A bioisosteric oligosaccharide mimetic based on isofagomine-type monomers

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A series of hydroxylated piperidine oligomers that resemble oligosaccharides is synthesised. Prepared were **5**, a mimic of 3-*O*-L-fucopyranosyl-D-galactopyranose, **6**, a mimic of 3-*O*-D-galactopyranosyl-D-galactopyranose and **7**, a mimic of 3-*O*-{3-*O*-[3-*O*-(L-fucopyranosyl)-D-galactopyranosyl]-D-galactopyranosyl}-D-galactopyranose. Conformational analysis of the new compounds using NMR spectroscopy showed them to have predominant conformations that were similar to those adopted by β-*O*-glycosides. The inhibition of a series of glycosidases by **5**–**7** was investigated and found to be inferior to that of the corresponding unsubstituted isofagomines.

Introduction

In the last few decades it has become increasingly clear that complex carbohydrates play a significant role in human biology. These molecules are an important factor in cell–cell, cell–virus, cell–antibody or virus–antibody interactions, typically through recognition of specific carbohydrate structures by proteins. Such interactions are potential targets for therapeutic action of inhibitors, and it is conceivable that many bacterial and viral infections and also inflammatory processes in the future may be treated with inhibitors that mimic these carbohydrates.[1] Consequently carbohydrate mimetics are subject to intense current interest.[2] Particularly desirable carbohydrate mimetics are those that have a repetitive nature so that different oligosaccharides can be mimicked by using appropriate building blocks. Recently a number of examples of such mimetics have been reported as having a peptide backbone.[3] This backbone gives several advantages: 1) efficient oligomerisation, because peptide synthesis is high-yielding, 2) the synthesis can be transferred to the solid phase for combinatorial chemistry, and 3) the peptide bond is stable and will not be hydrolysed physiologically. However, none of these 'carbopeptoids' have been bioisosteric with an oligosaccharide or consisted of more than one monomer. It was the goal of the present research to take the chemistry of carbohydrate mimetics one step further and to create a mimetic that would actually be bioisosteric with cell-surface oligosaccharides.

An attractive target for a carbohydrate mimetic is the oligosaccharide **1** associated with the breast tumor antigen (Fig. 1). This molecule is recognised by the mAb MBr1 and has shown promise in an anticancer vaccine.[4] Tumor immunotherapy is based on the theory that tumors possess specific antigens that can be recognised when presented to or processed by a properly trained immune system. This is supported by the observation that tumor cells commonly are characterised by unusual oligosaccharides on their cell surfaces which distinguish them from similar healthy cells. Training the immune system to recognise and attack these oligosaccharide motifs is thus a potential method of cancer treatment. A mimetic of **1** should equally well be applicable for anticancer vaccine creation and in addition afford advantages of stability, ready assembly, and linkage to carrier proteins. In the present work we have designed mimetics aimed at target **1**.

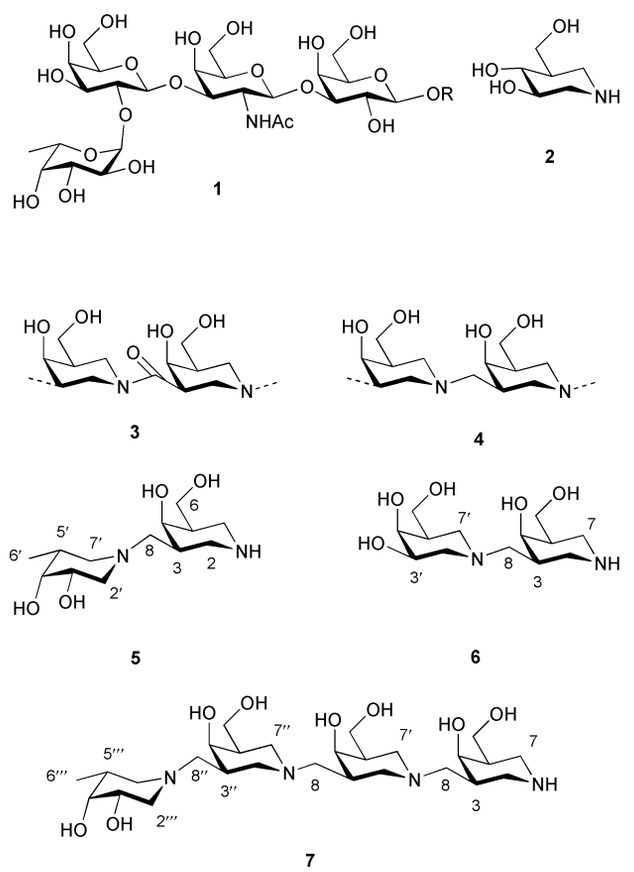

Fig. 1 Structure of cancer antigenic tetrasaccharide **1**, isofagomine **2**, two types of biomimetic based on the isofagomine structure, and polypiperidines **5**–**7**.

The mimetic we propose to mimic oligosaccharides is based on 1-aza-5a-carbasugar monomers of the isofagomine **2** type.[5] Isofagomine has a nitrogen atom in the 1-position, which allows it to be linked to another molecule in a glycoside-type fashion. By linking it to isofagomines with carboxylates in place of hydroxy groups the oligosaccharide mimetic **3** is obtained. Compounds **3** can also be globally reduced to compounds **4**.

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Compounds **4** would be expected to have a high degree of resemblance to a natural oligosaccharide. In this paper we report the stereoselective synthesis of di- and tetrasaccharide examples of **4**, *viz* the compounds **5**, **6** and **7** (Fig. 1).

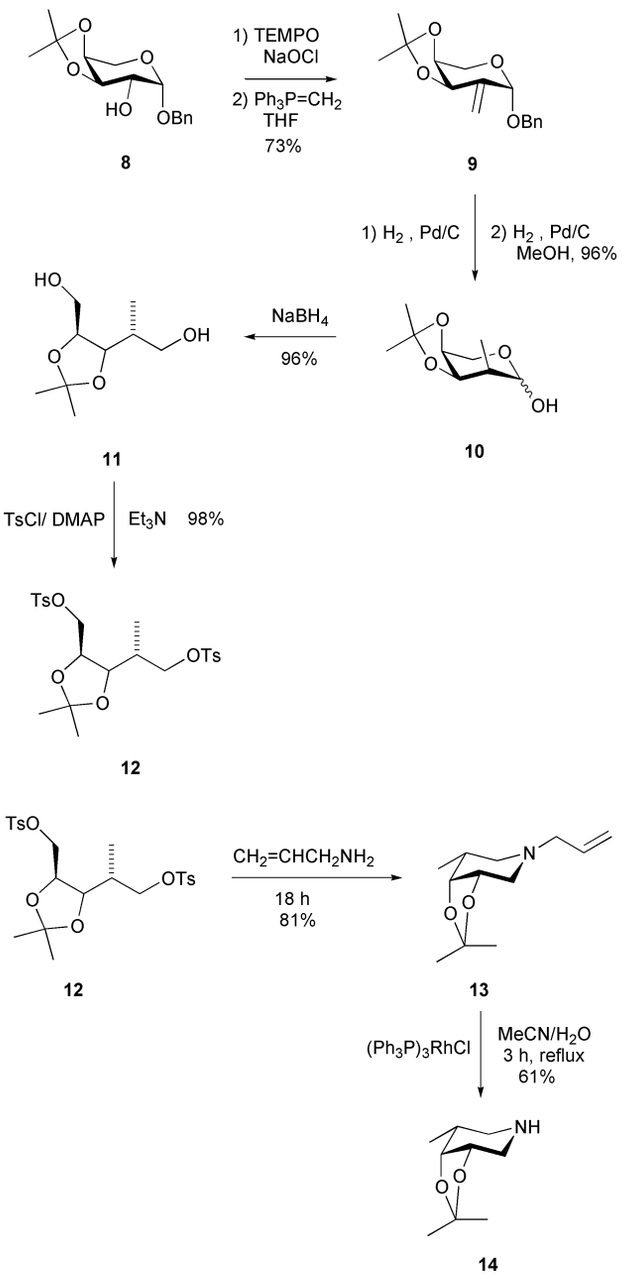
Results and discussion

Synthesis †

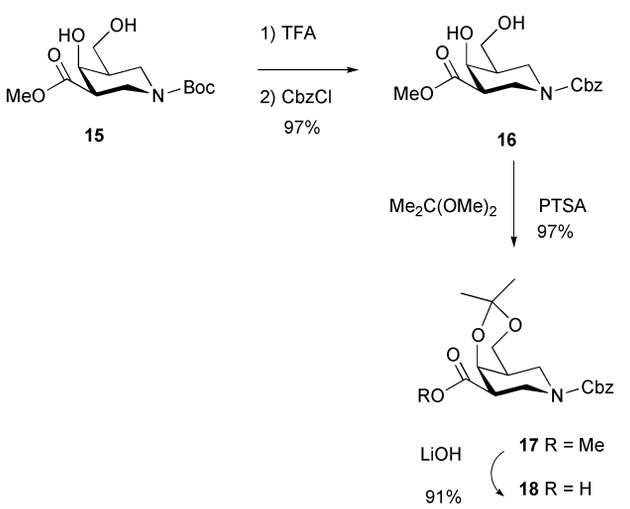
For the synthesis of targets **5**–**7** we needed two types of building blocks: a) a hydroxylated piperidine of either L-*fuco*- or D-*galacto* stereochemistry for the terminal residues and b) a piperidinecarboxylic acid with galactose stereochemistry for the remainder of the residues. From those two types of building blocks it should be possible to assemble the targets by peptide-coupling chemistry. Finally a reduction of all the peptide bonds would lead to the target amines.

