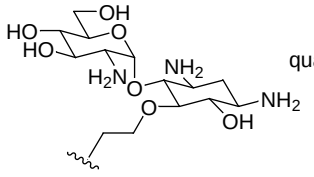
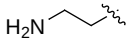
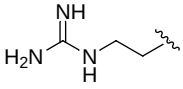
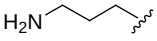
In conclusion, we have reported the preparation of a series of C-5 substituted paromamine derivatives representing a small library of paromomycin mimetics based on computer-aided design. In one series, the C-5 appendage consisted of a morpholino glycoside as a pseudo-sugar, in which the nitrogen atom was functionalized with a variety of chains deployed with hydrophobic or basic end-groups such as compounds **1a–e**, **1h** as well as dimeric aminoglycosides exemplified by **1f** and **1g** (Scheme 4). In another series, the C-5 appendage consisted of ω-amino or ω-guanidino alkyl ethers varying in



Scheme 3.



5 → **1a-h**

Starting Material	Product	R	Yield	Starting Material	Product	R	Yield
5a	**1a**	H	quant.	**5f**	**1f**		quant.
5b	**1b**		quant.				
5c	**1c**		quant.	**5g**	**1g**		quant.
5d	**1d**		quant.	**5h**	**1h**		quant.[a]
5e	**1e**		quant.				

Scheme 4. [a] Deprotection conditions involved: (1) TFA/H_2O (10:1) 2 h, (2) $H_2Pd(OH)_2$ in AcOH (80% in H_2O).

length such as compounds **2a**–**c**. This series also included an *N*-piperazinoethyl ether analog **2d** (Scheme 7).

Disappointingly, none of the above analogs exhibited any antibacterial activity when tested against a panel of resistant and sensitive strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus faecalis* at 50 μg/mL. Surprisingly, very closely related analogs based on the neamine core structure (Fig. 1) have been reported to exhibit significant antibacterial activity against *E. coli*.[10b,14a] Clearly, there is much to be learned about the functional and spacial requirements for effective ribosomal binding, as opposed to modeling based on an RNA substructure, in the design of such truncated aminoglycoside mimetics. The binding of our analogs to various RNAs, though of academic interest now, will be reported in due course.

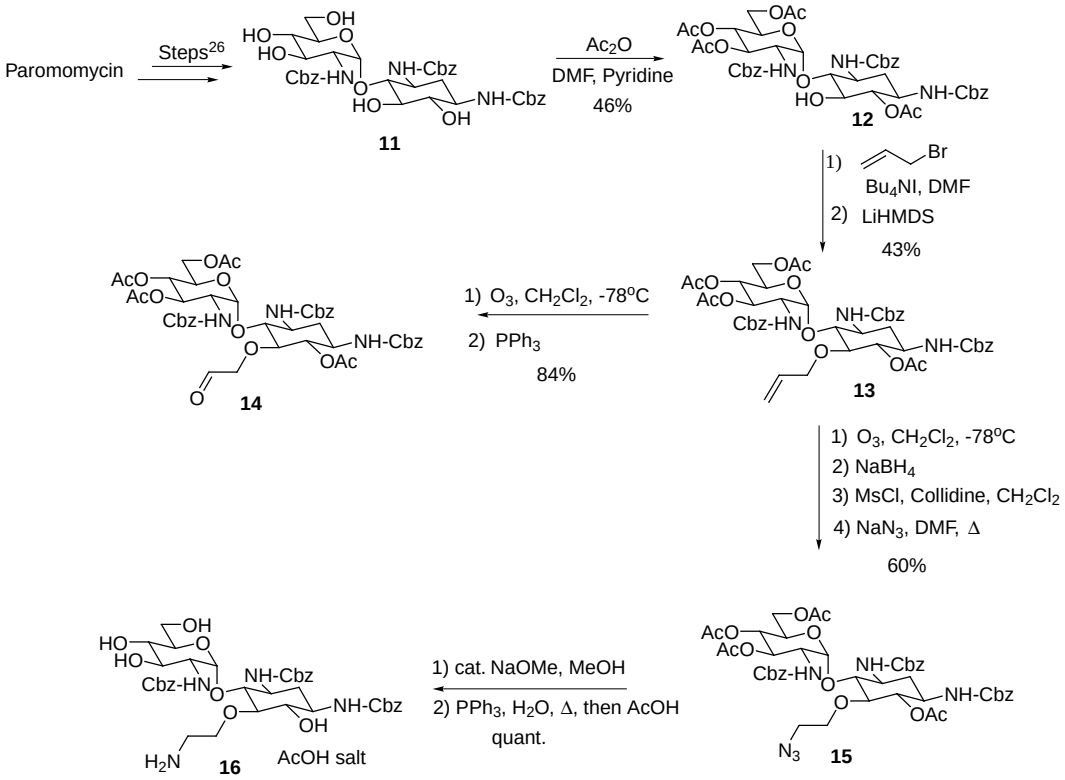
4. Experimental

4.1. General information

Solvents were distilled under positive pressure of dry nitrogen before use and dried by standard methods; THF and ether, from Na/benzophenone; and CH_2Cl_2, from $CaCl_2$. All commercially available reagents were used without further purification. All reactions were performed under nitrogen atmosphere. NMR (^1H, ^{13}C) spectra were recorded on AMX-300, ARX-400 and DMX-600 spectrometers. Low- and high-resolution mass spectra were recorded on VG Micromass, AEI-MS 902 or Kratos MS-50 spectrometers using fast atom bombardement (FAB) or electrospray techniques. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter in a 1 dm cell at ambient temperature. Analytical thin-layer chromatography was performed on Merck $60F_{254}$ pre-coated silica gel plates. Visualization was performed by ultraviolet light and/or by staining with ceric ammonium molybdate or ninhydrine. Flash column chromatography was performed using (40–60 μm) silica gel at increased pressure.

