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Stereodivergent synthesis of right- and left-handed iminoxylitol heterodimers and monomers. Study of their impact on β-glucocerebrosidase activity†

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A library of dimers and heterodimers of both enantiomers of 2-*O*-alkylated iminoxylitol derivatives has been synthesised and evaluated on β-glucocerebrosidase (GCase), the enzyme responsible for Gaucher disease (GD). Although the objective was to target simultaneously the active site and a secondary binding site of the glucosidase, the (−)-2-iminoxylitol moiety seemed detrimental for imiglucerase inhibition and no significant enhancement was obtained in G202R, N370S and L444P fibroblasts. However, all compounds having at least one (+)-2-*O*-alkyl iminoxylitol are GCase inhibitors in the nano molar range and are significant GCase activity enhancers in G202R fibroblats, as confirmed by a decrease of glucosylceramide levels and by co-localization studies.

Introduction

Gaucher disease (GD) is the most prevalent lysosomal storage disorder, characterized by decreased activity of the enzyme glucocerebrosidase (EC 3.2.1.45; GCase or GBA1) and the subsequent accumulation of glucosylceramide (GlcCer) in several organs including liver, spleen and, in some cases, brain. This enzyme also requires the coordinated action of saposin C and negatively-charged lipids for maximal activity.[1] Three major types of Gaucher disease have been described on the basis of the absence (type I) or presence and severity (types II and III) of primary central nervous system involvement. The disease is particularly frequent in the Ashkenazi Jewish population in which the incidence of GD is estimated as 1 in 850,[1] while in other populations the frequency ranges between 1 in 40 000 and 1 in 60 000.[1,2] Currently, 440 mutations have been described in the GCase gene, 386 of which causing GD (HGMD® professional 2016.3). Among them, the N370S and L444P missense mutations are the most common ones. Despite the imprecise genotype–phenotype correlation, the N370S mutation is the most prevalent among the Jewish patients and it is exclusively associated with the non-neuronopathic form (type I) of the disease. The L444P[3] is the most prevalent mutation associated with the neuronopathic forms of the disease (type II and III) when it appears in homozygosity or in heterozygosity with other severe mutations.[4] On the other hand, the G202R is a severe mutation found in homozygosity in patients presenting type II GD.[5] At the molecular level, the outcome of the N370S mutation is characterized by a GCase-reduced catalytic activity (Vmax of the N370S mutant enzyme is lower than that of the wild-type (WT) enzyme[6,7]) and a diminished amount of GCase in lysosomes, leading to an overall enzymatic activity reduced by 70%.[8] For L444P mutation, the residual activity is around 12% of the normal

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†Electronic supplementary information (ESI) available: Experimental procedures for the synthesis of starting materials, arms, and linkers. Analytical data and copies of NMR spectra activity of selected GCaseE enhancers towards glycosidases (Table S1); normalized GCase activity of human fibroblast cell lines after treatment with selected compounds at different concentrations (Fig. S54); synergism experiments with **11c** and **11d** (Fig. S55); inhibition of imiglucerase (double reciprocal plots) for **8a-e**, **9**, (+)-**10**, (−)-**10**, **11a-d**, **12a-c** (Fig. S56 to S69). See DOI: 10.1039/c7ob00443e

one[8] and for the rarer G202R mutation it is around 10% of the WT.[5,8] It is estimated that neurological disorders appear below a threshold of 11–15% of enzymatic activity[9,10] when GlcCer influx is higher than its degradation. All strategies aiming at reducing GlcCer accumulation in the lysosome, either by reducing its formation (SRT: Substrate Reduction Therapy),[11–13] infusing recombinant enzyme (ERT: Enzyme Replacement Therapy),[14–16] increasing GCase activity by stabilisation in the ER or lysosomes[7] or by promoting its folding and/or transport to lysosomes (PCT: Pharmacological Chaperone Therapy)[17–20] are suitable therapies that may be complementary with synergistic effects.[21] The chaperone therapy is based on the interaction between the mutated GCase and a small molecule, the pharmacological chaperone (PC), which is an inhibitor able to increase GCase activity in the lysosome at sub-inhibitory concentrations. PCs are believed to induce or stabilize the proper conformation of the misfolded but catalytically active enzyme, preventing its degradation by ER-associated degradation (ERAD).[19] This strategy holds great potential, since small molecules are amenable to cross the blood brain barrier.

Although many efforts have been carried out in the last fifteen years,[17,18] the search for new PCs to tackle specific GCase mutations and/or to be used in combination with ERT is still a hot point. The next generation of PCs would ideally be pure activators to avoid the drawbacks associated with GCase inhibition. Different strategies have already been used to discover non-inhibitory PCs. The high throughput screening approach using spleen homogenates from a Gaucher patient N370S homozygote and the profluorescent substrate 4-methylumbelliferyl-β-D-glucoside (4-MU), allowed the identification of a pyrazolopyrimidine showing a 50% GCase activation at a submicromolar concentration.[22] This compound promoted the transport of GCase to lysosomes and an enzyme activity increase on fibroblasts from patients homozygous for N370S and L444P.[22] Higher activity enhancements for pure activators are still needed. However, although structures for WT GCase (21 structures in the protein data bank (PDB)) and the N370S mutant (PBD codes 3KE0, 3KEH)[7] are known, there are no insights about potential allosteric sites, which have been discovered in many instances by serendipity, as in Fabry[23] and Pompe[24] diseases. For GCase, hot spots for secondary sites were identified on its surface from the multiple experimental solvent crystal structures method and computational fragment mapping simulations.[25] Furthermore, the structure–activity relationships (SAR) by NMR methods cannot be used, since they are only applicable to small biomolecules (MW < 30 kDa) that can be ^{15}N-labeled and obtained in relatively large quantities.[26,27] In this context, we describe herein our efforts towards the synthesis of unprecedented PCs based on heterodimeric inhibitors designed to target both the active site and a secondary binding site of GCase. One of the advantages of this approach is that the two ligand heads are enantiomers, allowing the development of heterodimeric PCs following an efficient divergent/convergent strategy.

Results and discussion

Heterodimeric concept

The starting point of our study was the observation made by Asano *et al.* that the (−)-enantiomer of isofagomine (+)-**1**, the most advanced PC clinical candidate for the treatment of GD, can also display chaperoning activity[28] although it is a non-competitive inhibitor of GCase (Fig. 1).[28–30] The same behaviour was observed by the groups of Kato and Fleet for (−)-DGJ and (+)-DGJ on α-galactosidase A (Fabry disease).[31]

In addition, this study showed that a dose–response synergistic effect on the enzyme activation could be obtained when both enantiomers were used.[31] Having in mind the double objectives of finding a new class of -hopefully better- chaperones and of identifying a secondary binding site, we designed heterodimers composed of both enantiomers of an appropriate iminosugar to target, at the same time, the GCase active

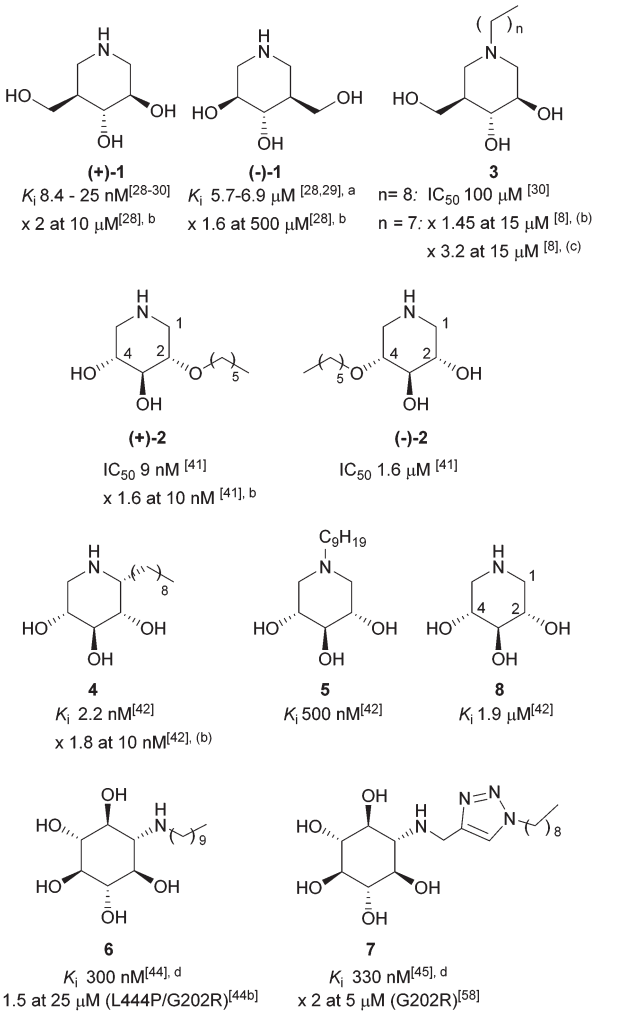


Fig. 1 Glycomimetic chaperones of GCase and their activity. K_i values measured on imiglucerase (Cerezyme®) with a competitive mode of inhibition except otherwise noted. (a) Non-competitive inhibition mode; (b) N370S-GCase activity increase; (c) G202R-GCase activity increase; (d) imiglucerase thermal stabilisation.

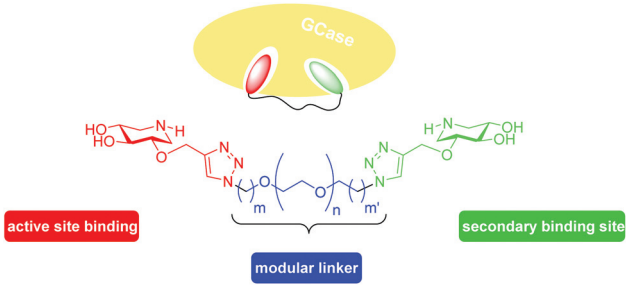


Fig. 2 Heterodimers as probes to search for a secondary GCase binding site.

and secondary binding sites (Fig. 2). For this purpose, a broad range of linker sizes have been explored in order to fine tune the binding of both enantiomers with an optimized distance (Fig. 3). Precedent works showed that the biological binding affinities are highly dependent on the optimal length and nature of the linkers for divalent[32–35] and heterodivalent inhibitors.[36–38] Even though the binding affinity of the (−) enantiomers of known iminosugars is known to be 1000 times lower than that of the corresponding (+) enantiomers for their respective binding sites, the anchoring of both ligands linked by the appropriate spacer was expected to result in an increased global binding of the heterodimeric inhibitor due to a chelate effect.[39,40] The strong fixation in the active site of the chosen nanomolar inhibitor was expected to drive the binding of its mirror-image analogue to the secondary site. Our idea was to find, by library screening, the most suitable arm length enabling the fixation of both enantiomers in order to identify a secondary binding site at a later stage by X-ray analysis or molecular dynamics measurements.

For the sake of comparison, and also to check the validity of this concept, we also prepared the (+) and (−) monomers, as well as the corresponding (+/+) and (−/−) homodimers, as shown in Fig. 3.

Target library

The choice of the iminosugar turned towards 1,5-dideoxy-1,5-imino-D-xylitol (DIX) since (+)-2-*O*-hexyl-DIX (+)-**2**[41] displays chaperone activity at a concentration 1000 times lower than IFG (+)-**1** and *N*-alkylated IFGs **3**[8] (Fig. 1). Moreover its (−)-enantiomer (−)-**2** is also a GCase inhibitor.[41] DIX derivatives are also known to be highly selective and to display no inhibition towards α-glucosidases, including intestinal α-glucosidases, and thus give the promise of less side effects in case of further development.[41,42] As a preliminary assay, we checked that (−)-**2** was indeed a non-competitive inhibitor (results not shown). The 2-*O*-alkyl chain of the DIX head targeting the active site was chosen to be composed of nine carbons, since GCase has a lipophilic binding pocket adapting well C9 chains, as deduced from the K_i values of 500 nM and 2.2 nM for compounds **5** and **4**, respectively[42] (Fig. 1). For the arm linked to the (−)-2-*O*-alkyl DIX, two different chains were chosen, the known C6 chain and an oligoethylene glycol-based chain to improve the solubility in aqueous medium (Fig. 3). For an efficient synthesis of the library of heterodimers, dimers and monomers of (+)- and (−)-2-*O*-alkyl DIX, the Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) reaction was chosen. The consequence is the presence of two 1,4-disubstituted 1,2,3-triazoles, which are known to be metabolically inert, to maintain a good pharmacokinetic profile and to be able to participate in binding interactions, *i.e.* hydrogen bonds and π–π stacking.[43] It is noteworthy that, at the beginning of this study, the presence of a triazole ring in the

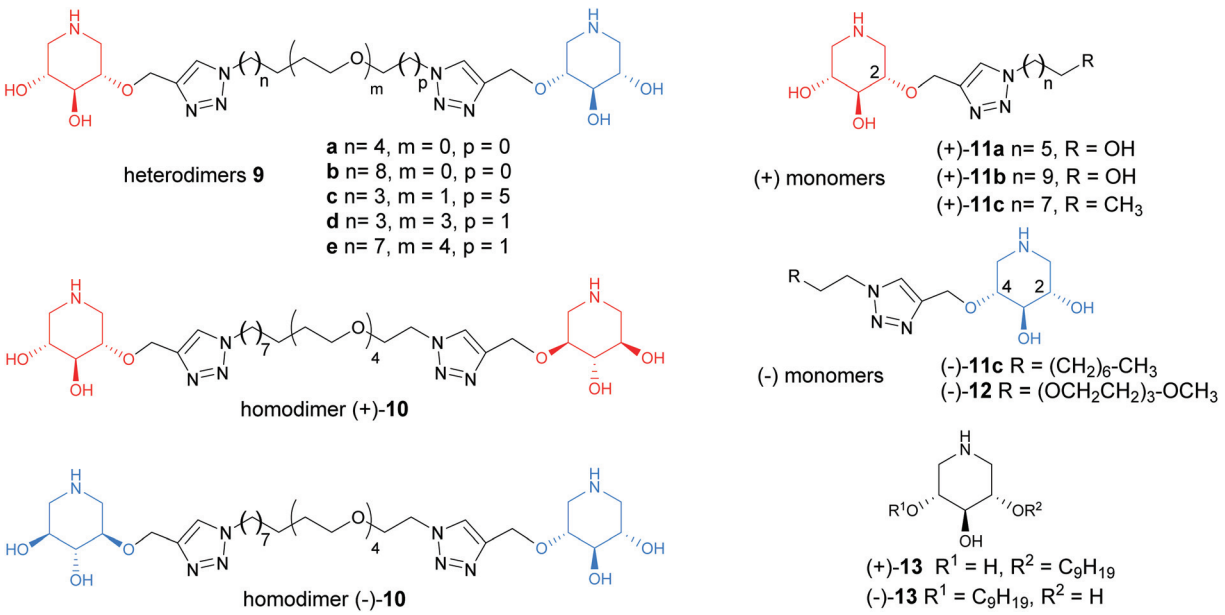


Fig. 3 Library of DIX-based monovalent, homodivalent and heterodivalent iminosugars.

aglycon part of GCase inhibitors seemed to have no impact on the inhibitory activity, as exemplified by the similar inhibition profile of compounds **6** and **7**[44,45] (Fig. 1). Based on these considerations, a small library of DIX-based monovalent, homodivalent and heterodivalent iminosugars was designed, as shown in Fig. 3.

Synthetic strategy

A single approach for both hetero and homodimers was established. Thus, propargylated DIX derivatives would be sequentially clicked in a convergent manner on an azido-armed linker bearing an azide precursor on the other side (Fig. 4 and Scheme 1). Moreover, the observation that 1,5-dideoxy-1,5-imino-D-xylitol **8** (Fig. 1) is a *meso* compound, and that alkylation of the hydroxyl group at C2 or C4 leads to enantiomers, prompted us to develop a stereodivergent approach for the synthesis of both enantiomers of **14**. Addition of a carbonyl group at C1 in **8** to give a lactam provides a convenient way to break the plane of symmetry in the *meso* imino-D-xylitol derivative. Having in mind that lactams are relevant precursors of iminosugars,[46,47] our first objective was to use a non-selective monopropargylation of lactam **A** (Scheme 1) leading to a pair of theoretically separable regioisomers as precursors of both heads. It is then easy to remove the carbonyl group by reduction of the lactam function. In order to guarantee a selective mono-alkylation at C2 or C4 in lactam **A**, the protection of the C3 hydroxyl group was thus a prerequisite. For this reason, diacetone D-glucose seemed to be the ideal starting material for this purpose (Scheme 2).[41,48–50]

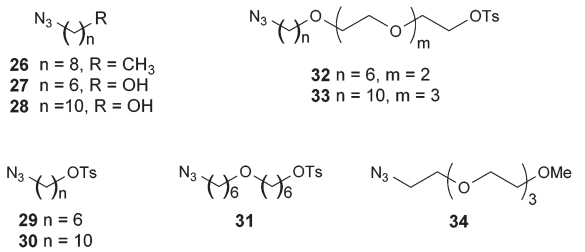
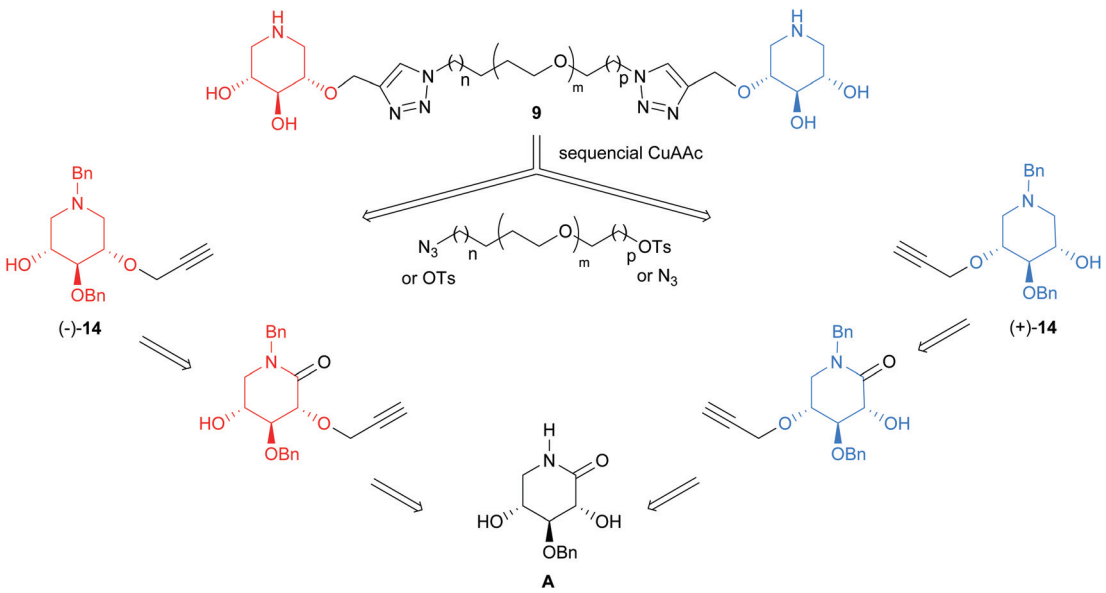


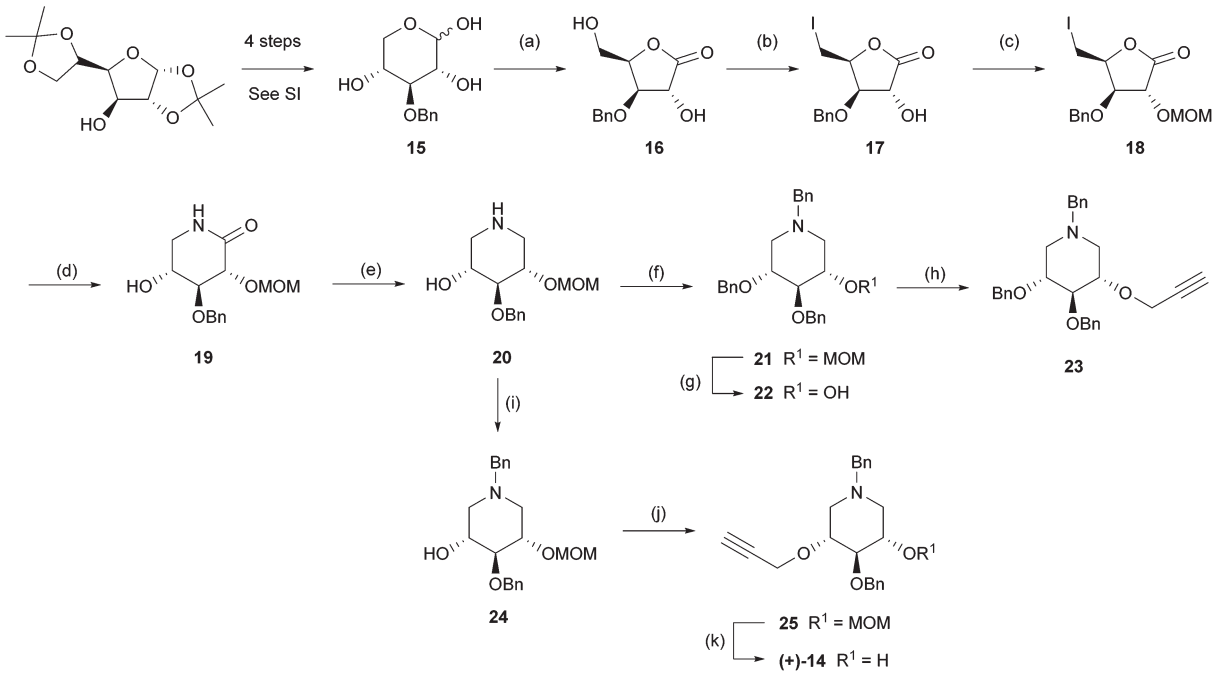
Fig. 4 Arm and linker precursors for the synthesis of heterodimers, dimers and monomers.

Stereodivergent synthesis of both inhibitor heads

A rapid, robust and efficient access to 3-*O*-benzyl-α-D-xylopyranose **15** (82% over 4 steps, Scheme S1, see ESI†) in a 20 g scale was possible by deprotecting 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose with a 1 M H_2SO_4 solution in THF at 60 °C instead of using very large amounts of Dowex (H^+) resin[41,48,50,51] (see ESI†). Formation of lactam **19** (Scheme 2) was inspired from the work of Lundt *et al.*, who synthesized lactams by aminolysis of γ-lactones using aqueous ammonia, followed by displacement of a leaving group in position 5 of the γ-lactone. This approach has been described for the unprotected D-arabino- and D-ribono-series, or for the corresponding protected L-lyxono- and D-xylono-analogues.[52] The γ-lactone **16** was directly formed by oxidation of lactol **15** by bromine in the presence of potassium carbonate.[53] Selective activation of the primary alcohol in **16** was first tested by mesitylene sulfonylation as described by Vasella on free D-xylonolactone.[54] However, hindrance arising from the benzyl group in position 3 limited the conversion, even after 4 days (26% yield, unpublished result). Selective iodination proved more successful under optimised conditions, leading to compound **17** in 90% yield (Scheme 2). However, it was necessary to protect the C2 hydroxyl group in **17** in order to avoid a 5-*exo*-tet irreversible cyclisation arising from intramolecular iodide displacement during the lactamization step. The free secondary



Scheme 1 Retrosynthetic approach for the synthesis of heterodimers.