The terminal residues are the L-*fuco*- or D-*galacto* analogues of **2** and they have previously been prepared.[6–8] We basically used our previous synthesis to prepare these building blocks. However, some changes were made to the synthesis of the L-*fuco* isofagomine and these have been outlined in Schemes 1 and 2. Starting with the acetonide **8**, which is available from L-arabinose in two steps,[9] we oxidised with 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) and NaOCl in place of Swern oxidation or CrO_3. This gave a higher yield and a purer product **9** after the subsequent Wittig reaction. The following two hydrogenation steps were carried out as previously described except that MeOH was used as solvent in the hydrogenolysis reaction, which improved the yield significantly. The yield in the following $NaBH_4$ reduction and ditosylation was also improved mainly due to a longer reaction time in the tosylation reaction and the addition of DMAP. (Direct reduction of the benzyl glycoside to the diol **11** using sodium in ammonia could also be carried out in good yield.) Cyclisation of the resulting ditosyl compound **12** was carried out with allylamine and not as previously reported benzylamine, because it was anticipated that the *N*-allyl group would be easier to remove than *N*-benzyl in the presence of the isopropylidene group. We have frequently seen that an *N*-benzyl group requires the addition of acid in order to undergo hydrogenolysis. The reaction of **12** with excess of allylamine gave the piperidine **13** in 81% yield. The allyl group was removed with Wilkinson's catalyst in aq. MeCN to give the piperidine derivative **14**.

The *galacto* amino acid building block was obtained from the chiral piperidine ester **15** (Scheme 2), an intermediate in the chemoenzymatic synthesis of isogalactofagomine **19**.[6] However, it was necessary to protect the hydroxy groups of **15**. Since it was found that the 4-hydroxy group was extremely sensitive to elimination when protected with a protecting group such as an ester, it was necessary to protect the diol with an isopropylidene group, which could be made in acidic solution. However, as the isopropylidene group was not entirely stable during trifluoroacetic acid (TFA)-catalysed cleavage of the *tert*-butoxycarbonyl (Boc) group, it was necessary to protect the amine function with a benzyloxycarbonyl (CBz) group. The Boc group of **15** was exchanged with Cbz by treatment with TFA followed by reaction with CBzCl (Scheme 2). This gave **16** in 97% yield. Treatment with 2,2-dimethoxypropane and toluene-*p*-sulfonic



Scheme 1 Synthesis of the fucosyl unit.

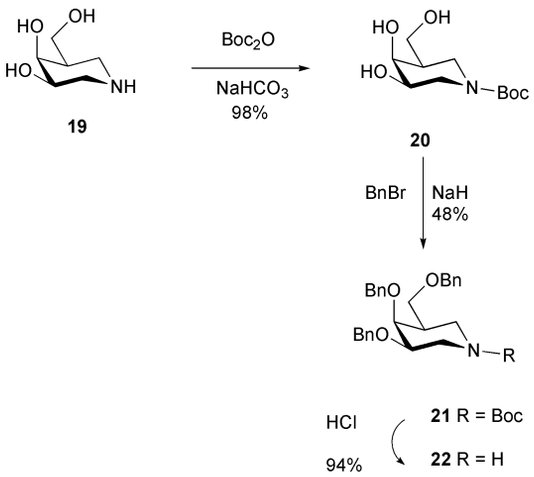


Scheme 2 Changes of protection groups of the galactosyl amino acid.

† Nomenclature. The oligopiperidines were named and numbered similarly to an oligosaccharide with the N-unsubstituted piperidine corresponding to the reducing end of an oligosaccharide. This piperidine unit was unlabelled, while subsequent piperidines were labelled with an increasing number of primes according to their position from the 'pseudo-reducing' terminal (Fig. 2). Individual atoms were numbered according to their similarity with a monosaccharide so that the nitrogen atom was numbered 1 and the exocyclic methyl or hydroxymethyl group was numbered 6. The carbon that replaces the ring-oxygen was numbered 7, while the carbon that replaces a glycosidic oxygen was numbered 8.

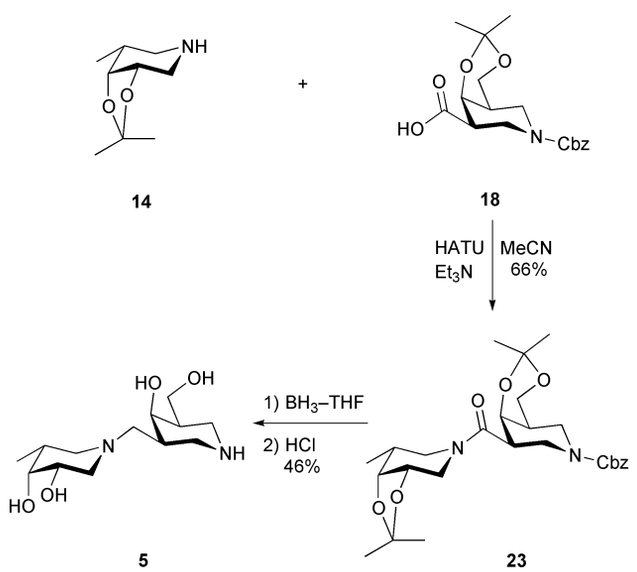
acid gave **17**, also in 97% yield, and saponification with LiOH led to the protected amino acid **18** in 91% yield.

Isogalactofagomine **19** was protected as outlined in Scheme 3. The Boc derivative **20** was obtained in 98% yield from **19** (trifluoroacetate) using di-*tert*-butyl dicarbonate and $NaHCO_3$. Benzylation of **20** with BnBr and NaH gave the tri(benzyl ether) **21** in 48% yield, and the Boc group was removed with hydrochloric acid to afford the amine **22** in 94% yield.



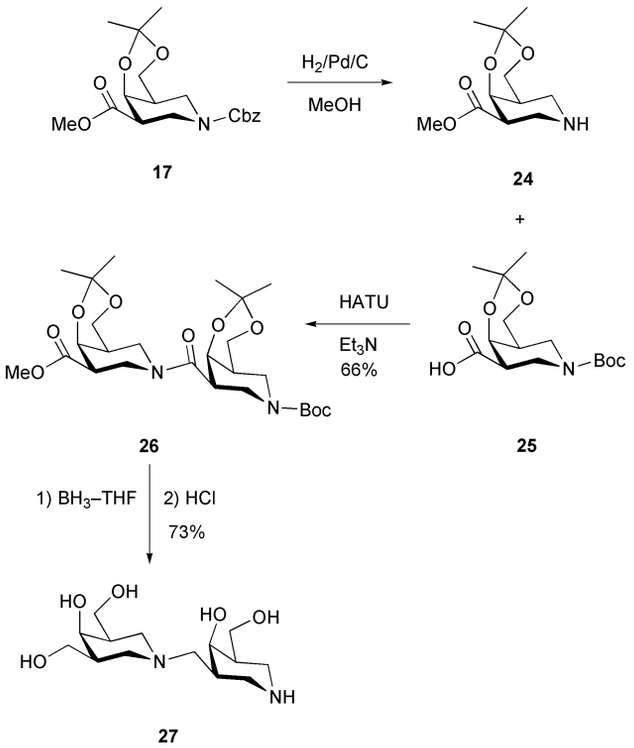
Scheme 3 Protection of isogalactofagomine **19**.

Now the stage was set for attempting a peptide coupling between the various sugar amino acids. In order to prepare **5**, which is also the left-half of **7**, the reaction between **14** and **18** in the presence of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and Et_3N in MeCN was carried out (Scheme 4). This gave the amide **23** in 69% yield. Now direct reduction of this amide with the borane–THF complex gave the amine **5** in 46% yield. However, the amide **23** could also be used as the left-hand part of tetrasaccharide analogue **7**.



Scheme 4 Synthesis of **5**.

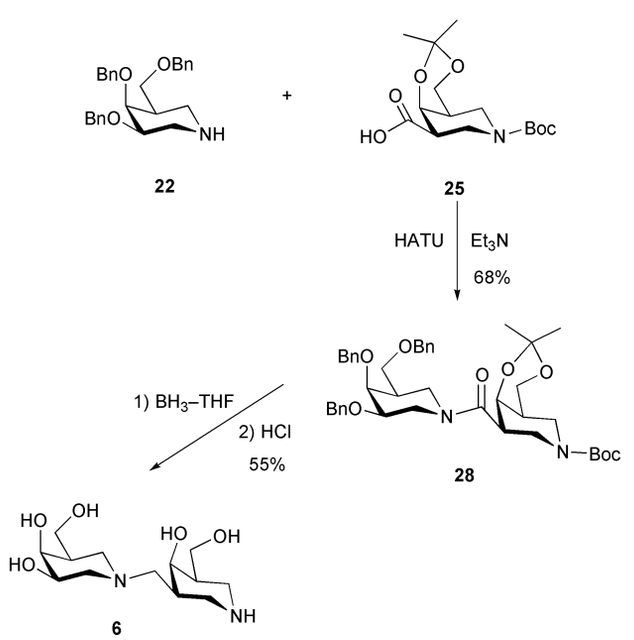
To obtain the right-hand part of target **7** the amino acid derivative **17** was converted to the amine **24** by hydrogenolysis with Pd/C in MeOH (Scheme 5). This amine **24** was now coupled with **25**, the Boc-protected analogue of **18**, a compound that has previously been prepared as an intermediate in the synthesis of **19**.[6] The coupling of **24** with **25** in the presence of HATU and Et_3N gave the amide **26** in 66% yield. Reduction



Scheme 5 Synthesis of **27**.

of this amide with borane–THF followed by acidic hydrolysis gave the deprotected diamine **27** in 73% yield.