4.2. General procedure for reductive amination (5a–h)

To a suspension of **3** (0.21 mmol) in THF (5 mL) at 0°C was added DMF (0.5 mL) until complete dissolution. After addition of periodic acid (0.32 mmol) and stirring for a few minutes, a white precipitate appeared. The reaction mixture was stirred overnight at 5°C then evaporated under vacuum and the resulting white solid was partitioned between water and EtOAc. The aqueous layer was extracted with EtOAc, and the combined organic extracts were dried over Na_2SO_4. The solvent was evaporated to give a white solid. The crude dialdehyde was suspended in MeOH (12 mL) and DMF (2 mL) was added until complete dissolution, followed by $NaBH_3CN$ (55 mg, 0.87 mmol). A solution containing the appropriate amine (0.17 mmol) and AcOH (0.90 mmol) in MeOH (2 mL) was added to the reaction mixture over a period of 1 h. After stirring at room temperature for 1 h, a few drops of Et_3N were added and the solvents were

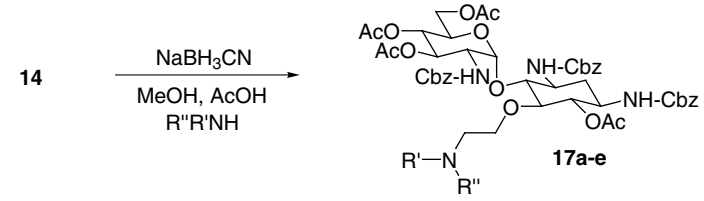


Scheme 5.

removed under vacuum. Na_2CO_3 (satd., 30 mL) was added, the suspension was extracted with EtOAc and the combined organic extracts were dried over Na_2SO_4. The mixture was concentrated under vacuum and the residue was purified by flash chromatography (CH_2Cl_2/MeOH, 9:1) to give **5a**–**h** as white solids.

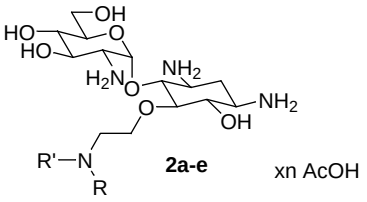
4.2.1. Compound 5a. 20%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44–7.07 (m, 24H), 5.46 (s, 1H), 5.18–4.98 (m, 8H), 4.79 (s, 1H), 4.25 (s, 1H), 3.98–3.32 (m, 18H), 2.30 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 159.0, 158.6, 158.1, 139.2, 138.9, 138.4, 138.3, 138.2, 131.1, 130.1, 129.9, 129.8, 129.5, 129.4, 129.3, 129.0, 128.9, 128.8, 128.6, 127.6, 127.4, 103.1, 102.9, 102.1, 85.2, 84.7, 83.5, 76.9, 75.4, 69.9, 67.7, 67.5, 65.5, 63.6, 62.8, 58.9, 56.3, 53.7, 52.7, 19.1; FAB for $C_{56}H_{64}N_4O_{15}$ calcd ($M+H^+$) 1034.4, found 1034.5.

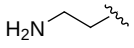
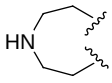
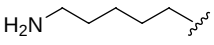
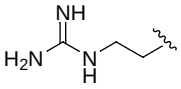
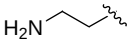
4.2.2. Compound 5b. 20%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49–6.90 (m, 25H), 5.54 (s, 1H), 5.13–4.89 (m, 8H), 4.21



Product	R'', (R'=H)	Yield	Product	R'', R'	Yield
17a	Cbz-HN	66%	**17d**	Cbz-N	94%
17b	Cbz-HN	73%			
17c	NBoc, BocHN, N, H	75%	**17e**	Cbz-HN	38%

Scheme 6.



Starting Material	Product	R, (R'=H)	Yield	Starting Material	Product	R, R'	Yield
17a	**2a**		quant.	**17d**	**2d**		quant.
17b	**2b**		quant.				
17c	**2c**		quant.[a]	**17e**	**2e**		quant.

Scheme 7. [a] Deprotection conditions involved: (1) TFA/H_2O (10:1) 1 h, (2) H_2, Pd/C in H_2O/dioxane/AcOH.

(m, 1H), 4.05–3.10 (m, 18H), 2.90 (m, 1H), 2.70 (m, 1H), 2.35 (m, 1H), 2.20–1.90 (m, 2H), 1.55 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 158.9, 158.7, 158.0, 139.2, 138.3, 138.2, 137.9, 129.9, 129.8, 129.7, 129.6, 129.5, 129.3, 129.1, 129.0, 128.9, 128.5, 127.7, 127.6, 127.4, 103.1, 102.9, 101.5, 85.6, 84.6, 83.5, 83.4, 77.1, 70.1, 67.9, 67.8, 67.5, 67.4, 65.5, 63.4, 60.4, 58.9, 53.8, 52.7, 34.8, 32.3; FAB for $C_{56}H_{64}N_4O_{15}$ calcd ($M+H^+$) 1034.1, found 1034.4.

4.2.3. Compound 5c. 25%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55–7.10 (m, 25H), 5.53 (s, 1H), 5.15–4.80 (m, 8H), 4.25 (m, 1H), 4.15–3.35 (m, 16H), 2.90 (m, 1H), 2.52 (t, J=7 Hz, 2H), 2.10–1.50 (m, 5H), 1.40 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 159.4, 158.6, 158.0, 143.2, 139.2, 138.3, 138.1, 130.5, 130.4, 130.3, 130.2, 129.9, 129.8, 129.7, 129.6, 129.3, 129.2, 128.8, 128.6, 128.5, 128.3, 128.2, 127.9, 127.3, 127.2, 103.2, 102.9, 102.7, 85.0, 76.2, 75.9, 70.0, 67.8, 67.7, 67.5, 63.8, 58.5, 52.9, 52.7, 52.6, 34.4, 29.0; FAB for $C_{57}H_{66}N_4O_{15}$ calcd ($M+H^+$) 1048.2, found 1048.5.

