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ARTICLE

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Amphiphilic 1-Deoxynojirimycin Derivatives through Click Strategies for Chemical Chaperoning in N370S Gaucher Cells

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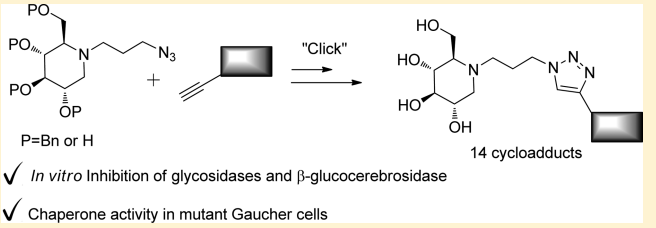
Supporting Information

ABSTRACT: In Gaucher disease (GD), mutant β-glucocerebrosidases (β-GCase) that are misfolded are recognized by the quality control machinery of the endoplasmic reticulum (ER) and degraded proteolytically. Hydrophobic iminosugars can be used as pharmacological chaperones to provide an improvement in the folding of the enzyme and promote trafficking from the ER. We have developed here an efficient click procedure to tether hydrophobic substituents to *N*-azidopropyl-1-deoxynojirimycin. A set of 14 original iminosugars was designed and evaluated for inhibition of commercially available glucosidases. Most of the compounds were micromolar inhibitors of those enzymes. In vitro inhibition assays with the N370S β-GCase revealed that the sublibrary containing the derivatives with aromatic aglycons displayed the highest inhibitory potency. Chaperone activity of the whole set of synthetic compounds was also explored in mutant Gaucher cells. The most active compound gave a nearly 2-fold increase in enzyme activity at 20 μM, a significantly higher value than the 1.33-fold recorded for the reference compound *N*-nonyl-1-deoxynojirimycin (*N*-nonyl-DNJ). As previously reported with bicyclic sp^2-iminosugars (Luan, Z.; Higaki, K.; Aguilar-Moncayo, M.; Ninomiya, H.; Ohno, K.; García-Moreno, M. I.; Ortiz Mellet, C.; García Fernández, J. M.; Suzuki, Y. ChemBioChem 2009, 10, 2780), in vitro inhibition of β-GCase measured for the compounds did not correlate with the cellular chaperone activity. The potency of new iminosugar chaperones is therefore not predictable from structure−activity relationships studies based on the in vitro β-GCase inhibition.



INTRODUCTION

Carbohydrates containing nitrogen in the hemiacetal ring, the so-called "imino-sugars", have been extensively studied since the first synthetic examples were reported in the 1960s[1−3] and the concomitant isolation of sugar-mimic alkaloids in microorganisms.[4] The formal replacement of the endocyclic oxygen by a protonated nitrogen atom leads to transition-state analogues of glycosidases, forming strong ionic interactions with the two carboxylic acid units catalyzing the cleavage of glycosidic bonds. Iminosugars are of particular interest in view of their therapeutic potential in the treatment of carbohydrate-mediated diseases such as viral infections,[5,6] tumor metastasis,[7] diabetes, and lysosomal storage diseases. Several synthetic methodologies have been developed to improve the potency of the naturally occurring classes of iminosugars such as indolizidines, pyrrolidines, or piperidines.[8] Research opportunities remain, and abundant chemical approaches to generate novel structures such as iminosugar *C*-glycosides,[9] seven-membered ring iminoalditols,[10] constrained bicyclic glycomimetics,[11] and multivalent deoxynojirimycin analogues[12,13] have been recently reported. In particular, efforts in the chemical synthesis of the piperidinic imino-sugars have been rewarded by the launch of Miglitol (Glyset) and *N*-butyl-1-deoxynojirimycin (Zavesca), respectively, approved for the treatment of type II diabetes and type 1 Gaucher disease (GD).[14,15]

GD is the most common of the glycosphingolipidoses, a group of inherited diseases caused by defects in the lysosomal degradation of glycosphingolipids. In GD, progressive accumulation of glucosylceramide in macrophages occurs because of a deficient activity of β-GCase, an enzyme which hydrolyzes glucosylceramide into ceramide and glucose. The most frequent mutation,

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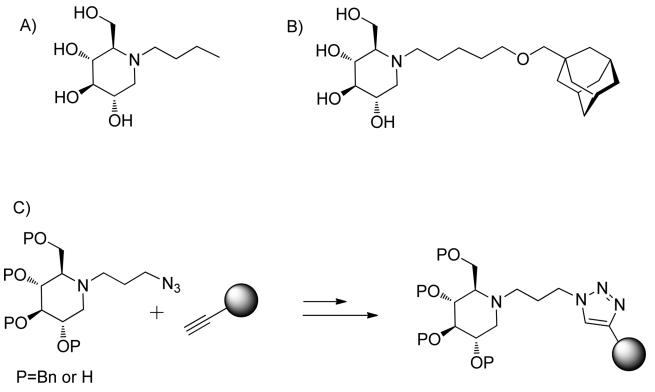
Chart 1. (A) *N*-Butyl-DNJ (Zavesca); (B) Potent GCS Inhibitor Designed by Overkleeft and Co-workers; (C) Click Chemical Strategy Adopted for the Functionalization of *N*-Azidopropyl-DNJ with a Set of Hydrophobic Substituents (in Gray)

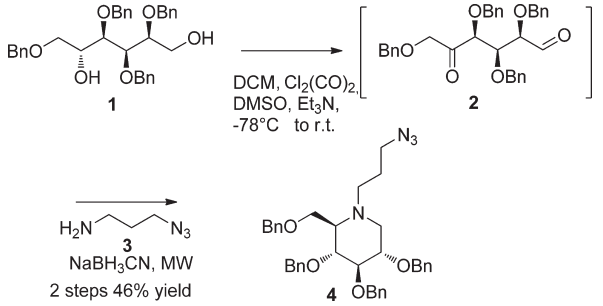


N370S β-GCase, is particularly prevalent in the Ashkenazi Jewish population. For type 1 GD, which does not affect the nervous central system, two treatments are currently available. Enzyme replacement therapy (ERT) is an effective treatment, although it is costly and ineffective for the neuronopathic form of GD, as the β-GCase fails to cross the blood−brain barrier. Alternatively, substrate reduction therapy (SRT) is aimed at inhibiting glycolipid biosynthesis to balance the deficient activity of β-GCase. *N*-Butyl-1-deoxynojirimycin (*N*-Bu-DNJ) was identified as a potent inhibitor of the glucosyltransferase-catalyzed biosynthesis of glucosylceramide and was shown to prevent lysosomal glycolipid storage,[16] allowing novel oral treatment for non-neuronopathic Gaucher's disease.[17] Potent glucosylceramide synthase (GCS) inhibitors have emerged after the registered success of *N*-Bu-DNJ marketed as Zavesca (Chart 1).[17] Aerts and co-workers have shown that DNJ derivatives bearing the hydrophobic adamantyl are submicromolar inhibitors of GCS using an in vitro assay.[18] Since structural information on the GCS enzyme is lacking, the search for new inhibitors proceeds through structure−activity relationship studies. Successful strategies consist of varying the nature[18] or the position[19] of the bulky substituent in the *N*-alkyl series or the synthesis of stereoisomer analogues of DNJ.[20,21]

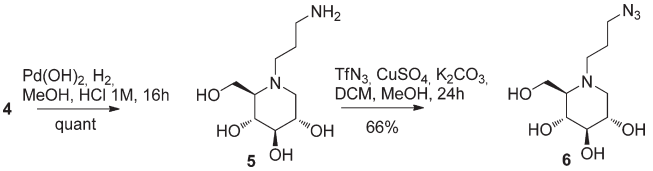
Chaperone-mediated therapy (CMT) has recently emerged as an appealing strategy for the treatment of lysosomal storage disorders. Mutant β-GCases that are misfolded are recognized by the quality control machinery of the endoplasmic reticulum (ER) and proteolytically degraded. Iminosugars can be used as pharmacological chaperones that bind to the active site of the enzyme to assist its folding and proper trafficking from the ER. In particular, iminosugar chaperones bearing hydrophobic groups have been shown to stabilize N370S β-GCase, the most common mutation causing Gaucher disease.[22] Considering the high potential of iminosugars for SRT and chaperone-mediated therapy (CMT) in a wide range of lysosomal disorders,[23] more potent and selective inhibitors are still required, and we are witnessing a rebirth of this field of research.

We present here an original strategy to develop a set of iminosugars bearing hydrophobic substituent. Considering the usefulness of the copper catalyzed azide−alkyne cycloaddition[24] (CuAAC) for the synthesis of sugar mimetics,[12,13,25−29] we

Scheme 1. Synthesis of Protected *N*-Alkyl-DNJ Derivative 4



Scheme 2. Synthesis of *N*-Azidopropyl-DNJ 6



implemented this reaction to connect alkynyl-armed substituents to an azido-functionalized *N*-propyl-DNJ core (Chart 1). The formed triazole should not impair the binding with β-GCase as recently observed with *N*-substituted aminocyclitols.[26] 1,2,3-Triazoles are interesting pharmacophores in view of their topological and electronic similarities with amide bonds and their inertness toward in vivo oxidation and reduction processes.[30] We first assessed the solution-phase binding affinities of the synthetic iminosugars for a set of commercial glucosidases (glu). Then, we specifically compared β-GCase inhibition profiles for the set of iminosugars with their potency to act as chaperones and increase the enzyme activity in Gaucher cells.

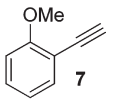
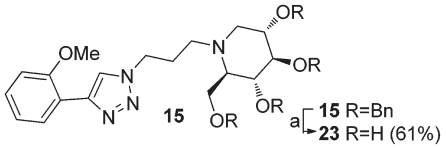
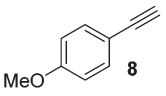
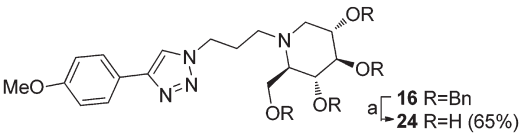
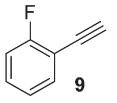
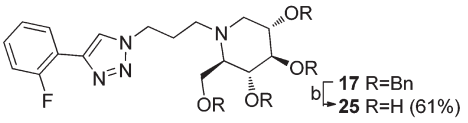
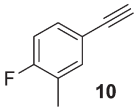
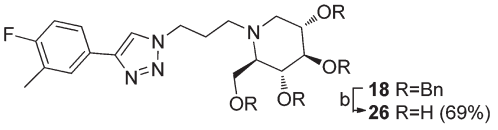
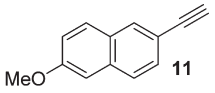
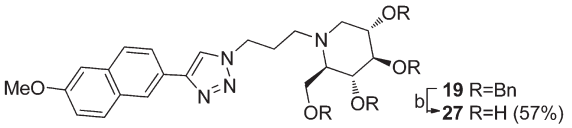
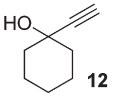
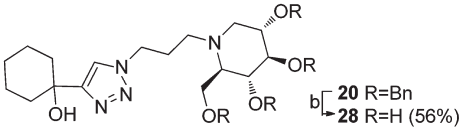
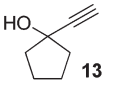
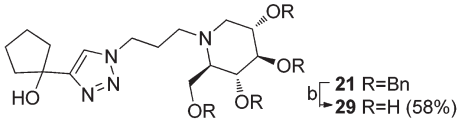
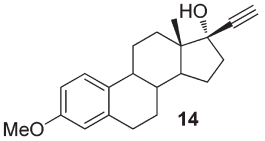
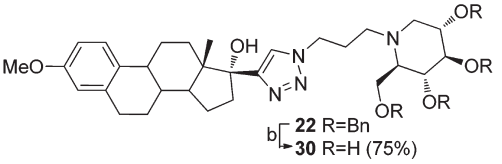
RESULTS AND DISCUSSION

Synthesis. We first synthesized the imino sugar core **4** using a procedure that we implemented for the synthesis of multivalent iminosugars.[12] Diol **1**, obtained from methyl α-D-glucopyranoside in five steps,[31−33] was converted in **2** using Swern conditions (Scheme 1).[34,35] A microwave-assisted double reductive amination of crude **2** in the presence of 3-azidopropylamine **3**[36,37] led exclusively to the expected diastereoisomer **4**.