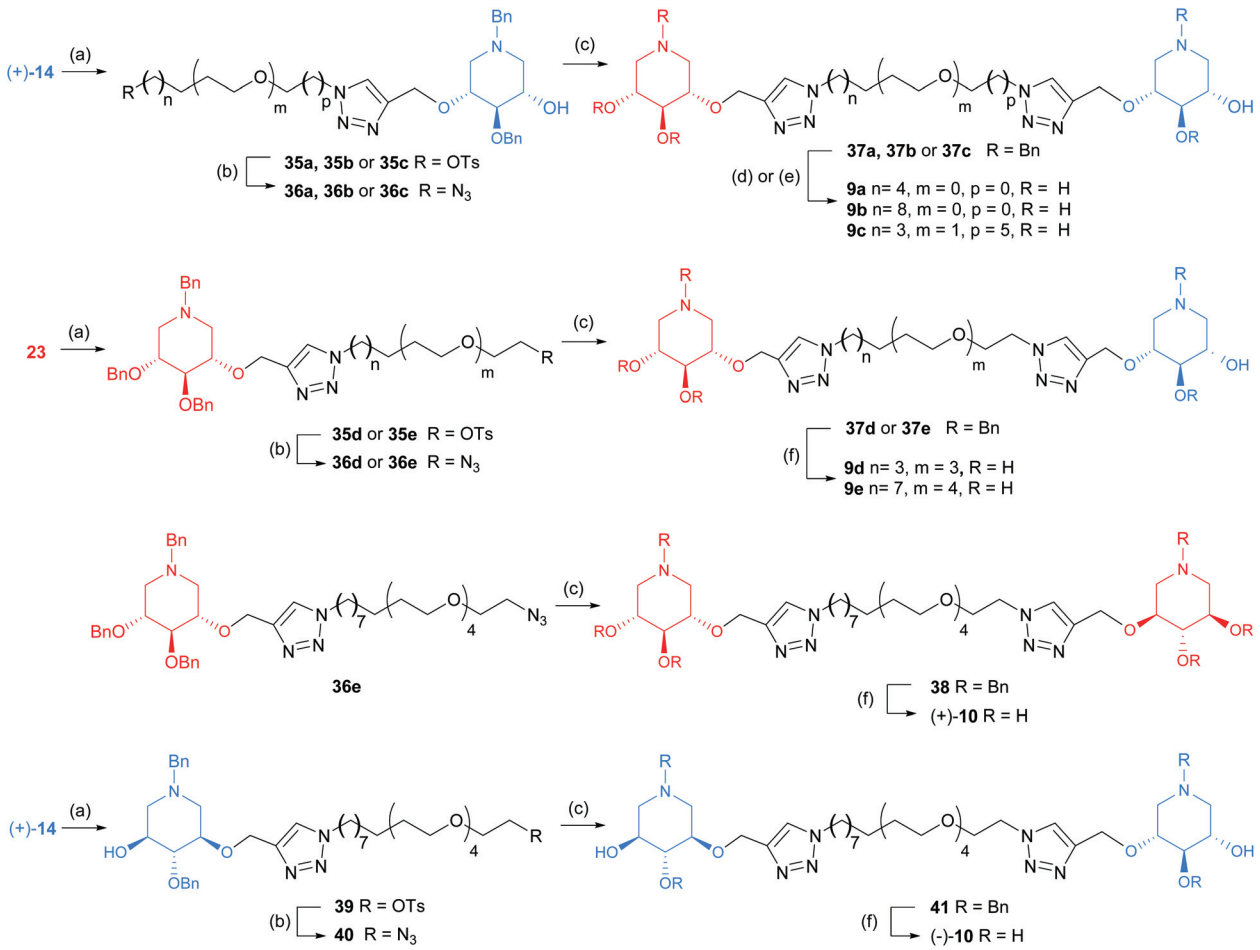
Scheme 2 Stereodivergent synthesis of both heads. Reaction conditions: (a) Br_2, K_2CO_3, THF, H_2O, 5 °C to r.t., 17 h, 78%; (b) PPh_3, I_2, imidazole, THF, 60 °C for 4 h, r.t. for 16 h, 65 °C for 2 h, 90%; (c) $H_2C(OMe)_2$, P_4O_{10}, r.t., 16 h, 85%; (d) 30% NH_4OH, DMF, r.t., 44 h, 84%; (e) $LiAlH_4$, THF, reflux, 2 h, 80%; (f) BnBr, NaH, KI, THF, r.t., 18 h, 87%; (g) 6 N HCl, MeOH, 65 °C, 2 h, 97%; (h) propargyl bromide, NaH, DMF, r.t., 16 h, 75%; (i) BnBr, K_2CO_3, DMF, 60 °C, 3 h, 93%; (j) propargyl bromide, NaH, DMF, r.t., 4 h, 87%; (k) 6 N HCl, MeOH, 65 °C, 2 h, 96%.

alcohol of γ-lactone **17** was thus protected as a methoxymethyl derivative. Treatment of fully protected γ-lactone **18** by aqueous ammonia in DMF led to the expected lactam **19** with a good yield. Since the synthesis of **19** required the protection of the C2 hydroxyl group, all the positions of the resulting lactam could be differentiated and, hence, a totally controlled alkylation approach, based on orthogonal protecting groups, was envisioned. In this way, lactam **19** was reduced by LAH in 80% yield to give piperidinol **20** as precursor of both heads. Selective *N*-benzylation of **20** allowed the propargylation of the free alcohol to yield (+)-**14** after MOM deprotection. The other required head **23** was also obtained from **20** through simulataneous *O*- and *N*-benzylation, followed by MOM deprotection and propargylation. These two sequences, based on protecting group manipulation, allowed the formation of the enantiomerically pure precursors of both heads.

Synthesis of the iminosugar library

Simultaneously, the synthesis of the suitable azido-armed linkers to be clicked by sequential CuAAC on propargylated heads was performed. The linkers were activated at one end by an azido group and on the other end with a tosyl group, for the subsequent azide substitution. Pure alkyl, diakyl ether and alkyl-oligoethyleneglycol arms were then prepared for the synthesis of heterodimers, homodimers and monomers, following classical functional group manipulation routes (Fig. 4 and ESI†).

Protected dimer and heterodimer derivatives were then synthesized following a three steps sequence with a first CuAAC coupling between the azido-armed linker and the first propargylated head under microwave irradiation at 80 °C, followed by the substitution of the tosyl group by sodium azide and a final CuAAC coupling with the second iminosugar head (Scheme 3). All these reactions were efficient with yields generally over 80%. The final compounds were first cautiously deprotected following a two-step approach using BCl_3 (*O*-debenzylation) and H_2 with Pd/C (*N*-debenzylation). It was found later that benzyl deprotection by direct hydrogenation using Pearlman's catalyst gave higher yields and was fully compatible with the presence of ether groups in the linker. All hydrogenations were performed in the presence of HCl, to avoid catalyst poisoning, and the free amines were obtained by treating the crude mixtures with Amberlite® IRA400 (HO^-) resin before column chromatography. The symmetry of compounds **9a–c** (Fig. 3) was nicely shown by simplified ^1H and ^{13}C NMR spectra and the absence of optical activity. On the contrary, non-symmetrical heterodimers **9d–e** have two different signals for triazole protons and carbon atoms linked to each enantiomeric head. However, heterodimers **9d–e** have also an α_D equal to zero. Compounds (+)-**10** and its enantiomer (−)-**10** were also prepared as the homodimer analogues of heterodimer **9e**, bearing the longest linker. They were synthesised following the same strategy (Scheme 3) with overall yields of 61% and 51% for (+)-**10** and (−)-**10**, respectively, from their corresponding precursors **23** and (+)-**14**. Satisfyingly, they showed identical NMR

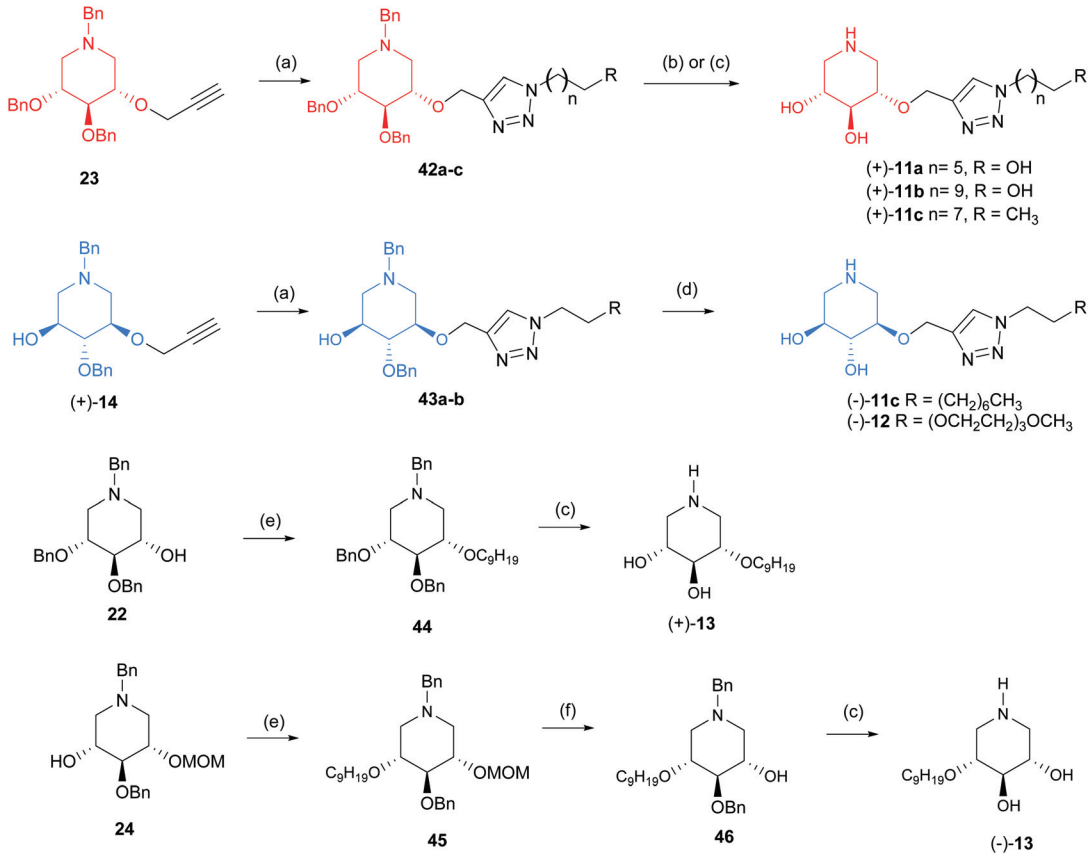


Scheme 3 Synthesis of heterodimers and dimers. Reaction conditions: (a) **29**, **30**, **31**, **32** or **33**, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, DMF, H_2O, 80 °C (μw), 30–60 min, 86% (**35a**), 87% (**35b**), 81% (**35c**), 86% (**35d**), 84% (**35e**), 78% (**39**); (b) NaN_3, DMF, r.t., overnight, 86% (**36a**), 81% (**36b**), 82% (**36c**), 88% (**36d**), 87% (**36e**), 89% (**40**); (c) **23** or (+)-**14**, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, DMF, H_2O, 80 °C (μw), 30–60 min, 64% (**37a**), 66% (**37b**), 61% (**37c**), 84% (**37d**), 83% (**37e**), 90% (**38**), 94% (**41**); (d) (1) BCl_3, CH_2Cl_2, −78 °C to r.t., overnight; (2) H_2, Pd/C, 1 N HCl, iPrOH, H_2O, r.t., 19 h, 70% (**9a**), 47% (**9c**); (e) (1) BCl_3, CH_2Cl_2, −78 °C to r.t., overnight; (2) H_2, $Pd(OH)_2$/C, 1 N HCl, iPrOH, H_2O, r.t., 42–91 h, 39% (**9b**); (f) H_2, $Pd(OH)_2$/C, 1 N HCl, iPrOH, THF, r.t., 20 h, 93% (**9d**), 90% (**9e**), 93% for (+)-**10**, 78% for (−)-**10**.

analyses and opposite specific optical rotations. Finally, monomers (+)-**11a–c**, (−)-**11c** and (−)-**12**, bearing the side chains of the different heterodimers, were also prepared as depicted in Scheme 4. For the sake of comparison, both enantiomeric alkylated monomers **13** were also synthesized to check the influence of the triazole moiety on the biological activity (Scheme 4).

Glucocerebrosidase inhibition

The ability of the synthesized iminoxylitols to act as GCase inhibitors was initially tested using Imiglucerase. Results, at acidic (pH = 5.2) and neutral conditions (pH = 7.0) are collected in Table 1. Interestingly, most of them exhibited higher K_i values at pH 5.2 than at pH 7.0, indicating a higher affinity towards GCase at the neutral pH of the endoplasmic reticulum. This is important to assist in protein folding and to enhance the enzyme transport to the lysosomes, while the compounds will dissociate at the lower pH of the lysosomal environment in the presence of an excess of substrate.[19] The best inhibitors at pH 7.0 were found to be (+)-monomers (+)-**11b–c** and (+)-**13** as well as heterodimer **9e** and dimer (+)-**10**, with inhibition in the low nM range. Mono- or divalent systems based on (−)-enantiomers of 2-alkylated-iminoxylitols led to an inhibitory potency decreased by at least 4 orders of magnitude. Heterodivalent systems based on right- and left-handed iminoxylitol heads displayed disappointing inhibition results, since compounds **9** were comparable or less potent inhibitors than the related (+)-monomers (+)-**11b–c** and (+)-**13**. It is worth mentioning that, in general, the experimental kinetic parameters are best explained by a non-competitive inhibition pattern for triazole-containing iminosugars. In contrast to previous results observed in the iminocyclitol series (Fig. 1), the presence of a triazole ring in the aglycon part of dextrorotatory monomeric iminoxylitols can lead to a switch of the inhibition mode from competitive to non-competitive.[55] The only exceptions were compounds (+)-**11b** and the corres-



Scheme 4 Synthesis of monomers. Reaction conditions: (a) **26**, **27**, **28** or **34**, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, DMF, H_2O, 80 °C (μw), 30–60 min, 98% (**42a**), 84% (**42b**), 88% (**42c**), 91% (**43a**), 90% (**43b**); (b) (1) BCl_3, CH_2Cl_2, −78 °C to r.t., overnight; (2) H_2, Pd/C, 1 N HCl, iPrOH, H_2O (for (+)-**11a**) or 1,4-dioxane (for (+)-**11b**), r.t., 47 h, 73% for (+)-**11a**, 81% for (+)-**11b**; (c) H_2, $Pd(OH)_2$/C, 1 N HCl, iPrOH, THF, r.t., 16–20 h, 76% for (+)-**11c**, quant. for (+)-**13**, 97% for (−)-**13**; (d) (1) BCl_3, CH_2Cl_2, −78 °C to r.t., overnight; (2) H_2, $Pd(OH)_2$/C, 1 N HCl, iPrOH, r.t., 42 h, 83% for (−)-**11c**, 65% for (−)-**12**; (e) 1-bromononane, NaH, DMF, r.t., 14–17 h, 88% (**44**), 89% (**45**); (f) 6 N HCl, MeOH, 65 °C, 2.5 h, 94%.

Table 1 Glucocerebrosidase inhibition evaluated on imiglucerase and activity enhancement in G202R/G202R fibroblasts

Compound	Structural type[b]	IC_{50} (nM) pH 5.2	IC_{50} (nM) pH 7.0	K_i (nM) pH 5.2	K_i (nM) pH 7.0	G202R/G202R[c] Fold increase	G202R/G202R[c] nM
9a	D (+/−)	$(1.8 \pm 0.1) \times 10^3$	223 ± 12	$(3.1 \pm 0.8) \times 10^3$	164.2 ± 23	1.5 ± 0.16	2×10^3
9b		23.6 ± 2.8	32.5 ± 2.4	19.9 ± 2.7	21.6 ± 3.3	1.7 ± 0.07	2×10^3
9c		57.1 ± 3.6	28.5 ± 2.2	28.9 ± 2.7	27.8 ± 2.1	2.2 ± 0.03	2×10^3
9d		$(1.1 \pm 0.04) \times 10^3$	362 ± 18	391 ± 19	260 ± 16	1.6 ± 0.02	2×10^3
9e		46.6 ± 3.8	17.0 ± 1.9	25.2 ± 3.9	7.3 ± 2.5	2.2 ± 0.03	100
(+)-**10**	D (+/+)	3.6 ± 0.1	3.3 ± 0.1	1.6 ± 0.1	2.1 ± 0.3	2.5 ± 0.22	100
(−)-**10**	D (−/−)	$(162 \pm 10) \times 10^3$	$(94 \pm 9) \times 10^3$	$(225 \pm 13) \times 10^3$	$(137 \pm 11) \times 10^3$	—	
(+)-**11a**	M (+)	292 ± 19	182 ± 14	341 ± 15	160 ± 13	1.6 ± 0.16	2×10^3
(+)-**11b**		24.3 ± 0.3	19.9 ± 0.3	13.3 ± 0.9[a]	8.2 ± 0.6[a]	3.6 ± 0.88	100
(+)-**11c**		4.8 ± 0.2	4.3 ± 0.2	2.1 ± 0.1	6.7 ± 0.2	2.2 ± 0.28	10
(+)-**13**		4.3 ± 0.2	5.7 ± 0.2	1.7 ± 0.1[a]	2.3 ± 0.1[a]	1.8 ± 0.23	1
(−)-**11c**	M (−)	$(58 \pm 4.1) \times 10^3$	$(19 \pm 2.3) \times 10^3$	$(71 \pm 9.2) \times 10^3$	$(15 \pm 5.2) \times 10^3$	—	2×10^3
(−)-**12**		$(129 \pm 7.1) \times 10^3$	$(59 \pm 3.9) \times 10^3$	$(144 \pm 9.9) \times 10^3$	$(59 \pm 4.9) \times 10^3$	—	2×10^3
(−)-**13**		$(75 \pm 5.2) \times 10^3$	$(42 \pm 3.8) \times 10^3$	$(69 \pm 6.2) \times 10^3$	$(42.3 \pm 4.1) \times 10^3$	—	300

All compounds behave as non-competitive inhibitors, unless otherwise noted (see ESI for the corresponding double reciprocal plots). [a] Competitive inhibitors. [b] D: dimeric compound; M: monomeric compound; (+) and (−) indicate the enantiomeric series of the iminosugar head. [c] Fold increase (treated cells/untreated cells) of GCase activity in homozygous fibroblasts for the G202R mutation at the indicated concentration. The most active G202R-GCase enhancers are shaded in grey.

ponding alkyl substituted iminoxylitol (+)-**13**, which behaved as competitive inhibitors. Comparison of inhibition constants at pH 7.0 of heterodimer **9e**, homodimer (+)-**10** and their corresponding monomer (+)-**11c**, shows that they bind GCase in a non-competitive mode with similar affinities, suggesting that their common part (the dextrorotatory head associated with the lipophilic chain of (+)-**11c**) is responsible for this behaviour. Duplication of the dextrorotatory head or the association with its enantiomer does not improve the binding. Interestingly, their clean non-competitive inhibition mode means that these inhibitors are able to bind the free enzyme, as well as the ESI complex, with the same affinity, suggesting that the inhibitor is not located in the active site. Although the location of this binding site is not known, we have evidenced several inhibitors binding a secondary site of GCase at the low nanomolar range.

GCase activity enhancement

A panel of fibroblasts from Gaucher patients with three different GD genotypes (N370S/N370S, L444P/L444P and G202R/G202R) has been used to screen the above compounds as GCase activity enhancers. GD fibroblasts were incubated with the compounds for 6 days and GCase activity was quantified using the fluorogenic substrate 4-MU. The results are collected in Fig. S54.† Significant activity enhancements for some compounds have been found only in fibroblasts bearing the genotype G202R/G202R (see also Table 1 for GCase enhancements in this mutation). Interestingly, the monomeric triazole-iminoxylitols ((+)-**11b** and (+)-**11c**) and the alkyliminoxylitol (+)-**13** were among the best GCase enhancers on this genotype, with increases of around 350% (for (+)-**11b** at 100 nM), 200% (for (+)-**11c** at 10 nM) and 180% (for (+)-**13** at 1 nM). The lack of GCase enhancement observed for (+)-**13** on the N370S mutation (Fig. S54†) is somewhat surprising if compared with the reported GCase enhancement for the structurally related (+) 2.[41] This variability can be attributed to the cell source or the number of cell transfers, *inter alia*.[56,57] As expected, the (−)-monomers (−)-**11c**, (−)-**12** (−)-**13**, corresponding to the enantiomeric series, that were less potent as GCase inhibitors, were also unable to enhance the enzyme activity at concentrations up to 2 μM (Table 1). In some cases, the lower affinity of the compounds for GCase at pH = 5.2 (lysosome) *vs.* pH = 7.0 (ER), as evidenced by the higher K_i values found at low pH (Table 1), was in agreement with the observed GCase enhancements. In the dimeric series, homodimer (+)-**10** and heterodimer **9e** were among the most potent enhancers, although they did not surpass their corresponding monomer (+)-**11b**. By comparing monomer (+)-**11c** with homodimer (+)-**10**, duplication seems detrimental for GCase activity enhancement, even though both compounds behave similarly as GCase inhibitors (Table 1). Heterodimers **9** are sensibly weaker GCase inhibitors and enhancers than homodimer (+)-**10**, with the exception of **9e**, a somewhat weaker inhibitor than (+)-**10**, but similar as GCase enhancer. The higher chaperone activity in the heterodimeric series (compounds **9**) observed for iminoxylitol **9e**, having the longer linker, stresses the impact of the spacer for enzyme stabilization. The fact that heterodimer **9e** and homodimer (+)-**10**, both having the same linker, displayed comparable GCase activity enhancement indicates that the enantiomeric nature of the second iminosugar head has almost no influence on the chaperone activity.

A direct evidence of the GCase activity enhancement elicited by some of the above mono and dimeric analogs was inferred from their effect on the metabolization of ω-azidosphingosine (ωN_3So), used as an external probe, which was added to the cell culture after the sixth day of incubation, following our reported methodology.[58] The probe ωN_3So behaves as a labelled sphingolipid that can be taken up and metabolized by WT and GD cells, similarly as the natural sphingolipids. The tested compounds (monomeric (+)-**11b–c**, (+)-**13**, homodimeric (+)-**10**, and heterodimeric **9b,c** and **9e**) lowered the levels of ωN_3GlcCer arising from the metabolization of the probe, thus indicating an increase of GCase activity (Fig. 5B). As expected, the levels of endogenous GlcCer were also diminished by some of the tested compounds (Fig. 5A).

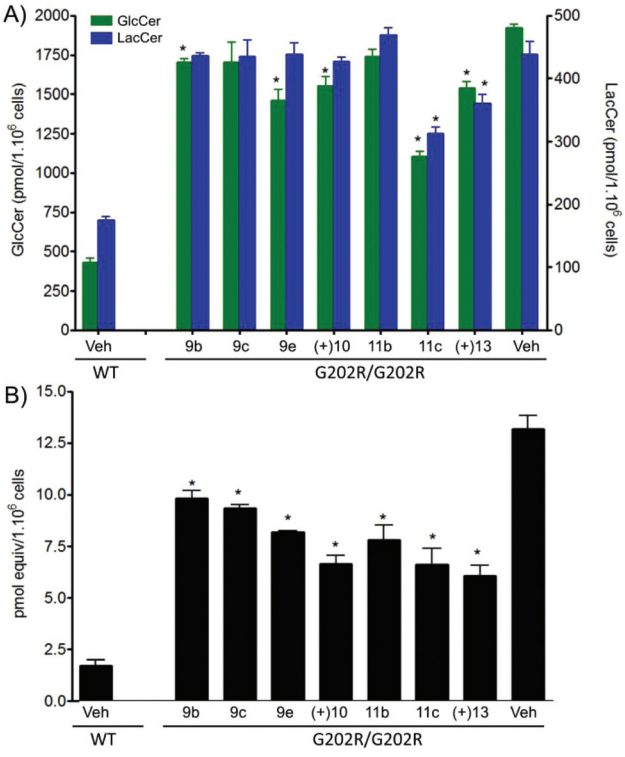


Fig. 5 (A) GlcCer and lactosylceramide (LacCer) levels after 6 days treatment of selected compounds on fibroblasts from GD patients bearing the G202R/G202R genotype. (B) Relative amounts of ωN3GlcCer after 24 h incubation with ωN_3Sph following a 6 days treatment with selected compounds on fibroblasts from GD patients bearing the G202R/G202R genotype and a 24 h washout. Compounds were tested at 100 nM, except (+)-**11c** and (+)-**13**, that were tested at 10 and 1 nM, respectively. Vehicle (Veh), 0.1% DMSO. Values are the mean ± SD of three separate experiments performed by triplicates. * Values statistically different from values of fibroblast with G202R/G202R genotype treated with vehicle ($p \leq 0.05$).

Finally, a possible synergistic chaperone effect between (+)-**11c** and (+)-**13** on G202R-GCase enzyme activity was also considered. Monomer (+)-**13** was chosen for this study, since it is also a potent GCase inhibitor (Table 1) but, unlike (+)-**11c**, it behaves as a competitive inhibitor. The combination of both types of inhibitors in a synergistic manner has already been explored successfully in the field of Fabry disease.[31] However, we have not been able to observe any synergistic effect by co-treatment of both compounds at different concentration ratios in G202R/G202R fibroblasts, the most sensitive genotype towards these compounds (Fig. S55†).