4.2.4. Compound 5d. 30%; ^{1}H NMR (400 MHz, CD_3OD) δ 7.50–7.20 (m, 25H), 5.54 (s, 1H), 5.14–4.80 (m, 10H), 4.25 (m, 1H), 4.10–3.35 (m, 16H), 3.10 (m, 1H), 2.92 (m, 1H), 2.55 (m, 1H), 2.15–1.70 (m, 4H), 1.42 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 159.1, 158.7, 158.6, 158.1, 139.2, 138.3, 138.2, 137.9, 130.2, 130.0, 129.9, 129.8, 129.4, 129.2, 128.7, 128.6, 127.9, 127.8, 127.3, 103.3, 102.9, 102.8, 84.9, 84.9, 83.7, 83.4, 76.3, 75.9, 70.0, 69.9, 69.9, 69.9, 69.8, 68.2, 67.9, 67.7, 67.6, 67.5, 67.2, 63.8; FAB for $C_{58}H_{67}N_5O_{17}$ calcd ($M+H^+$) 1107.2, found 1107.8.

4.2.5. Compound 5e. 41%; ^{1}H NMR (300 MHz, CD_3OD) δ 7.50–7.12 (m, 25H), 5.53 (s, 1H), 5.13–4.85 (m, 10H), 4.23 (m, 1H), 4.08–3.35 (m, 16H), 3.04 (t, J=6.5 Hz, 2H), 2.93 (m, 1H), 2.50 (m, 1H), 2.10–1.65 (m, 5H), 1.60–1.30 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 159.1, 158.7, 158.1, 158.0, 139.2, 138.4, 138.3, 138.2, 138.1, 129.9, 129.6, 129.5, 129.3, 128.9, 128.9, 127.6, 103.1, 102.9, 102.7, 85.2, 84.8, 83.5, 77.1, 76.1, 70.1, 69.9, 67.9, 67.7, 67.5, 67.4, 65.5, 63.9, 58.9, 57.1, 56.4, 54.2, 52.8, 40.0, 31.2, 27.6; FAB for $C_{59}H_{69}N_5O_{17}$ calcd ($M+H^+$) 1121.2, found 1121.6.

4.2.6. Compound 5f. 42%; ^{1}H NMR (300 MHz, CD_3OD) δ 7.50–7.25 (m, 20H), 5.54 (s, 1H), 5.13–4.90 (m, 9H), 4.30–4.15 (m, 2H), 3.96 (t, J=9.3 Hz, 1H), 3.92–3.21 (m, 24H), 3.17 (t, J=7.8 Hz, 1H), 3.00 (m, 1H), 2.65 (m, 1H), 2.30 (m, 1H), 2.15–1.85 (m, 3H), 1.65–1.50 (m, 1H), 1.48–1.30 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 159.1, 159.0, 158.6, 158.1, 139.2, 138.3, 138.1, 138.0, 129.9, 129.7, 129.5, 129.3, 129.0, 128.9, 128.9, 127.6, 104.4, 103.1, 102.8, 102.3, 85.1, 84.7, 83.51, 77.9, 77.7, 76.9, 75.7, 75.0, 73.2, 72.0, 71.6, 70.0, 69.9, 67.9, 67.5, 66.4, 65.4, 63.8, 62.7, 62.2, 58.9, 58.8, 58.3, 57.5, 53.7, 52.7, 35.7, 32.8, 20.3, 14.2; FAB for $C_{56}H_{70}N_4O_{21}$ calcd ($M+Na^+$) 1157.2, found 1157.4.

4.2.7. Compound 5g. 39%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.50–6.90 (m, 35H), 6.70–6.75 (broad s, 1H), 5.57 (s, 1H), 5.25–4.75 (m, 15H), 4.10–4.32 (broad m, 3H), 3.85–3.20 (m, 40H), 2.93–2.90 (m, 1H), 2.75–2.69 (m, 1H), 2.20–2.17 (m, 1H), 1.85–1.60 (m, 4H); FAB for $C_{94}H_{107}N_7O_{32}$ calcd ($M+Na^+$) 1680.7, found 1680.8.

4.2.8. Compound 5h. 47%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53–7.05 (m, 20H), 5.54 (s, 1H), 5.20–4.90 (m, 9H), 4.30–4.20 (m, 1H), 4.17–3.86 (m, 2H), 3.84–3.72 (m, 1H), 3.70–3.20 (m, 16H), 2.95–2.88 (m, 1H), 2.61–2.50 (m, 1H), 2.15–1.73 (m, 4H), 1.51 (s, 9H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD) δ 159.1, 158.6, 158.1, 157.5, 153.9, 139.2, 138.3, 138.2, 137.9, 129.9, 129.7, 129.5, 129.0, 128.9, 128.8, 127.6, 103.1, 102.7, 84.9, 84.4, 83.5, 80.3, 77.0, 76.3, 69.9, 68.0, 67.4, 65.4, 63.9, 58.9, 56.8, 56.5, 53.9, 52.6, 38.5, 35.7, 28.6, 28.3; FAB for $C_{61}H_{79}N_7O_{19}$ calcd ($M+H^+$) 1214.6, found 1214.7.

4.3. General procedure for deprotection (1a–g)

A solution of the starting material (0.026 mmol) in AcOH (80% in water, 2 mL) was heated at 60°C for 3 h and the solvents were removed under vacuum. To the resulting residue in AcOH (80% in water, 2 mL) was added 20% palladium hydroxide on carbon and stirred under 1 atm of H_2 at rt for 1 h. The mixture was filtered through a Celite pad, concentrated under vacuum, and the residue was dissolved in water and lyophilized to afford **1a–g** as fluffy white solids.

4.3.1. Compound 1a. Quantitative; ^{1}H NMR (400 MHz, D_2O) δ 5.50 (d, J=3.6 Hz, 1H), 5.31 (d, J=7.3 Hz, 1H), 4.05–3.22 (m, 18H), 2.93 (t, J=12.5 Hz, 1H), 2.84 (t, J=11.0 Hz, 1H), 2.40 (m, 1H), 1.88 (s, 12H), 1.70 (dd, J=8.4, 12.8 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) δ 179.5, 96.9, 95.4, 79.6, 79.4, 71.9, 71.7, 71.0, 67.7, 67.5, 59.0, 58.5, 52.4, 47.8, 47.6, 43.4, 40.9, 27.8, 21.4, 21.3; $[\alpha]_D$=+30.2 (c 1.10, H_2O).