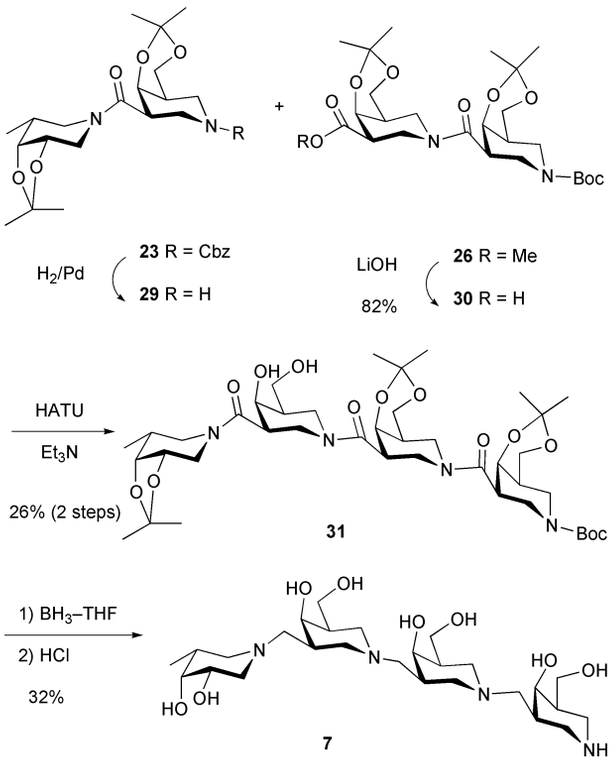
In order to obtain **6**, the protected amine **22** was coupled with **25** in the presence of HATU, which led to amide **28** in 68% yield (Scheme 6). Treatment of **28** with borane–THF under reflux



Scheme 6 Synthesis of **6**.

followed by reflux with hydrochloric acid led to both reduction and debenzylation and gave the deprotected diamine **6** in 55% yield.

For the target **7** the two dipiperidine building blocks **23** and **26** were prepared for coupling (Scheme 7). Amide **23** was hydrogenolysed in the presence of Pd/C to form the amine **29**. Similarly, compound **26** was hydrolysed with LiOH to give the acid **30** in 82% yield. Coupling of **29** and **30** in the presence of HATU gave the triamide **31** in 26% yield. However, triamide **31** gave a mass spectrum consistent with the loss of one of the

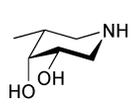


Scheme 7 Synthesis of **7**.

four acetonides. NMR analysis also suggested that an acetonide had been lost and TLC showed that the compound was extraordinarily polar. Owing to the complexity of the compound it was not possible to determine which of the 4 possible structures had been formed, and one of the possible compounds is shown in Scheme 7. Nevertheless, global reduction of this compound using refluxing borane–THF followed by hydrolysis with refluxing hydrochloric acid gave the target **7** in 32% yield.

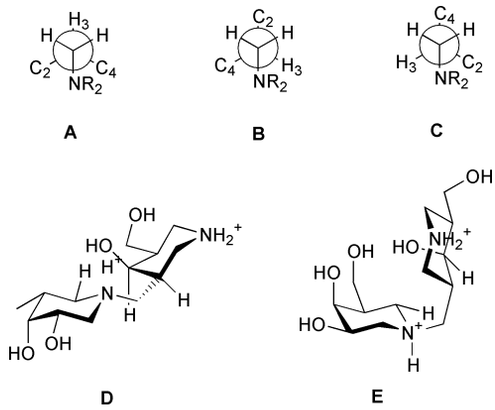
Conformational analysis

The conformation of *O*- and *C*-glycosides has been extensively studied.[10] *O*-Glycosides generally prefer a conformation where the C-1–C-2 bond and the O–R bond (R being the aglycone unit) are antiperiplanar to each other, and *C*-glycosides appear to adopt a similar conformation. The target compounds **5**–**7** differ in several respects from both *O*- and *C*-glycosides: Compounds **5**–**7** do not have the ring oxygen atom, but contain a nitrogen atom in place of the customary C-1 atom. Nor do they contain the exocyclic oxygen atom of *O*-glycosides. They should therefore not be affected by anomeric effects. However, at physiological pH the nitrogen atoms of **5**–**7** will be protonated, which may result in charge–charge and charge–dipole interactions between the different residues, and owing to the complexity of modelling these interactions in aqueous solution, such a study is beyond the scope of this work. Compounds **5**–**7** were, however, studied by 1D and 2D ^{1}H- and ^{13}C-NMR at 600 and 800 MHz (Tables 1 and 2), at pH 4.5–5.0 where the amino functions were protonated. The chemical-shift values of the terminal fucose residues of **5** and **7** and the terminal galactose residue of **6** were essentially identical with data from the spectra of the hydrochlorides of L-*fuco*-isofagomine [**14a**, (3*S*,4*R*,5*R*)-



14a

3,4-dihydroxy-5-methylpiperidine] and **19**, respectively.[7] Similarly the 3-C-substituted galactosyl units of all three compounds had chemical-shift values for the protons of C-2,



A **B** **C** **D** **E**

Fig. 2 Conformations of dipiperidines **5** and **6**.

C-4, C-5, C-6 and C-7 which were very similar to those of **19**. The internal ^{1}H–^{1}H couplings of the piperidine units of **5** and **6** were also consistent with these being in a $^{4}C_{1}$ conformation, which also could be seen from strong NOE's between H-3 and H-5, and H-3′ and H-5′ in both compounds (Table 1). In general the coupling constants could not be extracted in the case of compound **7** due to overlap. However, observed strong NOE's between H-3 and H-5, and H-3‴ and H-5‴ and the quite similar chemical-shift values to those of the similar parts of **5** and **6** strongly suggest a $^{4}C_{1}$ conformation for all units in **7** as well.

In all compounds the inter-piperidine CH_2 (C-8) is at quite a low field in the ^{13}C-NMR spectrum ($\delta_C \approx 58$) and considerably lower than for the similarly substituted C-7′ ($\delta_C \approx 50$–53). This suggests that the nitrogen substituents are predominantly equatorial, since it has previously been shown that an equatorial N-substituent is far more deshielded than an axial one in quaternary ammonium salts of isofagomine.[11] It is not likely that an axial N-substituent would result in such a low-field resonance.

In compounds **5** and **6** it was possible to obtain the coupling constants of the C-8 protons and some NOE's between different piperidine units, which could provide information about the relative orientation of the piperidine units. For both compounds $J_{3,8a}$ and $J_{3,8b}$ were 6 Hz, which shows that they exist as a mixture of several conformers **A**–**C** (Fig. 2); however, the strong NOE between H-8a and H-4 suggests that the number of populated rotamer conformations around the C-3–C-8 bond is limited. Of the three possible rotamers around this bond (**A**–**C**) the rotamer **B** having the N–C-8 bond *anti* to the C-3–C-2 bond has the largest distances between the H-8s and H-4 and appears therefore least likely given the strong NOE.

Compound **5** shows medium and weak NOE's between H-7′ eq and H-4, and between H-7′ eq and H-3, respectively. Since it is not possible to get close contacts between H-7′ eq and H-4 in the conformer **C** this suggest that this NOE comes from conformer **A** in the conformation **D** where the C-8–C-3 bond is antiperiplanar to the N–C-2′ bond. Compound **6** also shows an NOE between H-7′ eq and H-4. Similarly to the case above it is not possible to get close contacts between these two protons in the conformer **C**, suggesting that this NOE comes from conformer **A** in the conformation **E** where the C-8–C-3 bond is antiperiplanar to the N–H bond. Though **D** and **E** are not the only conformations that **5** and **6** adopt, it is noteworthy that β-*O*-glycosides are likely to attain similar conformations because the *exo*-anomeric effect is fulfilled.

No NOE or H-8 coupling constants could be extracted from the spectra of **7** so no firm conclusion regarding its conformation could be made. However, since the chemical shifts of **7** are similar to those of the substructures **5** and **6** for comparable positions it is likely that the conformation of **7** resembles that of the substructures, since any differences in conformation would affect chemical shifts.

Table 1 NMR data for compounds **5** and **6** at pH 4.5–5.0

	6				**5**			
Carbon	^{1}H	*J*	^{13}C	NOE	^{1}H	*J*	^{13}C	NOE
2	3.396, 3.068	4.0, 12.8, 12.8, 12.8	41.8		3.386, 3.054	3, 12.5, 12.5	41.9	
3	2.520		35.2		2.515		35.1	7′a(m)
4	4.175	<0.5	64.2	6a(m), 6b(w)	4.160	<0.5	64.2	7′a(w)
5	2.139		41.4	3(s)	2.129		41.5	3(s)
6	3.660, 3.556	6.8, 11.1, 7.0, 11.1	61.3	4(s), 7a(m)	3.655, 3.552	7.0, 11.3, 6.9, 11.3	61.4	4(s), 4(m)
7	3.308, 2.984	4.0, 12.8, 12.5, 12.8	41.1		3.301, 2.976	4.1, 12.7, 12.5, 12.7	41.2	
8	3.443, 3.181	6, 6.0, 13.7	58.0	4(s)	3.385, 3.140	6, 6.0, 13.7	57.9	4(s)
2′	3.418, 3.092	3.5, 12, 11, 12	52.1		3.389, 2.998	12, 11	51.5	7′b(m)
3′	3.993		66.8	5′(s)	4.001		67.1	5′(s)
4′	4.099	<0.5	66.5	6′a(s), 6′b(w)	3.899	<0.5	69.8	6′(s)
5′	2.192		40.2		2.095		32.8	4′(s)
6′	3.711, 3.588	6.4, 11.4, 7.1, 11.4	61.0		1.003	7.0	14.5	
7′	3.420, 2.983	3.5, 12.5, 12.5, 12.5	50.3	4(m)	3.236, 2.847	12, 11	53.6	