Removal of the benzyl protective groups after CuAAC should be problematic for compounds bearing substituents sensitive to hydrogenolysis. With this concern, we also decided to synthesize unprotected azido derivative **6** as a specific substrate (Scheme 2). Attempts to selectively remove benzyl groups by the use of BCl_3 were unsuccessful and led to a mixture of products. To achieve this goal, Pearlman's catalyst was used in acidic media to avoid poisoning of the palladium by the amino-groups.[38] The azido group was regenerated with a copper-catalyzed diazo transfer reaction with triflyl azide, a procedure previously reported by Wong and co-workers on glycosamines.[39]

CuAAC were first performed with **4** and a set of commercially available hydrophobic alkynes **7**−**14** (Table 1). The highest yields were obtained in a mixture of dioxane−water (4:1) with copper sulfate and sodium ascorbate as catalyst system. Microwave irradiation was previously described to significantly

Table 1. Chemical Structure of the Cycloadducts Obtained from Commercial Alkynes

Entry	Azide	Alkyne	Cycloadduct	Yield[c]
1	**4**	 **7**	 **15** a→ **15** R=Bn, **23** R=H (61%)	65%
2	**4**	 **8**	 a→ **16** R=Bn, **24** R=H (65%)	69%
3	**4**	 **9**	 b→ **17** R=Bn, **25** R=H (61%)	65%
4	**4**	 **10**	 b→ **18** R=Bn, **26** R=H (69%)	67%
5	**4**	 **11**	 b→ **19** R=Bn, **27** R=H (57%)	62%
6	**4**	 **12**	 b→ **20** R=Bn, **28** R=H (56%)	94%
7	**4**	 **13**	 b→ **21** R=Bn, **29** R=H (58%)	71%
8	**4**	 **14**	 b→ **22** R=Bn, **30** R=H (75%)	66%

[a] $Pd(OH)_2$, H_2, MeOH, HCl, rt. [b] $Pd(OH)_2$, H_2, MeOH, 65 °C. [c] Yields reported for the cyclization step after purification on silica gel or HPLC.

decrease the reaction times in CuAAC.[40] This method allowed a fast and exclusive formation of the expected 1,4-disubstituted 1,2,3-triazoles **15**−**22**, which were identified by the large $\Delta(\delta_{C4} - \delta_{C5})$ values (~20 ppm) observed by ^{13}C NMR spectroscopy for the cycloadducts.[41] We then performed a catalytic hydrogenolysis of the benzyl groups in aqueous media. Although these conditions allowed us to isolate **23** and **24** with satisfactory yields of 61 and 65% after HPLC purification, respectively, moderate yields were obtained with the other cycloadducts, and we observed a dehydration of the free hydroxyl groups in **20** and **21**. Compounds **15**−**22** were successfully deprotected at 65 °C with $Pd(OH)_2$ in methanol[42] leading to **23**−**30** with yields ranging from 56 to 75% after purification.

In order to increase the chemical diversity of our library of iminosugars, we have functionalized a set of commercial hydrophobic alcohols with propargyl bromide. The resulting compounds **31**−**34** and protected mannoside **35**[43] were subsequently subjected to CuAAc with **4** or when the substituents were sensitive to the subsequent hydrogenolysis with **6** (Table 2). As an illustrative example, attempts to selectively deprotect the benzyl groups of **36** led to the simultaneous removal of the two chlorine atoms in 75% yield. After deprotection,

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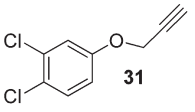
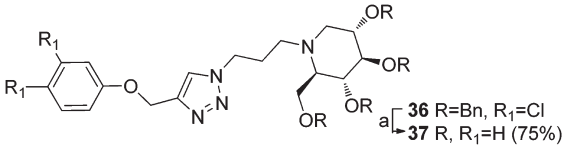
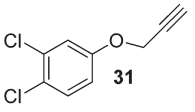
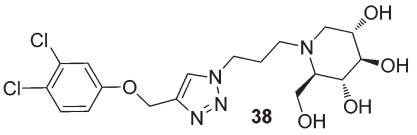
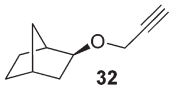
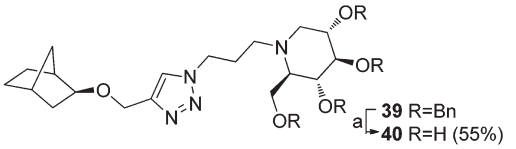
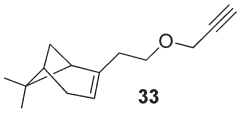
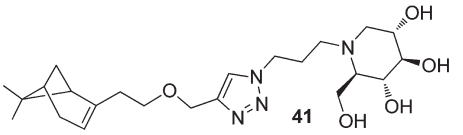
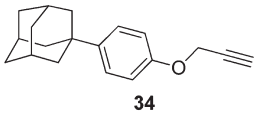
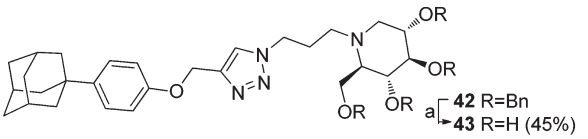
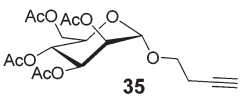
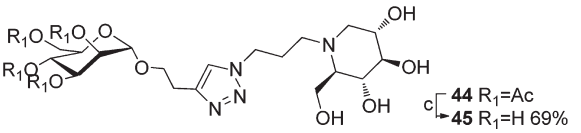
Table 2. Chemical Structure of the Cycloadducts Obtained from Alkynyl-Armed Synthetic Intermediates 31−35

Entry	Azide	Alkyne	Cycloadduct	Yield[d]
1	**4**	 **31**	 a → **36** R=Bn, R_1=Cl; **37** R, R_1=H (75%)	64%
2	**6**	 **31**	 **38**	59%
3	**4**	 **32**	 a → **39** R=Bn; **40** R=H (55%)	65%
4	**6**	 **33**	 **41**	60%
5	**4**	 **34**	 a → **42** R=Bn; **43** R=H (45%)	74%
6	**6**	 **35**	 c → **44** R_1=Ac; **45** R_1=H 69%	60%

[a] $Pd(OH)_2$, H_2, MeOH, HCl, rt. [b] $Pd(OH)_2$, H_2, MeOH, 65 °C. [c] MeONa, MeOH. [d] Yields reported for the cyclization step after purification on silica gel or by HPLC for **38**.

compounds **37**, **40**, **43**, and **45** were purified by preparative HPLC on a C18 column.

Glycosidases Inhibition. To test the selectivity profile, the new 1-DNJ derivatives (except **30** for solubility reasons) were first tested as inhibitors against a series of commercial glycosidases including α-glucosidase (yeast), β-glucosidase (almonds), β-glucosidase (bovine liver, cytosolic), and amyloglucosidase (*Aspergillus niger*). The corresponding inhibition constants (K_i) are collected in Table 3. Inhibition data for DNJ have been also included for comparative purposes. Weak or no inhibition of the yeast α-glucosidase was observed in all cases (excepting for **41** showing K_i = 20 μM). The values of K_i range from 9 to 669 μM for the β-glucosidase from bovine liver, 0.59 to 177 μM for almond β-glucosidase, 20 μM to >2 mM for baker's yeast α-glucosidase, and 1 to >2 mM for amyloglucosidase. A strong influence of the nature of the exocyclic substituent on the potency of binding β-glucosidases was observed. Amphiphilic derivatives bearing hydrophobic *N*′-substituents were much more potent than derivatives bearing polar substituents (i.e., compound **45** having an α-D-mannopyranosyl residue).[44] β-Glucosidase from almond and amyloglucosidase were efficiently inhibited by the whole set of aromatic derivatives (**23**−**27**). Subtle chemical modifications on the position of substituents in the aromatic substituents for **23**/**24** and **25**/**26** or in the size of cyclic alcohols substituents (from cyclopentanol to cyclohexanol) for **29**/**28** do not significantly affect the binding affinity, except for the latter couple with β-glucosidase from bovine liver leading to worse results. Compound **38**, chemically very closed to **37**, is a much more potent inhibitor of this enzyme (K_i 9 μM vs 317 μM, respectively). Interestingly, both compounds **37** and **38** are potent inhibitors of almond β-glucosidase, with **37** also being selective for this enzyme. With a binding constant of 590 nM for almond β-glucosidase, **43** displayed the lowest inhibitory value.

***β*-GCase Inhibition.** All compounds were initially screened for β-GCase inhibition at 100 μM. *N*-Nonyl-1-deoxynojirimycin (*N*-nonyl-DNJ), a potent chemical chaperone of N370S β-GCase, was included as a reference (Figure 1).[38] The whole set of compounds bearing aromatic aglycons **23**−**26**, **37**, **38**, **43** displayed strong inhibition of β-GCase, in the same order as *N*-nonyl-DNJ.

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Table 3. Glycosidase Inhibitory Activities (K_i, μM) for 1-DNJ and the Synthetic Derivative Inhibitors 5, 6, 23−29, 37, 38, 40−43, and 45 against Commercial Glycosidases[a]

compd	β-glucosidase (bovine liver)	β-glucosidase (almond)	α-glucosidase (baker's yeast)	amyloglucosidase (*Aspergillus niger*)
DNJ	42	47	25	2.1
5	623	177	872	81
6	256	58	n.i.	9.2
23	167	4.5	506	20
24	158	3.5	409	11
25	151	2.8	n.i.	13
26	66	3.6	255	9.9
27	27	1.9	28	1
28	669	88	n.i.	43
29	177	55	n.i.	41
37	317	3	684	20
38	9	1	57	16
40	390	5.1	n.i.	25
41	23	3.2	20	18
43	14	0.59	n.i.	6.9
45	242	124	n.i.	38

[a] Inhibition was competitive in all cases. n.i.: no inhibition was observed at 2 mM concentration of the inhibitor.

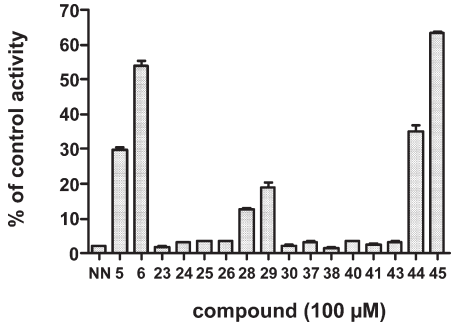

Figure 1. β-GCase inhibition at 100 μM. Compounds were assayed at 100 and 5 μM using purified enzyme and 0.5 mM 4-MU-β-glucoside as described in the text. Triplicate determinations were made, and the mean and standard deviations are shown as a percentage of control enzyme activity. NN: *N*-nonyl-DNJ.

Variation in the nature of the aromatic substituents exerted relatively few effects. A significant drop in the inhibitory potency was observed for compounds lacking "clicked" appends (**5**, **6**). Thus, the introduction of substituents is in general beneficial, and a large variety of chemical structures can be used to improve affinity. The more hydrophilic derivatives **28** and **29** with free hydroxyl groups are less potent inhibitors than the set of aromatic ones. Additionally, unprotected hydrophilic mannoside **45** is significantly less potent than the protected synthetic intermediate **44**. It seems, therefore, that the hydrophilic behavior, rather than the nature and topology of the substituents, has more influence on the binding affinity with β-GCase. These results are in accordance with recent observations made with hydrophilic aminocyclitols[45] and with bicyclic isoureas and guanidines.[46] The 10 most potent inhibitors **24**−**26**, **30**, **37**, **38**, **40**, and **41** were then evaluated for their inhibitory effect at the lower

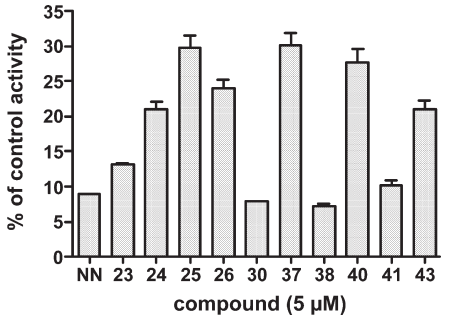

Figure 2. β-GCase inhibition at 5 μM. Compounds were assayed at 100 and 5 μM using purified enzyme and 0.5 mM 4-MU-β-glucoside as described in the text. Triplicate determinations were made, and the mean and standard deviations are shown as a percentage of control enzyme activity. NN: *N*-nonyl-DNJ.