4.3.2. Compound 1b. Quantitative; ^{1}H NMR (400 MHz, D_2O) δ 7.40–7.25 (m, 5H), 5.49 (d, J=3.7 Hz, 1H), 5.16 (d, J=7.8 Hz, 1H), 4.06–3.29 (m, 22H), 3.06 (d, J=11.6 Hz, 1H), 2.43 (m, 1H), 2.29 (t, J=11.3 Hz, 1H), 2.20 (t, J=11.6 Hz, 1H), 1.91 (s, 12H), 1.72 (dd, J=7.4, 11.9 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) δ 179.0, 137.5, 127.0, 126.9, 124.8, 98.5, 95.7, 95.7, 80.3, 79.2, 72.6, 72.0, 71.1, 67.7, 67.6, 59.8, 58.6, 57.1, 53.4, 52.4, 50.3, 47.9, 47.7, 29.6, 27.8; $[\alpha]_D$=+28.5 (c 1.00, H_2O).

4.3.3. Compound 1c. Quantitative; ^{1}H NMR (300 MHz, D_2O) δ 7.38–7.25 (m, 5H), 5.45 (d, J=3.4 Hz, 1H), 5.17 (d, J=9.4 Hz, 1H), 4.00–3.28 (m, 16H), 3.09 (d, J=12.2 Hz, 1H), 2.80–2.58 (m, 4H), 2.50–2.20 (m, 3H), 1.88 (s, 12H), 1.72 (dd, J=6.7, 12.5 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) δ 179.5, 139.9, 126.9, 126.7, 124.4, 98.1, 95.6, 80.2, 79.3, 72.3, 71.9, 71.1, 67.7, 67.6, 59.6, 58.5, 55.1, 53.0, 52.4, 50.1, 47.8, 47.7, 30.7, 27.8, 24.6; $[\alpha]_D$=+27.9 (c 1.20, H_2O).

4.3.4. Compound 1d. Quantitative; ^{1}H NMR (300 MHz, D_2O) δ 5.41 (d, J=3.8 Hz, 1H), 5.05 (d, J=7.0 Hz, 1H), 4.90–3.08 (m, 20H), 2.80 (d, J=10.9 Hz, 1H), 2.70 (t, J=6.2 Hz, 2H), 2.33 (m, 1H), 2.20 (dd, J=7.2, 10.3 Hz, 2H), 1.87 (s, 15H), 1.64 (dd, J=8.2, 12.4 Hz, 1H); ^{13}C NMR (75 MHz, D_2O) δ 179.6, 98.8, 95.8, 81.1, 79.1, 73.1, 71.9, 71.4, 67.9, 67.6, 59.9, 58.5, 53.9, 52.5, 51.6, 50.4, 47.9, 47.7, 34.0, 28.3, 21.4; $[\alpha]_D$=+33.1 (c 1.00, H_2O).

4.3.5. Compound 1e. Quantitative; ^{1}H NMR (300 MHz, D_2O) δ 5.45 (d, J=3.2 Hz, 1H), 5.08 (d, J=7.9 Hz, 1H), 4.00–3.10 (m, 20H), 3.10 (t, J=7.3 Hz, 2H), 2.88 (d, J=11.3 Hz, 1H), 2.56 (m, 2H), 2.40 (m, 1H), 2.05 (m, 2H), 1.90 (s, 15H), 1.70 (m, 1H); ^{13}C NMR (75 MHz, D_2O) δ 179.5, 98.7, 95.6, 80.0, 79.1, 72.8, 72.0, 71.0, 67.7, 67.6, 59.8, 58.6, 53.7, 52.4, 50.3, 47.8, 47.7, 35.9, 27.5, 21.5; $[\alpha]_D$=+32.8 (c 1.04, H_2O).

4.3.6. Compound 1f. Quantitative; ^{1}H NMR (400 MHz, D_2O) δ 5.49 (d, J=3.5 Hz, 1H), 5.13 (d, J=8.7 Hz, 1H), 4.46 (d, J=7.9 Hz, 1H), 4.12–3.25 (m, 24H), 2.97 (d, J=11.5 Hz, 1H), 2.81 (m, 2H), 4.30 (m, 1H), 2.13 (dd, J=11.5, 14.4 Hz, 2H), 1.91 (s, 12H), 1.76 (dd, J=12.1, 13.4 Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 182.2, 103.1, 101.1, 98.3, 83.4, 81.8, 76.8, 76.4, 75.4, 74.5, 73.9, 70.4, 70.2, 66.7, 62.5, 64.5, 61.2, 57.2, 56.6, 55.1, 52.9, 50.6, 50.4, 30.7, 24.0; $[\alpha]_D$=+10.0 (c 0.90, H_2O).

4.3.7. Compound 1g. Quantitative; ^{1}H NMR (400 MHz, D_2O) δ 5.62 (d, J=3.8 Hz, 1H), 5.49 (d, J=3.4 Hz, 1H), 5.11 (d, J=7.5 Hz, 1H), 4.10–3.20 (m, 38H), 3.00–2.90 (m, 1H), 2.80–2.70 (m, 2H), 2.52–2.38 (m, 2H), 2.20–2.09 (m, 2H), 1.93 (s, 21H), 1.85–1.70 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 181.6, 100.7, 97.9, 96.0, 83.2, 82.3, 81.8, 81.3, 79.0, 76.4, 74.9, 74.3, 73.3, 72.7, 70.4, 69.8, 69.6, 67.4, 62.0, 60.8, 60.7, 56.3, 54.6, 53.2, 50.3, 50.0, 49.9, 49.5, 30.6, 29.8, 23.5; $[\alpha]_D$=+20.7 (c 0.90, H_2O).