Table 2 NMR data for compound **7** at pH 4.5–5.0

	7								
Carbon	H	*J*	C	NOE	Carbon	^{1}H	*J*	^{13}C	NOE
2	3.407, 3.063		42.1		2″	3.408, 2.959		51.7	
3	2.499		35.3	5(s)	3″	2.572		35.9	
4	4.168		64.5	6a(s)	4″	4.133		64.1	6a″(s)
5	2.135		41.5		5″	2.195		42.1	
6	3.657, 3.554		61.4	7(s)	6″	3.665, 3.544		61.4	
7	3.305, 2.980		41.3	6a(m), 6a(s)	7″	3.408, 2.923		50.5	
8	3.326, 3.108		58.2		8″	3.374, 3.099		57.9	
2′	3.473, 3.009		51.7		2‴	3.386, 2.994		51.7	
3′	2.551		35.9		3‴	4.003		67.1	5‴(s)
4′	4.139		64.1	6a′(s)	4‴	3.896		69.8	6‴(s)
5′	2.206		42.1		5‴	2.094		32.8	
6′	3.670, 3.551		61.4		6‴	1.001	6.6	14.5	7a‴(m)
7′	3.378, 2.866		50.5		7‴	3.231, 2.841		53.6	6‴(s)
8′	3.374, 3.099		57.9						

Table 3 Inhibition of various enzymes by compounds **5**–**7** and **27**. Inhibition was competitive and values shown are K_i in μM

Enzyme	**14a**	**5**	**7**	**19**	**6**	**27**
α-Galactosidase (green coffee bean)				50	5.7	>1000
β-Galactosidase (*Aspergillus oryzae*)				0.004	0.9	
β-Galactosidase (*Eschericha coli*)				0.2	10	NI
β-Galactosidase (*Saccharomyces fragilis*)				0.3	287	
α-Fucosidase (human placenta)	6.4	144				
α-Fucosidase (bovine kidney)	4.0	30	11			

Glycosidase inhibition

Since isofagomines are potent glycosidase inhibitors it was of interest to investigate the interaction between the piperidine oligomers and glycosidases. The oligoazasugars **5** and **7** that had a terminal fucosyl unit were analysed for binding to the human placenta and bovine kidney α-fucosidases. The substrate specificity of the human fucosidase has been investigated.[12] This enzyme appears to have a preference for α-1,2-Gal linkages over α-1,3-Gal linkages, which in turn are better substrates than α-1,6-Gal linkages. The 4-nitrophenyl fucoside is, however, by far the best substrate for this enzyme, which suggests a limited importance of the aglycone-binding site. The substrate specificity of the bovine kidney enzyme is unclear, but the binding of a series of thiodisaccharides (having sulfur in place the interglycosidic oxygen) to the enzyme has been investigated.[13] This study showed that disaccharides bound about 10 times better than did the methyl analogue, although all inhibitors were rather poor (K_i = 0.65–17 mM). The best inhibitor of bovine kidney fucosidase was a disaccharide analogue having a fucose bound to the 3-position of *N*-Ac-glucosamine, a result that suggested that this enzyme had a preference for this structure.

Compound **5** was found to competitively inhibit L-fucosidase from human placenta with a K_i-value of 144 μM, which is 23-times weaker than the K_i obtained for the isofagomine **14a** (Table 3). This suggests that this enzyme does not prefer fucosides bound to the 3-position, which accords with the study of this enzyme's substrate specificity.[12] Compound **5** was more potent against the bovine kidney enzyme, which was competitively inhibited with a K_i-value of 30 μM, which is 8-times weaker than that for **14a**. This is rather surprising given the enzyme's apparent preference for a 1–3-linked disaccharide.[13] The binding was increased about three times when the chain was extended to the tetrameric analogue **7**.

Similarly the diazadicarbasugar **6** and its analogue **27** were analysed for binding to four galactosidases; α-galactosidase from green coffee bean and three β-galactosidases from *E. coli*, *Aspergillus oryzae* and *Saccharomyces fragilis*, respectively. The α-galactosidase is believed to have a lipophilic aglycone-binding site.[14] It was therefore not obvious that the inhibitor **6** is more potent against this enzyme than was the corresponding isofagomine **19**. Presumably the S1 binding site of the enzyme can accommodate both lipophilic and polar groups. The fact that **27** does not inhibit the enzyme is predictable as glycosidases are extremely sensitive to changes in the hydroxy groups of

an azacarbasugar ring.[15] Inhibition, by some *N*-alkyl- and *N*-hydroxyalkyl-substituted derivatives of **19**, of this enzyme has been investigated by Ichikawa and others.[16] Similarly it was found that substitution of **19** increased binding 5- to 10-times whether it was by lipophilic or polar groups.

Ichikawa and co-workers[16] also investigated the *N*-substituted analogues of **19** for inhibition of β-galactosidase from *A. oryzae* and found that these analogues were about 1000-times weaker than **19**. Similarly for **6**; while not a bad inhibitor, was about 500-times less potent than unsubstituted **19**. Inhibition of the two other β-galactosidases by **6** was also 50- to 1000-times smaller than that of **19**.

In summary, the inhibition data show that azaoligosaccharide inhibitors **5**–**7** are inferior inhibitors to the corresponding *N*-unsubstituted isofagomines when the latter are potent inhibitors. When the isofagomine is a weak inhibitor the oligosaccharide analogue may be more potent. This accords with previous results on maltose-type disaccharide analogues.[17] The only exception is for the enzyme glucoamylase where the disaccharide analogues are quite potent inhibitors.[17] These observations can be explained as follows: In a configurationally retaining glycosidase there seems to be too little space in the active site to accommodate both a aglycone group and a strong interaction to the anomeric nitrogen atom. In a configurationally inverting enzyme, like glucoamylase, there is much more space and strong interactions both to the azacarbasugar and to the aglycone are possible.

Conclusions

We have accomplished the synthesis a series of azacarbasugars oligomers of which **7** is the most complex azacarbasugar ever synthesised. The work shows that peptide synthesis and reduction works well on polyhydroxylated piperidines, and it will be straightforward to transfer this chemistry to the solid phase for combinatorial chemistry. Future work will also focus on investigating the binding of the **5**–**7** to antibodies.

Experimental

NMR Spectroscopy

Structural assignments were made as previously described.[18] In addition, ^{1}H spectra were recorded on a Varian Unity INOVA 800 spectrometer for measurement of coupling constants and nuclear Overhauser effects (NOEs) with a mixing time of 400 ms.

Polarimetry

Optical rotations were measured on a Perkin-Elmer 241 polarimeter; $[a]_D$-values are in units of 10^{-1} deg cm^1 g^{-1}.

General procedures: amide formation (GP 1)

To a secondary amine (1 mol equiv.), an acid (1.2 mol equiv.) and Et_3N (3 mol equiv.) in CH_3CN (concentration ≈ 0.1 mmol of starting material ml^{-1}) was added HATU (1.2 mol equiv.) at room temperature. The obtained solution was stirred for 2–4 h at the same temperature and then concentrated *in vacuo*. The residue was taken up in $CHCl_3$ and washed with saturated aq. $NaHCO_3$. The aqueous phase was extracted twice with $CHCl_3$. The combined organic phases were dried ($MgSO_4$), and concentrated *in vacuo*; the pure amide was obtained by chromatography on silica gel with the eluent specified.

Reduction of amide (GP 2)

To an amide (1 mol equiv.) was added BH_3·THF (20 mol equiv.) at room temperature. The obtained mixture was heated under reflux for 2–4 h. After the reaction, 2 ml of conc. HCl per mmol of amide was added, and THF was removed *in vacuo*. The aqueous mixture was heated under reflux for 2 h, then neutralised with saturated aq. $NaHCO_3$ and diluted with water. The aqueous solution was put on a column of ion-exchange resin (Amberlite 15, H^+), washed with water and eluted with 2.5% NH_4OH. The product was obtained by concentration *in vacuo*.

Petroleum ether refers to the fraction with distillation range 35–60 °C.