Table 4. β-GCase Activity in Mutant Gaucher Cells

	fold increase in β-GCase activity				
compd (μM)	0.1	1	10	20	β-GCase inhibition
NN[a]	1.2	1.36	1.4	1.33	IC_{50} = <5 μM
5	1.09	1.18	1.51	1.39	IC_{50} = >50 μM
6	0.97	1.05	1.13	1	IC_{50} = >100 μM
15	0.99	1.15	1.27	1.21	IC_{50} = <5 μM
23	1.26	1.39	1.25	1.45	IC_{50} = <5 μM
24	0.99	1.11	1.17	0.95	IC_{50} = <5 μM
25	1.15	1.18	1.2	1.28	IC_{50} = <5 μM
26	1.09	1.06	1.17	1.23	IC_{50} = <5 μM
28	1	1.04	0.95	0.97	IC_{50} = <50 μM
29	1.26	1.32	1.12	0.91	IC_{50} = <50 μM
30	0.81	0.87	1	0.94	IC_{50} = $\ll$5 μM
37	1.2	1.18	1.59	1.94	IC_{50} = <5 μM
38	0.85	0.94	1.21	1.37	IC_{50} = $\ll$5 μM
41	0.74	0.87	0.98	0.89	IC_{50} = $\ll$5 μM
43	0.71	0.72	0.36	0.28	IC_{50} = <5 μM
44	0.78	0.82	0.8	1.02	IC_{50} = ~50 μM
45	nd	nd	nd	nd	IC_{50} = >50 μM

[a] NN: *N*-nonyl-DNJ.

concentration of 5 μM (Figure 2). The selected derivatives retained their faculty to inhibit the enzyme. Compounds **23**, **30**, **38**, and **41** and *N*-nonyl-DNJ were able to decrease the β-GCase activity to less than 13% of control activity.

Ability To Chaperone β-GCase. Chaperone activity of the set of compounds in mutant Gaucher lymphoblasts with the most common N370S mutation[23] is reported in Table 4. These data show the fold increase in β-GCase activity compared to untreated cells. The IC_{50} of the compounds for the tested enzyme are also reported. At the highest concentration reported in activation assays with N370S Gaucher lymphoblasts, no cytotoxicity was observed as measured using a MTS assay.[47] The structurally related compounds **23** and **24** are both good inhibitors of β-GCase. However, only **23** was active at elevating mutant enzyme in cells. With almost a 2-fold increased activity at 20 μM, compound **37** was the most active compound but was not a better inhibitor than **23** or **24**. Compound **30** was one of the three most potent inhibitors of β-GCase in vitro but did not

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enhance enzyme activity in Gaucher cells. Compound **5**, a weak inhibitor, of β-GCase in vitro was a surprisingly good chaperone for mutant enzyme at 10 μM. Altogether, these results suggests that the best inhibitors were not necessary the best enzyme chaperones. The addition of an adamantlyl group to **37** (leading to **43**) did not significantly modulate inhibition of βGCase in vitro. However, a dramatic change in the inhibitory profile was observed in the cellular activity of mutant βGCase, **37** being the best chaperone of the series and **43** the most potent inhibitor. Therefore, in vitro inhibition of βGCase did not always correlate with the increase in mutant enzyme activity in Gaucher cells.

CONCLUSION

In summary, we have developed an efficient synthetic methodology relying on copper-mediated 1,3-dipolar cycloaddition for the design of iminosugars bearing hydrophobic aglycons. The procedure allowed the rapid obtention of a large set of compounds from a common intermediate. The "click iminosugars" were micromolar inhibitors of commercial β-glucosidase and amyloglucosidase and displayed a significant selectivity for these enzymes toward α-glucosidase. Compounds **30**, **38**, and **41** were particularly potent in vitro inhibitors of β-GCase with inhibitory activity in the same range as the reference compound *N*-nonyl-DNJ. The nearly 2-fold increase of β-GCase activity in mutant Gaucher cells at 20 μM for **37** was significantly higher than the reference compound *N*-nonyl-DNJ. This chaperoning effect is promising considering that a modest increase in activity may be clinically useful.[48] Interestingly, a discrepancy between the in vitro inhibition of β-GCase and the chaperone activity was observed. In vitro β-GCase inhibition results should therefore be interpreted with caution and not directly translated into ability for chaperoning the enzyme.

EXPERIMENTAL METHODS

General Chemical Reagents and Methods. All purchased materials were used without further purification. Dichloromethane was distilled from calcium hydride and tetrahydrofuran over sodium and benzophenone. Flash chromatography was performed on silica (0.040−0.060 mm pore size) using distilled solvents. Microwave irradiation experiments were performed using a CEM-Discover system in an open vessel. The temperature profiles were recorded using an infrared probe. NMR chemical shifts are reported in parts per million relative to tetramethylsilane or a residual solvent peak ($CHCl_3$: ^{1}H: δ = 7.26 ppm, ^{13}C: δ = 77.2 ppm). Assignments of ^{1}H and ^{13}C were assisted by 2D ^{1}H COSY and 2D ^{1}H−^{13}C CORR experiments. Optical rotations were measured at 20 °C in a 1 cm cell in the stated solvent; $[\alpha]_D$ values are given in 10^{-1} deg·cm^2 g^{-1} (concentration *c* given as g/100 mL). HPLC samples were purified on a preparative C18 (2.2 × 25 cm) or an NH_2 5 μM column using a PL-ELS 1000 photodiode array detector. Conditions 1 (C18 column): The mobile phase was H_2O (solvent A) and MeOH (solvent B). The gradient consisted of 100% A for 5 min then 100% A to 100% B in 35 min (22 mL/min flow rate). Conditions 2 (C18 column): The gradient consisted of 100% A for 1 min then 100% A to 100% B in 39 min (22 mL/min flow rate). Conditions 3 (NH_2 column): The gradient consisted of 100% A to 100% B in 40 min (22 mL/min flow rate). Conditions 4 (NH_2 column): the mobile phase was H_2O (solvent A) and CH_3CN. The gradient consisted of 10% A to 50% A in 50 min (22 mL/min flow rate). All of the HPLC samples were controlled by NMR and showed >95% purity.

***N*-(3-Aminopropyl)-1,5-dideoxy-1,5-imino-D-glucitol (5).** Conventional catalytic hydrogenation of compound **4** (200 mg, 0.330 mmol) was carried out with $Pd(OH)_2$ (220 mg) in MeOH−1 M HCl (4 mL, 1:1) at 1 atm for 16 h. The catalyst was filtered over a pad of Celite, and the solvent was removed to give **5** quantitatively as a colorless solid: $[\alpha]^{20}_D$ = +3 (*c* = 1, H_2O); ^{1}H NMR (300 MHz, D_2O) δ = 4.14 (1 H, dd, $J_{6a,6b}$ = 13.1 Hz, $J_{5,6a}$ = 1.9 Hz, H-6a), 4.02 (1 H, dd, $J_{5,6b}$ = 2.4 Hz, H-6b), 3.86 (1 H, ddd, *J* = 11.2, *J* 9.5 Hz, $J_{1a,2}$ = 5.0 Hz, H-2), 3.70 (1 H, t, $J_{4,5}$ = $J_{5,6}$ = 9.5 Hz, H-5), 3,63 (1 H, dd, $J_{1a,1b}$ = 12.2 Hz, H-1a), 3.55 (2 H, m, H-3, H-1′a), 3.43−3.29 (2H, m, H-4, H-1′b), 3.17 (3 H, m, H-1b, 2 × H-3′), 2.29−2.15 (2 H, m, 2 × H-2′); ^{13}C NMR (75 MHz, D_2O) δ = 75.7 (C-3), 67.0 (C-5), 65.9 (C-2), 65.8 (C-4), 53.8 (C-6), 53.1 (C-1), 49.8 (C-1′), 36.6 (C-3′), 21.2 (C-2′); HRMS (ES+) found 221.1499, $C_9H_{21}N_2O_4$ requires 221.1501.

***N*-(3-Azidopropyl)-1.5-dideoxy-1,5-imino-D-glucitol (6).** *Preparation of TfN_3 Dolution.* To a solution of sodium azide (289 mg, 4.45 mmol) in H_2O (0.5 mL) and DCM (1.5 mL), cooled to 0 °C, was added Tf_2O (148 μL, 0.98 mmol) dropwise. The mixture was stirred at 0 °C for 2 h. The organic layer was separated and the aqueous layer extracted with DCM (2 × 1.5 mL). The organic layers were combined and washed with saturated $NaHCO_3$ (4 mL). The organic solution containing TfN_3 was separated and used without further purification.

Diazo-Transfer Reaction. To a solution of **5** (100 mg, 0.454 mmol) in H_2O (1.5 mL) were added K_2CO_3 (94 mg, 0.681 mmol), copper sulfate (1 mg, 6.26 μmol), MeOH (15 mL), and the whole TfN_3 solution. (Caution! TfN_3 is explosive when not in solution.) The mixture was stirred at rt for 24 h and quenched with glycine (350 mg) for 5 h. The solvents were evaporated under reduced pressure, and the residue was purified by preparative HPLC (conditions 1) to give **6** (73 mg, 66%) as a white hygroscopic solid: $[\alpha]_D$ = +7 (*c* = 0.1, MeOH); t_R = 19.7 min; ^{1}H NMR (500 MHz, D_2O) δ = 3.94−3.81 (2 H, ddd, $J_{6a,6b}$ = $J_{6a,5}$ = 12.8 Hz, $J_{6b,5}$ 1.8 Hz, 2 × H-6), 3.55 (1 H, dt, $J_{1b,2}$ = $J_{2,3}$ = 9.5 Hz, $J_{1a,2}$ = 4.7 Hz, H-2), 3.41−3.35 (3 H, m, H-4, 2 × H-3′), 3.26 (1 H, t, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 3.02 (1H, dd, $J_{1a,1b}$ = 11.3 Hz, H-1a), 2.89−2.68 (2 H, m, 2 × H-1′), 2.36−2.26 (2 H, m, H-1b, H-5), 2.83−2.78 (2 H, m, 2 × H-2′); ^{13}C NMR (75 MHz, D_2O) δ = 78.3 (C-3), 70.0 (C-4), 68.8 (C-2), 64.9 (C-5), 57.5 (C-6), 55.3 (C-1), 49.4 (C-3′), 49.2 (C-1′), 22.5 (C-2′); HRMS (ES+) found 247.1417, $C_9H_{19}N_4O_4$ requires 247.1406.

General Procedure for the Preparation of Compounds 15−22, 36, 39, 42, and 44 by CuAAC. To a solution of the azido derivative **4** (80 mg, 0.132 mmol) and the alkyne **7** (19 mg, 0.145 mmol) in dioxane−H_2O (5 mL, 4:1) were added $CuSO_4$ (32 mg, 0.198 mmol) and sodium ascorbate (78 mg, 0.396 mmol), and the mixture was stirred under microwave irradiation at 80 °C for 45 min. The mixture was diluted with EtOAc (10 mL) and washed with NH_4Cl satd solution (2 × 8 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a silica gel column with 4:1 EtOAc−cyclohexane as eluent to afford **15** (63 mg, 65%).

***N*-(3-(4-(2-Methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)propyl)-2,3,4,6-tetra-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol (15):** $[\alpha]_D$ = +8 (*c* = 0.5, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.39 (1 H, dd, *J* = 7.7, 1.6 Hz, Har), 8.00 (1 H, s, Har), 7.36−7.08 (22 H, m, 21 × Har, C=CHN), 6.98 (1 H, d, *J* = 8.2 Hz, Har), 4.98−4.80 (3 H, 3 d, *J* =11.0, 10.8, 11.0 Hz, CH_2Ph, C*H*HPh), 4.67−4.60 (2 H, 2 d, *J* = 11.6, 11.4 Hz, CH_2Ph), 4.44−4.28 (5 H, m, CH_2Ph, CH*H*Ph, 2 × H-3′), 3.93 (3 H, s, CH_3), 3.67−3.48 (5 H, m, H-2, H-3, H-4, 2 × H-6), 3.03 (1 H, dd, $J_{1a,1b}$ = 11.0 Hz, $J_{1a,2}$ = 4.7 Hz, H-1a), 2.88−2.78 (1 H, m, H-1′a), 2.64−2.56 (1 H, m, H-1′b), 2.34 (1 H, d, $J_{5,4}$ = $J_{5,6}$ 8.9 Hz, H-5), 2.18 (1 H, t, $J_{1a,1b}$ = $J_{1b,2}$ = 11.0 Hz, H-1b), 2.06 (2 H, m, *J* = 7.0 Hz, 2 × H-2′); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 155.7 (CH_3OCar), 143.1 (NC=CH), 139.0, 138.5, 137.8 (Car), 129.0, 128.5, 128.4, 128.0, 127.8, 127.7, 127.6, 123.4 (CHar), 121.2 (NCH=C), 119.5 (NCH=CCar), 110.9 (CHar), 87.2 (C-3), 78.6, 78.4 (C-2, C-4), 75.4, 75.3, 73.4, 72.9 (CH_2Ph), 66.1 (C-6), 64.3 (C-5), 55.5 (CH_3), 54.6 (C-1), 49.3 (C-1′), 48.4 (C-3′), 25.8

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(C-2′); HRMS (ES+) found 761.3671, $C_{46}H_{50}N_4O_5Na$ requires 761.3679.