Cytotoxicity and selectivity towards glycosidases

None of the compounds have shown cytotoxicity (MTT assay) at the concentration used in GCase activity enhancement experiments. All compounds were non-toxic at concentrations up to 300 μM, with the exception of (−)-**11c** (CC_{50} 90 μM), (+)-**13** (CC_{50} 150 μM) and (+)-**11c** (CC_{50} 150 μM). The most active GCase enhancers (see below) were also tested as inhibitors of selected glycosidases (α- and β-galactosidases, α-glucosidase, and β-hexosaminidase) in WT fibroblasts after 6 days treatment (Table S1†). Compound (+)-**11b** was only a weak inhibitor on β-hexosaminidase (around 10% at 100 nM), while **9e**, and (+)-**10** were less selective with inhibitions of around 15–25% at 100 nM. It is interesting to note that compound **9b** (1 μM) acts as a β-galactosidase enhancer (around 15%). We speculate that it could act as an unspecific chaperone, since it is also able to enhance, albeit moderately, the activity of GCase (see Table 1). The GBA2 (non lysosomal β-glucorerebrosidase activity was not affected at the concentration tested (20 μM–2 pM)) (data not shown).

Endo-H assay and co-localization studies

The ability of the best GCase enhancer in G202R/G202R fibroblasts ((+)-**11b**) to increase the levels of mature GCase was assessed by the endoglycosidase-H (*endo*-H) assay.[59] This enzyme is an *endo* glycosidase that distinguishes high mannose containing *N*-linked glycans from a mature *N*-glycan complex, usually with only three mannose residues.[59,60] Only mature glycoproteins that have passed mid-Golgi after proper folding and processing become *endo*-H resistant.[59] Thus, an increase on the levels of *endo*-H resistant GCase, after incu-

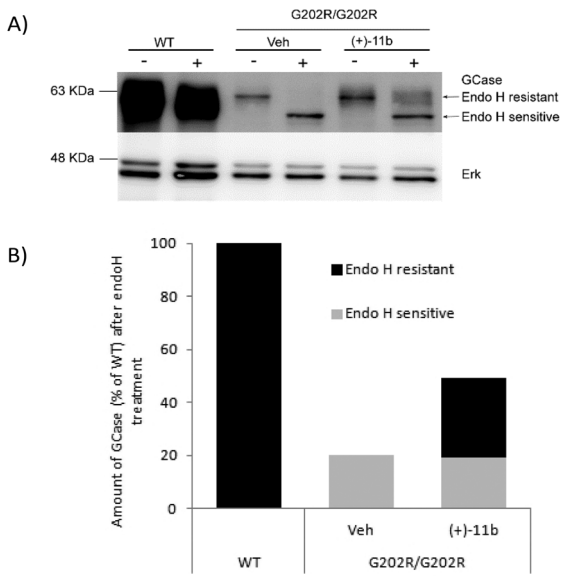


Fig. 6 Effect of compound (+)-**11b** on total GCase protein amount and mature GCase. (A) Proteins (18 μg) from fibroblasts of a healthy individual and from treated ((+)-**11b**) or non-treated (veh) fibroblasts of a GD patient (G202R/G202R) were subjected to *endo*-H digestion (+), then 10% SDS-PAGE and western blot analysis with anti-GCase and anti-Erk antibodies were performed. Samples not treated with *endo*-H (–) are shown for comparison. Mature GCase (~63 kDa) is *endo*-H resistant while ER-retained GCase/immature GCase (56 kDa) is *endo*-H sensitive. (B) Quantification of GCase bands (only *endo* H +) detected by western blot. The intensity of GCase band at each lane was divided by the intensity of the ErK band in order to normalize the results. To determine the *endo*-H resistant fraction, the amount of *endo*-H resistant GCase was divided by the entire amount of GCase in the same lane. The results were expressed in relation to the WT protein amount obtained in each experiment. The results shown are the mean of three experiments.

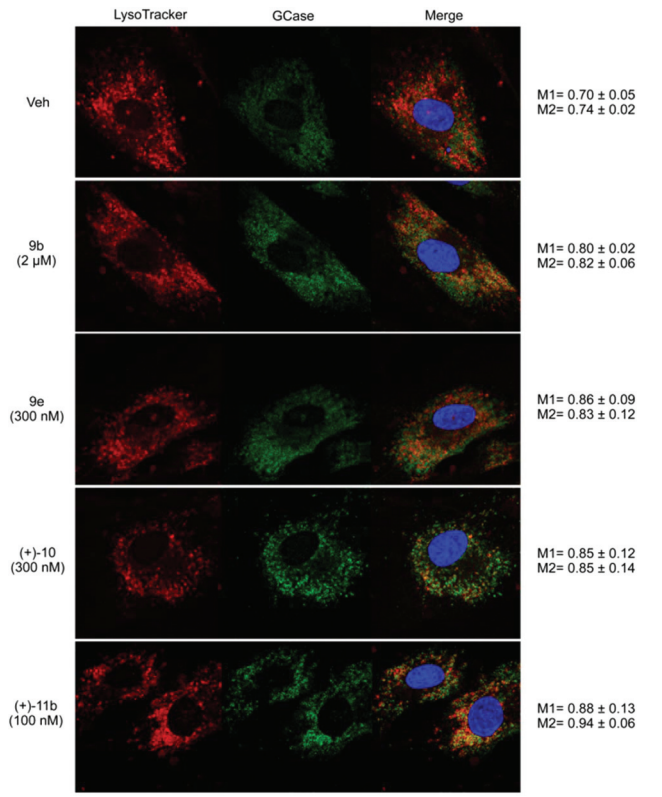


Fig. 7 Confocal laser microscope images of immunofluorescence staining of fibroblasts bearing the G202R/G202R genotype after the treatment with some compounds (**9b**, **9e**, (+)-**10** and (+)-**11b**). Veh, 0.1% DMSO. Detection of GCase was performed with the 8E34 monoclonal anti-GCase antibody (green) and LysoTracker® as a lysosome dye (red). The co-localization (Merge) was illustrated in orange-yellow by merging LysoTracker (red) and GCase (green) images. Manders' coefficients (M1 and M2) were calculated as a quantification of the co-localization. M1 corresponds to the fraction of lysosome overlapping with GCase, and M2 to the fraction of GCase overlapping with lysosome.

bation with a given compound, could be a valuable indirect proof of the ability of this compound to act as PC in a particular cell system. The resulting data indicate that G202R/G202R fibroblasts have very low GCase protein levels (around 20%) when compared with healthy fibroblasts (Fig. 6A and B). However, there was a significant increase in the amount of GCase levels after treatment of the G202R/G202R fibroblasts with compound (+)-**11b**. The levels of mature lysosomal GCase capable to pass the mid-Golgi were evaluated by the *endo*-H assay. The results revealed no evidence of the presence of mature GCase (*endo*-H resistant) in untreated (DMSO as a vehicle) G202R/G202R fibroblasts. However, around 60% of GCase became *endo*-H-resistant after treatment with compound (+)-**11b** (Fig. 6B). These results indicate that (+)-**11b** is able to promote the traffic of the G202R-GCase protein from the ER to the lysosome, thus preventing its premature degradation by the proteasomal machinery.

To evaluate in a different way the effect of our compounds on the trafficking of G202R-GCase to lysosomes, patient fibroblasts were incubated with a representative compound of each series ((+)-**11b** at 100 nM, **9b** at 2 μM, **9e** at 300 nM, and (+)-**10** at 300 nM) for six days (Fig. 7). WT fibroblasts treated with 0.1% DMSO were used as reference (not shown). Increased trafficking to the lysosome by the above compounds was confirmed by immunofluorescence staining and confocal microscopy imaging. In treated cells, the co-localization of the GCase mutated enzyme with a lysosomal marker (LysoTracker®) was increased for all tested compounds. Co-localization was quantified by the Manders' coefficients (M1 and M2), as described elsewhere,[58] showing a slight increase of both coefficients for all the compounds, in agreement with an increased trafficking of the mutated GCase to the lysosome.

Conclusions

The initial objective of this study was to design heterodimers that could target simultaneously the GCase active site and a putative secondary, non-catalytic binding site. The main risk associated with this approach was that no information was available concerning the localization of the above non-catalytic binding site. However, the design chosen for the two-headed ligands facilitated their synthesis and, thus, their rapid evaluation as GCase activity enhancers. The heterodimers were indeed based on two enantiomeric heads obtained by a stereodivergent approach and attached by CuAAC through linkers of various lengths. A series of monomeric and dimeric iminoxylitols of opposite enantiomeric series were thus efficiently synthesized and their inhibition potency was evaluated on imiglucerase. Some of these mono- or dimeric compounds were found to display GCase inhibition in the nM range, comparable to the reference iminoxylitol (+)-**2**. It was shown, however, that the presence of the triazole function may influence the observed binding mode, challenging our initial design hypothesis. If the triazole ring has no significant impact on the inhibitory activity (low nanomolar inhibition), it may indeed lead in some cases to a switch of the inhibition mode from competitive to non-competitive. Among the monomeric and the dimeric iminoxylitols evaluated, (+)-**11b** has been identified as the most potent GCase enhancer in fibroblasts bearing the G202R/G202R genotype, while the related enantiomeric monomeric series was devoid of activity. This enzyme enhancement effect was decreased by duplication of (+)-**11b** into homodimeric or heterodimeric analogues. Among the two-headed ligands, the best GCase inhibition and activity enhancements were observed for homodimer (+)-**10** and heterodimer **9e**, both bearing the longer spacer. Since the G202R-GCase variant has been characterized to be retained in the ER, the observed GCase activity increases are most likely due to an improved trafficking of this variant to the lysosome in the presence of our PC's.

Experimental/materials and methods

Chemistry

General methods. Solvents were reagent grade and further dried when necessary. Dichloromethane (CH_2Cl_2) was distilled over CaH_2 under argon and tetrahydrofuran (THF) was distilled over Na/benzophenone under argon. Pyridine was distilled over KOH under argon and stored over KOH. Dry DMF over molecular sieves was purchased from commercial vendors and used as such. All reactions were performed in standard glassware and microwave reactions were carried out using Biotage microwave reactor vials and Initiator microwave synthesizer. The reactions were monitored by Thin Layer Chromatography (TLC) on aluminium sheets coated with silica gel 60 F_{254} purchased from Merck KGaA. Visualization was accomplished with UV light (at 254 nm) and exposure to TLC stains, phosphomolybdic acid or potassium permanganate, followed by heating. Flash column chromatographies were carried out on silica gel 60 (230–400 mesh, 40–63 μm) purchased from Merck KGaA. ^{1}H and ^{13}C NMR experiments were carried out at 298 K on either a Bruker Avance 300 MHz or a Bruker Avance III HD 400 MHz spectrometer. The chemical shifts are reported as δ values in parts per million (ppm) relative to residual solvent signals used as an internal reference. The exponents "A" or "B" will be used for diastereotopic protons, "A" is assigned to the proton with the lowest chemical shift and "B" to the proton with the highest chemical shift. Assignments of ^{1}H and ^{13}C signals were made by DEPT, ^{1}H–^{1}H COSY, HSQC and HMBC experiments (see ESI† for the numbering chosen for ^{1}H and ^{13}C assignments). Optical rotations were measured at 589 nm (sodium lamp) and 20 °C on either a PerkinElmer 341 polarimeter or an Anton Paar MCP 200 polarimeter with a path length of 1 dm. The concentration (c) is indicated in gram per deciliter. Infrared (IR) spectra were recorded neat on a PerkinElmer Spectrum Two FT-IR spectrometer. High-resolution (HRMS) electrospray ionization-time-of-flight (ESI-TOF) mass spectra were recorded on a Bruker micrOTOF® mass spectrometer.

3-*O*-Benzyl-D-xylono-1,4-lactone (16)

Compound **15** (11.8 g, 49 mmol) was diluted in a mixture of THF (25 mL) and water (70 mL), and the solution was cooled to 5 °C. K_2CO_3 (1.2 eq., 8.28 g, 60 mmol) was added portion wise to the solution maintaining the temperature below 10 °C, and the solution was stirred for 30 min. Br_2 (1.2 eq., 2.90 mL, 56 mmol) was then added dropwise at 0 °C over a period of 1 h 30. The reaction mixture was maintained below 10 °C for a further 1 h and then stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure and the residue was co-evaporated with toluene. The residue was extracted with hot acetone (6×) and the organic extracts were concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (AcOEt/petroleum ether 50 : 50 to 70 : 30) to afford **16** (9.17 g, 78%) as a white solid. R_f 0.24 (AcOEt/petroleum ether 1 : 1). $[\alpha]_D^{20}$ = +40 (*c* 1.0, acetone). ^{1}H-NMR (acetone-d_6, 300 MHz) δ 3.82 (ddd, *J* = 12.4, 5.6, 3.1 Hz, 1H; H^A-5), 3.69 (ddd, *J* = 12.4, 4.9, 3.1 Hz, 1H; H^B-5), 4.08 (dd, *J* = 5.6, 4.9 Hz, 1H; OH), 4.40 (t, *J* = 7.5 Hz, 1H; H-3), 4.63 (dt, *J* = 7.5, 3.1 Hz, 1H; H-4), 4.72 (d, *J* = 11.9 Hz, 1H; OCH_2Ph), 4.75–4.81 (m, 1H; H-2), 4.84 (d, *J* = 11.9 Hz, 1H; OCH_2Ph), 5.24 (d, *J* = 4.8 Hz, 1H; OH), 7.26–7.44 (m, 5H; H_{Ar}). ^{13}C-NMR (acetone-d_6, 100 MHz) δ 60.5 (C-5), 72.8 (C-2), 72.9 (OCH_2Ph), 79.1 (C-4), 82.1 (C-3), 128.5, 129.2 (5 × CH_{Ar}), 139.1 (C_{Ar}), 175.2 (C-1). IR (neat) 3371 (O–H), 1770 cm^{-1} (C═O). HRMS (ESI) *m*/*z* calculated for $C_{12}H_{14}O_5Na$: 261.0733 $[M + Na]^+$; found 261.0712.

3-*O*-Benzyl-5-deoxy-5-iodo-D-xylono-1,4-lactone (17)

To a solution of **16** (81 mg, 0.34 mmol) in dry THF (2.5 mL) under argon were added PPh_3 (1.2 eq., 107 mg, 0.41 mmol), imidazole (1.5 eq., 35 mg, 0.51 mmol) and I_2 (1 eq., 88 mg, 0.35 mmol). The reaction mixture was heated at 60 °C for 4 h, then cooled to room temperature and stirred at this temperature for 16 h. Additional I_2 (0.2 eq., 18 mg, 0.071 mmol) and PPh_3 (0.2 eq., 16 mg, 0.061 mmol) were added and the mixture was heated at 65 °C for 2 h. The reaction mixture was diluted with AcOEt and successively washed with sat. $Na_2S_2O_3$, water and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (AcOEt/petroleum ether 25 : 75) to afford **17** (107 mg, 90%) as a colorless oil. R_f 0.21 (CH_2Cl_2). $[\alpha]_D^{20}$ = +16 (*c* 2.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.15 (d, *J* = 2.8 Hz, 1H; OH), 3.31 (dd, *J* = 10.7, 6.9 Hz, 1H; H^A-5), 3.53 (dd, *J* = 10.7, 6.1 Hz, 1H; H^B-5), 4.29 (dd, *J* = 6.5, 5.4 Hz, 1H; H-3), 4.62 (dd, *J* = 5.4, 2.8 Hz, 1H; H-2), 4.65 (d, *J* = 11.7 Hz, 1H; OCH_2Ph), 4.78 (d, *J* = 11.7 Hz, 1H; OCH_2Ph), 4.83 (ddd, *J* = 6.9, 6.5, 6.1 Hz, 1H; H-4), 7.30–7.42 (m, 5H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 0.1 (C-5), 71.9 (C-2), 73.0 (OCH_2Ph), 79.8 (C-3), 79.9 (C-4), 128.1, 128.5, 128.8 (5 × CH_{Ar}), 136.8 (C_{Ar}), 174.4 (C-1). IR (neat) 3431 (O–H), 1776 (C═O), 615 cm^{-1} (C–I). HRMS (ESI) *m*/*z* calculated for $C_{12}H_{13}IO_4Na$: 370.9751 $[M + Na]^+$; found 370.9740.

3-*O*-Benzyl-5-deoxy-5-iodo-2-*O*-(methoxymethyl)-D-xylono-1,4-lactone (18)

To a solution of **17** (4.94 g, 14.2 mmol) in dimethoxymethane (50 mL) was added P_4O_{10} (1.8 eq., 7.05 g, 24.8 mmol) and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was then slowly treated with sat. $NaHCO_3$ and extracted with Et_2O (3×). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (AcOEt/petroleum ether 20 : 80) to afford **18** (4.71 g, 85%) as a pale yellow oil. R_f 0.44 (AcOEt/petroleum ether 20 : 80). $[\alpha]_D^{20}$ = +21 (*c* 2.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.36 (dd, *J* = 9.9, 6.0 Hz, 1H; H^A-5), 3.42 (s, 3H; OCH_2OCH_3) 3.48 (dd, *J* = 9.9, 8.4 Hz, 1H; H^B-5), 4.22 (dd, *J* = 4.8, 2.9 Hz, 1H; H-3), 4.40 (d, *J* = 2.9 Hz, 1H; H-2), 4.61 (d, *J* = 11.4 Hz, 1H; OCH_2Ph), 4.67 (d, *J* = 6.7 Hz, 1H; OCH_2OCH_3), 4.71 (d, *J* = 11.4 Hz, 1H; OCH_2Ph), 4.81 (ddd, *J* = 8.4, 6.0, 4.8 Hz, 1H; H-4), 4.93 (d, *J* = 6.7 Hz, 1H; OCH_2OCH_3), 7.31–7.41 (m, 5H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ −1.2 (C-5), 56.3 (OCH_2OCH_3), 73.1 (OCH_2Ph), 74.2 (C-2), 78.5 (C-3), 81.3 (C-4), 96.0 (OCH_2OCH_3) 128.3, 128.6, 128.8 (5 × CH_{Ar}), 136.5 (C_{Ar}), 172.2 (C-1). IR (neat) 1786 (C═O), 614 cm^{-1} (C–I). HRMS (ESI) *m*/*z* calculated for $C_{14}H_{17}IO_5Na$: 415.0013 $[M + Na]^+$; found 415.0009.

5-Amino-3-*O*-benzyl-2-*O*-(methoxymethyl)-5-deoxy-D-xylono-1,5-lactam (19)

Compound **18** (5.0 g, 12.7 mmol) was dissolved in 30% NH_4OH aqueous solution (120 mL) and DMF (35 mL), and the reaction mixture was stirred at room temperature for 44 h. Solvents were evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (70 mL) and washed with water (4 × 70 mL) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 95 : 5) to afford **19** (3.0 g, 84%) as a white solid. R_f 0.29 (CH_2Cl_2/MeOH 95 : 5). $[\alpha]_D^{20}$ = +46 (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.10 (d, *J* = 4.8 Hz, 1H; OH), 3.18 (ddd, *J* = 12.3, 6.6, 2.6 Hz, 1H; H^A-5), 3.44 (s, 3H; OCH_2OCH_3), 3.47 (ddd, *J* = 12.3, 4.6, 2.8 Hz, 1H; H^B-5), 3.71 (dd, *J* = 7.2, 6.6 Hz, 1H; H-3), 3.96 (tdd, *J* = 6.6, 4.8, 4.6 Hz, 1H; H-4), 4.16 (d, *J* = 7.2 Hz, 1H; H-2), 4.70 (d, *J* = 11.5 Hz, 1H; OCH_2Ph), 4.81 (d, *J* = 6.6 Hz, 1H; OCH_2OCH_3), 4.88 (d, *J* = 11.5 Hz, 1H; OCH_2Ph), 5.09 (d, *J* = 6.6 Hz, 1H; OCH_2OCH_3), 6.18 (br s, 1H; NH), 7.28–7.39 (m, 5H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 44.7 (C-5), 56.5 (OCH_2OCH_3), 67.4 (C-4), 73.9 (OCH_2Ph), 74.5 (C-2), 81.0 (C-3), 97.4 (OCH_2OCH_3) 128.1, 128.3, 128.8 (5 × CH_{Ar}), 137.8 (C_{Ar}), 170.8 (C-1). IR (neat) 3274 (O–H), 1652 cm^{-1} (C═O). HRMS (ESI) *m*/*z* calculated for $C_{14}H_{19}NO_5Na$: 304.1155 $[M + Na]^+$; found 304.1118.

3-*O*-Benzyl-2-*O*-(methoxymethyl)-1,5-dideoxy-1,5-imino-D-xylitol (20)

To a suspension of $LiAlH_4$ (7.8 eq., 3.16 g, 83.3 mmol) in dry THF (120 mL) was added a solution of **19** (3.0 g, 10.7 mmol) in

dry THF (120 mL) at 0 °C under argon. The reaction mixture was heated under reflux for 2 h. The reaction mixture was cooled and water (3.2 mL) was cautiously added then 10% NaOH aqueous solution (6.4 mL) and water (9.6 mL) were successively added, the mixture was then stirred overnight. The insoluble material was filtered off and the filtrate was concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 90 : 10) to afford **20** (2.29 g, 80%) as a white solid. R_f 0.25 (CH_2Cl_2/MeOH 90 : 10). $[\alpha]_D^{20}$ = +43 (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.46–2.53 (br s, 2H; OH, NH), 2.61 (dd, *J* = 12.7, 7.6 Hz, 1H; H^A-5), 2.66 (dd, *J* = 13.0, 7.6 Hz, 1H; H^A-1), 3.14 (dd, *J* = 12.7, 3.9 Hz, 1H; H^B-5), 3.21 (dd, *J* = 13.0, 3.9 Hz, 1H; H^B-1), 3.37 (s, 3H; OCH_2OCH_3), 3.38 (dd, *J* = 7.0, 6.7 Hz, 1H; H-3), 3.56 (ddd, *J* = 7.6, 6.7, 3.9 Hz, 1H; H-4), 3.59 (ddd, *J* = 7.6, 7.0, 3.9 Hz, 1H; H-2), 4.68 (d, *J* = 11.7 Hz, 1H; OCH_2Ph), 4.69 (d, *J* = 6.7 Hz, 1H; OCH_2OCH_3), 4.74 (d, *J* = 6.7 Hz, 1H; OCH_2OCH_3), 4.84 (d, *J* = 11.7 Hz, 1H; OCH_2Ph), 7.27–7.39 (m, 5H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 48.5 (C-1), 49.6 (C-5), 55.8 (OCH_2OCH_3), 70.1 (C-4), 74.2 (OCH_2Ph), 77.4 (C-2), 82.4 (C-3), 96.4 (OCH_2OCH_3), 127.6, 128.0, 128.7 (5 × CH_{Ar}), 138.6 (C_{Ar}). IR (neat) 3268 cm^{-1} (O–H, N–H). HRMS (ESI) *m*/*z* calculated for $C_{14}H_{21}NO_4Na$: 290.136 $[M + Na]^+$; found 290.137.