Benzyl 2-deoxy-3,4-*O*-isopropylidene-2-methylene-β-L-*erythro*-pentopyranoside 9

To a mixture of **8** (14.0 g, 50.0 mmol), TEMPO (0.8 g, 5.1 mmol), NaBr (0.52 g, 5.1 mmol) and $NaHCO_3$ (6.3 g, 75.0 mmol) in CH_2Cl_2 (100 ml)–water (50 ml) was portionwise added NaOCl (12% w/w; *d* 1.2; 60 ml, 116.0 mmol) at room temperature until the starting material disappeared (TLC control, R_f = 0.46 for starting material and 0.73 for product). After separation of the phases, the aqueous phase was extracted with CH_2Cl_2 (50 ml × 2). The combined organic phases were washed with water (50 ml × 2), dried ($MgSO_4$), and concentrated *in vacuo* to provide crude benzyl 3,4-*O*-isopropylidene-β-L-*erythro*-pentopyranosid-2-ulose (15.2 g) as a yellow oil, which was used directly for the next step without further purification. NMR data of this material were identical with previously published data.[8]

To a mixture of $CH_3PPh_3^+I^-$ (30.3 g, 75 mmol) in dry THF (150 ml) was added dropwise *n*-butyllithium (1.6 M in *n*-hexane; 50 ml, 80 mmol) at −78 °C (N_2). After stirring of this mixture for 1 h at the same temperature, a solution of benzyl 3,4-*O*-isopropylidene-β-L-*erythro*-pentopyranosid-2-ulose (15.2 g, crude material) in THF (50 ml) was added. The reaction mixture was warmed slowly to room temperature (1 h) and stirred for 18 h at the same temperature. After the total conversion diethyl ether (200 ml) was added and the reaction mixture was filtered. The obtained solution was washed with aq. NH_4Cl (5% w/v; 100 ml), dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by chromatography (silica gel; petroleum ether–ethyl acetate 10 : 1, R_f = 0.32) to provide **9** (10.1 g, 73% over two steps) as a colorless oil. NMR data of this material were identical with previously published data.[8]

2-Deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-α/β-L-ribopyranose 10

To a solution of benzyl 2-deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-β-L-ribopyranoside[8] (2.0 g, 7.2 mmol, made from **9** by hydrogenation) in MeOH (50 ml) was added Pd/C (5%, 0.5 g). The mixture was hydrogenolysed under 1 atmosphere of H_2 and at room temperature for 18 h. After the total conversion, the reaction mixture was filtered through Celite and the solution was concentrated *in vacuo* to give **10** (1.3 g, 96%) as a colorless solid. NMR data of this material were identical with previously published data.[8]

2-Deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-α/β-L-ribopyranoside 10 and 2-deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-ribitol 11

To a solution of benzyl 2-deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-β-L-ribopyranoside[8] (2.0 g, 7.2 mmol) in THF (15 ml) were added liquid ammonia (*ca*. 20 ml) and solid sodium (2.0 g, 87.0 mmol) at −78 °C. The reaction mixture was stirred for 1 h, and then solid NH_4Cl (5 g) was added portionwise. After the mixture had been warmed to room temperature, water (20 ml) was added and the obtained mixture was extracted with CH_2Cl_2 (50 ml × 3). The combined solution was dried ($MgSO_4$), and concentrated *in vacuo*. The residue was separated by chromatography (silica gel; ethyl acetate, R_f = 0.63 for **10** and 0.32 for **11**) to give **10** (1.03 g, 76%) as a colorless solid and **11** (0.22 g, 16%) as a colorless oil. Physical data of these materials were identical with those previously published.[8]

2-Deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-L-ribitol 11

To a solution of **10** (1.03 g, 5.5 mmol) in EtOH (50 ml) was added $NaBH_4$ (0.83 g, 21.9 mmol) at 0 °C. After stirring of this mixture for 30 min at the same temperature, moist acidic ion-exchange resin (10 ml; Amberlite IR-120, H^+) was added. The obtained mixture was stirred for 20 min at 0 °C and filtered. The solution was concentrated *in vacuo* and the residue was filtered through a short column of silica gel (ethyl acetate, $R_f = 0.32$) to provide **11** (1.00 g, 96%) as a colorless oil. Physical data of this material were identical with those previously published.[18] Total yield from benzyl 2-deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-β-L-ribopyranoside was 89%.

2-Deoxy-3,4-*O*-isopropylidene-2-*C*-methyl-1,5-di-*O*-*p*-tolyl-sulfonyl-L-ribitol 12

To a solution of **11** (1.16 g, 6.1 mmol) and DMAP (*ca.* 10 mg) in Et_3N (10 ml)–CH_2Cl_2 (30 ml) was added TsCl (3.00 g, 15.7 mmol) at room temperature. The obtained mixture was stirred overnight, then concentrated *in vacuo* and taken up in water–CH_2Cl_2 (100 ml; 1 : 1). After separation of both phases the aqueous phase was extracted with CH_2Cl_2 (30 ml × 3). The combined organic phases were washed with water (50 ml), dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by chromatography, first with petroleum ether–ethyl acetate 3 : 1 (R_f = 0.17) and then with petroleum ether–ethyl acetate 2 : 1 (R_f = 0.59) to provide **12** (2.98 g, 98%) as a yellowish oil. Physical data of this material were identical with those previously published.[8]

(3*S*,4*R*,5*R*)-1-Allyl-3,4-isopropylidenedioxy-5-methylpiperidine 13

A solution of **12** (2.98 g, 6.0 mmol) in allylamine (40 ml) was heated under reflux overnight and then concentrated *in vacuo*. The residue was taken up in water–CH_2Cl_2 (100 ml; 1 : 1). After separation of both phases the aqueous phase was extracted with CH_2Cl_2 (50 ml × 2). The combined organic phases were dried ($MgSO_4$), and concentrated *in vacuo*. The residue was filtered through a short column of silica gel (Et_2O, $R_f = 0.38$) to give **13** (1.02 g, 81%) as a brownish oil; $[\alpha]_D^{22}$ −48.2 (*c* 3.0, $CHCl_3$); ^{1}H NMR (200 MHz; $CDCl_3$) δ 5.81 (dddd, *J* 17.1, 10.6, 6.5, 6.5 Hz, 1 H, olefin-H of allyl), 5.15 (ddd, *J* 1.7, 1.7 Hz, 1 H, olefin-H of allyl), 5.13 (ddd, 1 H, olefin-H of allyl), 4.14 (ddd, $J_{3,4}$ 4.7, $J_{2eq,3}$ 6.4 Hz, 1 H, H-3), 4.06 (dd, $J_{4,5}$ 4.7 Hz, 1 H, H-4), 2.96 (ddd, *J* 6.3 Hz, 2 H, CH_2 of allyl), 2.87 (ddd, $J_{2eq,2ax}$ 10.9, $J_{2eq,7eq}$ 1.8 Hz, 1 H, H-2eq), 2.55 (ddd, $J_{7eq,7ax}$ 10.7, $J_{5,7eq}$ 4.6 Hz, 1 H, H-7eq), 2.24 (m, 1 H, H-5), 1.88 (dd, $J_{5,7ax}$ 9.2 Hz, 1 H, H-7ax), 1.83 (dd, $J_{2ax,3}$ 10.9 Hz, 1 H, H-3ax), 1.47/1.32 (s, 6 H, 2 × CH_3 of isopropylidene), 1.03 (d, 3 H, H_3-6); ^{13}C NMR (50 MHz; $CDCl_3$) δ_C 134.4/118.3 (olefin of allyl), 108.3 [$(CH_3)_2C$], 75.4 (C-3*), 72.7 (C-4*), 61.3 (CH_2 of allyl), 55.2 (C-2**), 55.0 (C-7**), 31.7 (C-5), 28.3/26.3 [2 C, $(CH_3)_2$C], 14.8 (C-6); HRMS (ES) Calc. for $C_{12}H_{21}NO_2$ + Na^+: *m*/*z*, 234.1470. Found: *m*/*z*, 234.1469.

(3*S*,4*R*,5*R*)-3,4-Isopropylidenedioxy-5-methylpiperidine 14

Compound **13** (205 mg, 0.97 mmol) was dissolved in acetonitrile–water (50 ml; 84 : 16). The obtained solution was degassed with N_2 for 10 min, and then $(Ph_3P)_3RhCl$ (168 mg, 0.18 mmol) was added. The mixture was heated under reflux for 3 h. After starting material had disappeared (TLC control; Et_2O, R_f = 0.38 for starting material), the reaction mixture was concentrated *in vacuo*. The residue was purified by chromatography (ethyl acetate–methanol 5 : 2, R_f = 0.09) to provide **14** (101 mg, 61%) as a brownish oil; ^{1}H NMR (200 MHz; $CDCl_3$) δ 4.14 (dd, $J_{4,5} = J_{3,4}$ = 4.2 Hz, 1 H, H-4), 4.07 (dddd, $J_{2eq,3}$ 5.4 Hz, 1 H, H-3), 2.98 (dd, $J_{7eq,7ax}$ 13.0, $J_{5,7eq}$ 5.4 Hz, 1 H, H-7eq), 2.72 (dd, $J_{2eq,2ax}$ 12.3 Hz, 1 H, H-2eq), 2.55 (dd, $J_{5,7ax}$ 7.7 Hz, 1 H, H-7ax), 2.55 (NH), 2.48 (1 H, H-2ax), 1.82 (m, 1 H, H-5), 1.48/1.32 (s, 6 H, 2 × CH_3 of isopropylidene), 1.02 (d, 3 H, H_3-6); ^{13}C NMR (50 MHz; $CDCl_3$) δ_C 108.1 [$(CH_3)_2C$], 75.6 (C-3*), 72.1 (C-4*), 46.7 (C-2**), 46.5 (C-7**), 31.8 (C-5), 27.9/25.8 [2 C, $(CH_3)_2$C], 14.9 (C-6); HRMS (ES) Calc. for $C_9H_{17}NO_2$ + Na^+: *m*/*z*, 194.1157. Found: *m*/*z*, 194.1159.