***N*-(3-(4-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)propyl)-2,3,4,6-tetra-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol (16):** $[\alpha]^{20}_D$ = +11 (*c* = 0.6, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.72 (2 H, d, *J* = 8.8 Hz, 2 × Har), 7.53 (1 H, s, C═CHN), 7.30−7.12 (20 H, m, 20 × Har), 6.90 (2 H, d, *J* = 8.8 Hz, 2 × Har), 4.96−4.78 (3 H, 3 d, *J* = 11.0, 10.8, 11.0 Hz, CH_2Ph, C*H*HPh), 4.65−4.59 (2 H, 2 d, *J* = 11.2, 11.1 Hz, CH_2Ph), 4.43−4.19 (5 H, m, CH_2Ph, CH*H*Ph, 2 × H-3′), 3.82 (1 H, s, CH_3), 3.66−3.44 (5 H, m, H-2, H-3, H-4, 2 × H-6), 3.00 (1 H, dd, $J_{1a,1b}$ = 11.1 Hz, $J_{1a,2}$ = 4.4 Hz, H-1a), 2.86−2.75 (1 H, m, H-1′a), 2.54−2.50 (1 H, m, H-1′b), 2.33 (1 H, d, $J_{4,5} = J_{5,6}$ = 8.3 Hz, H-5), 2.15 (1 H, t, $J_{1a,1b} = J_{1b,2}$ = 11.1 Hz, H-1b), 2.01 (2 H, quint, *J* = 7.0 Hz, 2 × H-2′); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 159.6 (Car), 147.5 (*N*C═CH), 139.0, 138.5, 137.8 (Car), 128.5, 128.4, 128.3, 127.9, 127.8, 127.7, 127.6, 127.0 (CHar), 123.5 (NCH═C*C*ar), 119.2 (N*C*H═C), 114.3 (2 × CHar), 87.1 (C-3), 78.6, 78.4 (C-2, C-4), 75.4, 75.3, 73.3, 72.9 (CH_2Ph), 66.3 (C-6), 64.5 (C-5), 55.4 (OCH_3), 54.5 (C-1), 49.3 (C-1′), 48.5 (C-3′), 26.0 (C-2′); HRMS (ES+) found 739.3849, $C_{46}H_{51}N_4O_5$ requires 739.3859.

***N*-(3-(4-(2-Fluorophenyl)-1*H*-1,2,3-triazol-1-yl)propyl)-2,3,4,6-tetra-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol (17).** $[\alpha]_D$ = +13 (*c* = 0.5, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.61 (1 H, s, C═CHN), 7.57−7.50 (2 H, m, 2 × Har), 7.39−7.14 (21 H, m, 21 × Har), 7.02 (1 H, dt, *J* = 7.6, 2.6 Hz, Har), 4.97−4.79 (3 H, 3 d, *J* = 11.1, 10.9, 11.1 Hz, CH_2Ph, C*H*HPh), 4.70−4.60 (2 H, 2 d, *J* = 11.6 Hz, CH_2Ph), 4.47−4.27 (5 H, m, CH_2Ph, CH*H*Ph, 2 × H-3′), 3.67 (1 H, dd, $J_{6a,6b}$ = 10.5 Hz, $J_{6a,5}$ = 3.1 Hz, H-6a), 3.59−3.48 (4 H, m, H-2, H-3, H-4, H-6b), 3.01 (1 H, dd, $J_{1a,1b}$ = 11.0 Hz, $J_{1a,2}$ = 4.3 Hz, H-1a), 2.89−2.79 (1 H, m, H-1′a), 2.57−2.50 (1 H, m, H-1′b), 2.34 (1 H, d, $J_{4,5} = J_{5,6}$ = 8.4 Hz, H-5), 2.15 (1 H, t, $J_{1a,1b} = J_{1b,2}$ = 11.0 Hz, H-1b), 2.04 (2 H, m, *J* = 6.8 Hz, 2 × H-2′); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 163.3 (d, J_{C-F} = 243.7 Hz, FCar), 146.6 (NC═CH), 139.0, 138.5, 137.8 (Car), 130.6 (d, J_{C-F} = 8.2 Hz, CHar), 128.6, 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 127.6 (CHar), 121.4 (d, J_{C-F} = 2.6 Hz, CHar), 120.5 (NCH═C), 115.0 (d, J_{C-F} = 21.0 Hz, CHar), 112.7 (d, J_{C-F} = 23.3 Hz, CHar), 87.1 (C-3), 78.6, 78.4 (C-2, C-4), 75.4, 75.3, 73.4, 73.0 (CH_2Ph), 66.5 (C-6), 64.6 (C-5), 54.5 (C-1), 49.3 (C-1′), 48.7 (C3′), 26.2 (C-2′); HRMS (ESI+) found 749.3449, $C_{45}H_{47}N_4O_4FNa$ requires 749.3479.

***N*-(3-(4-(3-Fluoro-2-methylphenyl)-1*H*-1,2,3-triazol-1-yl)-propyl)-2,3,4,6-tetra-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol (18):** $[\alpha]_D$ = +7 (*c* = 0.3, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.66 (1 H, d, *J* = 8.3 Hz, Har), 7.58−7.52 (2 H, m, C═CHN, Har), 7.32−7.13 (20 H, m, 20 × Har), 7.04 (1 H, t, *J* = 8.7 Hz, Har), 4.98−4.83 (3 H, 3 d, *J* = 11.0, 10.8, 11.0 Hz, CH_2Ph, C*H*HPh), 4.70−4.60 (2 H, 2 d, *J* = 11.6, 11.6 Hz, CH_2Ph), 4.46−4.21 (5 H, m, CH_2Ph, CH*H*Ph, 2 × H-3′), 3.76−3.45 (5 H, m, H-2, H-3, H-4, 2 × H-6), 3.01 (1 H, dd, $J_{1a,1b}$ = 10.9 Hz, $J_{1a,2}$ = 4.3 Hz, H-1a), 2.89−2.80 (1 H, m, H-1′a), 2.63−2.51 (1 H, m, H-1′b), 2.36−2.32 (4 H, m, H-5, CH_3), 2.16 (1 H, t, $J_{1b,1a} = J_{1b,2}$ = 10.9 Hz, H-1b), 2.04 (2 H, m, *J* = 6.4 Hz, 2 × H-2′); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 161.3 (d, J_{C-F} = 244.5 Hz, Car), 147.0 (*N*C═CH), 139.0, 138.5, 137.8 (Car), 128.9, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6 (CHar), 124.7 (d, J_{C-F} = 7.5 Hz, CHar), 119.8 (NCH═C), 115.5 (d, J_{C-F} 22.5 Hz, CHar), 87.1 (C-3), 78.6, 78.4 (C-2, C-4), 75.4, 75.3, 73.4, 73.0 (CH_2Ph), 66.5 (C-6), 64.5 (C-5), 54.5 (C-1), 49.3 (C-1′), 48.6 (C-3′), 26.1 (C-2′), 14.6 (CH_3); HRMS (ESI+) found 741.3810, $C_{46}H_{50}N_4O_4F$ requires 741.3816.

***N*-(3-(4-(6-Methoxynaphtalen-2-yl)-1*H*-1,2,3-triazol-1-yl)-propyl)-2,3,4,6-tetra-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol (19):** $[\alpha]_D$ = +6 (*c* = 0.5, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.24 (1 H, s, Har), 7.87 (1 H, dd, *J* = .6 Hz, 1.7 Hz, Har), 7.81 (1 H, d, *J* = 4.8 Hz, Har), 7.78 (1 H, d, *J* = 5.0 Hz, Har), 7.71 (1 H, s, Har), 7.31−7.16 (22 H, m, 21 × Har, C═CHN), 4.97−4.79 (3 H, 3 d, *J* = 11.0, 10.8, 11.1 Hz, CH_2Ph, C*H*HPh), 4.66−4.58 (2 H, 2 d, *J* = 11.4, 11.6 Hz, CH_2Ph), 4.46−4.3 (5 H, m, CH_2Ph, CH*H*Ph, 2 × H-3′), 3.94 (3 H, s, CH_3), 3.69−3.57 (4 H, m, H-2, H-4, 2 × H-6), 3.51 (1 H, t, $J_{2,3} = J_{3,4}$ = 8.4 Hz, H-3), 3.02 (1 H, dd, $J_{1a,1b}$ = 11.0 Hz, $J_{1a,2}$ = 4.7 Hz, H-1a), 2.88−2.80 (1 H, m, H-1′a), 2.60−2.53 (1 H, m, H-1′b), 2.35 (1 H, d, $J_{4,5} = J_{5,6}$ = 8.7 Hz, H-5), 2.17 (1 H, t, $J_{1a,1b} = J_{1b,2}$ = 11.0 Hz, H-1b), 2.08 (2 H, m, *J* = 7.1 Hz, 2 × H-2′); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 158.0 (CH_3OC*a*r), 147.9 (N*C*═CH), 139.0, 138.5, 137.8, 134.4 (Car), 129.8 (CHar), 129.1 (Car), 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5 (CHar), 126.0 (Car), 124.5, 124.4, 120.0 (CHar), 119.4 (NCH═C), 105.9 (CHar), 87.2 (C-3), 78.6, 78.4 (C-2, C-4), 75.4, 75.3, 73.4, 72.9 (CH_2Ph), 66.4 (C-6), 64.5 (C-5), 55.4 (CH_3), 54.5 (C-1), 49.3 (C-1′), 48.6 (C-3′), 26.1 (C-2′); HRMS (ESI+) found 811.3826, $C_{50}H_{52}N_4O_5Na$ requires 811.3835.

***N*-(3-(4-(Cyclohexanol-1-yl)-1*H*-1,2,3-triazol-1-yl)propyl)-2,3,4,6-tetra-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol (20):** $[\alpha]_D$ = +12 (*c* = 0.6, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.33−7.14 (21 H, m, 20 × Har, C═CHN), 4.98−4.79 (3 H, 3 d, *J* = 11.0, 10.9, 11.1 Hz, CH_2Ph, C*H*HPh), 4.67−4.61 (2 H, 2 d, *J* = 11.3 Hz, 11.1 Hz, CH_2Ph), 4.46−4.41 (3 H, m, CH_2Ph, CH*H*Ph), 4.22−4.18 (2 H, m, 2 × H-3′), 3.68−3.53 (4 H, m, H-2, H-4, 2 × H-6), 3.50 (1 H, t, $J_{2,3} = J_{3,4}$ = 8.0 Hz, H-3), 3.01 (1 H, dd, $J_{1a,1b}$ = 10.8 Hz, $J_{1a,2}$ = 4.2 Hz, H-1a), 2.88−2.72 (1 H, m, H-1′a), 2.61−2.58 (1 H, m, H-1′b), 2.34 (1 H, d, $J_{4,5} = J_{5,6}$ = 8.3 Hz, H-5), 2.14 (1 H, t, $J_{1a,1b} = J_{1b,2}$ = 10.8 Hz, H-1b), 2.04−1.84 (4 H, m, 2 × H-2′, CH_2), 1.76−1.51 (5 H, m, 2 × CH_2, C*H*H), 1.37−1.27 (1 H, m, CH*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 155.5 (NC═CH), 138.9, 138.5, 137.8 (Car), 128.6, 128.5, 128.4, 128.1, 128.0, 127.8, 127.7, 127.6 (CHar), 120.1 (NCH═C), 87.1 (C-3), 78.5, 78.3 (C-2, C-4), 75.4, 75.3, 73.4, 72.9 (CH_2Ph), 69.6 (C), 66.4 (C-6), 64.4 (C-5), 54.3 (C-1), 49.4 (C-1′), 48.6 (C-3′), 38.3, 38.2 (CH_2), 25.9 (C-2′), 25.5 (CH_2), 22.1 (2 × CH_2); HRMS (ES+) found 731.4143, $C_{45}H_{55}N_4O_5$ requires 731.4172.