N-Benzyl-3,4-*O*-dibenzyl-2-*O*-(methoxymethyl)-1,5-dideoxy-1,5-imino-D-xylitol (21)

To a solution of **20** (1.20 g, 4.49 mmol) in dry THF (26 mL) at 0 °C was added NaH (6.8 eq., 1.22 g, 30 mmol, 60% on mineral oil). The suspension was stirred at room temperature for 30 min and BnBr (4.3 eq., 2.3 mL, 19.2 mmol) was added, followed by KI (0.5 eq., 0.37 g, 2.23 mmol). The mixture was stirred at room temperature for 18 h. For quenching, water (5 mL) was slowly added, and then the mixture was diluted with water and CH_2Cl_2. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (AcOEt/petroleum ether 10 : 90) to afford **21** (1.74 g, 87%) as a pale yellow oil. R_f 0.30 (AcOEt/petroleum ether 20 : 80). $[\alpha]_D^{20}$ = −30 (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.96 (dd, *J* = 10.9, 10.3 Hz, 1H; H^A-5), 2.04 (dd, *J* = 10.9, 10.3 Hz, 1H; H^A-1), 3.06 (ddd, *J* = 10.9, 5.0, 2.0 Hz, 1H; H^B-5), 3.09 (ddd, *J* = 10.9, 5.0, 2.0 Hz, 1H; H^B-1), 3.32 (s, 3H; OCH_2OCH_3), 3.37 (t, *J* = 9.0 Hz, 1H; H-3), 3.53 (d, *J* = 13.3 Hz, 1H; NCH_2Ph), 3.60 (ddd, *J* = 10.3, 9.0, 5.0 Hz, 1H; H-4), 3.62 (d, *J* = 13.3 Hz, 1H; NCH_2Ph), 3.72 (ddd, *J* = 10.3, 9.0, 5.0 Hz, 1H; H-2), 4.59 (d, *J* = 11.6 Hz, 1H; OCH_2Ph), 4.66 (d, *J* = 11.6 Hz, 1H; OCH_2Ph), 4.66 (d, *J* = 6.7 Hz, 1H; OCH_2OCH_3), 4.79 (d, *J* = 6.7 Hz, 1H; OCH_2OCH_3), 4.82 (d, *J* = 11.2 Hz, 1H; OCH_2Ph), 4.91 (d, *J* = 11.2 Hz, 1H; OCH_2Ph), 7.24–7.38 (m, 15H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 55.6 (OCH_2OCH_3), 55.8 (C-5), 57.1 (C-1), 62.1 (NCH_2Ph), 73.0 (OCH_2Ph), 75.5 (OCH_2Ph), 77.0 (C-2), 79.0 (C-4), 86.2 (C-3), 97.0 (OCH_2OCH_3), 127.3, 127.5, 127.7, 127.8, 127.9, 128.4, 128.5, 128.9 (15 × CH_{Ar}), 137.9, 138.7, 139.2 (3 × C_{Ar}). HRMS (ESI) *m*/*z* calculated for $C_{28}H_{33}NO_4Na$: 470.230 $[M + Na]^+$; found 470.230.

N-Benzyl-3,4-*O*-dibenzyl-1,5-dideoxy-1,5-imino-D-xylitol (22)

A solution of **21** (1.53 g, 3.43 mmol) in MeOH (43 mL) and 6 M HCl aqueous solution (36 mL) was heated at 65 °C for 2 h. After being cooled to room temperature, the solution was treated with sat. $NaHCO_3$ and extracted with AcOEt (2×). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (AcOEt/petroleum ether 30 : 70) to afford **22** (1.34 g, 97%) as a pale yellow oil. R_f 0.25 (AcOEt/petroleum ether 30 : 70). $[\alpha]_D^{20}$ = +7 (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.27–2.38 (m, 2H; H^A-5, H^A-1), 2.86–2.92 (m, 2H; H^B-5, H^B-1), 2.97 (br s, 1H, OH), 3.43 (t, *J* = 6.9 Hz, 1H; H-3), 3.54 (d, *J* = 13.3 Hz, 1H; NCH_2Ph), 3.62 (d, *J* = 13.3 Hz, 1H; NCH_2Ph), 3.66 (ddd, *J* = 7.8, 6.9, 3.9 Hz, 1H; H-4), 3.75 (ddd, *J* = 7.8, 6.9, 3.9 Hz, 1H; H-2), 4.55 (d, *J* = 11.6 Hz, 1H; OCH_2Ph), 4.59 (d, *J* = 11.6 Hz, 1H; OCH_2Ph), 4.69 (d, *J* = 11.6 Hz, 1H; OCH_2Ph), 4.88 (d, *J* = 11.6 Hz, 1H; OCH_2Ph), 7.22–7.41 (m, 15H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 54.4 (C-5), 56.7 (C-1), 62.2 (NCH_2Ph), 69.4 (C-2), 71.9 (OCH_2Ph), 74.0 (OCH_2Ph), 77.7 (C-4), 82.6 (C-3), 127.2, 127.69, 127.70, 127.77, 127.79, 128.3, 128.4, 128.5, 129.0 (15 × CH_{Ar}), 138.0, 138.3, 138.7 (3 × C_{Ar}). IR (neat) 3301 cm^{-1} (O–H). HRMS (ESI) *m*/*z* calculated for $C_{26}H_{30}NO_3$: 404.222 $[M + H]^+$; found 404.220.

N-Benzyl-3,4-*O*-dibenzyl-2-*O*-propargyl-1,5-dideoxy-1,5-imino-D-xylitol (23)

To a solution of **22** (1.50 g, 3.72 mmol) in dry DMF (16 mL) at 0 °C was added NaH (2.2 eq., 0.33 g, 8.25 mmol, 60% on mineral oil). The suspension was stirred at room temperature for 30 min then propargyl bromide (1.5 eq., 0.63 mL, 5.66 mmol, 80% in toluene) was added and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with AcOEt and washed with water (3×). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (AcOEt/petroleum ether 10 : 90) to afford **23** (1.23 g, 75%) as a yellow oil. R_f 0.31 (AcOEt/petroleum ether 10 : 90). $[\alpha]_D^{20}$ = −18 (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.95 (dd, *J* = 10.9, 3.8 Hz, 1H; H^A-5 or H^A-1), 1.98 (dd, *J* = 10.9, 3.8 Hz, 1H; H^A-1 or H^A-5), 2.37 (t, *J* = 2.4 Hz, 1H; H-8), 3.06 (ddd, *J* = 10.9, 5.1, 2.0 Hz, 1H; H^B-5 or H^B-1), 3.10 (ddd, *J* = 10.9, 5.1, 2.0 Hz, 1H; H^B-1 or H^B 5), 3.37 (t, *J* = 8.9 Hz, 1H; H-3), 3.53–3.64 (m, 4H; H-2, H-4, NCH_2Ph), 4.25 (dd, *J* = 15.7, 2.4 Hz, 1H; H^A-6), 4.30 (dd, *J* = 15.7, 2.4 Hz, 1H; H^B-6), 4.60 (d, *J* = 11.6 Hz, 1H; OCH_2Ph), 4.67 (d, *J* = 11.6 Hz, 1H; OCH_2Ph), 4.84 (d, *J* = 11.1 Hz, 1H; OCH_2Ph), 4.88 (d, *J* = 11.1 Hz, 1H; OCH_2Ph), 7.24–7.40 (m, 15H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 56.0, 56.1 (C-1, C-5), 58.6 (C-6), 62.1 (NCH_2Ph), 73.1 (OCH_2Ph), 74.3 (C-8), 75.5 (OCH_2Ph), 78.7, 78.9 (C-4, C-2), 80.4 (C-7), 86.4 (C-3), 127.3, 127.6, 127.7, 127.9, 128.3, 128.1, 128.4, 128.45, 128.49, 129.0 (15 × CH_{Ar}), 137.9, 138.7, 139.2 (3 × C_{Ar}). IR (neat) 3289 cm^{-1}

(≡C–H), 2114 cm^{-1} (C≡C). HRMS (ESI) *m/z* calculated for $C_{29}H_{32}NO_3$: 442.238 $[M + H]^+$; found 442.235.

N-Benzyl-3-*O*-benzyl-2-*O*-(methoxymethyl)-1,5-dideoxy-1,5-imino-D-xylitol (24)

Compound **20** (1.0 g, 3.74 mmol) was dissolved in dry DMF (25 mL), then K_2CO_3 (0.6 eq., 0.31 g, 2.24 mmol) was added, followed by BnBr (1.1 eq., 0.5 mL, 4.18 mmol) and the reaction mixture was stirred at 60 °C for 3 h. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in AcOEt and washed with water (3×). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (AcOEt/petroleum ether 30 : 70) to afford **24** (1.24 g, 93%) as a pale yellow oil. R_f 0.23 (AcOEt/petroleum ether 30 : 70). $[\alpha]_D^{20}$ = −19 (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.24 (dd, *J* = 11.0, 7.8 Hz, 1H; H^A-5), 2.33 (dd, *J* = 11.1, 7.8 Hz, 1H; H^A-1), 2.58–2.83 (br s, 1H; OH), 2.85 (dd, *J* = 11.0, 4.0 Hz, 1H; H^B-5), 2.92 (dd, *J* = 11.0, 4.0 Hz, 1H; H^B-1), 3.33 (s, 3H; OCH_2OCH_3), 3.34 (t, *J* = 7.0 Hz, 1H; H-3), 3.52 (d, *J* = 13.2 Hz, 1H; NCH_2Ph), 3.63 (d, *J* = 13.2 Hz, 1H; NCH_2Ph), 3.71 (ddd, *J* = 7.8, 7.0, 4.0 Hz, 1H; H-4), 3.77 (ddd, *J* = 7.8, 7.0, 4.0 Hz, 1H; H-2), 4.65 (d, *J* = 6.7 Hz, 1H; OCH_2OCH_3), 4.68 (d, *J* = 11.7 Hz, 1H; OCH_2Ph), 4.71 (d, *J* = 6.7 Hz, 1H; OCH_2OCH_3), 4.84 (d, *J* = 11.7 Hz, 1H; OCH_2Ph), 7.22–7.40 (m, 10H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 55.49 (C-1), 55.54 (OCH_2OCH_3), 56.4 (C-5), 62.2 (NCH_2Ph), 69.4 (C-4), 74.0 (OCH_2Ph), 75.9 (C-2), 82.8 (C-3), 96.2 (OCH_2OCH_3), 127.3, 127.8, 127.9, 128.4, 128.6, 129.0 (10 × CH_{Ar}), 137.9, 138.7 (2 × C_{Ar}). IR (neat) 3436 cm^{-1} (O–H). HRMS (ESI) *m/z* calculated for $C_{21}H_{27}NO_4Na$: 380.183 $[M + Na]^+$; found 380.184.

N-Benzyl-3-*O*-benzyl-2-*O*-(methoxymethyl)-4-*O*-propargyl-1,5-dideoxy-1,5-imino-D-xylitol (25)

To a solution of **24** (1.17 g, 3.27 mmol) in dry DMF (15 mL) at 0 °C was added NaH (1.3 eq., 0.17 g, 4.26 mmol, 60% on mineral oil). The resulting suspension was stirred at room temperature for 30 min then propargyl bromide (1.5 eq., 0.53 mL, 4.76 mmol, 80% in toluene) was added and the reaction mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with AcOEt and washed with water (3×). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (AcOEt/petroleum ether 10 : 90) to afford **25** (1.12 g, 87%) as a yellow oil. R_f 0.66 (AcOEt/petroleum ether 40 : 60). $[\alpha]_D^{20}$ = −19 (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.94 (dd, *J* = 11.1, 10.4 Hz, 1H; H^A-5 or H^A-1), 2.03 (dd, *J* = 11.1, 10.4 Hz, 1H; H^A-1 or H^A-5), 2.37 (t, *J* = 2.4 Hz, 1H; H-8), 3.05–3.14 (m, 2H; H^B-1, H^B-5), 3.32 (t, *J* = 8.9 Hz, 1H; H-3), 3.34 (s, 3H; OCH_2OCH_3), 3.54 (d, *J* = 13.5 Hz, 1H; NCH_2Ph), 3.55–3.76 (m, 2H; H-2, H-4), 3.65 (d, *J* = 13.5 Hz, 1H; NCH_2Ph), 4.23 (dd, *J* = 15.7, 2.4 Hz, 1H; H^A-6), 4.29 (dd, *J* = 15.7, 2.4 Hz, 1H; H^B-6), 4.67 (d, *J* = 6.6 Hz, 1H; OCH_2OCH_3), 4.79 (d, *J* = 6.6 Hz, 1H; OCH_2OCH_3), 4.81 (d, *J* = 11.1 Hz, 1H; OCH_2Ph), 4.87 (d, *J* = 11.1 Hz, 1H; OCH_2Ph), 7.24–7.45 (m, 10H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ 55.58 (OCH_2OCH_3), 55.67, 57.0 (C-1, C-5), 58.5 (C-6), 62.0 (NCH_2Ph), 74.3 (C-8), 75.4 (OCH_2Ph), 77.0, 78.7 (C-2, C-4), 80.3 (C-7), 86.0 (C-3), 96.9 (OCH_2OCH_3), 127.3, 127.6, 128.0, 128.41, 128.43, 129.0 (10 × CH_{Ar}), 137.9, 139.1 (2 × C_{Ar}). IR (neat) 3289 (≡C–H), 2117 cm^{-1} (C≡C). HRMS (ESI) *m/z* calculated for $C_{24}H_{30}NO_4$: 396.217 $[M + H]^+$; found 396.219.

N-Benzyl-3-*O*-benzyl-4-*O*-propargyl-1,5-dideoxy-1,5-imino-D-xylitol ((+)-14)

A solution of **25** (965 mg, 2.44 mmol) in MeOH (30 mL) and 6 M HCl aqueous solution (5 mL) was heated at 65 °C for 2 h. After being cooled to room temperature, the solution was treated with sat. $NaHCO_3$ and extracted with AcOEt (2×). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (AcOEt/petroleum ether 25 : 75) to afford (+)-**14** (826 mg, 96%) as a while solid. R_f 0.35 (AcOEt/petroleum ether 30 : 70). $[\alpha]_D^{20}$ = +4 (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.23 (dd, *J* = 11.1, 8.9 Hz, 1H; H^A-1), 2.28 (dd, *J* = 11.1, 8.2 Hz, 1H; H^A-5), 2.39 (t, *J* = 2.4 Hz, 1H; H-8), 2.62 (br s, 1H, OH), 2.86 (ddd, *J* = 11.1, 3.7, 1.0 Hz, 1H; H^B-1), 2.93 (ddd, *J* = 11.1, 2.8, 1.0 Hz, 1H; H^B-5), 3.34 (t, *J* = 7.0 Hz, 1H; H-3), 3.54 (d, *J* = 13.2 Hz, 1H; NCH_2Ph), 3.60 (d, *J* = 13.2 Hz, 1H; NCH_2Ph), 3.67–3.75 (m, 2H; H-2, H-4), 4.18 (dd, *J* = 15.8, 2.4 Hz, 1H; H^A-6), 4.23 (dd, *J* = 15.8, 2.4 Hz, 1H; H^B-6), 4.67 (d, *J* = 11.6 Hz, 1H; OCH_2Ph), 4.88 (d, *J* = 11.6 Hz, 1H; OCH_2Ph), 7.23–7.39 (m, 10H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 54.4 (C-1), 56.4 (C-5), 57.1 (C-6), 62.1 (NCH_2Ph), 69.3 (C-2), 74.0 (OCH_2Ph), 74.5 (C-8), 77.2 (C-4), 79.7 (C-7), 82.5 (C-3), 127.2, 127.80, 127.83, 127.3, 127.5, 128.9 (10 × CH_{Ar}), 137.8, 138.6 (2 × C_{Ar}). IR (neat) 3232 (≡C–H), 3090 (O–H), 2111 cm^{-1} (C≡C). HRMS (ESI) *m/z* calculated for $C_{22}H_{26}NO_3$: 352.191$[M + H]^+$; found 352.190.

General procedure A: CuAAC reaction

To a solution of alkyne in DMF (0.06 M) was added the azide and a yellow suspension of $CuSO_4 \cdot 5H_2O$ (0.1 eq.) and sodium ascorbate (0.2 eq.) in H_2O (DMF/H_2O 4 : 1). The resulting suspension was stirred and heated under microwave irradiation at 80 °C for 30–60 min. The reaction mixture was diluted with water and extracted with AcOEt (2×). The combined organic extracts were washed with water (2×), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel to afford the desired iminosugar click cluster.

General procedure B: synthesis of azides

Sodium azide (4 eq.) was added to a solution of *p*-toluene sulfonate ester in dry DMF (0.06 M), and the reaction mixture was stirred at room temperature overnight. The solvent was then evaporated under reduced pressure. The residue was diluted with AcOEt and washed with water (3×). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel to afford the desired azide.

General procedure C: optimized benzyl deprotection

Benzyl-protected compound was dissolved in a 1 : 1 mixture of iPrOH and THF (0.04 M), then 1 M HCl aqueous solution (8 eq.) and $Pd(OH)_2/C$ (0.2 eq., 20% Pd on carbon) were added. The flask was evacuated and backfilled with argon (four cycles) and then evacuated and backfilled with H_2 (four cycles). The reaction mixture was stirred under an atmosphere of H_2 (1 atm) at room temperature for 20 h. The reaction mixture was then filtered through a pad of Celite (previously washed with at least 250 mL of 1 M HCl aqueous solution) and rinsed with MeOH and water. The filtrate was concentrated under reduced pressure. The residue was dissolved in a 1 : 1 mixture of MeOH and water, then ion exchange resin Amberlite® IRA400 (OH^-) was added and the suspension was rotated at room temperature for 1 h. The resin was filtered, rinsed with MeOH and water and the filtrate was concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel ($MeCN/H_2O/NH_4OH$ 5 : 1 : 1 to 14 : 1 : 1) to afford the desired debenzylated compound.

General procedure D: *O*-debenzylation and *N*-debenzylation

To a solution of benzyl-protected compound in CH_2Cl_2 (0.025 M) at −60 °C was added dropwise 1 M BCl_3 solution in CH_2Cl_2 (2 eq. per benzyl group). The solution was allowed to raise room temperature and was stirred overnight. A mixture of $MeOH/H_2O$ (20 : 1, 12 mL per mmol of BCl_3) was added to the reaction mixture at room temperature and after 10 min stirring, the solvent was evaporated under reduced pressure. This step was repeated twice. For the synthesis of compounds **9b**, (−)-**11c**, and (−)-**12**: the partially debenzylated product was dissolved in iPrOH (0.05 M), then 1 M HCl aqueous solution (15 eq.) and $Pd(OH)_2/C$ (0.5 eq., 20% Pd on carbon) were added. For the synthesis of compounds **9a**, **9c**, (+)-**11a** and (+)-**11b**: the partially debenzylated product was dissolved in a 10 : 1 mixture of iPrOH and H_2O (0.05 M; **9a**, **9c** and (+)-**11a**) or a 10 : 1 mixture of iPrOH and 1,4-dioxane (0.05 M; (+)-**11b**), then 1 M HCl aqueous solution (1 éq.) and Pd/C (0.3 eq., 10% Pd on carbon) were added. The flask was evacuated and backfilled with argon (four cycles) and then evacuated and backfilled with H_2 (four cycles). The reaction mixture was stirred under an atmosphere of H_2 (1 atm) at room temperature for 19 to 91 h. The reaction mixture was then filtered through a pad of Celite (previously washed with at least 250 mL of 1 M HCl aqueous solution) and rinsed with MeOH and water. The filtrate was concentrated under reduced pressure. The residue was dissolved in a mixture of MeOH and water (1 : 1) then ion exchange resin Amberlite® IRA400 (OH^-) was added and the suspension was rotated at room temperature for 1 h. The resin was filtered, rinsed with MeOH and water and the filtrate was concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel ($MeCN/H_2O/NH_4OH$ 5 : 1 : 1 to 15 : 1 : 1) to afford the desired debenzylated compound.

Compound 35a

Compound **35a** (154 mg, 86%) was prepared by general procedure A, starting from (+)-**14** (97 mg, 0.28 mmol) and **29** (1.2 eq., 100 mg, 0.34 mmol). Colorless oil. R_f 0.16 (AcOEt/petroleum ether 80 : 20). $[\alpha]_D^{20}$ = −6 (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.20–1.36 (m, 4H; CH_2-11, CH_2-12), 1.56–1.64 (m, 2H; CH_2-13), 1.73–1.80 (m, 2H; CH_2-10), 2.16–2.29 (m, 2H; H^A-1, H^A-5), 2.44 (s, 3H; CH_3), 2.86–2.99 (m, 2H; H^B-1, H^B-5), 3.08 (br s, 1H; OH), 3.34 (t, *J* = 7.3 Hz, 1H; H-3), 3.53 (d, *J* = 13.2 Hz, 1H; NCH_2Ph), 3.60 (d, *J* = 13.2 Hz, 1H; NCH_2Ph), 3.64–3.74 (m, 2H; H-2, H-4), 3.99 (t, *J* = 6.3 Hz, 2H; CH_2-14), 4.19 (t, *J* = 7.2 Hz, 2H; CH_2-9), 4.66 (d, *J* = 12.3 Hz, 1H; H^A-6), 4.72 (d, *J* = 11.7 Hz, 1H; OCH_2Ph), 4.74 (d, *J* = 12.3 Hz, 1H; H^B-6), 4.85 (d, *J* = 11.7 Hz, 1H; OCH_2Ph), 7.23–7.40 (m, 13H; H-8, H_{Ar}), 7.78 (d, *J* = 8.2 Hz, 2H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ 21.5 (CH_3), 24.7, 25.7, 28.5, 29.8 (C-10, C-11, C-12, C-13), 50.4 (C-9), 54.6 (C-5), 56.7 (C-1), 62.0 (NCH_2Ph), 63.5 (C-6), 69.5 (C-4), 70.2 (C-14), 73.9 (OCH_2Ph), 77.8 (C-2), 83.3 (C-3), 122.1 (C-8), 127.1, 127.5, 127.7, 128.2, 128.3, 128.8, 129.8 (14 × CH_{Ar}), 133.0, 137.7, 138.8, 144.7 (4 × C_{Ar}), 145.2 (C-7). IR (neat) 3416 (O–H), 1356 (SO_2), 1175 cm^{-1} (SO_2). HRMS (ESI) *m*/*z* calculated for $C_{35}H_{45}N_4O_6S$: 649.305 $[M + H]^+$; found 649.306.

Compound 35b

Compound **35b** (174 mg, 87%) was prepared by general procedure A, starting from (+)-**14** (110 mg, 0.28 mmol) and **30** (1.1 eq., 110 mg, 0.31 mmol). Orange oil. R_f 0.19 (AcOEt/petroleum ether 80 : 20). $[\alpha]_D^{20}$ = −6 (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.18–1.33 (m, 12H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15, CH_2-16), 1.58–1.66 (m, 2H; CH_2-17), 1.78–1.86 (m, 2H; CH_2-10), 2.21–2.33 (m, 2H; H^A-1, H^A-5), 2.44 (s, 3H; CH_3), 2.70 (br s, 1H; OH), 2.84–2.95 (m, 2H; H^B-1, H^B-5), 3.35 (t, *J* = 6.9 Hz, 1H; H-3), 3.53 (d, *J* = 13.2 Hz, 1H; NCH_2Ph), 3.61 (d, *J* = 13.2 Hz, 1H; NCH_2Ph), 3.64–3.74 (m, 2H; H-2, H-4), 4.01 (t, *J* = 6.5 Hz, 2H; CH_2-18), 4.30 (t, *J* = 7.3 Hz, 2H; CH_2-9), 4.65 (d, *J* = 12.3 Hz, 1H; H^A-6), 4.66 (d, *J* = 11.7 Hz, 1H; OCH_2Ph), 4.73 (d, *J* = 12.3 Hz, 1H; H^B-6), 4.84 (d, *J* = 11.7 Hz, 1H; OCH_2Ph), 7.23–7.38 (m, 13H; H-8, H_{Ar}), 7.78 (d, *J* = 8.2 Hz, 2H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ 21.8 (CH_3), 25.4, 26.6, 28.95, 28.97, 29.33, 29.34, 30.4 (C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17), 50.4 (C-9), 54.6 (C-5), 56.6 (C-1), 62.2 (NCH_2Ph), 63.7 (C-6), 69.5 (C-4), 70.8 (C-18), 74.0 (OCH_2Ph), 77.8 (C-2), 82.8 (C-3), 122.2 (C-8), 127.4, 127.8, 127.9, 128.0, 128.5, 128.6, 129.1, 129.9 (14 × CH_{Ar}), 133.4, 137.8, 138.8, 144.7 (4 × C_{Ar}), 145.3 (C-7). IR (neat) 3400 (O–H), 1356 (SO_2), 1175 cm^{-1} (SO_2). HRMS (ESI) *m*/*z* calculated for $C_{39}H_{53}N_4O_6S$: 705.368 $[M + H]^+$; found 705.364.