4.3.8. Compound 1h. To a solution of the starting material (0.026 mmol) in TFA (2 mL) was added H_2O (5 drops), stirred for 2 h and the solvent was removed under vacuum. To the resulting residue in AcOH (80% in water, 2 mL) was added 20% palladium hydroxide on carbon at rt for 1 h. Then, the mixture was filtered through a Celite pad, concentrated under vacuum, diluted with water and lyophilized to afford **1h** (quantitative) as a fluffy white solid; ^{1}H NMR (400 MHz, D_2O) δ 5.56 (d, J=3.8 Hz, 1H), 5.35 (d, J=9.1 Hz, 1H), 4.12–3.20 (m, 26H), 2.88 (t, J=11.8 Hz, 1H), 2.79 (t, J=10.8 Hz, 1H), 2.55–2.43 (m, 1H), 1.85 (dd, J=12.7 Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 163.9, 163.6, 157.8, 121.5, 118.6, 115.7, 112.5, 99.2, 97.7, 82.2, 80.3, 74.9, 73.3, 73.1, 70.1, 69.9, 61.5, 61.2, 56.2, 54.8, 54.3, 52.1, 50.1, 36.9, 28.9; $[\alpha]_D$=+26.8 (c 1.00, H_2O).

4.3.9. Compound 7. To a solution of **6** (200 mg, 0.70 mmol) in anhydrous pyridine (7 mL) was added benzoyl chloride (0.39 mL, 3.34 mmol). The mixture was stirred at rt until reaction was complete, after which a few drops of MeOH were added. After stirring for a further 15 min, the solution was concentrated and the residue was dissolved in EtOAc and washed with water, brine and dried over $MgSO_4$. The solvent was removed under vacuum, and the resulting white solid was purified by flash chromatography (EtOAc/hexanes, 1:1) to give **7** (377 mg, 77%) as a white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00–7.87 (m, 7H), 7.70 (dd, J=1.5, 5.7 Hz, 1H), 7.60–7.25 (m, 13H), 7.04 (dd, J=1.5, 8.6 Hz, 1H), 6.90 (dd, J=4.9, 10.3 Hz, 1H), 6.63 (d, J=7.6 Hz, 1H), 6.03 (t, J=9.1 Hz, 1H), 5.91 (t, J=7.6 Hz, 1H), 5.79 (t, J=9.3 Hz, 1H), 4.64 (dd, J=3.2, 8.8 Hz, 1H), 4.60–4.35 (m, 2H), 3.74 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 165.9, 165.6, 165.0, 164.9, 151.5, 144.2, 136.6, 133.3, 133.1, 132.9, 132.8, 129.9, 129.7, 129.6, 129.5, 129.1, 128.7, 128.7, 128.3, 128.2, 128.1, 119.2, 118.9, 93.8, 72.9, 72.6, 71.2, 69.3, 62.9, 55.7; $[\alpha]_D$=+34,7 (c 2.3, $CHCl_3$); HRMS for $C_{40}H_{33}NO_{11}$ calcd ($M+H^+$) 704.21320, found 704.21440.

4.3.10. Compound 8. To a solution of **7** (400 mg, 0.57 mmol) in CH_2Cl_2 (8 mL) was added $HBF_4{\cdot}Et_2O$ (54% in Et_2O, 0.072 mL, 0.63 mmol). After the reaction was complete, a few drops of Et_3N were added, the mixture was concentrated, the residue was dissolved in EtOAc, washed with water, brine and the organic layer dried over $MgSO_4$. The solvent was removed under vacuum and the resulting crude solid was purified by flash chromatography (EtOAc/hexanes, 3:7) to give **8** (329 mg, 75%) as a white

solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06–8.01 (m, 10H), 7.97–7.25 (m, 15H), 5.92 (t, J=9.7 Hz, 1H), 5.69 (t, J=9.8 Hz, 1H), 5.52 (t, J=9.0 Hz, 1H), 5.20 (broad t, J=5.3 Hz, 1H), 4.94 (dd, J=12.3, 28.7 Hz, 2H), 4.83 (d, J=7.9 Hz, 1H), 4.68 (dd, J=3.0, 12.2 Hz, 1H), 4.46 (dd, J=5.2, 12.2 Hz, 1H), 4.15 (m, 1H), 3.96 (m, 1H), 3.49 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.9, 165.6, 165.1, 165.0, 156.2, 136.4, 133.4, 133.2, 133.1, 133.1, 129.7, 129.6, 129.3, 128.9, 128.5, 128.3, 128.2, 127.9, 101.4, 72.5, 72.2, 71.8, 69.4, 69.3, 66.4, 62.8, 40.7; $[\alpha]_D$=+22.3 (c 4.30, $CHCl_3$); HRMS for $C_{44}H_{39}NO_{12}$ calcd ($M+H^+$) 774.25507, found 774.25220.

4.3.11. Compound 9. To a solution of **8** (350 mg, 0.45 mmol) in MeOH (2 mL) was added a solution of NaOMe (0.5 M in MeOH, 0.45 mL) dropwise at rt. The reaction mixture was stirred at room temperature until completion (approximately 1 h) then the reaction mixture was neutralized by addition of Amberlite IR-120(H^+). The resin was filtered off, the solution was concentrated under vacuum, the residue was diluted with water and lyophilized to give **9** (145 mg, 90%) as a fluffy white solid; ^{1}H NMR (400 MHz, D_2O) δ 7.42 (m, 5H), 5.11 (s, 2H), 4.42 (d, J=8.0 Hz, 1H), 4.00–3.85 (m, 2H), 4.80–3.60 (m, 2H), 3.55–3.30 (m, 5H), 3.25 (t, J=8.5 Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 159.2, 137.2, 129.6, 129.2, 128.5, 103.3, 76.7, 76.4, 73.9, 70.4, 69.8, 67.7, 61.5, 41.2; $[\alpha]_D$=−18.1 (c 1.40, H_2O); HRMS for $C_{16}H_{23}NO_8$ calcd ($M+H^+$) 357.142367, found 357.141093.