(3*R*,4*S*,5*R*)-1-Benzyloxycarbonyl-4-hydroxy-5-(hydroxymethyl)-piperidine-3-carboxylic acid methyl ester 16

Compound **15** (0.74 g, 2.56 mmol) was added to CF_3COOH (3 ml) at room temperature. After stirring for 5 min at the same temperature, the reaction solution was concentrated *in vacuo*. The residue was taken up in water (15 ml) and then $NaHCO_3$ (3 g) and acetone (15 ml) were added at room temperature. Under vigorous stirring CbzCl (0.5 ml, 3.50 mmol) was added. The mixture was stirred for 1 h. After reaction acetone was removed *in vacuo*. The aqueous mixture was extracted with $CHCl_3$ (30 ml × 3). Drying ($MgSO_4$), concentration, and chromatography (pentane–ethyl acetate 1 : 2) provided compound **16** (0.80 g, 97%) as a colorless oil; $[\alpha]_D^{22}$ = +32.6 (*c* 0.57, $CHCl_3$); ^{1}H NMR (200 MHz; $CDCl_3$) δ 7.35–7.16 (m, 5 H, ArH), 5.05 (br s, 2 H, CH_2 of Bn), 4.44 (br s, 1 H, H-4), 4.20 (m, 1 H, H-2eq), 3.85 (m, 1 H, H-7eq), 3.60 (br s, 5 H, H-6a, H-6b, OCH_3), 3.20 (t, $J_{2ax,2eq} = J_{2ax,3}$ = 10.2 Hz, 1 H, H-2ax), 2.92 (t, $J_{5,7ax} = J_{7ax,7eq}$ = 12.0 Hz, 1 H, H-7ax), 2.47 (dm, 1 H, H-3), 1.65 (m, 1 H, H-5); ^{13}C NMR (50 MHz; $CDCl_3$) δ_C 172.6 (C=O of methyl ester), 155.6 (C=O of Cbz), 136.6/128.7/128.3/128.1 (6 C, arom.-C), 67.6 (C-4), 66.6 (CH_2 of Bn), 62.6 (C-6), 52.3 (CH_3 of methyl ester), 46.8 (C-2), 42.2 (C-7), 40.6 (C-3), 39.8 (C-5); HRMS (ES) Calc. for $C_{16}H_{21}NO_6$ + Na: *m*/*z*, 346.1267. Found: *m*/*z*, 346.1270.

(3*R*,4*S*,5*R*)-1-Benzyloxycarbonyl-4-hydroxy-5-hydroxymethyl-4,5′-*O*-isopropylidenepiperidine-3-carboxylic acid methyl ester 17

A solution of **16** (0.80 g, 2.48 mmol) and PTSA (10 mg) in 2,2-dimethoxypropane (50 ml) was stirred for 2 h at room temperature. After completion of the reaction the solution was washed with saturated aq. $NaHCO_3$ (10 ml). The aqueous phase was extracted with ethyl acetate (30 ml × 2). The combined organic phases were dried ($MgSO_4$), and concentrated *in vacuo* to give **17** (0.87 g, 97%) as a colorless oil; $[\alpha]_D^{22}$ +17.9 (*c* 0.26, $CHCl_3$); ^{1}H NMR (200 MHz; $CDCl_3$) δ 7.30–7.20 (m, 5 H, ArH), 5.05 (br s, 2 H, CH_2 of Bn), 4.55 (t, $J_{3,4} = J_{4,5}$ = 2.3 Hz, 1 H, H-4), 4.20 (m, 1 H, H-2eq), 4.03 (dd, $J_{5,6a}$ 3.0, $J_{6a,6b}$ 12.2 Hz, 1 H, H-6a), 3.88 (m, 1 H, H-7eq), 3.72 (s, 3 H, OCH_3), 3.54 (d, 1 H, H-6b), 3.30 (t, $J_{2ax,2eq} = J_{2ax,3}$ = 12.6 Hz, 1 H, H-2ax), 3.16 (t, $J_{5,7ax} = J_{7ax,7eq}$ = 12.6 Hz, 1 H, H-6ax), 2.45 (dt, $J_{2eq,3} = J_{3,4}$ = 3.0 Hz, 1 H, H-3), 1.46 (m, 1 H, H-5), 1.37/1.28 (s, 6 H, 2 × CH_3 of isopropylidene); ^{13}C NMR (50 MHz; $CDCl_3$) δ_C 171.1 (C=O of methyl ester), 155.5 (C=O of Cbz), 136.8/128.7/128.2/128.1 (6 C, arom.-C), 99.1 [$CH(CH_3)_2$ of isopropylidene], 67.4 (C-4), 66.4 (CH_2 of Bn), 62.2 (C-6), 52.0 (CH_3 of methyl ester), 45.4 (C-3), 41.4 (C-2), 39.6 (C-7), 33.8 (C-5), 29.6/18.8 [2 C, $CH(CH_3)_2$ of isopropylidene]; HRMS (ES) Calc. for $C_{19}H_{25}NO_6$ + Na^+: *m*/*z*, 386.1580. Found: *m*/*z*, 386.1581.

(3*R*,4*S*,5*R*)-1-Benzyloxycarbonyl-4-hydroxy-5-hydroxymethyl-4,5′-*O*-isopropylidenepiperidine-3-carboxylic acid 18

A mixture of **17** (0.40 g, 1.1 mmol) and $LiOH·H_2O$ (0.40 g, 9.5 mmol) in THF–water (20 ml; 1 : 1) was stirred for 4 h at room temperature. After the reaction was complete the mixture was neutralised with conc. HCl, washed with $CHCl_3$ (10 ml × 2), and then acidified to pH 1 with conc. HCl. The aqueous phase was extracted with ethyl acetate (30 ml × 2). The combined organic phases were dried ($MgSO_4$), and concentrated *in vacuo* to provide **18** (0.35 g, 91%) as a colorless foam; ^{1}H NMR (200 MHz; $CDCl_3$) δ 7.40–7.20 (m, 5 H, ArH), 5.05 (br s, 2 H, CH_2 of Bn), 4.57 (br s, 1 H, H-4), 4.20 (m, 1 H, H-2eq), 4.07

(dd, $J_{5,6a}$ 3.0, $J_{6a,6b}$ 12.3 Hz, 1 H, H-6a), 3.88 (m, 1 H, H-7eq), 3.57 (d, 1 H, 5′-H_b), 3.35 (t, $J_{2ax,2eq} = J_{2ax,3}$ = 12.6 Hz, 1 H, 2-H_{ax}), 3.18 (t, $J_{5,7ax} = J_{7ax,7eq}$ = 12.6 Hz, 1 H, H-7ax), 2.54 (dm, 1 H, H-3), 1.46 (m, 1 H, H-5), 1.37/1.28 (s, 6 H, 2 × CH_3 of isopropylidene); HRMS (ES) Calc. for $C_{18}H_{23}NO_6$ + Na^+: *m*/*z*, 372.1423. Found: *m*/*z*, 372.1426.

(3*R*,4*S*,5*R*)-1-*tert*-Butoxycarbonyl-3,4-dihydroxy-5-(hydroxymethyl)piperidine 20

To a mixture of **19** (trifluoroacetate, 60 mg, 0.26 mmol) and $NaHCO_3$ (200 mg, 2.38 mmol) in acetone–water (1 : 1; 10 ml) was added $(Boc)_2O$ (100 mg, 0.46 mmol) at room temperature. The reaction mixture was stirred for 1 h at the same temperature. After the reaction the acetone was removed *in vacuo* and the aqueous phase was extracted with ethyl acetate (20 ml × 3). The combined organic phases were dried ($MgSO_4$) and concentrated to provide **20** (63 mg, 98%) as a colorless foam; $[a]_D^{22}$ −2.5 (*c* 1.3, $CHCl_3$); ^{1}H NMR (200 MHz; $CDCl_3$) δ 4.08 (br s, 1 H, H-4), 3.85 (dd, $J_{2ax,3}$ 12.0, $J_{2eq,3}$ 3.8 Hz, 1 H, H-2eq), 3.80–3.50 (m, 4 H, H-3, H-6a, H-6b, H-7eq), 2.94 (t, $J_{2ax,2eq} = J_{2ax,3}$ = 12.0 Hz, 1 H, H-2ax), 2.78 (t, $J_{5,7ax} = J_{7ax,7eq}$ = 12.0 Hz, 1 H, H-7ax), 1.80 (m, 1 H, H-5), 1.47 [s, 9 H, $(CH_3)_3C$]; ^{13}C NMR (50 MHz; $CDCl_3$) δ_C 155.6 (C=O of Boc), 80.6 [$(CH_3)_3C$], 68.6 (C-3*), 67.9 (C-4*), 60.8 (C-6), 44.0 (C-2), 41.9 (C-5), 40.2 (C-7), 28.6 [3 C, $(CH_3)_3C$]; HRMS (ES) Calc. for $C_{11}H_{21}NO_5$ + Na^+: *m*/*z*, 270.1317. Found: *m*/*z*, 270.1310.

(3*R*,4*S*,5*R*)-3,4-Bis(benzyloxy)-5-benzyloxymethyl-1-(*tert*-butoxycarbonyl)piperidine 21

To a solution of **20** (60 mg, 0.24 mmol) in DMF (3 ml) was added NaH (60% in oil; 49 mg, 1.22 mmol) at room temperature. After the mixture had been stirred for 10 min, BnBr (0.15 ml, 1.26 mmol) was added. Stirring was continued for 1 h at the same temperature and then water (50 ml) was added. The aqueous mixture was extracted with $CHCl_3$ (50 ml, 5 ml × 2). The combined organic phases were washed with water (50 ml), dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by chromatography (pentane–ethyl acetate 10 : 1) to give **21** (61 mg, 48%) as a colorless oil; $[a]_D^{22}$ −8.1 (*c* 1.5, $CHCl_3$); ^{1}H NMR (200 MHz; $CDCl_3$) δ 7.42–7.24 (m, 15 H, ArH), 4.88/4.47 (d, *J* 11.8, 2 H, CH_2 of Bn), 4.58 (s, 2 H, CH_2 of Bn), 4.37 (s, 2 H, CH_2 of Bn), 4.00 (br s, 1 H, H-4), 4.15–3.80 (m, 1 H, H-2eq), 3.65 (m, 1 H, H-3), 3.50–3.25 (m, 1 H, H-7eq), 3.43 (t, $J_{5,5'a} = J_{5'a,5'b}$ = 8.3 Hz, 1 H, H-6a), 3.30 (dd, $J_{5,6b}$ 6.0 Hz, 1 H, H-6b), 3.25 (dd, $J_{2ax,2eq}$ 12.0, $J_{2ax,3}$ 10.9 Hz, 1 H, H-2ax), 2.84 (m, 1 H, H-7ax), 1.90 (m, 1 H, H-5), 1.39 [s, 9 H, $(CH_3)_3C$]; ^{13}C NMR (50 MHz; $CDCl_3$) δ_C 155.1 (C=O of Boc), 139.4/138.6/138.4/128.6/128.4/127.9/127.8/127.5 (18 C, arom.-C), 79.9 [$(CH_3)_3C$], 74.1 (C-3*), 73.5 (C-4*), 71.2 (3 C, CH_2 of Bn), 68.8 (C-6), 42.0 (2 C, C-2, C-7), 40.4 (C-5), 28.6 [3 C, $(CH_3)_3C$]; HRMS (ES) Calc. for $C_{32}H_{39}NO_5$ + Na^+: *m*/*z*, 540.2726. Found: *m*/*z*, 540.2729.