***N*-(3-(4-(Cyclopentanol-1-yl)-1*H*-1,2,3-triazol-1-yl)propyl)-2,3,4,6-tetra-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol (21):** $[\alpha]_D$ = +5 (*c* = 0.5, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.35−7.14 (21 H, m, 20 × Har, C═CHN), 4.97−4.79 (3 H, 3 d, *J* = 11.0 Hz, 10.8 Hz, 11.0 Hz, CH_2Ph, C*H*HPh), 4.70−4.60 (2 H, 2 d, *J* = 11.4 Hz, 11.6 Hz, CH_2Ph), 4.50−4.36 (3 H, m, CH_2Ph, CH*H*Ph), 4.21 (2 H, dt, *J* = 6.9 Hz, 6.8 Hz, 2 × H-3′), 3.68−3.46 (6 H, m, H-2, H-3, H-4, 2 × H-6), 3.00 (1 H, dd, $J_{1a,1b}$ = 11.1 Hz, $J_{1a,2}$ = 4.3 Hz, H-1a), 2.89−2.75 (1H, m, H-1′a), 2.56−2.43 (1 H, m, H-1′b), 2.32 (1 H, d, $J_{4,5} = J_{5,6}$ = 8.7 Hz, H-5), 2.15−1.90 (9 H, m, H-1b, 2 × H-2′, 3 × CH_2), 1.82 (2 H, m, CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 154.1 (NC═CH), 138.9, 138.5, 137.8 (Car), 128.6, 128.5, 128.5, 128.4, 128.1, 128.0, 127.8, 127.7, 127.6 (CHar), 120.3 (NCH═C), 87.2 (C-3), 78.6, 78.4 (C-2, C-4), 75.4, 75.3 (CH_2Ph), 73.2 (C), 73.4, 72.9 (CH_2Ph), 66.4 (C-6), 64.5 (C-5), 54.3 (C-1), 49.4 (C-1′), 48.6 (C-3′), 41.5, 41.3 (CH_2), 26.1 (C-2′), 23.7 (2 × CH_2); HRMS (ESI+) found 739.3859, $C_{44}H_{52}N_4O_5Na$ requires 739.3835.

***N*-(3-(4-(3-Methoxyestradiol)-1-yl-1*H*-1,2,3-triazol-1-yl)-propyl)-2,3,4,6-tetra-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol (22):** $[\alpha]_D$ = +14 (*c* = 0.5, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.33−7.10 (22 H, m, 21 × Har, C═CHN), 6.69−6.62 (2 H, m, 2 × Har), 4.98−4.80 (3 H, 3 d, *J* = 11.1 Hz, 10.9 Hz, 11.1 Hz, CH_2Ph, C*H*HPh), 4.72−4.62 (2 H, 2 d, *J* = 11.2 Hz, 11.1 Hz, CH_2Ph), 4.48−4.42 (3 H, m, CH_2Ph, CH*H*Ph), 4.24 (2 H, dt, *J* = 7.0 Hz, 6.8 Hz, 2 × H-3′), 3.77 (3 H, s, OCH_3), 3.70−3.57 (4 H, m, H-2, H-4, 2 × H-6), 3.51 (1 H, t, $J_{2,3} = J_{3,4}$ = 6.7 Hz, H-3), 3.03 (1 H, dd, $J_{1a,1b}$ = 11.3 Hz, $J_{1a,2}$ = 4.7 Hz, H-1a), 2.91−2.80 (3 H, m, CH_2, H-1′a), 2.61−2.52 (1 H, m, H-1′b), 2.47−2.35 (2 H, m, H-5, C*H*H), 2.23−2.14 (3 H, m, H-1b, C*H*H, CH*H*), 2.05−1.93 (6 H, m, 2 × H-2′, 2 × C*H*H, CH*H*, CH), 1.65−1.31 (5 H, m, 2 × CH*H*, C*H*H, 2 × CH), 1.28 (3 H, s, CH_3), 1.01 (1 H, dt, *J* = 12.8 Hz, 2.8 Hz, CH*H*); ^{13}C NMR (75 MHz,

$CDCl_3$) δ = 157.5 (CH_3OCar), 153.7 (NC=CH), 138.5, 138.3, 138.0, 137.0, 132.6 (Car), 128.6, 128.5, 128.4, 128.0, 127.8, 127.7, 127.6, 126.4 (CHar), 121.4 (NCH=C), 113.8, 111.5 (CHar), 87.2 (C-3), 82.5 (C), 78.5, 78.3 (C-2, C-4), 75.4, 75.2, 73.3, 72.9 (CH_2Ph), 66.3 (C-6), 64.3 (C-5), 55.2 (OCH_3), 54.5 (C-1), 49.3 (C-1′), 48.5 (C-3′, CH_2), 47.4, 43.5, 39.6 (CH), 38.1, 33.1, 30.0, 27.5, 26.4 (CH_2), 25.9 (C-2′), 23.5 (CH_2), 14.4 (CH_3); HRMS (ES+) found 917.5207, $C_{58}H_{69}N_4O_6$ requires 917.5217.

General Procedure for the Preparation of 23, 24, and 43. Conventional catalytic hydrogenation of compound **15** (35 mg, 0.0474 mmol) was carried out with $Pd(OH)_2$ (25 mg) in EtOH−1 M HCl (2 mL, 1:1) at 1 atm for 19 h. The catalyst was filtered over a pad of Celite, the solvent was removed, and the residue was purified by preparative HPLC (conditions 2) to afford **23** (11 mg, 61%) as a white solid.

***N*-(3-(4-(2-Methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)propyl)-1,5-dideoxy-1,5-imino-D-glucitol (23):** $[\alpha]_D$ = −5 (c = 0.1, MeOH); t_R = 24.7 min; ^{1}H NMR (300 MHz, D_2O) δ = 8.25 (1 H, s, C=CHN), 7.92 (1 H, d, J = 7.8 Hz, Har), 7.42 (1 H, t, J = 8.1 Hz, Har), 7.13 (2 H, d, J = 7.6 Hz, 2 × Har), 4.43 (2 H, t, J = 6.9 Hz, 2 × H-3′), 3.90 (3 H, s, CH_3), 3.75 (2 H, s, 2 × H-6), 3.51 (1 H, dt, $J_{1b,2}$ = $J_{2,3}$ = 9.5 Hz, $J_{1a,2}$ = 5.0 Hz, H-2), 3.35 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.24 (1 H, t, $J_{2,3}$ = $J_{3,4}$, H-3), 2.96 (1 H, dd, $J_{1a,1b}$ = 11.5 Hz, H-1a), 2.85−2.56 (2 H, m, 2 × H-1′), 2.33−2.23 (2 H, m, H-1b, H-5), 2.12 (2 H, m, J = 6.9 Hz, 2 × H-2′); ^{13}C NMR (75.5 MHz, D_2O) δ = 155.7 (CH_3OCar), 143.0 (NC=CH), 130.0, 127.2 (CHar), 124.7 (NCH=C), 121.1 (CHar), 118.0 (NCH=CCar), 111.9 (CHar), 78.1 (C-3), 69.9 (C-4), 68.7 (C-2), 64.7 (C-5), 57.3 (C-6), 55.4 (OCH_3), 55.2 (C-1), 48.7 (C-1′), 48.4 (C-3′), 23.7 (C-2′); HRMS (ESI+) found 379.1993, $C_{18}H_{27}N_4O_5$ requires 379.1981.

***N*-(3-(4-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)propyl)-1,5-dideoxy-1,5-imino-D-glucitol (24):** $[\alpha]_D$ = −6 (c = 0.2, MeOH); t_R = 23.4 min (conditions 2); ^{1}H NMR (300 MHz, D_2O) δ = 8.03 (1 H, s, C=CHN), 7.57 (2 H, d, J = 8.6 Hz, 2 × Har), 6.96 (2 H, d, J = 8.6 Hz, 2 × Har), 4.36 (2 H, t, J = 7.1 Hz, 2 × H-3′), 3.81 (3 H, s, CH_3), 3.74 (2 H, s, 2 × H-6), 3.51 (1 H, dt, $J_{1b,2}$ = $J_{2,3}$ = 9.6 Hz, $J_{1a,2}$ = 4.9 Hz, H-2), 3.34 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 3.23 (1 H, t, $J_{2,3}$ = $J_{3,4}$, H-3), 2.93 (1 H, dd, $J_{1a,1b}$ = 11.0 Hz, H-1a), 2.79−2.56 (2 H, m, 2 × H-1′), 2.28−2.21 (2 H, m, H-1b, H-5), 2.04 (2 H, m, J = 7.1 Hz, 2 × H-2′); ^{13}C NMR (75.5 MHz, D_2O) δ = 159.0 (CH_3OCar), 147.1 (NC=CH), 126.9 (2 × CHar), 122.4 (Car), 121.2 (NCH=C), 114.4 (2 × CHar), 78.2 (C-3), 69.9 (C-4), 68.8 (C-2), 64.7 (C-5), 57.4 (C-6), 55.3 (CH_3), 55.2 (C-1), 48.6 (C-1′), 48.5 (C-3′), 23.8 (C-2′); HRMS (ESI+) found 379.1987, $C_{18}H_{27}N_4O_5$ requires 379.1981.

General Procedure for the Preparation of 25−30, 37, and 40. Compound **17** (36 mg, 0.0496 mmol) was dissolved in methanol (10 mL) at 50 °C. Palladium hydroxide (55 mg) was added, and the mixture was stirred at 65 °C for 24 h. The mixture was filtered over a pad of Celite, and the filtrate evaporated under reduced pressure. The residue was purified by preparative HPLC (conditions 2) to afford **25** (11 mg, 61%) as a white solid.

***N*-(3-(4-(2-Fluorophenyl)-1*H*-1,2,3-triazol-1-yl)propyl)-1,5-dideoxy-1,5-imino-D-glucitol (25):** $[\alpha]_D$ = −4 (c = 0.2, MeOH); t_R = 24.0 (conditions 2); ^{1}H NMR (300 MHz, D_2O) δ = 8.17 (1 H, s, C=CHN), 7.44−7.34 (3 H, m, 3 × Har), 7.08 (1 H, dt, J = 8.2 Hz, 3.2 Hz, Har), 4.41 (2 H, t, J = 7.2 Hz, 2 × H-3′), 3.76 (2 H, s, 2 × H-6), 3.53 (1 H, dt, $J_{1b,2}$ = $J_{2,3}$ = 9.7 Hz, $J_{1a,2}$ = 4.8 Hz, H-2), 3.35 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4), 3.24 (1 H, t, $J_{2,3}$ = $J_{3,4}$, H-3), 2.96 (1 H, dd, $J_{1a,1b}$ = 11.3 Hz, H-1a), 2.81−2.61 (2 H, m, 2 × H-1′), 2.33−2.23 (2 H, m, H-1b, H-5), 2.10 (2 H, quint, J = 7.2 Hz, 2 × H-2′); ^{13}C NMR (75 MHz, D_2O) δ = 162.8 (d, J_{C-F} 241.3 Hz, FCar), 146.3 (NC=CH), 131.5 (Car), 130.8 (d, J_{C-F} = 8.6 Hz, CHar), 122.3 (NCH=C), 121.2 (d, J_{C-F} = 2.7 Hz, CHar), 115.2 (d, J_{C-F} = 21.1 Hz, CHar), 112.1 (d, J_{C-F} = 23.1 Hz, CHar), 78.2 (C-3), 70.4 (C-4), 68.7 (C-2), 64.7 (C-5), 57.4 (C-6), 55.2 (C-1), 48.7 (C-1′), 48.6 (C-3′), 23.7 (C-2′); HRMS (ES+) found 367.1781, $C_{17}H_{24}N_4O_4F$ requires 367.1782.

***N*-(3-(4-(3-Fluoro-2-methylphenyl)-1*H*-1,2,3-triazol-1-yl)propyl)-1,5-dideoxy-1,5-imino-D-glucitol (26):** $[\alpha]_D$ = +3 (c = 0.2, MeOH); t_R = 26.9 (conditions 2); ^{1}H NMR (300 MHz, D_2O) δ = 7.84 (1 H, s, C=CHN), 7.24 (2 H, d, J = 7.6 Hz, 2 × Har), 6.81 (1 H, t, J = 9.2 Hz, Har), 4.26 (2 H, m, 2 × H-3′), 3.81−3.68 (2 H, m, 2 × H-6), 3.52 (1 H, dt, $J_{1b,2}$ = $J_{2,3}$ = 9.8 Hz, $J_{1a,2}$ = 4.9 Hz, H-2), 3.36 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 3.21 (1 H, t, $J_{2,3}$ = $J_{3,4}$, H-3), 2.94 (1 H, dd, $J_{1a,1b}$ = 10.8 Hz, H-1a), 2.74−2.66 (1 H, m, H-1′a), 2.63−2.58 (1 H, m, H-1′b), 2.24−2.14 (2 H, m, H-1b, H-5), 2.00−1.92 (5 H, m, 2 × H-2′, CH_3); ^{13}C NMR (75 MHz, D_2O): δ = 160.8 (d, J_{C-F} = 243.7 Hz, Car), 146.4 (NC=CH), 128.3, 125.3 (d, J_{C-F} = 17.2 Hz, CHar), 124.3 (Car), 121.1 (NCH=C), 115.2 (d, J_{C-F} = 23.2 Hz, CHar), 122.0 (CHar), 78.2 (C-3), 69.2 (C-4), 68.7 (C-2), 64.9 (C-5), 57.2 (C-6), 55.3 (C-1), 48.7 (C-1′), 48.4 (C-3′), 24.0 (C-2′), 13.5 (CH_3); HRMS (ESI+) found 403.1767, $C_{18}H_{25}FN_4O_4Na$ requires 403.1758.