Compound 35c

Compound **35c** (103 mg, 81%) was prepared by general procedure A, starting from (+)-**14** (60 mg, 0.17 mmol) and **31** (1.1 eq., 75 mg, 0.19 mmol). Orange oil. R_f 0.23 (AcOEt/petroleum ether 70 : 30). $[\alpha]_D^{20}$ = −6 (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.24–1.41 (m, 8H; CH_2-11, CH_2-12, CH_2-17,

CH_2-18), 1.47–1.59 (m, 4H; CH_2-13, CH_2-16), 1.60–1.69 (m, 2H; CH_2-19), 1.79–1.88 (m, 2H; CH_2-10), 2.18–2.34 (m, 2H; H^A-1, H^A-5), 2.45 (s, 3H; CH_3), 2.75 (br s, 1H; OH), 2.84–2.96 (m, 2H; H^B-1, H^B-5), 3.31–3.40 (m, 5H; H-3, CH_2-14, CH_2-15), 3.53 (d, J = 13.2 Hz, 1H; NCH_2Ph), 3.61 (d, J = 13.2 Hz, 1H; NCH_2Ph), 3.64–3.74 (m, 2H; H-2, H-4), 4.01 (t, J = 6.4 Hz, 2H; CH_2-20), 4.25 (t, J = 7.3 Hz, 2H; CH_2-9), 4.65 (d, J = 12.3 Hz, 1H; H^A-6), 4.67 (d, J = 11.7 Hz, 1H; OCH_2Ph), 4.73 (d, J = 12.3 Hz, 1H; H^B-6), 4.85 (d, J = 11.7 Hz, 1H; OCH_2Ph), 7.23–7.39 (m, 13H; H-8, H_{Ar}), 7.78 (d, J = 8.2 Hz, 2H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ 21.8 (CH_3), 25.4, 25.7, 25.8, 26.5 (C-11, C-12, C-17, C-18), 28.9, 29.63, 29.64, 30.3 (C-10, C-13, C-16, C-19), 50.3 (C-9), 54.6 (C-5), 56.6 (C-1), 62.2 (NCH_2Ph), 63.7 (C-6), 69.5 (C-4), 70.70, 70.72, 70.78 (C-14, C-15, C-20), 74.1 (OCH_2Ph), 77.9 (C-2), 82.9 (C-3), 122.2 (C-8), 127.4, 127.8, 127.9, 128.0, 128.4, 128.6, 129.1, 129.9 (14 × CH_{Ar}), 133.4, 137.9, 138.8, 144.8 (4 × C_{Ar}), 145.3 (C-7). IR (neat) 3410 (O–H), 1357 (SO_2), 1175 cm^{-1} (SO_2). HRMS (ESI) m/z calculated for $C_{41}H_{57}N_4O_7S$: 749.394 $[M + H]^+$; found 749.390.

Compound 35d

Compound **35d** (54 mg, 86%) was prepared by general procedure A, starting from **23** (32 mg, 0.072 mmol) and **32** (1.5 eq., 45 mg, 0.105 mmol). Colorless oil. R_f 0.29 (AcOEt/petroleum ether 70 : 30). $[\alpha]_D^{20}$ = +7 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.24–1.41 (m, 4H; CH_2-11, CH_2-12), 1.51–1.61 (m, 2H; CH_2-13), 1.73–1.83 (m, 2H; CH_2-10), 1.93–2.02 (m, 2H; H^A-1, H^A-5), 2.45 (s, 3H; CH_3), 3.04–3.13 (m, 2H; H^B-1, H^B-5), 3.40 (t, J = 8.9 Hz, 1H; H-3), 3.43 (t, J = 6.6 Hz, 2H; CH_2-14), 3.52–3.74 (m, 14H; H-2, H-4, CH_2-15, CH_2-16, CH_2-17, CH_2-18, CH_2-19, NCH_2Ph), 4.12–4.22 (m, 4H; CH_2-9, CH_2-20), 4.61 (d, J = 11.5 Hz, 1H; OCH_2Ph), 4.68 (d, J = 11.5 Hz, 1H; OCH_2Ph), 4.75 (d, J = 12.6 Hz, 1H; H^A-6), 4.79 (d, J = 12.6 Hz, 1H; H^B-6), 4.84 (d, J = 11.2 Hz, 1H; OCH_2Ph), 4.93 (d, J = 11.2 Hz, 1H; OCH_2Ph), 7.23–7.40 (m, 18H; H-8, H_{Ar}), 7.80 (d, J = 8.2 Hz, 2H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 21.8 (CH_3), 25.7, 26.4, 29.5, 30.3 (C-10, C-11, C-12, C-13), 50.2 (C-9), 55.9, 56.0 (C-1, C-5), 62.1 (NCH_2Ph), 64.5 (C-6), 68.8 (C-19), 69.3 (C-20), 70.2, 70.6, 70.7, 70.9, (C-15, C-16, C-17, C-18), 71.2 (C-14), 73.0 (OCH_2Ph), 75.3 (OCH_2Ph), 78.7, 78.9 (C-2, C-4), 86.3 (C-3), 122.2 (C-8), 127.3, 127.5, 127.7, 127.8, 127.9, 128.1, 128.40, 128.44, 128.9, 129.9 (19 × CH_{Ar}), 133.1, 137.8, 138.6, 139.3, 144.9 (5 × C_{Ar}), 145.6 (C-7). IR (neat) 1357 (SO_2), 1176 cm^{-1} (SO_2). HRMS (ESI) m/z calculated for $C_{48}H_{63}N_4O_9S$: 871.431 $[M + H]^+$; found 871.431.

Compound 35e

Compound **35e** (278 mg, 84%) was prepared by general procedure A, starting from **23** (150 mg, 0.34 mmol) and **33** (1.1 eq., 198 mg, 0.37 mmol). Colorless oil. R_f 0.16 (AcOEt/petroleum ether 60 : 40). $[\alpha]_D^{20}$ = +6 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.20–1.35 (m, 12H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15, CH_2-16), 1.52–1.62 (m, 2H; CH_2-17), 1.72–1.81 (m, 2H; CH_2-10), 1.92–2.02 (m, 2H; H^A-1, H^A-5), 2.45 (s, 3H; CH_3), 3.04–3.12 (m, 2H; H^B-1, H^B-5), 3.39 (t, J = 8.9 Hz, 1H; H-3), 3.44 (t, J = 6.8 Hz, 2H; CH_2-18), 3.53–3.71 (m, 18H; H-2, H-4, CH_2-19, CH_2-20, CH_2-21, CH_2-22, CH_2-23, CH_2-24, CH_2-25, NCH_2Ph), 4.13–4.21 (m, 4H; CH_2-9, CH_2-26), 4.61 (d, J = 11.5 Hz, 1H; OCH_2Ph), 4.68 (d, J = 11.5 Hz, 1H; OCH_2Ph), 4.75 (d, J = 12.8 Hz, 1H; H^A-6), 4.78 (d, J = 12.8 Hz, 1H; H^B-6), 4.84 (d, J = 11.2 Hz, 1H; OCH_2Ph), 4.92 (d, J = 11.2 Hz, 1H; OCH_2Ph), 7.23–7.39 (m, 18H; H-8, H_{Ar}), 7.80 (d, J = 8.3 Hz, 2H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 21.8 (CH_3), 26.2, 26.6, 29.1, 29.4, 29.52, 29.6, 29.7, 30.3 (C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17), 50.3 (C-9), 55.9, 56.0 (C-1, C-5), 62.1 (NCH_2Ph), 64.5 (C-6), 68.8 (C-25), 69.3 (C-26), 70.2, 70.6, 70.7, 70.8, 70.9 (C-19, C-20, C-21, C-22, C-23, C-24), 71.6 (C-18), 73.0 (OCH_2Ph), 75.3 (OCH_2Ph), 78.7, 78.9 (C-2, C-4), 86.3 (C-3), 122.2 (C-8), 127.3, 127.5, 127.7, 127.79, 127.84, 128.1, 128.4, 128.9, 129.0, 129.9 (19 × CH_{Ar}), 133.1, 137.8, 138.6, 139.3, 144.9 (5 × C_{Ar}), 145.6 (C-7). IR (neat) 1358 (SO_2), 1176 cm^{-1} (SO_2). HRMS (ESI) m/z calculated for $C_{54}H_{75}N_4O_{10}S$: 971.520 $[M + H]^+$; found 971.518.

Compound 36a

Compound **36a** (103 mg, 86%) was prepared by general procedure B, starting from **35a** (150 mg, 0.23 mmol). Colorless oil. R_f 0.29 (AcOEt/petroleum ether 90 : 10). $[\alpha]_D^{20}$ = −9 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.27–1.44 (m, 4H; CH_2-11, CH_2-12), 1.53–1.61 (m, 2H; CH_2-13), 1.81–1.90 (m, 2H; CH_2-10), 2.19–2.35 (m, 2H; H^A-1, H^A-5), 2.70 (br s, 1H; OH), 2.84–2.97 (m, 2H; H^B-1, H^B-5), 3.25 (t, J = 6.8 Hz, 2H; CH_2-14), 3.35 (t, J = 7.0 Hz, 1H; H-3), 3.54 (d, J = 13.2 Hz, 1H; NCH_2Ph), 3.61 (d, J = 13.2 Hz, 1H; NCH_2Ph), 3.65–3.75 (m, 2H; H-2, H-4), 4.26 (t, J = 7.2 Hz, 2H; CH_2-9), 4.66 (d, J = 12.3 Hz, 1H; H^A-6), 4.67 (d, J = 11.7 Hz, 1H; OCH_2Ph), 4.74 (d, J = 12.3 Hz, 1H; H^B-6), 4.85 (d, J = 11.7 Hz, 1H; OCH_2Ph), 7.23–7.40 (m, 11H; H-8, H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 26.2, 26.3, 28.8, 30.2 (C-10, C-11, C-12, C-13), 50.2 (C-9), 51.4 (C-14), 54.6 (C-5), 56.6 (C-1), 62.3 (NCH_2Ph), 63.7 (C-6), 69.5 (C-4), 74.1 (OCH_2Ph), 77.9 (C-2), 82.9 (C-3), 122.2 (C-8), 127.4, 127.8, 127.9, 128.5, 128.3, 128.6, 129.1 (10 × CH_{Ar}), 137.9, 138.8 (2 × C_{Ar}), 145.4 (C-7). IR (neat) 3383 (O–H), 2093 cm^{-1} (N_3). HRMS (ESI) m/z calculated for $C_{28}H_{38}N_7O_3$: 520.303 $[M + H]^+$; found 520.302.

Compound 36b

Compound **36b** (113 mg, 81%) was prepared by general procedure B, starting from **35b** (170 mg, 0.24 mmol). Orange oil. R_f 0.23 (AcOEt/petroleum ether 70 : 30). $[\alpha]_D^{20}$ = −7 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.21–1.39 (m, 12H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15, CH_2-16), 1.54–1.63 (m, 2H; CH_2-17), 1.78–1.88 (m, 2H; CH_2-10), 2.19–2.35 (m, 2H; H^A-1, H^A-5), 2.72 (br s, 1H; OH), 2.83–2.95 (m, 2H; H^B-1, H^B-5), 3.25 (t, J = 6.9 Hz, 2H; CH_2-18), 3.35 (t, J = 6.9 Hz, 1H; H-3), 3.53 (d, J = 13.2 Hz, 1H; NCH_2Ph), 3.61 (d, J = 13.2 Hz, 1H; NCH_2Ph), 3.64–3.76 (m, 2H; H-2, H-4), 4.25 (t, J = 7.3 Hz, 2H; CH_2-9), 4.65 (d, J = 12.3 Hz, 1H; H^A-6), 4.67 (d, J = 11.7 Hz, 1H; OCH_2Ph), 4.74 (d, J = 12.3 Hz, 1H; H^B-6), 4.84 (d, J = 11.7 Hz, 1H; OCH_2Ph), 7.23–7.39 (m, 11H; H-8, H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 26.6, 26.8, 28.9, 29.1, 29.2, 29.4, 29.5, 30.4 (C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17), 50.4 (C-9), 51.6 (C-18), 54.6 (C-5), 56.6 (C-1), 62.2 (NCH_2Ph), 63.7 (C-6), 69.5 (C-4), 74.0 (OCH_2Ph), 77.9 (C-2), 82.8 (C-3), 122.2 (C-8), 127.4, 127.8,

127.9, 128.4, 128.6, 129.1 (10 × CH_{Ar}), 137.9, 138.8 (2 × C_{Ar}), 145.3 (C-7). IR (neat) 3400 (O–H), 2093 cm^{-1} (N_3). HRMS (ESI) *m*/*z* calculated for $C_{32}H_{45}N_7O_3Na$: 598.348 $[M + Na]^+$; found 598.343.

Compound 36c

Compound **36c** (68 mg, 82%) was prepared by general procedure B, starting from **35c** (100 mg, 0.13 mmol). Colorless oil. R_f 0.45 (AcOEt/petroleum ether 80 : 20). $[\alpha]_D^{20}$ = −6 (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.25–1.46 (m, 8H; CH_2-11, CH_2-12, CH_2-17, CH_2-18), 1.51–1.70 (m, 6H; CH_2-13, CH_2-16, CH_2-19), 1.79–1.90 (m, 2H; CH_2-10), 2.18–2.34 (m, 2H; H^A-1, H^A-5), 2.75–2.99 (m, 3H; H^B-1, H^B-5, OH), 3.27 (t, *J* = 6.8 Hz, 2H; CH_2-20), 3.33–3.44 (m, 5H; H-3, CH_2-14, CH_2-15), 3.54 (d, *J* = 13.2 Hz, 1H; NCH_2Ph), 3.61 (d, *J* = 13.2 Hz, 1H; NCH_2Ph), 3.65–3.75 (m, 2H; H-2, H-4), 4.26 (t, *J* = 7.2 Hz, 2H; CH_2-9), 4.67 (d, *J* = 12.3 Hz, 1H; H^A-6), 4.70 (d, *J* = 11.7 Hz, 1H; OCH_2Ph), 4.75 (d, *J* = 12.3 Hz, 1H; H^B-6), 4.86 (d, *J* = 11.7 Hz, 1H; OCH_2Ph), 7.24–7.41 (m, 11H; H-8, H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 25.7, 25.9, 26.4, 26.6 (C-11, C-12, C-17, C-18), 28.9, 29.6, 29.7, 30.3 (C-10, C-13, C-16, C-19), 50.3 (C-9), 51.5 (C-20), 54.6 (C-5), 56.6 (C-1), 62.2 (NCH_2Ph), 63.6 (C-6), 69.5 (C-4), 70.7, 70.8 (C-14, C-15), 74.0 (OCH_2Ph), 77.8 (C-2), 83.0 (C-3), 122.2 (C-8), 127.3, 127.7, 127.8, 128.4, 128.6, 129.0 (10 × CH_{Ar}), 137.8, 138.8 (2 × C_{Ar}), 145.3 (C-7). IR (neat) 3411 (O–H), 2094 cm^{-1} (N_3). HRMS (ESI) *m*/*z* calculated for $C_{34}H_{50}N_7O_4$: 620.392 $[M + H]^+$; found 620.385.

Compound 36d

Compound **36d** (62 mg, 88%) was prepared by general procedure B, starting from **35d** (83 mg, 0.095 mmol). Colorless oil. R_f 0.28 (AcOEt/petroleum ether 60 : 40). $[\alpha]_D^{20}$ = +7 (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.23–1.42 (m, 4H; CH_2-11, CH_2-12), 1.52–1.62 (m, 2H; CH_2-13), 1.73–1.84 (m, 2H; CH_2-10), 1.93–2.03 (m, 2H; H^A-1, H^A-5), 3.04–3.14 (m, 2H; H^B-1, H^B-5), 3.36–3.47 (m, 5H; H-3, CH_2-14, CH_2-20), 3.54–3.71 (m, 14H; H-2, H-4, CH_2-15, CH_2-16, CH_2-17, CH_2-18, CH_2-19, NCH_2Ph), 4.14–4.22 (m, 2H; CH_2-9), 4.62 (d, *J* = 11.5 Hz, 1H; OCH_2Ph), 4.68 (d, *J* = 11.5 Hz, 1H; OCH_2Ph), 4.76 (d, *J* = 12.8 Hz, 1H; H^A-6), 4.79 (d, *J* = 12.8 Hz, 1H; H^B-6), 4.85 (d, *J* = 11.2 Hz, 1H; OCH_2Ph), 4.93 (d, *J* = 11.2 Hz, 1H; OCH_2Ph), 7.24–7.40 (m, 16H; H-8, H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 25.6, 26.4, 29.5, 30.3 (C-10, C-11, C-12, C-13), 50.2 (C-9), 50.8 (C-20), 55.9, 56.0 (C-1, C-5), 62.1 (NCH_2Ph), 64.5 (C-6), 70.1, 70.2, 70.7, 70.78, 70.80 (C-15, C-16, C-17, C-18, C-19), 71.2 (C-14), 73.0 (OCH_2Ph), 75.3 (OCH_2Ph), 78.6, 78.9 (C-2, C-4), 86.3 (C-3), 122.2 (C-8), 127.3, 127.5, 127.7, 127.78, 127.83, 128.39, 128.43, 128.9 (15 × CH_{Ar}), 137.8, 138.6, 139.3 (3 × C_{Ar}), 145.6 (C-7). IR (neat) 2101 cm^{-1} (N_3). HRMS (ESI) *m*/*z* calculated for $C_{41}H_{56}N_7O_6$: 742.429 $[M + H]^+$; found 742.437.

Compound 36e

Compound **36e** (208 mg, 87%) was prepared by general procedure B, starting from **35e** (276 mg, 0.28 mmol). Colorless oil. R_f 0.31 (AcOEt/petroleum ether 60 : 40). $[\alpha]_D^{20}$ = +7 (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.21–1.39 (m, 12H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15, CH_2-16), 1.54–1.64 (m, 2H; CH_2-17), 1.73–1.83 (m, 2H; CH_2-10), 1.94–2.03 (m, 2H; H^A-1, H^A-5), 3.05–3.15 (m, 2H; H^B-1, H^B-5), 3.37–3.50 (m, 5H; H-3, CH_2-18, CH_2-26), 3.54–3.71 (m, 18H; H-2, H-4, CH_2-19, CH_2-20, CH_2-21, CH_2-22, CH_2-23, CH_2-24, CH_2-25, NCH_2Ph), 4.14–4.23 (m, 2H; CH_2-9), 4.63 (d, *J* = 11.6 Hz, 1H; OCH_2Ph), 4.69 (d, *J* = 11.6 Hz, 1H; OCH_2Ph), 4.77 (d, *J* = 12.8 Hz, 1H; H^A-6), 4.80 (d, *J* = 12.8 Hz, 1H; H^B-6), 4.86 (d, *J* = 11.2 Hz, 1H; OCH_2Ph), 4.94 (d, *J* = 11.2 Hz, 1H; OCH_2Ph), 7.24–7.41 (m, 16H; H-8, H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 26.1, 26.5, 29.0, 29.4, 29.45, 29.49, 29.7, 30.2 (C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17), 50.2 (C-9), 50.7 (C-26), 55.8, 55.9 (C-1, C-5), 62.0 (NCH_2Ph), 64.4 (C-6), 70.05, 70.08, 70.62, 70.64, 70.7 (C-19, C-20, C-21, C-22, C-23, C-24, C-25), 71.5 (C-18), 72.9 (OCH_2Ph), 75.2 (OCH_2Ph), 78.6, 78.8 (C-2, C-4), 86.2 (C-3), 122.1 (C-8), 127.3, 127.4, 127.6, 127.7, 127.8, 128.3, 128.4, 128.8 (15 × CH_{Ar}), 137.7, 138.5, 139.3 (3 × C_{Ar}), 145.5 (C-7). IR (neat) 2101 cm^{-1} (N_3). HRMS (ESI) *m*/*z* calculated for $C_{47}H_{67}N_7O_7Na$: 864.499 $[M + Na]^+$; found 864.510.

Compound 37a

Compound **37a** (119 mg, 64%) was prepared by general procedure A, starting from **36a** (100 mg, 0.19 mmol) and **23** (1.1 eq., 96 mg, 0.22 mmol). Pale yellow oil. R_f 0.20 (CH_2Cl_2/MeOH 96 : 4). $[\alpha]_D^{20}$ = 0 (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.25–1.29 (m, 4H; CH_2-11, CH_2-12), 1.69–1.84 (m, 4H; CH_2-10, CH_2-13), 1.93–2.02 (m, 2H; H^A-1, H^A-5), 2.19–2.34 (m, 2H; H^A-1′, H^A-5′), 2.79 (br s, 1H; OH), 2.84–2.98 (m, 2H; H^B-1′, H^B-5′), 3.04–3.13 (m, 2H; H^B-1, H^B-5), 3.35 (t, *J* = 7.1 Hz, 1H; H-3′), 3.39 (t, *J* = 8.9 Hz, 1H; H-3), 3.51–3.74 (m, 8H; H-2, H-2′, H-4, H-4′, 2 × NCH_2Ph), 4.14 (t, *J* = 7.2 Hz, 2H; CH_2-9 or CH_2-14), 4.22 (t, *J* = 7.0 Hz, 2H; CH_2-9 or CH_2-14), 4.61 (d, *J* = 11.6 Hz, 1H; OCH_2Ph), 4.66 (d, *J* = 12.3 Hz, 1H; H^A-6′), 4.67 (d, *J* = 11.6 Hz, 1H; OCH_2Ph), 4.68 (d, *J* = 11.7 Hz, 1H; OCH_2Ph), 4.742 (d, *J* = 12.3 Hz, 1H; H^B-6′), 4.744 (d, *J* = 12.6 Hz, 1H; H^A-6), 4.78 (d, *J* = 12.6 Hz, 1H; H^B-6), 4.83 (d, *J* = 11.2 Hz, 1H; OCH_2Ph), 4.84 (d, *J* = 11.7 Hz, 1H; OCH_2Ph), 4.92 (d, *J* = 11.2 Hz, 1H; OCH_2Ph), 7.22–7.39 (m, 27H; H-8, H-8′, H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 25.9, 30.0, 30.1 (C-10, C-11, C-12, C-13), 50.0, 50.1 (C-9, C-14), 54.6 (C-5′), 55.9, 56.0 (C-1, C-5), 56.7 (C-1′), 62.1, 62.2 (2 × NCH_2Ph), 63.6 (C-6′), 64.5 (C-6), 69.5 (C-4′), 73.0, 74.1, 75.3 (3 × OCH_2Ph), 77.8 (C-2′), 78.7, 78.9 (C-2, C-4), 83.0 (C-3′), 86.3 (C-3), 122.3 (C-8, C-8′), 127.4, 127.5, 127.7, 127.80, 127.82, 127.9, 128.41, 128.44, 128.5, 128.6, 128.9, 129.1 (25 × CH_{Ar}), 137.8, 137.9, 138.6, 138.8, 139.4 (5 × C_{Ar}), 145.4 (C-7′), 145.8 (C-7). IR (neat) 3416 cm^{-1} (O–H). HRMS (ESI) *m*/*z* calculated for $C_{57}H_{69}N_8O_6$: 961.533 $[M + H]^+$; found 961.529.

Compound 37b

Compound **37b** (128 mg, 66%) was prepared by general procedure A, starting from **36b** (110 mg, 0.19 mmol) and **23** (1.1 eq., 93 mg, 0.21 mmol). Orange oil. R_f 0.27 (CH_2Cl_2/MeOH 98 : 2). $[\alpha]_D^{20}$ = +1 (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.21–1.33 (m, 12H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15, CH_2-16), 1.71–1.87 (m, 4H; CH_2-10, CH_2-17), 1.93–2.01 (m, 2H; H^A-1, H^A-5), 2.20–2.36 (m, 2H; H^A-1′, H^A-5′), 2.76 (br s, 1H;

OH), 2.84–2.97 (m, 2H; H^B-1′, H^B-5′), 3.04–3.13 (m, 2H; H^B-1, H^B-5), 3.36 (t, J = 6.9 Hz, 1H; H-3′), 3.39 (t, J = 8.9 Hz, 1H; H-3), 3.52–3.75 (m, 8H; H-2, H-2′, H-4, H-4′, 2 × NCH_2Ph), 4.14–4.19 (m, 2H; CH_2-9 or CH_2-18), 4.25 (t, J = 7.2 Hz, 2H; CH_2-9 or CH_2-18), 4.60 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.66 (d, J = 12.3 Hz, 1H; H^A-6′), 4.67 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.68 (d, J = 11.7 Hz, 1H; OCH_2Ph), 4.746 (d, J = 12.3 Hz, 1H; H^B-6′), 4.749 (d, J = 12.6 Hz, 1H; H^A-6), 4.79 (d, J = 12.6 Hz, 1H; H^B-6), 4.837 (d, J = 11.2 Hz, 1H; OCH_2Ph), 4.842 (d, J = 11.7 Hz, 1H; OCH_2Ph), 4.92 (d, J = 11.2 Hz, 1H; OCH_2Ph), 7.22–7.40 (m, 27H; H-8, H-8′, H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 26.5, 26.6, 29.0, 29.4, 30.3, 30.4 (C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17), 50.3, 50.4 (C-9, C-18), 54.5 (C-5′), 55.9, 56.0 (C-1, C-5), 56.6 (C-1′), 62.1, 62.2 (2 × NCH_2Ph), 63.7 (C-6′), 64.6 (C-6), 69.5 (C-4′), 73.0, 74.0, 75.3 (3 × OCH_2Ph), 77.8 (C-2′), 78.7, 78.9 (C-2, C-4), 83.0 (C-3′), 86.3 (C-3), 122.2 (C-8, C-8′), 127.4, 127.5, 127.7, 127.77, 127.82, 127.9, 128.4, 128.45, 128.47, 128.6, 129.0, 129.1 (25 × CH_{Ar}), 137.8, 138.6, 138.8, 139.4 (5 × C_{Ar}), 145.3 (C-7′), 145.7 (C-7). IR (neat) 3400 cm^{-1} (O–H). HRMS (ESI) m/z calculated for $C_{61}H_{77}N_8O_6$: 1017.596 $[M + H]^+$; found 1017.587.