4.3.12. Compound 10. To a solution of **9** (233 mg, 0.65 mmol) in AcOH (2 mL, 80% in water) was added 10% Pd/C (approximately 10 mg) and stirred under 1 atm of hydrogen at rt for 2 h. The mixture was filtered through a Celite pad, concentrated, diluted with water and lyophilized to give **10** (175 mg, 95%) as a fluffy white solid; ^{1}H NMR (300 MHz, D_2O) δ 4.49 (d, J=7.9 Hz, 1H), 4.17–4.03 (m, 1H), 4.00–3.84 (m, 2H), 3.70 (dd, J=5.6, 15.0 Hz, 1H), 3.52–3.15 (m, 6H), 1.90 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ 102.9, 76.7, 76.3, 73.8, 70.3, 66.6, 61.4, 40.2, 24.0; $[\alpha]_D$=−18.2 (c 0.85, H_2O).

4.3.13. Compound 12. To a suspension of **11** (2.00 g, 0.24 mmol) in pyridine (0.40 mL) were added DMF (0.30 mL) and acetic anhydride (0.104 mL, 1.1 mmol). The mixture was stirred overnight, neutralized with HCl (2N) and extracted with EtOAc. The combined organic extracts were washed with a solution of $CuSO_4$ (satd.), water, brine and dried over Na_2SO_4. The solvent was evaporated under vacuum and the residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5) to give **12** (1.127 g, 46%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30–7.25 (m, 15H), 5.85 (broad s, 1H), 5.51 (broad s, 1H), 5.35–4.85 (m, 10H), 4.75 (broad s, 1H), 4.20–3.85 (m, 4H), 3.80–3.50 (m, 4H), 3.14 (broad s, 1H), 2.28 (d, J=12.5 Hz, 1H), 1.96 (s, 3H), 1.94 (s, 3H), 1.93 (s, 3H), 1.85 (s, 3H), 1.70–1.50 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 171.8, 170.7, 169.2, 156.1, 155.7, 136.3, 135.9, 128.4, 128.3, 128.1, 127.9, 127.8, 99.5, 82.6, 75.9, 74.5, 71.0, 68.2, 67.9, 66.8, 66.5, 61.7, 54.0, 50.3, 49.4, 33.5, 29.5, 20.7, 20.4, 19.2, 14.3; $[\alpha]_D$=+53.2 (c 2.00, $CHCl_3$); HRMS for $C_{44}H_{51}N_3O_{17}$ calcd ($M+H^+$) 894.32965, found 894.32810.

4.3.14. Compound 13. To a solution of **12** (517 mg, 0.58 mmol) in DMF (3 mL) was added allyl bromide (0.10 mL, 1.16 mmol) and tetrabutylammonium iodide (428 mg, 0.116 mmol). After stirring at 20°C for 15 min, LiHMDS (1.0 M in THF, 0.695 mL) was added and the resulting mixture was stirred for a further 1 h, quenched with a few drops of 1N NH_4Cl and the solvent was evaporated. The residue was dissolved in EtOAc and washed with 1N HCl, $NaHCO_3$ (satd.), water, brine and dried over Na_2SO_4. The solvent was evaporated under vacuum and the crude solid was purified by flash chromatography (EtOAc/hexanes, 45:55) to give **13** (230 mg, 43%) as a white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.26 (m, 15H), 5.82–5.60 (m, 1H), 5.47 (broad s, 1H), 5.34–4.75 (m, 10H), 4.25–3.92 (m, 6H), 3.90–3.40 (m, 4H), 2.37 (m, 1H), 2.01 (s, 3H), 1.96 (s, 3H), 1.93 (s, 3H), 1.85 (s, 3H), 1.70–1.50 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 170.9, 170.7, 170.5, 169.2, 155.6, 155.5, 136.3, 136.2, 136.0, 133.5, 128.4, 128.3, 128.3, 128.1, 128.0, 127.9, 127.8, 117.1, 98.7, 81.6, 80.0, 45.9, 73.8, 71.1, 68.3, 67.9, 66.8, 66.6, 66.5, 62.2, 53.6, 50.3, 49.8, 34.0, 20.7, 20.5, 20.4, 20.3; $[\alpha]_D$=+38.2 (c 2.45, $CHCl_3$); HRMS for $C_{44}H_{51}N_3O_{17}$ calcd ($M+H^+$) 934.35900, found 934.36096.

4.3.15. Compound 14. A solution of **13** (705 mg, 0.76 mmol) in CH_2Cl_2 (14 mL) was cooled at −78°C, ozone was bubbled until the solution turned light blue, after which argon was bubbled through. The mixture was treated with PPh_3 (297 mg, 1.13 mmol) and warmed up to room temperature. The solvent was removed under vacuum and the crude solid purified by flash chromatography (EtOAc/hexanes, 1:1) to give **14** (590 mg, 84%) as a white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.20 (s, 1H), 7.40–7.20 (m, 15H), 5.45–4.80 (m, 11H), 4.32–3.90 (m, 5H), 3.88–3.45 (4H), 2.40 (broad d, J=12.4 Hz, 1H), 2.01 (s, 3H), 1.93 (s, 3H), 1.85 (s, 3H), 1.84 (s, 3H), 1.50–1.33 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 198.1, 170.8, 170.6, 169.1, 155.6, 155.4, 155.3, 136.2, 136.1, 135.9, 132.0, 131.9, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 99.1, 83.7, 80.0, 77.9, 71.0, 68.1, 67.9, 66.9, 66.8, 66.6, 61.9, 53.5, 49.8, 34.0, 29.5, 20.6, 20.5, 20.4, 20.3; $[\alpha]_D$=+34.8 (c 1.51, $CHCl_3$); HRMS for $C_{46}H_{53}N_3O_{18}$ calcd ($M+H^+$) 936.34021, found 936.34500.