(3*R*,4*S*,5*R*)-3,4-Bis(benzyloxy)-5-(benzyloxymethyl)piperidine hydrochloride 22

A mixture of **21** (72 mg, 0.14 mmol) in MeOH–conc. HCl (5 : 2; 7 ml) was stirred for 1 h at room temperature. Concentration gave **22** (59 mg, 94%) as a yellowish oil; $[a]_D^{22}$ +5.4 (*c* 1.18, MeOH); ^{1}H NMR (200 MHz; $CDCl_3$) δ 7.40–7.00 (m, 15 H, ArH), 4.70/4.40 (d, *J* 11.3, 2 H, CH_2 of Bn), 4.57 (s, 2 H, CH_2 of Bn), 4.32 (s, 2 H, CH_2 of Bn), 4.02 (br s, 1 H, H-4), 3.76 (dm, 1 H, H-3), 3.47–2.96 (m, 4 H, H-2eq, H-6a, H-6b, H-7eq), 3.08 (t, $J_{2ax,2eq} = J_{2,3}$ = 11.0 Hz, 1 H, H-2ax), 2.80 (t, $J_{5,7ax} = J_{7ax,7eq}$ = 11.0 Hz, 1 H, H-7ax), 2.20 (m, 1 H, H-5); ^{13}C NMR (50 MHz; $CDCl_3$) δ_C 137.9/137.5/137.4/127.8/127.7/127.6/127.4/127.3/127.1/127.0 (18 C, arom.-C), 74.0 (C-3*), 73.7 (C-4*), 72.4/71.5/71.0 (3 C, CH_2 of Bn), 67.3 (C-6), 41.3 (C-2), 40.4 (C-7), 37.5 (C-5); HRMS (ES) Calc. for $C_{27}H_{31}NO_3$ + H^+: *m*/*z*, 418.2382. Found: *m*/*z*, 418.2381.

(3*S*,4*R*,5*R*)-1-[(3*R*,4*S*,5*R*)-1-Benzyloxycarbonyl-4-hydroxy-5-hydroxymethyl-4,5′-*O*-isopropylidenepiperidine-3-carbonyl]-3,4-isopropylidenedioxy-5-methylpiperidine 23

Amine **14** (79 mg, 0.46 mmol) and acid **18** (193 mg, 0.55 mmol) were coupled according to *GP 1*. Chromatography (pentane–ethyl acetate 1 : 1) provided **23** (173 mg) with some impurity. The crude product was subjected to further chromatography (ethyl acetate) to give **23** (153 mg, 66%) as a colorless foam; $[a]_D^{22}$ −4.5 (*c*2.1, $CHCl_3$); ^{1}H NMR (200 MHz; $CDCl_3$) δ 7.35–7.15 (m, 5 H, ArH), 5.05 (br s, 2 H, CH_2 of Bn), 4.34 (br s, 1 H, H-4), 4.25–4.10 (m, 2 H, H-3′, H-4′), 4.00 (dd, $J_{5,6a}$ 3.0, $J_{6a,6b}$ 12.8 Hz, 1 H, H-6a), 4.04–3.78 (m, 2 H, H-2eq, H-7eq), 3.74–3.20 (m, 7 H, H-7′eq, H-2′ax, H-2′eq, H-7′ax, H-2ax, H-7ax, H-6), 2.60 (m, 1 H, H-3), 1.85 (m, 1 H, H-5), 1.43 (m, 1 H, H-5′), 1.30/1.25 [s, 12 H, 2 × $(CH_3)_2C$], 0.98 (d, $J_{5',6'}$ 6.7 Hz, 3 H, H_3-6′); ^{13}C NMR (50 MHz; $CDCl_3$) δ_C 169.3 (C=O of amide), 155.5 (C=O of Cbz), 136.8/128.7/128.2/128.1 (6 C, arom.-C), 108.6 [$(CH_3)_2C'$], 99.2 [$(CH_3)_2C$], 74.7 (C-3′), 72.5 (C-4′), 67.4 (CH_2 of Bn), 65.8 (C-4), 62.3 (C-6), 44.0–40.0 (m, 5 C, C-2′,-7′, -2,-3,-7), 34.1 (C-5), 31.5 (C-5′), 29.7/19.1 [$(CH_3)_2C$], 27.1/24.8 [$(CH_3)_2C'$], 14.9 (C-6′); HRMS (ES) Calc. for $C_{27}H_{38}N_2O_7$ + Na^+: *m*/*z*, 525.2577. Found: *m*/*z*, 525.2578.

(3*S*,4*R*,5*R*)-1-[(3*S*,4*R*,5*R*)-4-Hydroxy-5-(hydroxymethyl)-3-piperidylmethyl]-3,4-dihydroxy-5-methylpiperidine dihydrochloride 5

Compound **23** (60 mg, 0.12 mmol) was treated according to *GP 2* to provide **5**, which was acidified to pH 1 with conc. HCl. After concentration, 17 mg (46%) of the $(HCl)_2$ salt of **5** were obtained. ^{1}H and ^{13}C NMR (see Table 1); HRMS (ES) Calc. for $C_{13}H_{26}N_2O_4$ + H^+: *m*/*z*, 275.1971. Found: *m*/*z*, 275.1969.

(3*R*,4*S*,5*R*)-1-[(3*R*,4*S*,5*R*)-1-*tert*-Butoxycarbonyl-4-hydroxy-5-hydroxymethyl-4,5′-*O*-isopropylidenepiperidine-3-carbonyl]-4-hydroxy-5-hydroxymethyl-4,5′-*O*-isopropylidenepiperidine-3-carboxylic acid methyl ester 26

A solution of **17** (0.42 g, 1.16 mmol) in methanol was treated with H_2 in the presence of 10% Pd/C (100 mg) under 1 atmosphere for 0.5 h at room temperature. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to provide crude ester **24**, which was used directly to the next step with further purification. The coupling of **24** with acid **25** (0.44 g, 1.40 mmol) was proceeded according to *GP 1*. Chromatography pentane–ethyl acetate 1 : 1) provided compound **26** with some impurity. The crude product was subject to further chromatography (ethyl acetate) to give **26** (0.42 g, 69% based on **17**) as a colorless foam; $[a]_D^{22}$ +18.3 (*c* 1.1, $CHCl_3$); ^{1}H NMR (200 MHz; $CDCl_3$) δ 4.55 (t, $J_{3,4} = J_{4,5}$ = 2.3 Hz, 1 H, H-4), 4.30 (m, 1 H, H-2eq), 4.20–3.70 (m, 6 H, H-2′eq, H-7eq, H-6a, H-4′, H-7′eq, H-6′a), 3.62 (s, 3 H, OCH_3), 3.56 (d, $J_{6'a,6'b}$ 12.2 Hz, 1 H, H-6′b), 3.51 (d, $J_{6a,6b}$ 12.8 Hz, 1 H, H-6b), 3.50–2.80 (m, 2 H, H-2′, H-7′ax), 3.31 (t, $J_{2ax,2eq} = J_{2ax,3}$ = 11.8 Hz, 1 H, H-2ax), 3.25 (t, $J_{5,7ax} = J_{7ax,7eq}$ = 11.8 Hz, 1 H, H-7ax), 2.67 (m, 1 H, H-3′), 2.40 (m, 1 H, H-3), 1.54–1.20 (m, 2 H, H-5′, H-5), 1.33 [s, 9 H, $(CH_3)_3C$], 1.33/1.25 [s, 12 H, 2 × $(CH_3)_2C$]; ^{13}C NMR (50 MHz; $CDCl_3$) δ_C 170.8 (C=O of ester), 169.1 (C=O of amide), 155.0 (C=O of Boc), 99.3 [2 × $(CH_3)_2C$], 79.9 [$(CH_3)_3C$], 66.4 (2 C, C-4′, C-4), 62.3 (C-6′*), 62.2 (C-6*), 52.1 (CH_3 of ester), 46.6 (C-3′**), 45.0 (C-3**), 42.9/41.3/40.7/39.4 (4 C, C-2′, C-7′, C-2, C-7), 34.0 (2 C, C-5′, C-5), 29.6/19.0/18.8 [4 C, 2 × $(CH_3)_3C$], 28.6 [3 C, $(CH_3)_3C$]; HRMS (ES) Calc. for $C_{26}H_{42}N_2O_9$ + Na^+: *m*/*z*, 549.2788. Found: *m*/*z*, 549.2786.