Compound 37c

Compound **37c** (69 mg, 61%) was prepared by general procedure A, starting from **36c** (66 mg, 0.11 mmol) and **23** (1.1 eq., 52 mg, 0.12 mmol). Pale yellow oil. R_f 0.24 (CH_2Cl_2/MeOH 96 : 4). $[\alpha]_D^{20}$ = +1 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.24–1.44 (m, 8H; CH_2-11, CH_2-12, CH_2-17, CH_2-18), 1.51–1.61 (m, 4H; CH_2-13, CH_2-16), 1.74–1.89 (m, 4H; CH_2-10, CH_2-19), 1.94–2.03 (m, 2H; H^A-1, H^A-5), 2.19–2.35 (m, 2H; H^A-1′, H^A-5′), 2.85–3.01 (m, 3H; H^B-1′, H^B-5′, OH), 3.06–3.15 (m, 2H; H^B-1, H^B-5), 3.33–3.45 (m, 6H; H-3, H-3′, CH_2-14, CH_2-15), 3.52–3.77 (m, 8H; H-2, H-2′, H-4, H-4′, 2 × NCH_2Ph), 4.16–4.21 (m, 2H; CH_2-9 or CH_2-20), 4.26 (t, J = 7.2 Hz, 2H; CH_2-9 or CH_2-20), 4.63 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.68 (d, J = 12.3 Hz, 1H; H^A-6′), 4.69 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.71 (d, J = 11.7 Hz, 1H; OCH_2Ph), 4.76 (d, J = 12.3 Hz, 1H; H^B-6′), 4.77 (d, J = 12.6 Hz, 1H; H^A-6), 4.80 (d, J = 12.6 Hz, 1H; H^B-6), 4.86 (d, J = 11.2 Hz, 1H; OCH_2Ph), 4.87 (d, J = 11.7 Hz, 1H; OCH_2Ph), 4.95 (d, J = 11.2 Hz, 1H; OCH_2Ph), 7.23–7.42 (m, 27H; H-8, H-8′, H_{Ar}). ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ 25.7, 26.4 (C-11, C-12, C-17, C-18), 29.6, 30.22, 30.24 (C-10, C-13, C-16, C-19), 50.15, 50.23 (C-9, C-20), 54.6 (C-5′), 55.9, 56.0 (C-1, C-5), 56.7 (C-1′), 62.1, 62.2 (2 × NCH_2Ph), 63.6 (C-6′), 64.5 (C-6), 69.5 (C-4′), 70.7 (C-14, C-15), 72.9, 74.0, 75.3 (3 × OCH_2Ph), 77.8 (C-2′), 78.6, 78.9 (C-2, C-4), 83.0 (C-3′), 86.2 (C-3), 122.1 (C-8, C-8′), 127.3, 127.4, 127.6, 127.68, 127.74, 127.8, 128.3, 128.35, 128.40, 128.5, 128.9, 129.0 (25 × CH_{Ar}), 137.7, 137.9, 138.6, 138.8, 139.3 (5 × C_{Ar}), 145.3 (C-7′), 145.6 (C-7). IR (neat) 3400 cm^{-1} (O–H). HRMS (ESI) m/z calculated for $C_{63}H_{81}N_8O_7$: 1061.622 $[M + H]^+$; found 1061.612.

Compound 37d

Compound **37d** (74 mg, 84%) was prepared by general procedure A, starting from **36d** (60 mg, 0.081 mmol) and (+)-**14** (1.1 eq., 31 mg, 0.088 mmol). Colorless oil. R_f 0.21 (CH_2Cl_2/MeOH 98 : 2). $[\alpha]_D^{20}$ = +3 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.22–1.42 (m, 4H; CH_2-11, CH_2-12), 1.50–1.62 (m, 2H; CH_2-13), 1.71–1.84 (m, 2H; CH_2-10), 1.93–2.04 (m, 2H; H^A-1, H^A-5), 2.19–2.36 (m, 2H; H^A-1′, H^A-5′), 2.83–2.99 (m, 2H; H^B-1′, H^B-5′), 3.04–3.15 (m, 2H; H^B-1, H^B-5), 3.36 (t, J = 7.0 Hz, 1H; H-3′), 3.40 (t, J = 8.9 Hz, 1H; H-3), 3.43 (t, J = 6.4 Hz, 2H; CH_2-14), 3.50–3.76 (m, 16H; H-2, H-2′, H-4, H-4′, CH_2-15, CH_2-16, CH_2-17, CH_2-18, 2 × NCH_2Ph), 3.83 (t, J = 5.1 Hz, 2H; CH_2-19), 4.11–4.23 (m, 2H; CH_2-9), 4.48 (t, J = 5.1 Hz, 2H; CH_2-20), 4.62 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.67 (d, J = 12.3 Hz, 1H; H^A-6′), 4.68 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.69 (d, J = 11.7 Hz, 1H; OCH_2Ph), 4.75 (d, J = 12.3 Hz, 1H; H^B-6′), 4.73–4.82 (m, 2H; CH_2-6), 4.85 (d, J = 11.2 Hz, 1H; OCH_2Ph), 4.86 (d, J = 11.7 Hz, 1H; OCH_2Ph), 4.94 (d, J = 11.2 Hz, 1H; OCH_2Ph), 7.22–7.41 (m, 26H; H-8, H_{Ar}), 7.63 (s, 1H; H-8′). ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ 25.6, 26.4 (C-11, C-12), 29.5, 30.2 (C-10, C-13), 50.1, 50.3 (C-9, C-20), 54.6 (C-5′), 55.9, 56.0 (C-1, C-5), 56.6 (C-1′), 62.1, 62.2 (2 × NCH_2Ph), 63.5 (C-6′), 64.5 (C-6), 69.47 (C-4′), 69.51 (C-19), 70.2, 70.5, 70.6, 70.7 (C-15, C-16, C-17, C-18), 71.2 (C-14), 72.9, 74.0, 75.3 (3 × OCH_2Ph), 77.8 (C-2′), 78.7, 78.9 (C-2, C-4), 82.9 (C-3′), 86.3 (C-3), 122.2 (C-8), 123.6 (C-8′), 127.28, 127.30, 127.5, 127.7, 127.76, 127.78, 127.81, 128.36, 128.37, 128.41, 128.6, 128.9, 129.0 (25 × CH_{Ar}), 137.8, 137.9, 138.6, 138.8, 139.3 (5 × C_{Ar}), 145.1 (C-7′), 145.6 (C-7). IR (neat) 3416 cm^{-1} (O–H). HRMS (ESI) m/z calculated for $C_{63}H_{81}N_8O_9$: 1093.612 $[M + H]^+$; found 1093.609.

Compound 37e

Compound **37e** (115 mg, 83%) was prepared by general procedure A, starting from **36e** (98 mg, 0.12 mmol) and (+)-**14** (1.1 eq., 45 mg, 0.13 mmol). Colorless oil. R_f 0.22 (CH_2Cl_2/MeOH 98 : 2). $[\alpha]_D^{20}$ = +2 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.20–1.39 (m, 12H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15, CH_2-16), 1.53–1.65 (m, 2H; CH_2-17), 1.72–1.84 (m, 2H; CH_2-10), 1.94–2.05 (m, 2H; H^A-1, H^A-5), 2.19–2.35 (m, 2H; H^A-1′, H^A-5′), 2.85–3.00 (m, 2H; H^B-1′, H^B-5′), 3.05–3.16 (m, 2H; H^B-1, H^B-5), 3.37 (t, J = 7.1 Hz, 1H; H-3′), 3.42 (t, J = 8.9 Hz, 1H; H-3), 3.46 (t, J = 6.8 Hz, 2H; CH_2-18), 3.52–3.77 (m, 20H; H-2, H-2′, H-4, H-4′, CH_2-19, CH_2-20, CH_2-21, CH_2-22, CH_2-23, CH_2-24, 2 × NCH_2Ph), 3.84 (t, J = 5.1 Hz, 2H; CH_2-25), 4.13–4.24 (m, 2H; CH_2-9), 4.49 (t, J = 5.1 Hz, 2H; CH_2-26), 4.63 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.69 (d, J = 12.3 Hz, 1H; H^A-6′), 4.70 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.70 (d, J = 11.7 Hz, 1H; OCH_2Ph), 4.76 (d, J = 12.3 Hz, 1H; H^B-6′), 4.74–4.84 (m, 2H; CH_2-6), 4.87 (d, J = 11.2 Hz, 1H; OCH_2Ph), 4.88 (d, J = 11.7 Hz, 1H; OCH_2Ph), 4.95 (d, J = 11.2 Hz, 1H; OCH_2Ph), 7.22–7.42 (m, 26H; H-8, H_{Ar}), 7.65 (s, 1H; H-8′). ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ 26.1, 26.5, 29.0, 29.36, 29.44, 29.5, 29.6, 30.2 (C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17), 50.2 (C-9, C-26), 54.6 (C-5′), 55.85, 55.92 (C-1, C-5), 56.6 (C-1′), 62.0, 62.1 (2 × NCH_2Ph), 63.4 (C-6′), 64.4 (C-6), 69.5 (C-4′, C-25), 70.1, 70.5, 70.56, 70.59, 70.63 (C-19, C-20, C-21, C-22, C-23, C-24), 71.5 (C-18), 72.9, 74.0, 75.2 (3 × OCH_2Ph), 77.8 (C-2′), 78.6, 78.8 (C-2, C-4), 83.0 (C-3′), 86.2 (C-3), 122.1 (C-8), 123.6 (C-8′), 127.2, 127.3, 127.4, 127.6, 127.70, 127.74, 127.8, 128.30, 128.32, 128.4, 128.5, 128.8, 128.9 (25 × CH_{Ar}), 137.7, 137.9, 138.5, 138.8, 139.3 (5 × C_{Ar}), 145.0

(C-7′), 145.5 (C-7). IR (neat) 3433 cm^{-1} (O–H). HRMS (ESI) *m*/*z* calculated for $C_{69}H_{93}N_8O_{10}$: 1193.701 $[M + H]^+$; found 1193.696.

Compound 9a

Compound **9a** (44 mg, 70%) was prepared by general procedure D, starting from **37a** (119 mg, 0.124 mmol). White amorphous solid. R_f 0.33 ($MeCN/H_2O/NH_4OH$ 5 : 1 : 1). $[\alpha]_D^{20} = 0$ (*c* 1.0, H_2O). ^{1}H-NMR (D_2O, 400 MHz) δ 1.19–1.29 (m, 4H; CH_2-11, CH_2-11′), 1.82–1.92 (m, 4H; CH_2-10, CH_2-10′), 2.67 (dd, *J* = 12.6, 10.2 Hz, 2H; H^A-5, H^A-5′), 2.68 (dd, *J* = 12.6, 10.2 Hz, 2H; H^A-1, H^A-1′), 3.28 (dd, *J* = 12.6, 4.8 Hz, 2H; H^B-5, H^B-5′), 3.39 (dd, *J* = 12.6, 4.8 Hz, 2H; H^B-1, H^B-1′), 3.51 (t, *J* = 8.2 Hz, 2H; H-3, H-3′), 3.59 (ddd, *J* = 10.2, 8.2, 4.8 Hz, 2H; H-2, H-2′), 3.67 (ddd, *J* = 10.2, 8.2, 4.8 Hz, 2H; H-4, H-4′), 4.41 (t, *J* = 6.8 Hz, 4H; CH_2-9, CH_2-9′), 4.79–4.85 (m, 4H; CH_2-6, CH_2-6′), 8.04 (s, 2H; H-8, H-8′). ^{13}C-NMR (D_2O, 75.5 MHz) δ 24.9 (C-11, C-11′), 29.0 (C-10, C-10′), 45.5 (C-1, C-1′), 47.7 (C-5, C-5′), 50.3 (C-9), 63.0 (C-6, C-6′), 69.0 (C-4, C-4′), 75.1 (C-3, C-3′), 76.9 (C-2, C-2′), 125.0 (C-8, C-8′), 143.9 (C-7, C-7′). IR (neat) 3188 cm^{-1} (O–H, N–H). HRMS (ESI) *m*/*z* calculated for $C_{22}H_{38}N_8O_6Na$: 533.281 $[M + Na]^+$; found 533.281.

Compound 9b

Compound **9b** (27 mg, 39%) was prepared by general procedure D, starting from **37b** (125 mg, 0.123 mmol). White amorphous solid. R_f 0.20 ($MeCN/H_2O/NH_4OH$ 10 : 1 : 1). $[\alpha]_D^{20}$ = 0 (*c* 1.0, H_2O). ^{1}H-NMR (D_2O, 400 MHz) δ 1.13–1.27 (m, 12H; CH_2-11, CH_2-11′, CH_2-12, CH_2-12′, CH_2-13, CH_2-13′), 1.80–1.90 (m, 4H; CH_2-10, CH_2-10′), 2.80 (dd, *J* = 12.7, 9.8 Hz, 2H; H^A-5, H^A-5′), 2.795 (dd, *J* = 12.7, 9.8 Hz, 2H; H^A-1, H^A-1′), 3.36 (dd, *J* = 12.7, 4.6 Hz, 2H; H^B-5, H^B-5′), 3.45 (dd, *J* = 12.7, 4.6 Hz, 2H; H^B-1, H^B-1′), 3.55 (t, *J* = 8.2 Hz, 2H; H-3, H-3′), 3.64 (ddd, *J* = 9.8, 8.2, 4.6 Hz, 2H; H-2, H-2′), 3.73 (ddd, *J* = 9.8, 8.2, 4.6 Hz, 2H; H-4, H-4′), 4.40 (t, *J* = 6.8 Hz, 4H; CH_2-9, CH_2-9′), 4.78–4.84 (m, 4H; CH_2-6, CH_2-6′), 8.03 (s, 2H; H-8, H-8′). ^{13}C-NMR (D_2O, 100 MHz) δ 25.4, 27.8, 28.2 (C-11, C-11′, C-12, C-12′, C-13, C-13′), 29.2 (C-10, C-10′), 44.8 (C-1, C-1′), 46.9 (C-5, C-5′), 50.5 (C-9), 63.0 (C-6, C-6′), 68.0 (C-4, C-4′), 74.2 (C-3, C-3′), 75.8 (C-2, C-2′), 125.0 (C-8, C-8′), 143.7 (C-7, C-7′). IR (neat) 3280 cm^{-1} (O–H, N–H). HRMS (ESI) *m*/*z* calculated for $C_{26}H_{46}N_8O_6Na$: 589.3433 $[M + Na]^+$; found 589.3398.

Compound 9c

Compound **9c** (18 mg, 47%) was prepared by general procedure D, starting from **37c** (66 mg, 0.062 mmol). White amorphous solid. R_f 0.24 ($MeCN/H_2O/NH_4OH$ 8 : 1 : 1). $[\alpha]_D^{20} = 0$ (*c* 1.0, H_2O). ^{1}H-NMR (D_2O, 400 MHz) δ 1.18–1.35 (m, 8H; CH_2-11, CH_2-11′, CH_2-12, CH_2-12′), 1.45–1.54 (m, 4H; CH_2-13, CH_2-13′), 1.83–1.93 (m, 4H; CH_2-10, CH_2-10′), 2.38–2.50 (m, 4H; H^A-1, H^A-1′, H^A-5, H^A-5′), 3.09–3.16 (m, 2H; H^B-5, H^B-5′), 3.20–3.27 (m, 2H; H^B-1, H^B-1′), 3.33–3.49 (m, 8H; H-2, H-2′, H-3, H-3′, CH_2-14, CH_2-14′), 3.50–3.58 (m, 2H; H-4, H-4′), 4.42 (t, *J* = 6.8 Hz, 4H; CH_2-9, CH_2-9′), 4.75–4.79 (m, 4H; CH_2-6, CH_2-6′), 8.03 (s, 2H; H-8, H-8′). ^{13}C-NMR (D_2O, 100 MHz) δ 24.6, 25.2 (C-11, C-11′, C-12, C-12′), 28.2 (C-13, C-13′), 29.2 (C-10, C-10′), 46.4 (C-1, C-1′), 48.6 (C-5, C-5′), 50.4 (C-9), 62.9 (C-6, C-6′), 70.25 (C-14), 70.28 (C-4, C-4′), 76.5 (C-3, C-3′), 78.3 (C-2, C-2′), 125.0 (C-8, C-8′), 144.0 (C-7, C-7′). IR (neat) 3292 cm^{-1} (O–H, N–H). HRMS (ESI) *m*/*z* calculated for $C_{28}H_{50}N_8O_7Na$: 633.3695 $[M + Na]^+$; found 633.3720.

Compound 9d

Compound **9d** (23 mg, 93%) was prepared by general procedure C, starting from **37d** (42 mg, 0.038 mmol). White amorphous solid. R_f 0.31 ($MeCN/H_2O/NH_4OH$ 6 : 1 : 1). $[\alpha]_D^{20}$ = 0 (*c* 1.0, H_2O). ^{1}H-NMR (D_2O, 400 MHz) δ 1.20–1.39 (m, 4H; CH_2-11, CH_2-12), 1.48–1.59 (m, 2H; CH_2-13), 1.84–1.95 (m, 2H; CH_2-10), 2.72–2.86 (m, 4H; H^A-1, H^A-1′, H^A-5, H^A-5′), 3.31–3.39 (m, 2H; H^B-5, H^B-5′), 3.42–3.52 (m, 4H; H^B-1, H^B-1′, CH_2-14), 3.52–3.69 (m, 12H; H-2, H-2′, H-3, H-3′, CH_2-15, CH_2-16, CH_2-17, CH_2-18), 3.69–3.77 (m, 2H; H-4, H-4′), 3.98 (t, *J* = 4.6 Hz, 2H; CH_2-19), 4.43 (t, *J* = 6.8 Hz, 2H; CH_2-9), 4.64 (t, *J* = 4.6 Hz, 2H; CH_2-20), 4.80–4.86 (m, 4H; CH_2-6, CH_2-6′), 8.05 (s, 1H; H-8), 8.11 (s, 1H; H-8′). ^{13}C-NMR (D_2O, 100 MHz) δ 24.6 (C-11), 25.3 (C-12), 28.3 (C-13), 29.2 (C-10), 45.0 (C-1, C-1′), 47.08, 47.10 (C-5, C-5′), 50.1 (C-20), 50.4 (C-9), 63.0 (C-6, C-6′), 68.16, 68.19 (C-4, C-4′), 68.7, 69.1, 69.4, 69.6, 69.7 (C-15, C-16, C-17, C-18, C-19), 70.8 (C-14), 74.37, 74.41 (C-3, C-3′), 76.0, 76.1 (C-2, C-2′), 125.1 (C-8), 125.5 (C-8′), 143.8, 143.9 (C-7, C-7′). IR (neat) 3285 cm^{-1} (O–H, N–H). HRMS (ESI) *m*/*z* calculated for $C_{28}H_{51}N_8O_9$: 643.3774 $[M + H]^+$; found 643.3753.

Compound 9e

Compound **9e** (43 mg, 90%) was prepared by general procedure C, starting from **37e** (77 mg, 0.064 mmol). White amorphous solid. R_f 0.24 ($MeCN/H_2O/NH_4OH$ 8 : 1 : 1). $[\alpha]_D^{20}$ = 0 (*c* 1.0, MeOH). ^{1}H-NMR (CD_3OD, 400 MHz) δ 1.24–1.38 (m, 12H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15, CH_2-16), 1.49–1.58 (m, 2H; CH_2-17), 1.85–1.94 (m, 2H; CH_2-10), 2.39–2.54 (m, 4H; H^A-1, H^A-1′, H^A-5, H^A-5′), 3.05–3.15 (m, 2H; H^B-5, H^B-5′), 3.19–3.28 (m, 2H; H^B-1, H^B-1′), 3.33–3.64 (m, 20H; H-2, H-2′, H-3, H-3′, H-4, H-4′, CH_2-18, CH_2-19, CH_2-20, CH_2-21, CH_2-22, CH_2-23, CH_2-24), 3.89 (t, *J* = 5.0 Hz, 2H; CH_2-25), 4.39 (t, *J* = 7.1 Hz, 2H; CH_2-9), 4.58 (t, *J* = 5.1 Hz, 2H; CH_2-26), 4.74–4.81 (m, 4H; CH_2-6, CH_2-6′), 8.00 (s, 1H; H-8), 8.06 (s, 1H; H-8′). ^{13}C-NMR (CD_3OD, 100 MHz) δ 27.2, 27.4, 30.0, 30.4, 30.5, 30.6, 30.7 (C-11, C-12, C-13, C-14, C-15, C-16, C-17), 31.3 (C-10), 48.2, 48.3 (C-1, C-1′), 50.7 (C-5, C-5′), 51.36, 51.44 (C-9, C-26), 64.52, 64.55 (C-6, C-6′), 70.4 (C-25), 71.2, 71.4, 71.51, 71.54, 71.56 (C-19, C-20, C-21, C-22, C-23, C-24), 71.8 (C-4, C-4′), 72.3 (C-18), 78.1, 78.2 (C-3, C-3′), 79.8 (C-2, C-2′), 124.9 (C-8), 125.8 (C-8′), 146.1, 146.2 (C-7, C-7′). IR (neat) 3294 cm^{-1} (O–H, N–H). HRMS (ESI) *m*/*z* calculated for $C_{34}H_{63}N_8O_{10}$: 743.4662 $[M + H]^+$; found 743.4743.

Compound 38

Compound **38** (142 mg, 90%) was prepared by general procedure A, starting from **36e** (104 mg, 0.123 mmol) and **23** (1.1 eq., 60 mg, 0.136 mmol). Pale yellow oil. R_f 0.25 (CH_2Cl_2/MeOH 98 : 2). $[\alpha]_D^{20}$ = +7 (*c* 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.22–1.42 (m, 12H; CH_2-11, CH_2-12, CH_2-13, CH_2-

14, CH_2-15, CH_2-16), 1.55–1.67 (m, 2H; CH_2-17), 1.74–1.86 (m, 2H; CH_2-10), 1.96–2.07 (m, 4H; H^A-1, H^A-1′, H^A-5, H^A-5′), 3.06–3.18 (m, 4H; H^B-1, H^B-1′, H^B-5, H^B-5′), 3.44 (t, J = 8.9 Hz, 2H; H-3, H-3′), 3.47 (t, J = 6.8 Hz, 2H; CH_2-18), 3.55–3.72 (m, 20H; H-2, H-2′, H-4, H-4′, CH_2-19, CH_2-20, CH_2-21, CH_2-22, CH_2-23, CH_2-24, 2 × NCH_2Ph), 3.82 (t, J = 5.2 Hz, 2H; CH_2-25), 4.15–4.25 (m, 2H; CH_2-9), 4.42–4.49 (m, 2H; CH_2-26), 4.64 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.65 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.71 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.72 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.76–4.87 (m, 4H; CH_2-6, CH_2-6′), 4.89 (d, J = 11.2 Hz, 1H; OCH_2Ph), 4.90 (d, J = 11.2 Hz, 1H; OCH_2Ph), 4.94 (d, J = 11.2 Hz, 1H; OCH_2Ph), 4.97 (d, J = 11.2 Hz, 1H; OCH_2Ph), 7.23–7.45 (m, 31H; H-8, H_{Ar}), 7.62 (s, 1H; H-8′). ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ 26.1, 26.5, 29.0, 29.3, 29.4, 29.5, 29.6, 30.2 (C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17), 50.1, 50.2 (C-9, C-26), 55.8, 55.9 (C-1, C-1′, C-5, C-5′), 62.0 (2 × NCH_2Ph), 64.4 (C-6, C-6′), 69.4 (C-25), 70.0, 70.4, 70.5, 70.56, 70.61 (C-19, C-20, C-21, C-22, C-23, C-24), 71.5 (C-18), 72.9, 75.2 (4 × OCH_2Ph), 78.6, 78.7, 78.77, 78.81 (C-2, C-2′, C-4, C-4′), 86.18, 86.23 (C-3, C-3′), 122.1 (C-8), 123.5 (C-8′), 127.20, 127.22, 127.4, 127.55, 127.57, 127.68, 127.73, 127.83, 128.27, 128.33, 128.8 (30 × CH_{Ar}), 137.70, 137.73, 138.52, 138.54, 139.20, 139.27 (6 × C_{Ar}), 145.3, 145.5 (C-7, C-7′). HRMS (ESI) m/z calculated for $C_{76}H_{99}N_8O_{10}$: 1283.748 $[M + H]^+$; found 1283.741.