4.3.16. Compound 15. A solution of **13** (410 mg, 0.44 mmol) in CH_2Cl_2 (9 mL) was cooled at −78°C, ozone was bubbled until the solution turned light blue, after which argon was bubbled through. The reaction mixture was warmed up to 0°C and treated with $NaBH_4$ (66 mg, 1.76 mmol) in MeOH (2 mL). The mixture was stirred for 30 min, warmed up to rt and a few drops of AcOH were added to quench the reaction. The solvents were removed under vacuum, the residue was dissolved in EtOAc, washed with water, brine and dried over Na_2SO_4. The solvent was removed and to a solution of the crude white solid in CH_2Cl_2 (4 mL) was added 2,4,6-collidine (0.074 mL, 0.56 mmol) followed by MsCl (0.043 mL, 0.56 mmol). After stirring for 36 h, the mixture was diluted with CH_2Cl_2, washed with $CuSO_4$ (satd.), water, brine, dried over Na_2SO_4 and the solvent was evaporated. To a solution of the resulting residue in DMF (5 mL) was added NaN_3 (217 mg, 3.35 mmol). After stirring at 50°C for 8 h, the solvent was removed under vacuum, the residue was

dissolved in EtOAc, washed with water, brine, dried over Na_2SO_4 and the solvent was evaporated to give **15** (253 mg, 60%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.20 (m, 15H), 5.65–5.50 (m, 1H), 5.22–4.90 (m, 12H), 4.84 (t, J=9.9 Hz, 1H), 4.20–3.95 (m, 4H), 3.87–3.40 (m, 6H), 3.33–3.20 (m, 1H), 2.90–2.80 (m, 1H), 2.37–2.25 (m, 1H), 2.00 (s, 6H), 1.96 (s, 3H), 1.93 (s, 3H), 1.51–1.36 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.7, 170.6, 169.1, 155.6, 155.4, 136.2, 136.1, 135.9, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 99.0, 81.7, 80.4, 75.5, 71.8, 70.9, 68.2, 68.1, 66.9, 66.8, 66.6, 62.2, 53.9, 50.4, 49.9, 34.2, 20.6, 20.5, 20.4, 20.3; IR cm^{-1} 3325, 3065, 2953, 2106, 1731, 1531, 1229, 1033; $[\alpha]_D$=+40.1 (c 3.50, $CHCl_3$); HRMS for $C_{46}H_{54}N_6O_{17}$ calcd (M+H$^+$) 963.36237, found 963.36020.

4.3.17. Compound 16. A solution of **15** (70 mg, 0.07 mmol) in MeOH (0.7 mL) was treated with a catalytic amount of NaOMe, stirred for 15 min and a few drops of DMF were added to dissolve the white precipitate. After stirring for 1 h, Amberlite IR-120(H$^+$) was added to neutralize the reaction mixture, the solvent was filtered and evaporated under vacuum to give a white solid. This was dissolved in DMF (0.6 mL), PPh_3 (38 mg, 0.14 mmol) added, followed by one drop of water. After stirring at 60°C for 24 h, one drop of AcOH was added, the solvents were removed under vacuum, the resulting white solid was triturated with EtOAc and filtered to give **16** (57 mg, quantitative) as a white solid; ^{1}H NMR (400 MHz, CD_3OD) δ 7.45–7.23 (m, 12H), 7.15 (d, J=7.0 Hz, 1H), 7.03 (d, J=9.5 Hz, 1H), 6.95–6.85 (m, 1H), 5.22–4.85 (m, 10 H), 3.72–3.15 (m, 18 H), 2.62–2.50 (m, 2H), 1.85 (s, 3H), 1.80–1.68 (m, 1H), 1.40–1.25 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.2, 157.7, 156.8, 156.5, 138.3, 138.1, 138.0, 134.4, 134.2, 133.1, 132.6, 132.5, 130.0, 129.9, 129.8, 129.7, 129.4, 128.9, 128.8, 128.6, 128.3, 98.5, 87.4, 79.0, 75.4, 73.5, 73.2, 71.7, 70.9, 66.5, 66.3, 66.1, 61.3, 56.9, 52.1, 51.0, 35.8; $[\alpha]_D$=+32.6 (c 3.70, DMSO); HRMS for $C_{38}H_{48}N_4O_{13}$ calcd (M+H$^+$) 769.32959, found 769.32660.

4.4. General procedure for reductive amination (17a–e)

To a mixture of **14** (0.034 mmol) and the appropriate amine (0.1 mmol) in MeOH (3 mL) was added AcOH (0.1 mL) followed by $NaBH_3CN$ (1.0 M in THF, 0.1 mL). The mixture was stirred at room temperature overnight until disappearance of **14**. The reaction mixture was diluted with EtOAc (15 mL) and washed with a solution of $NaHCO_3$ (satd., 10 mL) and dried over Na_2SO_4. After evaporation of the solvents, the residue was purified by flash chromatography ($CHCl_3$/EtOAc/MeOH, 7:2:1) to give **17a**–**e** as white solids.

4.4.1. Compound 17a. 66%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.22 (m, 20H), 5.60–5.45 (m, 1H), 5.30–4.72 (m, 10H), 4.25–3.96 (m, 8H), 3.85–3.00 (6H), 2.70–2.20 (m, 4H), 2.20 (s, 3H), 1.96 (s, 3H), 1.93 (s, 3H), 1.87 (s, 3H); FAB for $C_{56}H_{67}N_5O_{19}$ calcd (M+H$^+$) 1114.4, found 1114.4.

4.4.2. Compound 17b. 73%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.22 (m, 20H), 5.35–4.70 (m, 16H), 4.25–4.97 (m, 4H), 3.80–3.35 (m, 6H), 3.23–3.10 (m, 2H), 2.60 (broad s, 1H), 2.40 (broad s, 1H), 2.25 (broad s, 1H), 2.20 (s, 3H), 1.96 (s, 3H), 1.93 (s, 3H), 1.87 (s, 3H), 1.55–1.10 (m, 8H); FAB $C_{59}H_{73}N_5O_{19}$ calcd (M+H$^+$) 1156.5, found 1156.5.