(3,4-*cis*,4,5-*cis*)-1-[(3*S*,4*R*,5*R*)-4-Hydroxy-5-(hydroxymethyl)-3-piperidylmethyl]-4-hydroxy-3,5-bis(hydroxymethyl)piperidine 27

Compound **26** (100 mg, 0.19 mmol) was elaborated according to *GP 2* to provide diamine **27** (42 mg, 73%) as a colorless solid;

^{1}H NMR (200 MHz; $CDCl_3$) δ 4.20 (s, 1 H, H-4′*), 4.15 (s, 1 H, H-4*), 3.90–2.86 (m, 16 H), 2.60/2.43–2.05 (m, 4 H, H-3′, H-5′, H-3, H-5); ^{13}C NMR (50 MHz; $CDCl_3$) δ_C 62.8 (C-4′), 61.8 (C-4), 59.9 (2 C, C-6′, C-8′), 59.7 (C-6), 56.7 (2 C, C-2′, C-7′), 49.8 (C-8), 40.7/40.5/39.9/39.7 (5 C, C-3′, C-5′, C-2, C-3, C-7), 33.5 (C-5); HRMS (ES) Calc. for $C_{14}H_{28}N_2O_5$ + H^+: *m*/*z*, 305.2076. Found: *m*/*z*, 305.2078.

(3*R*,4*S*,5*R*)-1-[(3*R*,4*S*,5*R*)-1-*tert*-Butoxycarbonyl-4-hydroxy-5-hydroxymethyl-4,5′-*O*-isopropylidenepiperidine-3-carbonyl]-3,4-bis(benzyloxy)-5-(benzyloxymethyl)piperidine 28

The coupling of amine **22** (hydrochloride, 59 mg, 0.13 mmol) with acid **25** (51 mg, 0.16 mmol) was allowed to proceed according to *GP 1*. Chromatography (pentane–ethyl acetate 3 : 1) provided **28** (63 mg, 68%) as a colorless foam; $[\alpha]_D^{22}$ +13.5 (*c* 1.3, $CHCl_3$); ^{1}H NMR (200 MHz; $CDCl_3$) δ 7.40–7.16 (m, 15 H, ArH), 4.89 (d, 1 H, CH_2 of Bn), 4.80–4.33 (m, 5 H, H-4′, H-4, CH_2 of Bn), 4.38 (s, 2 H, CH_2 of Bn), 4.34–4.07 (m, 2 H, H-3′, H-2eq), 4.04–3.60 (m, 4 H, H-2′eq, H-7′eq, H-6a, H-7eq), 3.60–3.00 (m, 7 H, H-2ax, H-7′ax, H-2′ax, H-7ax, H-6b, H-6′a, H-6′b), 2.60 (m, 1 H, H-3), 2.00–1.60 (m, 2 H, H-5′, H-5), 1.40 [s, 9 H, $(CH_3)_3C$], 1.28 [s, 6 H, $(CH_3)_2C$]; ^{13}C NMR (50 MHz; $CDCl_3$) δ_C 171.8/170.9 (1 C, C=O of amide), 153.8 (C=O of Boc), 138.0–136.0/128.0–126.0 (m, 18 C, arom.-C), 97.7 [$(CH_3)_2C$], 79.1/78.9/78.7 [m, 1 C, $(CH_3)_3C$], 73.3/72.3/70.1 (3 C, CH_2 of Bn), 67.4 (C-3′), 66.3 (C-4′), 65.0 (C-4), 63.0/62.9 (C-6′), 61.0 (C-6), 44.6–37.0 (m, 6 C, C-2′, C-5′, C-7′, C-2, C-3, C-7), 32.6 (C-5), 29.9/17.6 [2 C, $(CH_3)_2C$], 27.5 [3 C, $(CH_3)_3C$]; HRMS (ES) Calc. for $C_{42}H_{54}N_2O_8$ + Na^+: *m*/*z*, 737.3778. Found: *m*/*z*, 737.3782.

(3*R*,4*S*,5*R*)-1-[(3*S*,4*R*,5*R*)-4-Hydroxy-3-(hydroxymethyl)-3-piperidylmethyl]-3,4-dihydroxy-5-(hydroxymethyl)piperidine 6

Compound **28** (63 mg, 0.088 mmol) was treated according to *GP 2* to provide **6** (14 mg, 55%) as a colorless foam. ^{1}H and ^{13}C NMR (see Table 1); HRMS (ES) Calc. for $C_{13}H_{26}N_2O_5$ + H^+: *m*/*z*, 291.1920. Found: *m*/*z*, 291.1918.

(3*R*,4*S*,5*R*)-1-[(3*R*,4*S*,5*R*)-1-*tert*-Butoxycarbonyl-4-hydroxy-5-hydroxymethyl-4,5′-*O*-isopropylidenepiperidine-3-carbonyl]-4-hydroxy-5-hydroxymethyl-4,5′-*O*-isopropylidenepiperidine-3-carboxylic acid 30

A mixture of ester **26** (105 mg, 0.20 mmol) and $LiOH\cdot H_2O$ (100 mg, 2.38 mmol) in THF–water (10 ml; 1 : 1) was stirred for 0.5 h at room temperature. After the reaction was complete the mixture was neutralised with conc. HCl and THF was removed *in vacuo*. The aqueous phase was washed with $CHCl_3$ (10 ml × 2), acidified to pH 1 with conc. HCl, and extracted with ethyl acetate (30 ml × 2). The combined ethyl acetate phases were dried ($MgSO_4$) and concentrated *in vacuo* to provide acid **30** acid (82 mg, 80%) as a colorless foam; ^{1}H NMR (200 MHz; $CDCl_3$) δ 4.60 (s, 1 H, H-4), 4.30 (m, 1 H, H-2eq), 4.20–3.70 (m, 7 H, H-2′eq, H-7′eq, H-6′a, H-4′, H-2′ax, H-7eq, H-6a), 3.70–.00 (m, 5 H, H-6′b, H-7′ax, H-2ax, H-6b, H-7ax), 2.80–2.40 (m, 2 H, H-3′, H-3), 1.54–1.20 (m, 2 H, H-5′, H-5), 1.33 [s, 9 H, $(CH_3)_3C$], 1.33/1.25 [s, 12 H, 2 × $(CH_3)_2C$]; ^{13}C NMR (50 MHz; $CDCl_3$) δ_C 168.0 (C=O of amide), 154.2 (C=O of Boc), 98.2 [2 C, 2 × $(CH_3)_2C$], 79.2 [$(CH_3)_3C$], 66.6 (C-4′), 65.1 (C-4), 61.1 (2 C, C-6′, C-6), 44.0–36.0 (m, 6 C, C-2′, C-3′, C-7′, C-2, C-3, C-7), 32.7 (2 C, C-5′, C-5), 28.4/28.1/17.6 [4 C, 2 × $(CH_3)_3C$], 27.5 [3 C, $(CH_3)_3C$]; HRMS (ES) Calc. for $C_{25}H_{40}N_2O_9$ + Na^+: *m*/*z*, 535.2631. Found: *m*/*z*, 535.2629.

Coupling of 29 with 30: product 31

A solution of **23** (51 mg, 0.1 mmol) in THF–water was treated with H_2 in the presence of 10% Pd/C (35 mg) under 1 atmosphere for 4 h at room temperature. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to provide crude free amine **29**, which was used directly in the next step with further purification.

The coupling of **29** with **30** (72 mg, 0.14 mmol) in the presence of HATU (53 mg, 0.14 mmol) was conducted according to *GP 1*. Chromatography (ethyl acetate–MeOH, 20 : 1) provided tetracycle **31** (22 mg, 26%) as a yellowish oil; ^{1}H NMR (200 MHz; $CDCl_3$) δ 4.40–3.00 (m, 27 H), 2.80–2.40 (m, 3 H), 2.20–1.70 (m, 3 H), 1.55 (m, 1 H), 1.50–1.25 (m, 27 H), 1.00 (br s, 3 H); ^{13}C NMR (100.6 MHz; $CDCl_3$) δ 108.6 [$(CH_3)_2C$], 99.6 [2 C, 2 × $(CH_3)_2C$], 74.6 (C-3‴), 72.6 (C-4‴), 65.8/64.2 (3 C, C-4, C-4′, C-4″), 62.5/62.3/62.2 (3C, C-6, C-6′, C-6″), 44.0–38.0 (m, 11 C, C-2‴, C-7‴, C-2″, C-3″, C-7″, C-2′, C-3′, C-7′, C-2, C-3, C-7), 35.7/35.2/33.7 (3C, C-5″, C-5′, C-5), 30.5 (C-5‴), 29.8/29.7 and 19.1/19.0 [4 C, 2 × $(CH_3)_2C$], 28.7 [3 C, $(CH_3)_3C$], 27.0/24.6 [2 C, $(CH_3)_2C$], 15.0 (C-6‴); HRMS (ES) Calc. for $C_{41}H_{66}N_4O_{13}$ + Na^+: *m*/*z*, 845.4524. Found: *m*/*z*, 845.4529.

(3*S*,4*R*,5*R*)-1-{(3*S*,4*R*,5*R*)-1-[(3*S*,4*R*,5*R*)-4-Hydroxy-5-(hydroxymethyl)-3-piperidylmethyl]-4-hydroxy-5-(hydroxymethyl)-3-piperidylmethyl}-3-[(3*S*,4*R*,5*R*)-3,4-dihydroxy-5-methylpiperidinomethyl]-4-hydroxy-5-(hydroxymethyl)piperidine 7

Compound **31** (22 mg, 0.027 mmol) was elaborated according to *GP 2* to provide tetraamine **7**, which was acidified to pH 1 with conc. HCl. After concentration, 6 mg (32%) of a hydrochloride of **7** were obtained. ^{1}H and ^{13}C NMR (see Table 2); HRMS (ES) Calc. for $C_{27}H_{52}N_4O_8$ + H^+: *m*/*z*, 561.3863. Found: *m*/*z*, 561.3856.

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