Compound (+)-10

Compound (+)-**10** (49 mg, 93%) was prepared by general procedure C, starting from **38** (91 mg, 0.071 mmol). R_f 0.27 ($MeCN/H_2O/NH_4OH$ 8 : 1 : 1). $[\alpha]_D^{20}$ = +7 (c 1.0, MeOH). ^{1}H-NMR (CD_3OD, 400 MHz) δ 1.24–1.38 (m, 12H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15, CH_2-16), 1.50–1.58 (m, 2H; CH_2-17), 1.85–1.94 (m, 2H; CH_2-10), 2.55–2.65 (m, 4H; H^A-1, H^A-1′, H^A-5, H^A-5′), 3.13–3.20 (m, 2H; H^B-5, H^B-5′), 3.25–3.32 (m, 2H; H^B-1, H^B-1′), 3.41–3.52 (m, 6H; H-2, H-2′, H-3, H-3′, CH_2-18), 3.53–3.64 (m, 14H; H-4, H-4′, CH_2-19, CH_2-20, CH_2-21, CH_2-22, CH_2-23, CH_2-24), 3.89 (t, J = 5.0 Hz, 2H; CH_2-25), 4.40 (t, J = 7.1 Hz, 2H; CH_2-9), 4.58 (t, J = 5.0 Hz, 2H; CH_2-26), 4.75–4.82 (m, 4H; CH_2-6, CH_2-6′), 8.01 (s, 1H; H-8), 8.07 (s, 1H; H-8′). ^{13}C-NMR (CD_3OD, 100 MHz) δ 27.2, 27.4, 30.0, 30.4, 30.5, 30.6, 30.7 (C-11, C-12, C-13, C-14, C-15, C-16, C-17), 31.3 (C-10), 47.53, 47.56 (C-1, C-1′), 49.92, 49.93 (C-5, C-5′), 51.36, 51.44 (C-9, C-26), 64.45, 64.48 (C-6, C-6′), 70.4 (C-25), 70.98, 71.00 (C-4, C-4′), 71.1, 71.4, 71.48, 71.51, 71.54 (C-19, C-20, C-21, C-22, C-23, C-24), 72.3 (C-18), 76.69, 76.68 (C-3, C-3′), 78.90, 78.87 (C-2, C-2′), 125.0 (C-8), 125.9 (C-8′), 145.9, 146.0 (C-7, C-7′). IR (neat) 3263 cm^{-1} (O–H, N–H). HRMS (ESI) m/z calculated for $C_{34}H_{62}N_8O_{10}Na$: 765.4481 $[M + Na]^+$; found 765.4460.

Compound 39

Compound **39** (117 mg, 78%) was prepared by general procedure A, starting from (+)-**14** (60 mg, 0.171 mmol) and **33** (1.1 eq., 100 mg, 0.189 mmol). Colorless oil. R_f 0.12 (AcOEt/petroleum ether 85 : 15). $[\alpha]_D^{20}$ = −5 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.22–1.34 (m, 12H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15, CH_2-16), 1.52–1.61 (m, 2H; CH_2-17), 1.77–1.86 (m, 2H; CH_2-10), 2.19–2.34 (m, 2H; H^A-1, H^A-5), 2.45 (s, 3H; CH_3), 2.74 (br s, 1H; OH), 2.84–2.96 (m, 2H; H^B-1, H^B-5), 3.35 (t, J = 7.1 Hz, 1H; H-3), 3.44 (t, J = 6.8 Hz, 2H; CH_2-18), 3.51–3.73 (m, 18H; H-2, H-4, CH_2-19, CH_2-20, CH_2-21, CH_2-22, CH_2-23, CH_2-24, CH_2-25, NCH_2Ph), 4.16 (t, J = 4.8 Hz, 2H; CH_2-26), 4.25 (t, J = 7.4 Hz, 2H; CH_2-9), 4.66 (d, J = 12.4 Hz, 1H; H^A-6), 4.67 (d, J = 11.8 Hz, 1H; OCH_2Ph), 4.74 (d, J = 12.4 Hz, 1H; H^B-6), 4.85 (d, J = 11.8 Hz, 1H; OCH_2Ph), 7.23–7.38 (m, 13H; H-8, H_{Ar}), 7.80 (d, J = 8.3 Hz, 2H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ 21.8 (CH_3), 26.2, 26.6, 29.1, 29.45, 29.53, 29.6, 29.8, 30.4 (C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17), 50.4 (C-9), 54.6 (C-5), 56.6 (C-1), 62.2 (NCH_2Ph), 63.7 (C-6), 68.8 (C-25), 69.4 (C-26), 69.5 (C-4), 70.2, 70.6, 70.7, 70.8, 70.9 (C-19, C-20, C-21, C-22, C-23, C-24), 71.6 (C-18), 74.0 (OCH_2Ph), 77.9 (C-2), 82.9 (C-3), 122.2 (C-8), 127.3, 127.75, 127.84, 128.1, 128.4, 128.6, 129.0, 129.9 (14 × CH_{Ar}), 133.2, 137.9, 138.8, 144.9 (4 × C_{Ar}), 145.3 (C-7). IR (neat) 3424 (O–H), 1355 (SO_2), 1176 cm^{-1} (SO_2). HRMS (ESI) m/z calculated for $C_{47}H_{68}N_4O_{10}SNa$: 903.455 $[M + Na]^+$; found 903.454.

Compound 40

Compound **40** (87 mg, 89%) was prepared by general procedure B, starting from **39** (114 mg, 0.129 mmol). Pale yellow oil. R_f 0.45 (AcOEt/petroleum ether 85 : 15). $[\alpha]_D^{20}$ = −5 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.20–1.38 (m, 12H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15, CH_2-16), 1.52–1.63 (m, 2H; CH_2-17), 1.76–1.88 (m, 2H; CH_2-10), 2.15–2.32 (m, 2H; H^A-1, H^A-5), 2.82–3.01 (m, 3H; H^B-1, H^B-5, OH), 3.35 (t, J = 7.2 Hz, 1H; H-3), 3.39 (t, J = 5.0 Hz, 2H; CH_2-26), 3.45 (t, J = 6.7 Hz, 2H; CH_2-18), 3.51–3.74 (m, 18H; H-2, H-4, CH_2-19, CH_2-20, CH_2-21, CH_2-22, CH_2-23, CH_2-24, CH_2-25, NCH_2Ph), 4.24 (t, J = 7.3 Hz, 2H; CH_2-9), 4.66 (d, J = 12.4 Hz, 1H; H^A-6), 4.70 (d, J = 11.8 Hz, 1H; OCH_2Ph), 4.74 (d, J = 12.4 Hz, 1H; H^B-6), 4.85 (d, J = 11.8 Hz, 1H; OCH_2Ph), 7.22–7.41 (m, 11H; H-8, H_{Ar}). ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ 26.1, 26.5, 29.0, 29.3, 29.41, 29.44, 29.6, 30.2 (C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17), 50.3 (C-9), 50.7 (C-26), 54.6 (C-5), 56.7 (C-1), 62.1 (NCH_2Ph), 63.6 (C-6), 69.5 (C-4), 70.0, 70.1, 70.60, 70.62, 70.63, 70.7 (C-19, C-20, C-21, C-22, C-23, C-24, C-25), 71.5 (C-18), 74.0 (OCH_2Ph), 77.8 (C-2), 83.0 (C-3), 122.1 (C-8), 127.2, 127.6, 127.7, 128.3, 128.5, 128.9 (10 × CH_{Ar}), 137.8, 138.8 (2 × C_{Ar}), 145.2 (C-7). IR (neat) 3426 (O–H), 2102 cm^{-1} (N_3). HRMS (ESI) m/z calculated for $C_{40}H_{62}N_7O_7$: 752.471$[M + H]^+$; found 752.476.

Compound 41

Compound **41** (131 mg, 94%) was prepared by general procedure A, starting from **40** (95 mg, 0.126 mmol) and (+)-**14** (1.1 eq., 49 mg, 0.139 mmol). Pale yellow oil. R_f 0.20 (CH_2Cl_2/MeOH 98 : 2). $[\alpha]_D^{20}$ = −7 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.22–1.38 (m, 12H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15, CH_2-16), 1.53–1.63 (m, 2H; CH_2-17), 1.78–1.88 (m, 2H; CH_2-10), 2.17–2.35 (m, 4H; H^A-1, H^A-1′, H^A-5, H^A-5′), 2.83–3.06 (m, 6H; H^B-1, H^B-1′, H^B-5, H^B-5′, 2 × OH), 3.36 (t, J = 7.0 Hz, 2H; H-3, H-3′), 3.45 (t, J = 6.7 Hz, 2H; CH_2-18), 3.51–3.77 (m, 20H; H-2, H-2′, H-4, H-4′, CH_2-19, CH_2-20, CH_2-21, CH_2-22, CH_2-23, CH_2-24, 2 × NCH_2Ph), 3.83 (t, J = 5.1 Hz,

2H; CH_2-25), 4.25 (t, J = 7.1 Hz, 2H; CH_2-9), 4.47 (t, J = 5.1 Hz, 2H; CH_2-26), 4.64–4.78 (m, 6H; CH_2-6, CH_2-6′, OCH_2Ph), 4.86 (d, J = 11.8 Hz, 1H; OCH_2Ph), 4.87 (d, J = 11.8 Hz, 1H; OCH_2Ph), 7.24–7.40 (m, 21H; H-8, H_{Ar}), 7.64 (s, 1H; H-8′). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 26.0, 26.5, 29.0, 29.3, 29.40, 29.44, 29.6, 30.2 (C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17), 50.2, 50.3 (C-9, C-26), 54.6 (C-5, C-5′), 56.6, 56.7 (C-1, C-1′), 62.1 (2 × NCH_2Ph), 63.4, 63.6 (C-6, C-6′), 69.4 (C-25), 69.5 (C-4, C-4′), 70.0, 70.4, 70.50, 70.54, 70.6 (C-19, C-20, C-21, C-22, C-23, C-24), 71.5 (C-18) 74.0 (2 × OCH_2Ph), 77.8 (C-2, C-2′), 83.1 (C-3, C-3′), 122.1 (C-8), 123.6 (C-8′), 127.19, 127.20, 127.6, 127.66, 127.67, 127.71, 128.3, 128.4, 128.9 (20 × CH_{Ar}), 137.75, 137.78, 138.74, 138.76 (4 × C_{Ar}), 145.0, 145.2 (C-7, C-7′). IR (neat) 3413 cm^{-1} (O–H). HRMS (ESI) m/z calculated for $C_{62}H_{87}N_8O_{10}$: 1103.654 $[M + H]^+$; found 1103.645.

Compound (−)-10

Compound (−)-**10** (69 mg, 78%) was prepared by general procedure C, starting from **41** (131 mg, 0.119 mmol). $[\alpha]_D^{20}$ = −7 (c 1.0, MeOH).

Compound 42a

Compound **42a** (265 mg, 98%) was prepared by general procedure A, starting from **23** (205 mg, 0.46 mmol) and **27** (1.1 eq., 73 mg, 0.51 mmol). Pale yellow oil. R_f 0.11 (AcOEt/petroleum ether 80 : 20). $[\alpha]_D^{20}$ = +8 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.25–1.41 (m, 4H; CH_2-11, CH_2-12), 1.50–1.57 (m, 2H; CH_2-13), 1.60 (br s, 1H; OH), 1.76–1.83 (m, 2H; CH_2-10), 1.95–2.01 (m, 2H; H^A-1, H^A-5), 3.06–3.12 (m, 2H; H^B-1, H^B-5), 3.40 (t, J = 8.9 Hz, 1H; H-3), 3.55–3.65 (m, 6H; H-2, H-4, CH_2-14, NCH_2Ph), 4.19 (t, J = 7.1 Hz, 2H; CH_2-9), 4.61 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.68 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.75 (d, J = 12.6 Hz, 1H; H^A-6), 4.79 (d, J = 12.6 Hz, 1H; H^B-6), 4.84 (d, J = 11.1 Hz, 1H; OCH_2Ph), 4.93 (d, J = 11.1 Hz, 1H; OCH_2Ph), 7.24–7.39 (m, 16H; H-8, H_{Ar}). ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ 25.2, 26.3, 30.2, 32.5 (C-10, C-11, C-12, C-13), 50.2 (C-9), 55.9, 56.0 (C-1, C-5), 62.1 (NCH_2Ph), 62.6 (C-14), 64.5 (C-6), 73.0 (OCH_2Ph), 75.3 (OCH_2Ph), 78.6, 78.9 (C-2, C-4), 86.3 (C-3), 122.3 (C-8), 127.4, 127.5, 127.7, 127.8, 127.9, 128.4, 128.46, 128.48, 129.0 (15 × CH_{Ar}), 137.7, 138.6, 139.4 (3 × C_{Ar}), 145.7 (C-7). IR (neat) 3396 cm^{-1} (O–H). HRMS (ESI) m/z calculated for $C_{35}H_{44}N_4O_4Na$: 607.325 $[M + Na]^+$; found 607.322.

Compound 42b

Compound **42b** (260 mg, 84%) was prepared by general procedure A, starting from **23** (213 mg, 0.48 mmol) and **28** (1.1 eq., 106 mg, 0.53 mmol). Pale yellow oil. R_f 0.15 (AcOEt/petroleum ether 70 : 30). $[\alpha]_D^{20}$ = +8 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.22–1.39 (m, 12H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15, CH_2-16), 1.50–1.69 (m, 3H; CH_2-17, OH), 1.72–1.83 (m, 2H; CH_2-10), 1.93–2.02 (m, 2H; H^A-1, H^A-5), 3.04–3.14 (m, 2H; H^B-1, H^B-5), 3.40 (t, J = 8.9 Hz, 1H; H-3), 3.55–3.66 (m, 6H; H-2, H-4, CH_2-18, NCH_2Ph), 4.18 (t, J = 7.3 Hz, 2H; CH_2-9), 4.61 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.68 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.75 (d, J = 12.7 Hz, 1H; H^A-6), 4.80 (d, J = 12.7 Hz, 1H; H^B-6), 4.84 (d, J = 11.1 Hz, 1H; OCH_2Ph), 4.93 (d, J = 11.2 Hz, 1H; OCH_2Ph), 7.23–7.40 (m, 16H; H-8, H_{Ar}). ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ 25.8, 26.5, 29.0, 29.36, 29.42, 29.5, 30.3, 32.9 (C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17), 50.3 (C-9), 55.9, 56.0 (C-1, C-5), 62.1 (NCH_2Ph), 63.1 (C-18), 64.5 (C-6), 73.0 (OCH_2Ph), 75.3 (OCH_2Ph), 78.7, 78.9 (C-2, C-4), 86.3 (C-3), 122.2 (C-8), 127.4, 127.5, 127.7, 127.8, 127.9, 128.4, 128.5, 128.9 (15 × CH_{Ar}), 137.8, 138.6, 139.4 (3 × C_{Ar}), 145.7 (C-7). IR (neat) 3398 cm^{-1} (O–H). HRMS (ESI) m/z calculated for $C_{39}H_{53}N_4O_4$: 641.406 $[M + H]^+$; found 641.400.

Compound 42c

Compound **42c** (62 mg, 88%) was prepared by general procedure A, starting from **23** (51 mg, 0.11 mmol) and **26** (1.1 eq., 22 mg, 0.13 mmol). Pale yellow oil. R_f 0.29 (CH_2Cl_2/MeOH 98 : 2). $[\alpha]_D^{20}$ = +9 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.92 (t, J = 6.8 Hz, 3H; CH_3-17), 1.23–1.37 (m, 12H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15, CH_2-16), 1.76–1.84 (m, 2H; CH_2-10), 1.97–2.04 (m, 2H; H^A-1, H^A-5), 3.07–3.15 (m, 2H; H^B-1, H^B-5), 3.42 (t, J = 8.9 Hz, 1H; H-3), 3.57–3.67 (m, 4H; H-2, H-4, NCH_2Ph), 4.20 (t, J = 7.1 Hz, 2H; CH_2-9), 4.64 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.70 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.78 (d, J = 12.6 Hz, 1H; H^A-6), 4.82 (d, J = 12.6 Hz, 1H; H^B-6), 4.87 (d, J = 11.2 Hz, 1H; OCH_2Ph), 4.96 (d, J = 11.2 Hz, 1H; OCH_2Ph), 7.26–7.42 (m, 16H; H-8, H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 14.2 (C-17), 22.7 (C-16), 26.6 (C-11), 29.1, 29.3, 29.4 (C-12, C-13, C-14), 30.3 (C-10), 31.9 (C-15), 50.3 (C-9), 55.9, 56.0 (C-1, C-5), 62.1 (NCH_2Ph), 64.5 (C-6), 73.0 (OCH_2Ph), 75.3 (OCH_2Ph), 78.7, 78.9 (C-2, C-4), 86.3 (C-3), 122.2 (C-8), 127.3, 127.5, 127.7, 127.79, 127.84, 128.39, 128.44, 128.9 (15 × CH_{Ar}), 137.8, 138.6, 139.4 (3 × C_{Ar}), 145.7 (C-7). HRMS (ESI) m/z calculated for $C_{38}H_{51}N_4O_3$: 611.3956 $[M + H]^+$; found 611.3922.

Compound (+)-11a

Compound (+)-**11a** (30 mg, 73%) was prepared by general procedure D, starting from **42a** (76 mg, 0.13 mmol). White amorphous solid. R_f 0.35 (MeCN/H_2O/NH_4OH 8 : 1 : 1). $[\alpha]_D^{20}$ = +8 (c 1.0, MeOH). ^{1}H-NMR (CD_3OD, 400 MHz) δ 1.28–1.46 (m, 4H; CH_2-11, CH_2-12), 1.47–1.57 (m, 2H; CH_2-13), 1.86–1.97 (m, 2H; CH_2-10), 2.59–2.72 (m, 2H; H^A-1, H^A-5), 3.14–3.21 (m, 1H; H^B-5), 3.25–3.31 (m, 1H; H^B-1), 3.45–3.62 (m, 5H; H-2, H-3, H-4, CH_2-14), 4.41 (t, J = 7.1 Hz, 2H; CH_2-9), 4.74–4.81 (m, 2H; CH_2-6), 8.01 (s, 1H; H-8). ^{13}C-NMR (CD_3OD, 100 MHz) δ 26.3 (C-12), 27.2 (C-11), 31.3 (C-10), 33.3 (C-13), 47.2 (C-1), 49.6 (C-5), 51.3 (C-9), 62.7 (C-14), 64.4 (C-6), 70.7 (C-4), 75.8 (C-3), 78.5 (C-2), 125.0 (C-8), 146.0 (C-7). IR (neat) 3268 cm^{-1} (O–H, N–H). HRMS (ESI) m/z calculated for $C_{14}H_{27}N_4O_4$: 315.2027 $[M + H]^+$; found 315.2022.

Compound (+)-11b

Compound (+)-**11b** (39 mg, 81%) was prepared by general procedure D, starting from **42b** (83 mg, 0.13 mmol). White amorphous solid. R_f 0.20 (MeCN/H_2O/NH_4OH 11 : 1 : 1). $[\alpha]_D^{20}$ = +7 (c 1.0, MeOH). ^{1}H-NMR (CD_3OD, 400 MHz) δ 1.25–1.41 (m, 12H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15, CH_2-16), 1.47–1.57 (m, 2H; CH_2-17), 1.85–1.95 (m, 2H; CH_2-10), 2.70–2.84 (m, 2H; H^A-1, H^A-5), 3.20–3.26 (m, 1H; H^B-5),

3.30–3.36 (m, 1H; H^B-1), 3.51–3.59 (m, 4H; H-2, H-3, CH_2-18), 3.61–3.68 (m, 1H; H-4), 4.40 (t, J = 7.1 Hz, 2H; CH_2-9), 4.75–4.82 (m, 2H; CH_2-6), 8.01 (s, 1H; H-8). ^{13}C-NMR (CD_3OD, 100 MHz) δ 26.9, 27.4, 30.0, 30.45, 30.50, 30.6 (C-11, C-12, C-13, C-14, C-15, C-16), 31.3 (C-10), 33.6 (C-17), 46.7 (C-1), 49.0 (C-5), 51.4 (C-9), 63.0 (C-18), 64.4 (C-6), 70.1 (C-4), 74.7 (C-3), 77.8 (C-2), 125.0 (C-8), 145.8 (C-7). IR (neat) 3341 cm^{-1} (O–H, N–H). HRMS (ESI) m/z calculated for $C_{18}H_{34}N_4O_4Na$: 393.2472 $[M + Na]^+$; found 393.2494.

Compound (+)-11c

Compound (+)-**11c** (26 mg, 76%) was prepared by general procedure C, starting from **42c** (61 mg, 0.1 mmol). White amorphous solid. R_f 0.40 ($MeCN/H_2O/NH_4OH$ 14 : 1 : 1). $[\alpha]_D^{20}$ = +7 (c 1.0, MeOH). ^{1}H-NMR (CD_3OD, 400 MHz) δ 0.89 (t, J = 6.8 Hz, 3H; CH_3-17), 1.23–1.39 (m, 12H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15, CH_2-16), 1.84–1.95 (m, 2H; CH_2-10), 2.30–2.39 (m, 2H; H^A-1, H^A-5), 3.00–3.06 (m, 1H; H^B-5), 3.14–3.20 (m, 1H; H^B-1), 3.26–3.31 (m, 2H; H-2, H-3), 3.36–3.46 (m, 1H; H-4), 4.39 (t, J = 7.1 Hz, 2H; CH_2-9), 4.73–4.80 (m, 2H; CH_2-6), 7.97 (s, 1H; H-8). ^{13}C-NMR (CD_3OD, 100 MHz) δ 14.4 (C-17), 23.7, 27.5, 30.1, 30.3, 30.5, 31.3, 33.0 (C-10, C-11, C-12, C-13, C-14, C-15, C-16), 49.0 (C-1), 51.36 (C-9), 51.44 (C-5), 64.5 (C-6), 72.7 (C-4), 79.2 (C-3), 80.8 (C-2), 124.8 (C-8), 146.4 (C-7). IR (neat) 3350 cm^{-1} (O–H, N–H). HRMS (ESI) m/z calculated for $C_{17}H_{33}N_4O_3$: 341.2547 $[M + H]^+$; found 341.2542.