4.4.3. Compound 17c. 75%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.20 (m, 15H), 5.75–5.60 (m, 1H), 5.40–4.70 (m, 10H), 4.30–3.82 (m, 4H), 3.78–3.25 (m, 4H), 2.70–2.22 (m, 3H), 2.24 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.89 (s, 3H), 1.49 (s, 9H), 1.47 (s, 9H); FAB $C_{59}H_{79}N_7O_{21}$ calcd (M+H$^+$) 1222.5, found 1222.7.

4.4.4. Compound 17d. 94%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.22 (m, 20H), 5.45–4.75 (m, 14H), 4.23–4.00 (m, 3H), 3.83–3.30 (m, 10H), 2.85 (broad s, 4H), 2.50–2.15 (m, 4H), 2.22 (s, 3H), 1.95 (s, 3H), 1.90 (s, 3H), 1.88 (s, 3H); FAB $C_{58}H_{69}N_5O_{19}$ calcd (M+H$^+$) 1140.5, found 1140.5.

4.4.5. Compound 17e. 38%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.22 (m, 25H), 5.65 (broad s, 1H), 5.42–4.82 (m, 12H), 4.80–4.60 (m, 1H), 4.75–3.94 (m, 4H), 3.80–3.30 (m, 6H), 3.37–2.95 (m, 6H), 2.60–2.30 (m, 4H), 1.96 (s, 3H), 1.92 (s, 3H), 1.90 (s, 3H), 1.83 (s, 3H); FAB $C_{66}H_{78}N_6O_{21}$ calcd (M+H$^+$) 1291.5, found 1291.6.

4.5. General procedure for deprotection (2a,b and 2d,e)

The appropriate pseudo-disaccharide (**17a**,**b** and **17d**,**e**) was treated with a catalytic amount of NaOMe in MeOH/THF (1:1, 2 mL). After stirring for 3 h at rt, the mixture was concentrated. To the resulting residue in H_2O/dioxane/AcOH (20:20:1, 2 mL) was added 10% Pd/C (approximately 10 mg) and the mixture was stirred under 1 atm of hydrogen at rt for 3 h. The mixture was filtered through a Celite pad, concentrated under vacuum, diluted with water and lyophilized to afford **2a**,**b** and **2d**,**e** as fluffy white solids.

4.5.1. Compound 2a. Quantitative; ^{1}H NMR (400 MHz, D_2O) δ 5.64 (d, J=4.0 Hz, 1H), 4.25–4.12 (m, 1H), 4.08–3.87 (m, 4H), 3.85–3.62 (m, 5H), 3.58–3.41 (m, 2H), 4.38–3.20 (m, 8H), 2.47–4.39 (m, 1H), 1.95 (s, 15H), 1.78 (dd, J=6.5, 11.5 Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 181.7, 95.8, 83.3, 77.0, 74.8, 73.5, 70.3, 69.8, 67.7, 60.9, 54.4, 50.6, 49.8, 48.7, 45.4, 36.7, 29.3, 23.7; $[\alpha]_D$=+42.0 (c 1.00, H_2O).

4.5.2. Compound 2b. Quantitative; ^{1}H NMR (400 MHz, D_2O) δ 5.63 (d, J=3.8 Hz, 1H), 4.22–4.12 (m, 1H), 4.10–3.60 (m, 9H), 3.55–3.42 (m, 3H), 3.40–3.20 (m, 4H), 3.04 (t, J=8.1 Hz, 2H), 2.95 (t, J=7.5 Hz, 2H), 2.43–2.35 (m, 1H), 2.00–1.60 (m, 25H), 1.48–1.32 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 181.8, 95.0, 83.3, 76.0, 74.8, 73.3, 70.1, 69.7, 66.6, 60.9, 54.4, 50.6, 49.7, 48.2, 39.9, 29.0, 27.0, 25.7, 23.8, 23.6; $[\alpha]_D$=+26.6 (c 1.00, H_2O).

4.5.3. Compound 2c. Quantitative; ^{1}H NMR (400 MHz, D_2O) δ 5.77 (d, J=3.7 Hz, 2/7H), 5.74 (d, J=3.7 Hz, 5/7H), 4.45–3.30 (m, 22H), 2.55–2.45 (m, 1H), 1.86 (dd, J=6.4, 12.6 Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 164.3, 163.9, 163.6, 163.2, 157.8, 121.5, 118.6, 115.7, 94.3, 83.4, 75.2, 73.2, 69.6, 69.5, 66.5, 63.3, 60.7, 54.2, 50.7, 50.5, 49.7, 48.7, 46.8, 38.2, 28.6; $[\alpha]_D$=+31.1 (c 1.00, H_2O).

4.5.4. Compound 2d. Quantitative; ^{1}H NMR (400 MHz, D_2O) δ 5.64 (d, J=3.8 Hz, 1H), 4.10–4.00 (m, 1H), 3.98–3.84 (m, 4H), 3.82–3.60 (m, 5H), 3.56–3.20 (m, 10H), 3.18–3.08 (broad s, 1H), 2.95–2.70 (m, 7H), 2.48–2.40 (m, 1H), 1.91 (s, 18H), 1.77 (t, J=12.6 Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 181.8, 95.4, 83.4, 76.8, 74.9, 73.1, 69.9, 69.7, 67.9, 60.9, 57.8, 54.4, 50.5, 50.1, 49.8, 43.4, 42.7, 29.1, 23.8; $[\alpha]_D$=+31.4 (c 1.00, H_2O).

4.5.5. Compound 2e. Quantitative; ^{1}H NMR (400 MHz, D_2O) δ 5.71 (d, J=3.8 Hz, 1H), 4.12–4.03 (m, 1H), 4.00–3.60 (m, 9H), 3.56–3.22 (m, 4H), 3.15–3.05 (m, 4H), 2.97–2.75 (m, 5H), 2.48–2.37 (m, 1H), 1.75 (dd, J=6.8, 12.4 Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 181.8, 94.6, 83.6, 75.5, 74.7, 73.6, 70.3, 69.7, 69.4, 60.9, 54.5, 52.9, 51.5, 50.8, 50.4, 49.8, 37.5, 29.3, 23.8; $[\alpha]_D$=+32.0 (c 1.00, H_2O).

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