***N*-(3-(4-(6-Methoxynaphtalen-2-yl)-1*H*-1,2,3-triazol-1-yl)propyl)-1,5-dideoxy-1,5-imino-D-glucitol (27):** $[\alpha]_D$ = +29 (c = 0.1, MeOH); t_R = 29.5 (conditions 1); ^{1}H NMR (300 MHz, CD_3OD) δ = 8.43 (1 H, s, C=CHN), 8.23 (1 H, s, Har), 7.90−7.79 (4 H, m, Har), 7.25 (1 H, d, J = 2.1 Hz, Har), 7.16 (1 H, dd, J = 8.9 Hz, 2.5 Hz, Har), 4.52 (2 H, t, J = 6.8 Hz, 2 × H-3′), 3.93 (3 H, s, CH_3), 3.84 (2 H, ddd, J = 12.7, 11.9, 3.1 Hz, 2 × H-6), 3.45 (1 H, dt, $J_{1b,2}$ = $J_{2,3}$ = 9.5 Hz, $J_{1a,2}$ = 5.2 Hz, H-2), 3.34 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.23 (1 H, t, $J_{2,3}$ = $J_{3,4}$, H-3), 3.05−2.92 (2 H, m, H-1a, H-1′a), 2.65−2.55 (1 H, m, H-1′b), 2.21−2.10 (4 H, m, H-1b, H-5, 2 × H-2′); ^{13}C NMR (75 MHz, D_2O) δ = 159.8 (CH_3OCar), 149.3(NC=CH), 136.2 (Car), 130.8 (CHar), 130.6 (Car), 128.7 (CHar), 125.4 (2 × CHar), 122.5 (NCH=C), 120.5 (CHar), 80.7 (C-3), 72.2 (C-4), 70.9 (C-2), 68.1 (C-5), 59.7 (CH_3), 57.8 (C-6), 56.0 (C-1), 50.5 (C-1′), 50.0 (C-3′), 27.2 (C-2′); HRMS (ES+) found 429.2129, $C_{22}H_{29}N_4O_5$ requires 429.2138.

***N*-(3-(4-(Cyclohexanol-1-yl)-1*H*-1,2,3-triazol-1-yl)propyl)-1,5-dideoxy-1,5-imino-D-glucitol (28):** $[\alpha]_D$ = +4 (c = 0.2, MeOH); t_R = 18.8 (conditions 2); ^{1}H NMR (300 MHz, D_2O) δ = 7.98 (1 H, s, C=CHN), 4.45 (2 H, m, 2 × H-3′), 3.81−3.72 (2 H, m, 2 × H-6), 3.53 (1 H, dt, $J_{1b,2}$ = $J_{2,3}$ = 9.7 Hz, $J_{1a,2}$ = 4.6 Hz, H-2), 3.37 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 3.26 (1 H, t, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3), 2.98 (1 H, dd, $J_{1a,1b}$ 11.5 Hz, H-1a), 2.86−2.56 (2 H, m, 2 × H-1′), 2.38−2.28 (2 H, m, H-1b, H-5), 2.15−2.06 (4 H, m, 2 × H-2′, CH_2), 1.83−1.79 (2 H, m, CH_2), 1.71−1.67 (2 H, m, CH_2), 1.50−1.33 (4 H, m, 2 × CH_2); ^{13}C NMR (75 MHz, D_2O) δ = 159.4 (NC=CH), 122.7 (NCH=C), 114.7, 112.5 (CHar), 78.0 (C-3), 69.7 (C-4), 69.05 (C), 68.5 (C-2), 64.7 (C-5), 57.1 (C-6), 55.4 (C-1), 48.6 (C-1′), 48.2 (C-3′), 37.0 (2 × CH_2), 0.24.7 (CH_2), 23.7 (C-2′), 22.0 (2 × CH_2); HRMS (ES+) found 393.2120, $C_{17}H_{30}N_4O_5Na$ requires 393.2114.

***N*-(3-(4-(Cyclopentanol-1-yl)-1*H*-1,2,3-triazol-1-yl)propyl)-1,5-dideoxy-1,5-imino-D-glucitol (29):** $[\alpha]_D$ = +9 (c = 0.1, MeOH); t_R = 16.2 (conditions 2); ^{1}H NMR (300 MHz, D_2O) δ = 7.96 (1 H, s, C=CHN), 4.46 (2 H, dt, J = 6.7, 6.8 Hz, 2 × H-3′), 3.78−3.68 (2 H, m, 2 × H-6), 3.55−3.49 (1 H, dt, $J_{1b,2}$ = $J_{2,3}$ = 9.5 Hz, $J_{1a,2}$ = 5.1 Hz, H-2), 3.34 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.23 (1 H, t, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3), 2.94 (1 H, dd, $J_{1a,1b}$ = 11.0 Hz, H-1a), 2.79−2.63 (2 H, m, 2 × H-1′), 2.28−2.09 (2 H, m, H-1b, H-5), 2.14−2.04 (6 H, m, 2 × H-2′, 2 × CH_2), 1.90−1.80 (4 H, m, 2 × CH_2); ^{13}C NMR (75 MHz, D_2O) δ = 157.8 (NCCH), 122.2 (NCHC), 78.6 (C), 78.2 (C-3), 69.9 (C-4), 68.8 (C-2), 64.6 (C-5), 58.7 (C-6), 55.2 (C-1), 48.6 (C-1′), 48.5 (C-3′), 39.9 (2 × CH_2), 23.7 (C-2′), 22.9 (2 × CH_2); HRMS (ES+) found 379.1952, $C_{16}H_{28}N_4O_5Na$ requires 379.1957.

***N*-(3-(4-(3-Methoxyestradiol-1-yl)-1*H*-1,2,3-triazol-1-yl)propyl)-1,5-dideoxy-1,5-imino-D-glucitol (30):** $[\alpha]_D$ = +36 (c = 0.5, MeOH); t_R = 35.6 (conditions 2); ^{1}H NMR (500 MHz, CD_3OD) δ = 7.85 (1 H, s, C=CHN), 7.83 (1 H, d, J = 8.6 Hz, Har), 6.64 (1 H, dd, J = 8.5, 2.6 Hz, Har), 6.60 (1 H, d, J = 2.5 Hz, Har), 4.48 (2 H, t, J = 6.7 Hz, 2 × H-3′), 3.84 (2 H, s, 2 × H-6), 3.74 (3 H, s, OCH_3), 3.49 (1 H, dt,

$J_{1b,2} = J_{2,3} = 9.7$ Hz, $J_{1a,2} = 4.2$ Hz, H-2), 3.38 (1 H, t, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 3.18 (1 H, t, $J_{2,3} = J_{3,4}$, H-3), 3.04 (1 H, dd, $J_{1a,1b} = 11.2$ Hz, H-1a), 2.94 (1 H, m, H-1′a), 2.86−2.77 (2 H, m, CH_2), 2.60−2.45 (2 H, m, H-1′b, C*H*H), 2.23−2.07 (6 H, m, H-1b, H-5, 2 × H-2′, CH*H*, C*H*H), 2.05−1.88 (3 H, m, C*H*H, C*H*H, CH), 1.71−1.65 (2 H, m, C*H*H, CH), 1.62−1.53 (1 H, m, CH*H*), 1.47−1.32 (3 H, CH*H*, CH*H*, CH), 0.92 (3 H, s, CH_3), 0.74 (1 H, dt, *J* 12.8, 3.8 Hz, CH*H*); ^{13}C NMR (125 MHz, CD_3OD) δ = 159.1 (CH_3OCar), 155.4 (NC═CH), 138.9, 133.8 (Car), 127.1 (CHar), 124.1 (NCH═C), 114.7, 112.5 (CHar), 83.5 (C), 80.4 (C-3), 72.0 (C-4), 70.6 (C-2), 67.9 (C-5), 59.6 (C-6), 57.6 (C-1), 55.6 (OCH_3), 49.7 (C-1′), 49.5 (CH), 49.3 (C-3′), 48.5, 48.4 (CH), 44.5, 41.1, 38.5, 34.4, 28.7, 27.2, 27.1 (CH_2), 24.6 (C-2′), 14.8 (CH_3); HRMS (ES+) found 557.3351, $C_{30}H_{45}N_4O_6$ requires 557.3339.

***N*-(3-(4-(3,4-Dichlorophenoxymethyl)-1*H*-1,2,3-triazol-1-yl)-propyl)-2,3,4,6-tetra-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol (36):** $[\alpha]_D$ = +11 (*c* = 0.4, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.10 (1 H, s, C═CHN), 7.33−7.10 (23 H, m, 23 × Har), 5.23 (2 H, s, OCH_2), 4.99−4.80 (3 H, 3 d, *J* = 11.0, 10.9, 11.0 Hz, CH_2Ph, C*H*HPh), 4.69−4.62 (2 H, 2 d, *J* = 11.6, 11.4 Hz, CH_2Ph), 4.47−4.37 (3 H, m, CH_2Ph, CH*H*Ph), 4.31−4.19 (2 H, m, 2 × H-3′), 3.68−3.49 (5 H, m, H-2, H-3, H-4, 2 × H-6), 3.01 (1 H, dd, $J_{1a,1b} = 10.9$ Hz, $J_{1a,2} = 4.5$ Hz, H-1a), 2.85−2.77 (1 H, m, H-1′a), 2.55−2.47 (1 H, m, H-1′b), 2.32 (1 H, d, $J_{4,5} = J_{5,6} = 8.4$ Hz, H-5), 2.14 (1 H, t, $J_{1a,1b} = J_{1b,2}$, H-1b), 2.01 (2 H, q, *J* = 6.8 Hz, 2 × H-2′); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 8.10 (1 H, s, C═CHN), 7.33−7.10 (23 H, m, 23 × Har), 5.23 (2 H, s, OCH_2), 4.99−4.80 (3 H, 3 d, *J* 11.0, 10.9, 11.0 Hz, CH_2Ph, C*H*HPh), 4.69−4.62 (2 H, 2 d, *J* = 11.6, 11.4 Hz, CH_2Ph), 4.47−4.37 (3 H, m, CH_2Ph, CH*H*Ph), 4.31−4.19 (2 H, m, 2 × H-3′), 3.68−3.49 (5 H, m, H-2, H-3, H-4, 2 × H-6), 3.01 (1 H, dd, $J_{1a,1b}$ = 10.9 Hz, $J_{1a,2}$ = 4.5 Hz, H-1a), 2.85−2.77 (1 H, m, H-1′a), 2.55−2.47 (1 H, m, H-1′b), 2.32 (1 H, d, $J_{4,5} = J_{5,6} = 8.4$ Hz, H-5), 2.14 (1 H, t, $J_{1a,1b} = J_{1b,2}$, H-1b), 2.01 (2 H, q, *J* = 6.8 Hz, 2 × H-2′); HRMS (ES+), found 807.3091, $C_{46}H_{49}N_4O_5Cl_2$ requires 807.3080.

***N*-(3-(4-(Phenoxymethyl)-1*H*-1,2,3-triazol-1-yl)propyl)- 1,5-dideoxy-1,5-imino-D-glucitol (37):** $[\alpha]_D$ = 36 (c= 0.5, MeOH); t_R = 22.5 (conditions 2); ^{1}H NMR (300 MHz, D_2O) δ = 7.95 (1H, s, C═CHN), 7.31 (2H, d, *J* = 8.0 Hz, 2 × Har), 7.03−6.91 (3H, m, 3 × Har), 5.16 (2H, s, OCH_2), 4.41−4.34 (2H, m, 2 × H-3′), 3.78−3.68 (2H, m, 2 × H-6), 3.52 (1H, m, dt, $J_{1b,2} = J_{2,3} = 9.7$ Hz, $J_{1a,2} = 4.6$ Hz, H-2), 3.35 (1H, t, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 3.27 (1H, t, $J_{2,3} = J_{3,4}$, H-3), 2.90 (1H, dd, $J_{1a,1b}$ = 11.1 Hz, H-1a), 2.75−2.50 (2H, m, 2 × H-1′), 2.24−2.17 (2H, m, H-1b, H-5), 2.03 (2H, quint, *J* = 7.2 Hz, 2 × H-2′); ^{13}C NMR (75 MHz, D_2O): δ = 157.2 (OCar), 143.4 (NC═CH), 129.9 (2 × CHar), 125.1 (NCH═C), 122.0 (CHar), 115.3 (2 × CHar), 78.1 (C-3), 69.2 (C-4), 68.7 (C-2), 64.7 (C-5), 61.0 (OCH_2), 57.2 (C-6), 55.4 (C-1), 48.5 (C-1′), 48.2 (C-3′), 23.8 (C-2′); HRMS (ES+) found 379.1985, $C_{18}H_{27}N_4O_5$ requires 379.1981.