Compound 43a

Compound **43a** (94 mg, 91%) was prepared by general procedure A, starting from (+)-**14** (70 mg, 0.20 mmol) and **26** (1.1 eq., 37 mg, 0.22 mmol). Yellow oil. R_f 0.17 (AcOEt/petroleum ether 60 : 40). $[\alpha]_D^{20}$ = −8 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.89 (t, J = 6.8 Hz, 3H; CH_3-17), 1.21–1.34 (m, 12H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15, CH_2-16), 1.78–1.88 (m, 2H; CH_2-10), 2.21–2.35 (m, 2H; H^A-1, H^A-5), 2.75 (br s, 1H; OH), 2.84–2.96 (m, 2H; H^B-1, H^B-5), 3.36 (t, J = 6.9 Hz, 1H; H-3), 3.54 (d, J = 13.2 Hz, 1H; NCH_2Ph), 3.61 (d, J = 13.2 Hz, 1H; NCH_2Ph), 3.65–3.75 (m, 2H; H-2, H-4), 4.25 (t, J = 7.3 Hz, 2H; CH_2-9), 4.66 (d, J = 12.3 Hz, 1H; H^A-6), 4.68 (d, J = 11.7 Hz, 1H; OCH_2Ph), 4.74 (d, J = 12.3 Hz, 1H; H^B-6), 4.85 (d, J = 11.7 Hz, 1H; OCH_2Ph), 7.23–7.40 (m, 11H; H-8, H_{Ar}). ^{13}C-NMR ($CDCl_3$, 75.5 MHz) δ 14.2 (C-17), 22.8, 26.6, 29.1, 29.3, 29.5, 30.4, 31.9 (C-10, C-11, C-12, C-13, C-14, C-15, C-16), 50.5 (C-9), 54.6 (C-5), 56.6 (C-1), 62.2 (NCH_2Ph), 63.7 (C-6), 69.5 (C-4), 74.0 (OCH_2Ph), 77.9 (C-2), 82.8 (C-3), 122.2 (C-8), 127.4, 127.75, 127.84, 128.4, 128.6, 129.1 (10 × CH_{Ar}), 137.9, 138.8 (2 × C_{Ar}), 145.3 (C-7). IR (neat) 3416 cm^{-1} (O–H). HRMS (ESI) m/z calculated for $C_{31}H_{44}N_4O_3Na$: 543.331 $[M + Na]^+$; found 543.331.

Compound 43b

Compound **43b** (60 mg, 90%) was prepared by general procedure A, starting from (+)-**14** (40 mg, 0.11 mmol) and **34** (1.2 eq., 32 mg, 0.14 mmol). Pale yellow oil. R_f 0.27 (CH_2Cl_2/MeOH 96 : 4). $[\alpha]_D^{20}$ = −4 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.18–2.35 (m, 2H; H^A-1, H^A-5), 2.74 (br s, 1H; OH), 2.83–2.96 (m, 2H; H^B-1, H^B-5), 3.35 (t, J = 7.0 Hz, 1H; H-3), 3.36 (s, 3H; OCH_3), 3.51–3.65 (m, 14H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15, CH_2-16, NCH_2Ph), 3.66–3.73 (m, 2H; H-2, H-4), 3.83 (t, J = 5.1 Hz, 2H; CH_2-10), 4.48 (t, J = 5.1 Hz, 2H; CH_2-9), 4.66 (d, J = 11.7 Hz, 1H; OCH_2Ph), 4.67 (d, J = 12.3 Hz, 1H; H^A-6), 4.74 (d, J = 12.3 Hz, 1H; H^B-6), 4.85 (d, J = 11.7 Hz, 1H; OCH_2Ph), 7.23–7.37 (m, 10H; H_{Ar}), 7.63 (s, 1H; H-8). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 50.4 (C-9), 54.7 (C-5), 56.6 (C-1), 59.1 (OCH_3), 62.2 (NCH_2Ph), 63.5 (C-6), 69.5 (C-4), 69.6 (C-10), 70.60, 70.64, 70.66, 70.72 (C-11, C-12, C-13, C-14, C-15), 72.0 (C-16), 74.0 (OCH_2Ph), 77.8 (C-2), 82.9 (C-3), 123.8 (C-8), 127.3, 127.8, 128.4, 128.6, 129.0 (10 × CH_{Ar}), 137.9, 138.8 (2 × C_{Ar}), 145.1 (C-7). IR (neat) 3423 cm^{-1} (O–H). HRMS (ESI) m/z calculated for $C_{31}H_{44}N_4O_7Na$: 607.310 $[M + Na]^+$; found 607.311.

Compound (−)-11c

Compound (−)-**11c** (51 mg, 83%) was prepared by general procedure D, starting from **43a** (94 mg, 0.18 mmol). $[\alpha]_D^{20}$ = −9 (c 1.0, MeOH).

Compound (−)-12

Compound (−)-**12** (27 mg, 65%) was prepared by general procedure D, starting from **43b** (60 mg, 0.10 mmol). R_f 0.51 ($MeCN/H_2O/NH_4OH$ 7 : 1 : 1). $[\alpha]_D^{20}$ = −2 (c 1.0, MeOH). ^{1}H-NMR (CD_3OD, 400 MHz) δ 2.96–3.03 (m, 1H; H^A-5), 3.06–3.13 (m, 1H; H^A-1), 3.33–3.35 (m, 3H, CH_3-17), 3.35–3.45 (m, 2H; H^B-1, H^B-5), 3.51–3.55 (m, 2H; CH_2-16), 3.56–3.64 (m, 10H; CH_2-11, CH_2-12, CH_2-13, CH_2-14, CH_2-15), 3.69–3.76 (m, 2H; H-2, H-3), 3.77–3.84 (m, 1H; H-4), 3.90 (t, J = 5.1 Hz, 2H; CH_2-10), 4.60 (t, J = 5.1 Hz, 2H; CH_2-9), 4.77–4.85 (m, 2H; CH_2-6), 8.11 (s, 1H; H-8). ^{13}C-NMR (CD_3OD, 100 MHz) δ 31.3 (C-10), 33.0 (C-15), 45.2 (C-1), 47.5 (C-5), 51.5 (C-9), 59.1 (C-17), 64.4 (C-6), 68.3 (C-4), 70.4 (C-10), 71.3, 71.41, 71.43, 71.5 (C-11, C-12, C-13, C-14, C-15), 72.0 (C-3), 72.9 (C-16), 75.8 (C-2), 126.1 (C-8), 145.4 (C-7). IR (neat) 3341 cm^{-1} (O–H, N–H). HRMS (ESI) m/z calculated for $C_{17}H_{32}N_4O_7Na$: 427.2163 $[M + Na]^+$; found 427.2197.

Compound 44

To a solution of **22** (39 mg, 0.097 mmol) in dry DMF (2 mL) was added NaH (2.6 eq., 10 mg, 0.25 mmol, 60% on mineral oil) at 0 °C. The mixture was stirred at room temperature for 30 min then 1-bromononane (3.2 eq., 0.06 mL, 0.31 mmol) was added and the reaction mixture was stirred at room temperature for 17 h. The reaction was quenched by slow addition of water, and the reaction mixture was extracted with AcOEt (2×). The combined organic extracts were washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (AcOEt/petroleum ether 10 : 90) to afford **44** (45 mg, 88%) as a colorless oil. R_f 0.35 (AcOEt/petroleum ether 10 : 90). $[\alpha]_D^{20}$ = +1 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.80 (t, J = 6.8 Hz, 3H; CH_3-14), 1.11–1.27 (m, 12H; CH_2-8, CH_2-9, CH_2-10, CH_2-11, CH_2-12, CH_2-13), 1.41–1.49 (m, 2H; CH_2-7), 1.83 (dd, J = 11.2, 10.2 Hz, 1H; H^A-5), 1.88 (dd, J = 11.2, 10.2 Hz, 1H; H^A-1), 2.92–3.00 (m, 2H; H^B-1, H^B-5), 3.25 (t, J = 8.9 Hz, 1H; H-3), 3.35 (ddd, J = 10.2, 8.9, 4.9 Hz, 1H; H-2), 3.42–3.53 (m, 5H; H-4,

CH_2-6, NCH_2Ph), 4.51 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.60 (d, J = 11.6 Hz, 1H; OCH_2Ph), 4.76 (d, J = 11.1 Hz, 1H; OCH_2Ph), 4.81 (d, J = 11.1 Hz, 1H; OCH_2Ph), 7.13–7.31 (m, 15H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 14.3 (C-14), 22.8 (C-13), 26.3, 29.4, 29.65, 29.70, 30.5, 32.0 (C-7, C-8, C-9, C-10, C-11, C-12), 56.2, 56.3 (C-1, C-5), 62.2 (NCH_2Ph), 71.3 (C-6), 73.1, 75.5 (2 × OCH_2Ph), 78.8 (C-4), 79.3 (C-2), 86.5 (C-3), 127.3, 127.5, 127.6, 127.8, 128.0, 128.36, 128.44, 128.9 (15 × CH_{Ar}), 138.0, 138.8, 139.4 (3 × C_{Ar}). IR (neat) 2924 cm^{-1} (C–H). HRMS (ESI) m/z calculated for $C_{35}H_{48}NO_3$: 530.3629 $[M + H]^+$; found 530.3603.

Compound (+)-13

Compound **44** (43 mg, 0.081 mmol) was dissolved in iPrOH (1 mL) and THF (1 mL), then 1 M HCl aqueous solution (0.6 mL) and $Pd(OH)_2/C$ (0.2 eq., 21 mg, 0.015 mmol, 20% Pd on carbon, nominally 50% water) were added. The flask was evacuated and backfilled with argon (four cycles) and then evacuated and backfilled with H_2 (four cycles). The reaction mixture was stirred under an atmosphere of H_2 (1 atm) at room temperature for 17 h. The reaction mixture was then filtered through a pad of Celite (previously rinsed with at least 250 mL of 1 M HCl aqueous solution) and rinsed with MeOH. The filtrate was concentrated under reduced pressure. The residue was dissolved in MeOH then ion exchange resin Amberlite® IRA400 (OH^-) was added and the suspension was rotated at room temperature for 1 h. The resin was filtered, rinsed with MeOH and the filtrate was concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 80 : 20) to afford (+)-**13** (21 mg, quant.) as a white solid. R_f 0.28 (CH_2Cl_2/MeOH 80 : 20). $[\alpha]_D^{20}$ = +16 (c 1.0, MeOH). ^{1}H-NMR (CD_3OD, 400 MHz) δ 0.90 (t, J = 6.8 Hz, 3H; CH_3-14), 1.23–1.40 (m, 12H; CH_2-8, CH_2-9, CH_2-10, CH_2-11, CH_2-12, CH_2-13), 1.53–1.62 (m, 2H; CH_2-7), 2.27–2.35 (m, 1H; H^A-1), 2.36 (dd, J = 12.4, 10.2 Hz, 1H; H^A-5), 3.03 (ddd, J = 12.4, 4.9, 1.0 Hz, 1H; H^B-5), 3.11–3.19 (m, 2H; H^B-1, H-2), 3.24 (t, J = 8.4 Hz, 1H; H-3), 3.40 (ddd, J = 10.2, 8.4, 4.9 Hz, 1H; H-4), 3.59 (t, J = 6.7 Hz, 2H; CH_2-6). ^{13}C-NMR (CD_3OD, 100 MHz) δ 14.4 (C-14), 23.7 (C-13), 27.2, 30.4, 30.6, 30.7, 31.2, 33.1 (C-7, C-8, C-9, C-10, C-11, C-12), 48.8 (C-1), 51.4 (C-5), 71.9 (C-6), 72.6 (C-4), 78.9 (C-3), 80.8 (C-2). IR (neat) 3286 (O–H, N–H), 2920 cm^{-1} (C–H). HRMS (ESI) m/z calculated for $C_{14}H_{30}NO_3$: 260.2220 $[M + H]^+$; found 260.2219.

Compound 45

To a solution of **24** (30 mg, 0.084 mmol) in dry DMF (1.2 mL) was added NaH (3.9 eq., 13 mg, 0.32 mmol, 60% on mineral oil) at 0 °C. The mixture was stirred at room temperature for 30 min then 1-bromononane (3.1 eq., 0.05 mL, 0.26 mmol) was added and the reaction mixture was stirred at room temperature for 14 h. The reaction was quenched by slow addition of water, and the reaction mixture was extracted with AcOEt (2×). The combined organic extracts were washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (AcOEt/petroleum ether 10 : 90) to afford **45** (36 mg, 89%) as a colorless oil. R_f 0.28 (AcOEt/petroleum ether 10 : 90). $[\alpha]_D^{20}$ = −24 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.80 (t, J = 6.8 Hz, 3H; CH_3-14), 1.11–1.26 (m, 12H; CH_2-8, CH_2-9, CH_2-10, CH_2-11, CH_2-12, CH_2-13), 1.39–1.49 (m, 2H; CH_2-7), 1.80 (dd, J = 11.0, 10.3 Hz, 1H; H^A-5), 1.92 (dd, J = 10.9, 10.5 Hz, 1H; H^A-1), 2.93–3.01 (m, 2H; H^B-1, H^B-5), 3.18 (t, J = 8.9 Hz, 1H; H-3), 3.23 (s, 3H; OCH_2OCH_3), 3.35 (ddd, J = 10.3, 8.9, 4.9 Hz, 1H; H-4), 3.45 (t, J = 6.7 Hz, 2H; CH_2-6), 3.46 (d, J = 13.3 Hz, 1H; NCH_2Ph), 3.53 (d, J = 13.3 Hz, 1H; NCH_2Ph), 3.35 (ddd, J = 10.5, 8.9, 5.0 Hz, 1H; H-2), 4.56 (d, J = 6.6 Hz, 1H; OCH_2OCH_3), 4.690 (d, J = 11.2 Hz, 1H; OCH_2Ph), 4.694 (d, J = 6.6 Hz, 1H; OCH_2OCH_3), 4.80 (d, J = 11.2 Hz, 1H; OCH_2Ph), 7.16–7.31 (m, 10H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 14.3 (C-14), 22.8 (C-13), 26.3, 29.4, 29.65, 29.69, 30.5, 32.0 (C-7, C-8, C-9, C-10, C-11, C-12), 55.5 (OCH_2OCH_3), 55.9 (C-5), 57.1 (C-1), 62.2 (NCH_2Ph), 71.2 (C-6), 75.4 (OCH_2Ph), 76.8 (C-2), 79.3 (C-4), 86.1 (C-3), 97.0 (OCH_2OCH_3), 127.3, 127.5, 127.9, 128.36, 128.44, 128.9 (10 × CH_{Ar}), 138.0, 139.3 (2 × C_{Ar}). IR (neat) 2925 cm^{-1} (C–H). HRMS (ESI) m/z calculated for $C_{30}H_{46}NO_4$: 484.3421 $[M + H]^+$; found 484.3464.

Compound 46

A solution of **45** (34 mg, 0.07 mmol) in MeOH (1 mL) and 6 M HCl aqueous solution (0.5 mL) was heated at 65 °C for 2 h 30. After being cooled to room temperature, the solution was treated with sat. $NaHCO_3$ and extracted with AcOEt (2×). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (AcOEt/petroleum ether 20 : 80) to afford **46** (29 mg, 94%) as a colorless oil. R_f 0.28 (AcOEt/petroleum ether 20 : 80). $[\alpha]_D^{20}$ = −1 (c 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.81 (t, J = 6.8 Hz, 3H; CH_3-14), 1.12–1.29 (m, 12H; CH_2-8, CH_2-9, CH_2-10, CH_2-11, CH_2-12, CH_2-13), 1.42–1.51 (m, 2H; CH_2-7), 2.12–2.26 (m, 2H; H^A-1, H^A-5), 2.50–2.96 (m, 1H; OH), 2.77 (dd, J = 11.0, 3.7 Hz, 2H; H^B-1, H^B-5), 3.25 (dd, J = 7.1, 6.5 Hz, 1H; H-3), 3.30–3.44 (m, 3H; H-4, CH_2-6), 3.43 (d, J = 13.3 Hz, 1H; NCH_2Ph), 3.54 (d, J = 13.3 Hz, 1H; NCH_2Ph), 3.30–3.44 (ddd, J = 7.4, 7.1, 3.7 Hz, 1H; H-2), 4.58 (d, J = 11.7 Hz, 1H; OCH_2Ph), 4.78 (d, J = 11.7 Hz, 1H; OCH_2Ph), 7.15–7.30 (m, 10H; H_{Ar}). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 14.3 (C-14), 22.8 (C-13), 26.3, 29.4, 29.6, 29.7, 30.3, 32.0 (C-7, C-8, C-9, C-10, C-11, C-12), 54.5 (C-5), 56.6 (C-1), 62.3 (NCH_2Ph), 69.4 (C-2), 70.2 (C-6), 73.9 (OCH_2Ph), 78.2 (C-4), 82.4 (C-3), 127.3, 127.9, 128.4, 128.6, 129.0 (10 × CH_{Ar}), 138.1, 139.9 (2 × C_{Ar}). IR (neat) 3427 (O–H), 2924 cm^{-1} (C–H). HRMS (ESI) m/z calculated for $C_{28}H_{42}NO_3$: 440.3159 $[M + H]^+$; found 440.3215.

Compound (−)-13

Compound **46** (27 mg, 0.061 mmol) was dissolved in iPrOH (0.7 mL) and THF (0.7 mL), then 1 M HCl aqueous solution (0.5 mL) and $Pd(OH)_2/C$ (0.1 eq., 11 mg, 0.008 mmol, 20% Pd on carbon, nominally 50% water) were added. The flask was evacuated and backfilled with argon (four cycles) and then

evacuated and backfilled with H_2 (four cycles). The reaction mixture was stirred under an atmosphere of H_2 (1 atm) at room temperature for 16 h. The reaction mixture was then filtered through a pad of Celite (previously rinsed with at least 250 mL of 1 M HCl aqueous solution) and rinsed with MeOH. The filtrate was concentrated under reduced pressure. The residue was dissolved in MeOH then ion exchange resin Amberlite® IRA400 (OH^-) was added and the suspension was rotated at room temperature for 1 h. The resin was filtered, rinsed with MeOH and the filtrate was concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 90 : 10) to afford (−)-**13** (15.5 mg, 97%) as a white solid. R_f 0.33 (CH_2Cl_2/MeOH 90 : 10). $[\alpha]_D^{20} = -18$ (c 1.0, MeOH).

Biochemical and cellular studies

IC_{50} and K_i were determined as previously reported.[61]

Fibroblasts culture assay

Skin fibroblasts derived from GD patients with distinct genotypes: N370S/N370S (non-neurological), L444P/L444P and G202R/G202R (neurological) were obtained from the Institute of Child Health, Athens. Fibroblasts from healthy individuals (WT) were used as controls. The study had the approval of the Ethics Committees. Fibroblast cultures were established following routine procedures in Dulbeco Eagle's minimal essential medium (D-MEM) with 10% fetal bovine serum (FBS).

Lysosomal glucocerebrosidase (GCase) activity in fibroblasts from GD patients

To determine the GCase activity in intact cells, 5000 cells were plated into 96-well black plate with clear bottom (Corning Incorporated, NY, USA) during 6 days in D-MEM, 10% FBS at 37 °C under 5% CO_2, either with or without the tested compounds at several concentrations. Culture media was replaced every 3 days with fresh media supplemented with the corresponding compound dissolved in dimethyl sulfoxide (DMSO). After washing with phosphate-buffered saline (PBS), the enzyme assay was performed. Fibroblasts were incubated in PBS (8 μl) and acetate buffer (8 μl, 0.1 M, pH 5.2). The reaction was started by addition of 4-MU (10 μl, 5 mM), followed by incubation at 37 °C for 1 h. Enzyme reaction was stopped with NaOH-glycine buffer (200 μl, 0.2 M, pH 10.2) and the released 4-methylumbelliferone was measured. Fluorescence was read at 340 nm (excitation) and 410–460 nm (emission) on a Turner Biosystems plate reader. For each experiment, untreated (0.1% DMSO) and treated cells were plated in quadruplicate.

Cytotoxicity assay

The cytotoxicity of the selected compounds and cell viability was tested by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. WT fibroblasts were seeded into 96-well plates at a density of 5000 cells per well. Cells were incubated at 37 °C in 5% CO_2 for six days with the different compounds (at the concentration range 300–0.7 μM). Media supplemented with compounds were replaced the third day. The last day, the cell viability was tested by the MTT assay. Absorbance was measured at 570 nm on a Turner Biosystems plate reader.

Co-localization of GCase and lysosome

For immunofluorescence, G202R/G202R and WT fibroblasts (5000 cells) were seeded overnight on 10 mm glass coverslips in 24-well plates. GD fibroblasts were then incubated in the absence or presence of the tested compounds: (+)-**11b** (100 nM), **9b** (2 μM) **9e** (300 nM) and (+)-**10** (300 nM) for 6 days. WT and untreated GD fibroblasts were incubated with 0.1% DMSO as a vehicle. Culture media was replaced every 3 days with fresh media supplemented with the compounds (or DMSO). After 6 days of treatment, the cells were incubated for 1 h with 75 nM LysoTracker® Red DND-99 (L7528; Invitrogen) at 37 °C. Then, cells were washed twice with PBS and fixed for 10 min with 4% paraformaldehyde at room temperature. The cover slips were then washed 4 times with 0.3 M glycine/PBS and the cells were treated with 0.1% Tween in PBS. Cells were then incubated with the primary antibody (mouse monoclonal anti-GCase 8E4, diluted 1 : 100)[62] in a solution of PBS with 10% of normal donkey serum (NDS; Millipore, Billerica, MA) and 0.1% Tween at 4 °C overnight. The coverslips were then washed three times with 0.3 M glycine/PBS and incubated for 1 h with the secondary antibody Cy2-conjugated AffinPure donkey anti-mouse IgG (diluted 1 : 100; Jackson Immuno Research Laboratories, Inc.) followed by DAPI staining (1 : 10 000 dilution in a solution of PBS with 10% NDS and 0.1% Tween for 10 min; Invitrogen). Staining was viewed with a Leica TCS-SP2, and the images were analyzed using Fiji-Image J software. Co-localization between lysosome and GCase was quantified using Mander's coefficients with the "JACoP" plug-in of the Image J software.

Endo-H treatment

Sample of cell lysates, containing 18 μg of total protein, were subjected to an overnight incubation with endoglycosidase H (*endo*-H) digestion (New England BioLab) at 37 °C overnight, according to the manufacturer's instructions.

SDS-PAGE and western blotting

G202R/G202R fibroblasts, grown on 60 mm diameter plates, were treated with the compound (+)-**11b** (100 nM) or DMSO (untreated cells) during 6 days. Fibroblasts from healthy individuals were used as a reference. Cell monolayers were washed twice with PBS 1× and lysed at 4 °C in TGH buffer (1% Triton X-100, 10% glycerol, 50 mM Hepes, pH 7.2, 200 mM NaCl,). Lysates were clarified by centrifugation (13 000 rpm, 10 min) and protein concentration was determined by the Lowry protein assay. The same amount of protein from each sample were electrophoresed through 10% SDS–PAGE and transferred onto Immobilon-P transfer membrane (Millipore, Billerica, MA, USA). Membranes were blocked in 5% non-fat milk in PBS and Tween 20 (PBS-T) for 1 h at room temperature and

then incubated with the primary antibodies, anti-GCase and anti-Erk (Sigma) at 4 °C overnight. The membranes were washed three times for 10 minutes in PBS-T and incubated with corresponding secondary antibodies, conjugated to horseradish peroxidase (Sigma). Anti-rabbit (or anti-mouse) immunoglobulin G (IgG) in 5% non-fat milk in PBS-T for 1 h at room temperature, followed by 3 × 10 min washings. Immuno reactivity was detected by enhanced chemiluminescence Western blotting detection reagents (Amersham ECL Plus; GE Healthcare Life Sciences, Piscataway, NJ, USA). The Western blot quantitation was performed using Image J software (http://rsb.info.nih.gov/ij/download.html; National Institutes of Health, Bethesda, MD, USA).

Sphingolipid analysis by UPLC/MS

Fibroblasts from GD patients with G202R/G202R genotype (100 000 cells) were plated into 12-well assay plates during 6 days in D-MEM, 10% FBS at 37 °C under 5% CO_2 with **9c** (100 nM), **9b** (100 nM), **9e** (100 nM), (+)-**10** (100 nM), (+)-**13** (1 nM), (+)-**11c** (10 nM). DMSO (0.1%) was used as a vehicle control for untreated cells. Fibroblasts from healthy individuals were used as reference. Culture media was replaced every 3 days with fresh media supplemented with the corresponding compound in DMSO. Following 6 days of treatment, and after a 24 h washout period, cells were cultured for additional 24 h in the presence of ωN_3Sph (5 μM). After PBS washings, the cells were trypsinized, counted and transferred to glass vials. Sphingolipid extracts were prepared and analysed by liquid chromatography–mass spectrometry using a Waters Aquity UPLC system connected to a Waters LCT Premier orthogonal accelerated time of flight mass spectrometer (Waters, Millford, MA), operating in positive electrospray ionization mode in the conditions reported previously.[63] Identification of tagged sphingolipids was based on accurate mass measurement (<5 ppm error) and LC relative retention time compared to ωN3Cer-C16 standard. Quantification was carried out with the extracted ion chromatogram of each compound, using 50 mDa windows.

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