***N*-(3-(4-(3,4-Dichlorophenoxymethyl)-1*H*-1,2,3-triazol-1-yl)-propyl)-1,5-dideoxy-1,5-imino-D-glucitol (38).** To a solution of the azido derivative **6** (15 mg, 0.061 mmol) and the alkyne **31** (14 mg, 0.067 mmol) in dioxane−H_2O (2 mL, 4:1) were added $CuSO_4$ (15 mg, 0.0915 mmol) and sodium ascorbate (36 mg, 0.183 mmol), and the mixture was stirred under microwave irradiation at 80 °C for 45 min. The mixture was diluted with EtOAc (10 mL) and washed with NH_4Cl satd solution (2 × 8 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was residue purified by preparative HPLC (conditions 2) to afford **38** (16 mg, 59%): $[\alpha]_D$ = −5 (*c* = 0.3, MeOH); t_R = 28.7 (conditions 2); ^{1}H NMR (500 MHz, D_2O) δ = 7.89 (1 H, s, C═CHN), 6.97 (1 H, s, Har), 6.88−6.84 (2 H, m, 2 × Har), 5.04 (2 H, s, OCH_2), 4.28 (2 H, s, 2 × H-3′), 3.78−3.69 (2 H, m, 2 × H-6), 3.52−3.58 (1 H, m, H-2), 3.40 (1 H, t, $J_{3,4} = J_{4,5} = 9.3$ Hz, H-4), 3.25 (1 H, t, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3), 2.91−2.98 (1 H, m, H-1a), 2.77−2.70 (1 H, m, H-1′a), 2.58−2.50 (1 H, m, H-1′b), 2.17 (1 H, t, $J_{1a,1b} = J_{1b,2} = 10.3$ Hz, H-1b), 2.14 (1 H, d, $J_{4,5} = J_{5,6} = 9.5$ Hz, H-5), 2.02−1.91 (2 H, m, 2 × H-2′); ^{13}C NMR (125 MHz, D_2O) δ = 154.5 (CH_2OCar), 142.6 (NC═CH), 133.1 (Car), 127.8 (CHar), 124.8 (NCH═C), 122.7 (CHar), 121.0 (Car), 112.2 (CHar), 78.2 (C-3), 69.7 (C-4), 68.7 (C-2), 62.5 (C-5), 64.9 (OCH_2), 57.2 (C-6), 55.4 (C-1), 48.7 (C-1′), 48.5 (C-3′), 24.2 (C-2′); HRMS (ES+) found 469.1029, $C_{18}H_{24}N_4O_5Cl_2Na$ requires 469.1021.

4-{[(1*S*,2*S*,4*R*)-Bicyclo[2.2.1]heptan-2-yloxy]methyl}-1-[3-(2,3,4,6-tetra-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol-*N*-yl)propyl]-1,2,3-triazole (39): $[\alpha]_D$ = +2 (*c* = 0.4, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.40 (1 H, s, C═CHN), 7.31−7.11 (20 H, m, 20 × Har), 4.94−4.79 (3 H, 3 d, *J* = 11.0, 10.8, 11.1 Hz, CH_2Ph, C*H*HPh), 4.68−4.39 (9 H, m, 2 × CH_2Ph, CH*H*Ph, 2 × H-3′, OCH_2), 3.72−3.44 (6 H, m, H-2, H-3, H-4, 2 × H-6, CH), 3.01 (1 H, dd, $J_{1a,1b}$ = 11.1 Hz, $J_{1a,2}$ = 4.7 Hz, H-1a), 2.86−2.75 (1 H, m, H-1′a), 2.61−2.46 (1 H, m, H-1′b), 2.38−1.97 (6 H, m, H-1b, H-5, 2 × H-2′, 2 × CH), 1.56−1.42 (5 H, m, CH_2, 3 × C*H*H), 1.12−0.99 (3 H, m, 3 × CH*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 146.0 (NC═CH), 139.0, 138.5, 137.8 (Car), 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 127.6 (CHar), 122.6 (NCH═C), 87.2 (C-3), 82.7 (CH), 78.6, 78.4 (C-2, C-4), 75.4, 75.3, 73.4, 72.9 (CH_2Ph), 66.3 (C-6), 64.5 (C-5), 62.1 (OCH_2), 54.5 (C-1), 49.2 (C-1′), 48.4 (C-3′), 40.4 (CH), 39.6 (CH_2), 35.3 (CH), 35.0 (CH_2), 28.6 (CH_2), 26.1 (C-2′), 24.7 (CH_2); HRMS (ES+) found 757.4339, $C_{47}H_{57}N_4O_5$ requires 757.4329.

4-{[(1*S*,2*S*,4*R*)-Bicyclo[2.2.1]heptan-2-yloxy]methyl}-1-[3-(1,5-dideoxy-1,5-imino-D-glucitol-*N*-yl)propyl]-1,2,3-triazole (40): $[\alpha]_D$ = −5 (*c* = 0.3, MeOH); t_R = 25.9 (conditions 2); ^{1}H NMR (300 MHz, D_2O) δ = 8.03 (1 H, s, C═CHN), 4.62 (2 H, s, OCH_2), 4.49−4.45 (2 H, m, 2 × H-3′), 3.75 (2 H, s, 2 × H-6), 3.62 (1 H, d, *J* = 6.4 Hz, CH), 3.52 (1 H, m, dt, $J_{1b,2} = J_{2,3} = 9.6$ Hz, $J_{1a,2} = 4.9$ Hz, H-2), 3.38 (1 H, t, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 3.27 (1 H, t, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3), 2.93 (1 H, dd, $J_{1a,1b}$ = 11.1 Hz, H-1a), 2.85−2.50 (2 H, m, 2 × H-1′), 2.34−2.15 (3 H, m, H-1b, H-5, CH), 2.12 (2 H, quint, *J* 6.9 Hz, 2 × H-2′), 1.68−1.62 (1 H, m, C*H*H), 1.46−1.37 (4 H, m, CH*H*, 3 × C*H*H), 1.14 (1 H, d, *J* 9.8 Hz, CH*H*), 1.04−0.99 (2 H, m, 2 × CH*H*); ^{13}C NMR (125 MHz, D_2O) δ = 144.5 (NC═CH), 125.0 (NCH═C), 83.0 (CH), 78.2 (C-3), 69.2 (C-4), 68.8 (C-2), 64.7 (C-5), 60.3 (OCH_2), 57.4 (C-6), 55.3 (C-1), 48.6 (C-1′), 48.5 (C-3′), 40.1 (CH), 38.9 (CH_2), 35.1 (CH), 34.3 (CH_2), 27.6 (CH_2), 24.1 (C-2′), 23.7 (CH_2); HRMS (ES+) found 397.2431, $C_{19}H_{33}N_4O_5$ requires 397.2451.

1-[3-(1,5-Dideoxy-1,5-imino-D-glucitol-*N*-yl)propyl)]-4-{[2-(6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethoxy]methyl}-1,2,3-triazole (41). Compound **6** (10 mg, 0.0406 mmol), compound **33** (9 mg, 0.0447 mmol), $CuSO_4$ (9 mg, 0.0610 mmol), and sodium ascorbate (24 mg, 0.122 mmol) were dissolved in dioxane (0.8 mL) and water (0.11 mL) The mixture was stirred under microwave irradiation at 80 °C for 45 min. The mixture was evaporated under reduced pressure, and the residue purified by preparative HPLC (conditions 2) to afford **41** (11 mg, 60%): $[\alpha]_D$ = −9 (*c* = 0.1, MeOH); t_R = 33.5; ^{1}H NMR (500 MHz, D_2O) δ = 8.11 (1 H, s, CCHN), 5.40 (1 H, s, CH_2CHC), 4.71 (2 H, s, OCH_2C), 4.59 (2 H, m, 2 × H-3′), 3.98−3.92 (2 H, m, 2 × H-6), 3.75−3.69 (3 H, m, H-2, CH_2), 3.57 (1 H, t, $J_{3,4} = J_{4,5} = 9.3$ Hz, H-4), 3.45 (1 H, t, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3), 3.22 (1 H, dd, $J_{1a,1b}$ = 11.0 Hz, $J_{1a,2}$ = 4.9 Hz, H-1a), 2.90−3.02 (1 H, m, H-1′a), 2.90−2.83 (1 H, m, H-1′b), 2.52−2.47 (2 H, m, H-1b, H-5), 2.41−2.27 (7H, m, 2 × H-2′, 2 × CH_2, C*H*H), 2.18 (2 H, s, CH), 1.39 (3 H, s, CH_3), 1.25 (1 H, d, *J* = 8.2 Hz, CH*H*), 0.94 (3 H, s, CH_3); ^{13}C NMR (125 MHz, CD_3OD): δ = 145.4 (C), 144.8 (NC═CH), 124.9 (NCH═C), 118.3 (CH_2CHC), 78.5(C-3), 70.0 (C-4), 68.9 (C-2, CH_2), 65.6 (C-5), 63.7 (OCH_2), 57.4 (C-6), 55.7 (C-1), 49.3 (C-1′), 48.7 (C-3′), 45.8, 41.0 (CH), 38.1 (C), 31.8, 31.6 (CH_2), 26.5 (CH_3), 24.7 (C-2′), 21.4 (CH_3); HRMS (ES+) found 451.2906, $C_{23}H_{39}N_4O_5$ requires 451.2920.

4-[4-Adamantylphenoxymethyl]-1-[3-(2,3,4,6-tetra-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol-*N*-yl)propyl]-1,2,3-

triazole (42): $[\alpha]_D$ = +6 (*c* = 0.3, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.48 (1 H, s, C═CHN), 7.33−7.16 (22 H, m, 22 × Har), 6.94 (2 H, d, *J* = 8.9 Hz, Har), 5.28 (2 H, s, OCH_2), 4.98−4.80 (3 H, 3 d, *J* = 11.0, 10.9, 11.0 Hz, CH_2Ph, C*H*HPh), 4.65−4.59 (2 H, 2 d, *J* = 11.6, 11.6 Hz, CH_2Ph), 4.46−4.35 (3 H, m, CH_2Ph, CH*H*Ph), 4.28−4.21 (2 H, m, 2 × H-3′), 3.64−3.50 (5 H, m, H-2, H-3, H-4, 2 × H-6), 3.00 (1 H, dd, $J_{1a,1b}$ = 11.2 Hz, $J_{1a,2}$ = 4.6 Hz, H-1a), 2.86−2.77 (1 H, m, H-1′a), 2.60−2.46 (1 H, m, H-1′b), 2.32 (1 H, d, $J_{4,5}$ = $J_{5,6}$ = 8.7 Hz, H-5), 2.17−2.04 (4 H, m, H-1b, 3 × CH), 2.00 (2 H, m, *J* = 7.2 Hz, 2 × H-2′), 1.89 (6 H, d, *J* = 2.6 Hz, 3 × CH_2) 1.82−1.72 (6 H, m, 3 × CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 156.2 (OCar), 144.4 (NC═CH), 139.0, 138.5, 137.8 (Car), 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6 (CHar), 126.0 (2 × CHar), 123.0 (NCH═C), 114.3 (2 × CHar), 87.2 (C-3), 78.6, 78.4 (C-2, C-4), 75.4, 75.3, 73.3, 72.9 (CH_2Ph), 66.4 (C-6), 64.6 (C-5), 62.2 (OCH_2), 54.5 (C-1), 49.2 (C-1′), 48.5 (C-3′), 43.4 (3 × CH_2), 36.8 (3 × CH_2), 35.7 (C), 29.1 (3 × CH), 26.1 (C-2′); HRMS (ES+) found 873.4977, $C_{56}H_{65}N_4O_5$ requires 873.4955.

***N*-(3-(4-(4-Adamantylphenoxymethyl)-1*H*-1,2,3-triazol-1-yl)propyl)-1,5-dideoxy-1,5-imino-D-glucitol (43).** Conventional catalytic hydrogenation of compound **42** (40 mg, 0.0459 mmol) was carried out with $Pd(OH)_2$ (30 mg) in MeOH−1 M HCl (3 mL, 1:1) at 1 atm for 18 h. The catalyst was filtered over a pad of Celite and the solvent was removed. The residue was purified by preparative HPLC (conditions 2) to afford **43** (11 mg, 45%) as a white solid: $[\alpha]_D$ = +55 (*c* = 0.1, MeOH); t_R = 38.0; ^{1}H NMR (300 MHz, CD_3OD) δ = 8.08 (1 H, s, C═CHN), 7.28 (2 H, d, *J* = 8.9 Hz, 2 × Har), 6.94 (2 H, d, *J* = 8.9 Hz, 2 × Har), 5.15 (2 H, s, OCH_2), 4.47 (2 H, t, *J* = 6.9 Hz, 2 × H-3′), 3.82 (2 H, ddd, *J* = 12.7, 12.1, 3.4 Hz, 2 × H-6), 3.46 (1 H, dt, $J_{1b,2}$ = $J_{2,3}$ = 9.5 Hz, $J_{1a,2}$ = 4.8 Hz, H-2), 3.33 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.23 (1 H, t, $J_{2,3}$ = $J_{3,4}$, H-3), 2.99 (1 H, dd, $J_{1a,1b}$ = 11.1 Hz, H-1a), 2.92−2.85 (1 H, m, H-1′a), 2.57−2.48 (1 H, m, H-1′b), 2.17−2.08 (7 H, m, H-1b, H-5, 2 × H-2′, 3 × CH), 1.91 (6 H, d, *J* = 2.7 Hz, 3 × CH_2), 1.85−1.75 (6 H, m, 3 × CH_2); ^{13}C NMR (75 MHz, CD_3OD) δ = 158.3 (OCar), 146.3, 146.2 (NC═CH, Car), 127.7 (2 × CHar), 126.2 (NCH═C), 116.3 (2 × CHar), 81.3 (C-3), 72.8 (C-4), 71.6 (C-2), 68.7 (C-5), 63.3 (OCH_2), 60.4 (C-6), 58.5 (C-1), 50.4 (C-1′), 49.4 (C-3′), 45.4 (3 × CH_2), 38.7 (3 × CH_2), 37.5 (C), 31.4 (3 × CH), 27.9 (C-2′); HRMS (ES+) found 513.3058, $C_{28}H_{41}N_4O_5$ requires 513.3077.

***N*-(3-(4-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyloxyethyl)-1*H*-1,2,3-triazol-1-yl)propyl)-2,3,4,6-tetra-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol (44):** $[\alpha]_D$ = +30 (*c* = 0.2, MeOH); t_R = 22.2 (conditions 2); ^{1}H NMR (300 MHz, D_2O) δ = 7.90 (1 H, s, C═CHN), 5.25−5.20 (2 H, m, H-2″, H-3″), 4.96 (1 H, d, $J_{1,2}$ = 1.3 Hz, H-1″), 4.47 (2 H, t, *J* = 6.9 Hz, 2 × H-3′), 4.32 (1 H, dd, $J_{6a',6b'}$ = 12.7 Hz, $J_{5',6a'}$ = 4.0 Hz, H-6a″), 4.09 (1 H, dd, $J_{5',6b'}$ = 1.9 Hz, H-6b″), 3.97 (1 H, m, OC*H*HCH$_2$), 3.85−3.76 (3 H, m, 2 × H-6, OCH*H*CH$_2$), 3.63−3.53 (3 H, m, H-2, H-4″, H-5″), 3.39 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4), 3.27 (1 H, t, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3), 3.06−2.99 (3 H, m, H-1a, OCH_2CH_2), 2.92−2.62 (2 H, m, 2 × H-1′), 2.40−2.25 (2 H, m, H-1b, H-5), 2.20−2.11 (11 H, 3 s, m, 3 × CH_3, 2 × H-2′), 2.04 (3 H, s, CH_3); ^{13}C NMR (75.5 MHz, D_2O) δ = 173.5, 172.9, 172.8, 172.7 (C═O), 151.5 (NC═CH), 124.1 (NCH═C), 100.1 (C-1″), 87.1 (C-3), 69.7, 69.6 (C-4,), 69.4 (), 68.6 (C-2), 68.0 (CHsucre), 66.7 (OCH_2CH_2), 65.6 (CHsucre), 64.9 (C-5), 61.9 (C-6″), 57.1 (C-6), 55.1 (C-1), 48.8 (C-1′), 48.3 (C-3′), 25.1 (OCH_2CH_2), 24.0 (C-2′), 20.2 (CH_3); HRMS (ESI+) found 669.2591, $C_{27}H_{42}N_4O_{14}Na$ requires 669.2595.

***N*-(3-(4-(α-D-Mannopyranosyloxyethyl)-1*H*-1,2,3-triazol-1-yl)propyl)-2,3,4,6-tetra-*O*-benzyl-1,5-dideoxy-1,5-imino-D-glucitol (45).** To a solution of **44** (20 mg, 0.0301 mmol) in dry MeOH (1 mL) was added methanolic NaMeO (1 M, 0.1 equiv per mol of acetate). The reaction mixture was stirred at rt for 3 h, then neutralized with Amberlite IRA-120 (H^+) ion-exchange resin, concentrated, and the resulting residue was purified by preparative HPLC (conditions 1) to afford **45** (10 mg, 65%) as a white solid: $[\alpha]_D$ = +30 (*c* = 0.2, MeOH); t_R = 11.2; ^{1}H NMR (500 MHz, D_2O) δ = 7.92 (1 H, s, C═CHN), 4.89 (1 H, d, $J_{1,2}$ = 1.4 Hz, H-1″), 4.47 (2 H, quad, *J* = 6.8 Hz, 2 × H-3′), 4.03−3.98 (1 H, m, OC*H*HCH$_2$), 3.92 (1 H, s, H?), 3.86−3.81 (4 H, m, 2 × H-6, H-6a″, OCH*H*CH$_2$), 3.76−3.71 (3H, m, 2 × H?, H-6b″), 3.65 (1 H, t, *J* = 9.3 Hz, H?), 3.57 (1 H, dt, $J_{1b,2}$ = $J_{2,3}$ = 9.4 Hz, $J_{1a,2}$ = 4.9 Hz, H-2), 3.40 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 3.35−3.32 (1 H, m, H?), 3.29 (1 H, t, $J_{2,3}$ = $J_{3,4}$, H-3), 3.06 (2 H, t, *J* = 6.0 Hz, OCH_2CH_2), 3.00 (1 H, dd, $J_{1a,1b}$ = 11.0 Hz, $J_{1a,2}$ = 4.5 Hz, H-1a), 2.85−2.77 (1 H, m, H-1′a), 2.75−2.66 (1 H, m, H-1′b), 2.37−2.30 (2 H, m, H-1b, H-5), 2.15 (2 H, m, *J* = 6.8 Hz, 2 × H-2′); ^{13}C NMR (75.5 MHz, D_2O) δ = 145.8 (NC═CH), 125.0 (NCH═C), 100.0 (C-1″), 78.6 (C-3), 73.2 (C?), 71.0, 70.4 (C?), 70.3 (C-4), 69.1 (C-2), 67.0 (C?), 66.7 (OCH_2CH_2), 65.1 (C-5), 61.2 (C-6″), 57.8 (C-6), 55.6 (C-1), 49.1 (C-1′), 48.8 (C-3′), 25.6 (OCH_2CH_2), 24.3 (C-2′); HRMS (ESI+) found 501.2169, $C_{19}H_{34}N_4O_{10}Na$ requires 501.2173.

General Procedures for Inhibition Assay. The glycosidases β-glucosidase (from bovine liver, cytosolic), α-galactosidase (from *Aspergillus niger*), α-galactosidase (from green coffee beans), β-glucosidase (from almonds), amyloglucosidase (from *Aspergillus niger*), α-glucosidase (from yeast), isomaltase (from yeast), naringinase (*Penicillium decumbes*), β-mannosidase (from *Helix pomatia*), and α-mannosidase (from jack bean) used in the inhibition studies, as well as the corresponding *o*- and *p*-nitrophenyl glycoside substrates, were purchased from a commercial supplier. Inhibitory potencies were determined by spectrophotometrically measuring the residual hydrolytic activities of the glycosidases against the respective *o*- (for β-glucosidase/β-galactosidase from bovine liver) or *p*-nitrophenyl α- or β-D-glycopyranoside, in the presence of the corresponding iminosugar derivative. Each assay was performed in phosphate or phosphate-citrate (for α- or β-mannosidase or amyloglucosidase) buffer at the optimal pH for each enzyme. The K_m values for the different glycosidases used in the tests and the corresponding working pHs are listed herein: β-glucosidase (bovine liver), K_m = 2.0 mM (pH 7.3); α-glucosidase (yeast), K_m = 0.35 mM (pH 6.8); β-glucosidase (almonds), K_m = 3.5 mM (pH 7.3); α-galactosidase (coffee beans), K_m = 2.0 mM (pH 6.8); amyloglucosidase (*Aspergillus niger*), K_m = 3.0 mM (pH 5.5); naringinase (*Penicillium decumbes*), K_m = 2.7 mM (pH 6.8); β-mannosidase (*Helix pomatia*), K_m = 0.6 mM (pH 5.5); α-mannosidase (jack bean), K_m = 2.0 mM (pH 5.5). The reactions were initiated by addition of enzyme to a solution of the substrate in the absence or presence of various concentrations of inhibitor. After the mixture was incubated for 10−30 min at 37 °C the reaction was quenched by addition of 1 M Na_2CO_3. The absorbance of the resulting mixture was determined at 405 or 505 nm. The K_i value and enzyme inhibition mode were determined from the slope of Lineweaver−Burk plots and double reciprocal analysis using a Microsoft Office Excel 2003 program. Inhibition mode was competitive in all cases.

Cell Culture. HL60 cells and Gaucher lymphoblasts (N370S) were cultured in RPMI1640 medium supplemented with 10% or 15% (v/v) fetal bovine serum, respectively, 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/mL streptomycin at 37 °C and 5% CO_2.

β-Glucocerebrosidase Inhibition Assay. Human placental β-glucocerebrosidase was isolated and partially purified from modified procedure.[49] Enzyme activity was measured in 50 μL of 5 mM 4-methylumbelliferyl-β-glucoside in 0.1 M citrate phosphate buffer, pH 5.2 containing 0.25% sodium taurocholate, 0.1% TX100 at 37 °C for 15−60 min. The reaction was stopped by the addition of 200 μL 0.5 M sodium carbonate and the fluorescence measured at ex 350 nm, em 460 nm. Inhibitory activity was generated for placental β-glucocerebrosidase (Km for 4-MU-β-glucoside, 1.9 ± 0.3 mM) using 0.5 mM substrate concentrations to obtain IC_{50} values.

β-Glucocerebrosidase Activation Assay. Gaucher lymphoblasts (N370S) were cultured in the presence of various concentrations of inhibitor (0−50 μM) for 3 days before β-glucocerebrosidase activity was measured. Cells were washed twice in phosphate-buffered saline, homogenized in water using a small dounce

homogenizer, centrifuged at 800*g* for 5 min and the supernatant taken for protein and ss-glucocerebrosidase activity. Protein concentration was determined using the BCA assay (Pierce, UK) according to manufacturer's instructions. All enzyme activation measurements were made using aliquots of homogenate and 5 mM 4-methylumbelliferyl-β-glucoside in 0.1 M citrate phosphate buffer, pH 5.2 containing 0.25% sodium taurocholate, 0.1% TX100 as described above. Bromoconduritol (500 μM to 2.5 mM) was added to some enzyme activity determinations to confirm the specific hydrolysis of substrate by β-glucocerebrosidase. Enzyme activation is defined as the fold increase in enzyme activity (U/mg protein) in treated cells compared to untreated cells.

Cell Toxicity Assay. The effect of compounds on Gaucher lymphoblast cell proliferation (cytotoxicity) was determined using a Promega CellTiter 96 AQ_{ueous} Cell Proliferation Assay as described in the manufacturers instructions and previously published.[47] This assay utilizes 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) which is bioreduced in actively proliferating cells to a formazan. Cells were seeded at a density of 5 $\times$ 10^5 per ml in 96 well plates with or without a range of concentrations of compound and incubated at 37 °C for 16 h. MTS reagent (20 mL) was added to each well, and the plates were mixed and incubated at 37 °C for 1 h. The absorbance was read at 490 nm using a UV max Kinetic microplate reader to determine the amount of formazan product, which is directly proportional to the number of living cells in culture.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra of compounds **15**−**30** and **36**−**45** and examples of Lineweaver−Burk plots for K_i determinations of **38** and **43**. This material is available free of charge via the Internet at http://pubs.acs.org.

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