Combinatorial Approach to N-Substituted Aminocyclitol Libraries by Solution-Phase Parallel Synthesis and Preliminary Evaluation as Glucocerebrosidase Inhibitors

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Received June 15, 2006

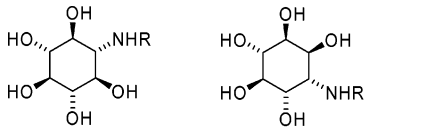
Libraries of N-substituted aminocyclitol derivatives of the *scyllo* and racemic *chiro* series by means of parallel solution-phase methodology with the help of robotic technology are described. Chemical diversity has been introduced by reaction of selected scaffolds with a set of aldehydes, acyl chlorides, sulfonyl chlorides, chloroformates, and amines to afford the corresponding amines, amides, sulfonamides, carbamates and ureas, respectively. The optimized methodology has proven excellent, in terms of overall purities of the resulting libraries, for the production of amides. Sulfonamides and carbamates have been obtained in slightly lower purities, while amines afforded modest results. Selected library members have been evaluated as inhibitors of recombinant glucocerebrosidase with K_i values ranging in the low micromolar scale for the most active members.

Introduction

Aminocyclitols represent a wide class of compounds with interesting biological properties.[1,2] Over the last years, there have been continuous efforts to obtain synthetic analogues with enhanced or more selective cellular profiles.[3] Thus, in addition to the classical aminocyclitol family of antibiotics,[4,5] certain aminocyclitol derivatives have been shown to be promising candidates for the development of therapeutic agents involved in the regulation of glucose metabolism,[6] as enzyme inhibitors with diverse biomedical applications,[7] and also as pharmacological tools for the study of the inositol phosphate cycle and related processes.[8]

As part of our studies on aminocyclitol derivatives as modulators of glycosphingolipid metabolism, we have recently developed a general regio- and diastereoselective synthetic strategy leading, inter alia, to aminocyclitols of the *scyllo* and *chiro* families.[9] In order to widen the scope of our methodology, we became interested in its extension to solution-phase parallel synthetic protocols to exploit the chemical diversity of this interesting kind of compounds.

In this context, within the frame of our ongoing projects related to aminocyclitol-derived glucocerebrosidase inhibitors as potential chemical chaperones for the treatment of Gaucher disease,[10] we focused on the development and optimization of solution-phase strategies amenable to robotic automation in a 96-well plate format. In this work, we report on the implementation of a combinatorial approach for the synthesis of aminocyclitol libraries of the *scyllo* (**1**) and *chiro* (**2**) series of aminocyclitols with different substitution patterns at the nitrogen atom (Figure 1). In addition, preliminary enzyme inhibition studies carried out on recombinant glucocerebrosidase will be presented.



1a: R=alkyl
1b: R=acyl
1c: R=sulfamoyl
1d: R=alkoxycarbonyl
1e: R=ureido

2a: R=alkyl
2b: R=acyl
2c: R=sulfamoyl
2d: R=alkoxycarbonyl

Figure 1. General structure of aminocyclitol libraries.

Library Design and Synthesis of Scaffolds

Libraries were designed by functionalization of scaffolds **A**−**E** (Scheme 1), which were synthesized by regio- and stereocontrolled opening from epoxide **(±)-3**, as previously reported.[9,11] Thus, azidolysis of **(±)-3** afforded azido alcohols **4** and **6**, whose reduction led to scaffolds **A** and **D**, respectively. Benzylation of the above azido alcohols followed by azide reduction of intermediate perbenzylated azido cyclitols **5** and **7** led to scaffolds **B** and **E**, respectively. Finally, scaffold **C** was obtained from **B** by treatment with phosgene following standard protocols (Scheme 1).[12]

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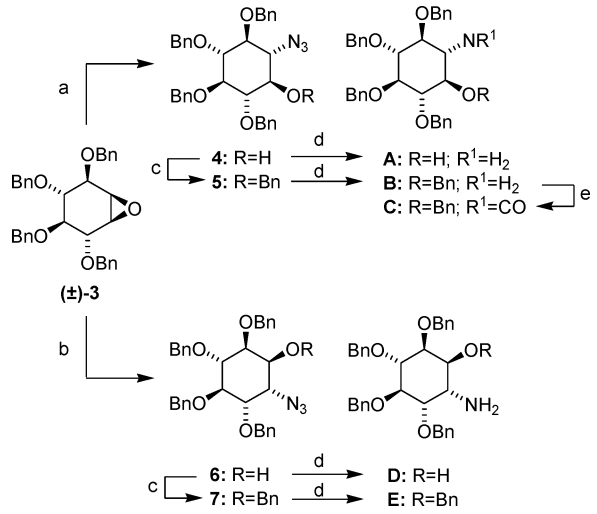
10.1021/cc060080o CCC: $37.00
Published on Web 11/18/2006

Scheme 1[a]



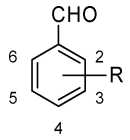
[a] Reagents and conditions: (a) NaN_3, 2*N* $LiClO_4$ in THF; (b) NaN_3, NH_4Cl in $MeOH/H_2O$; (c) BnCl, NaH, THF; (d) $LiAlH_4$, THF; (e) phosgene, DCM.

With scaffolds **A**−**E** in hand, cyclitol libraries were obtained by combination of each scaffold with a suitable building block collection.[13] Thus, a set of 85 aldehydes (**a1**−**a85**, Figure 2 and Tables 1−3) were submitted to reductive amination with scaffolds **A** and **D** to give libraries of amines **A(a1)**−**A(a85)** and **D(a1)**−**D(a85)**, respectively, whereas a library of ureas **C(e1)**−**C(e54)** (Scheme 2) was obtained by condensation of scaffold **C** with a collection of 54 amines (**e1**−**e54**, Figures 3 and 4). On the other hand, condensation of scaffolds **B** and **E** with a set of 43 acyl chlorides (**b1**−**b43**, Figure 5), 21 sulfonyl chlorides (**c1**−**c21**, Figure 6), and 17 chloroformates (**d1**−**d17**, Figure 7) led to the corresponding aminocyclitol libraries with amide, sulfonamide, and carbamate functionalities, respectively (Schemes 3 and 4). In all cases, massive removal of benzyloxy protecting groups (see below) was required to afford the final libraries of aminocyclitols **1** (*scyllo*) or **2** (*chiro*) (Schemes 2−4).

Library Production

An automated process was developed for library production. Reactions were carried out in 2 mL polypropylene 96-well plates, and reagents were added from stock solutions by means of a four-needle robot. This plate format avoided errors derived from sample handling since manipulation was reduced to a minimum. After addition of reagents, the plates

Table 1. Substituted Benzaldehydes Used in Reductive Amination with Scaffolds **A** and **D** (see also Figure 2 and Tables 2 and 3)



aldehyde	C(2)	C(3)	C(4)	C(5)	C(6)
a2	H	Me	OH	Me	H
a3	H	NO_2	OH	H	H
a4	H	F	MeO	H	H
a5	H	Me	OH	H	H
a6	H	H	OH	H	H
a7	H	F	F	H	H
a12	H	OH	MeO	H	H
a13	H	H	tBu	H	H
a14	H	H	H	H	H
a15	H	H	AcO	H	H
a16	H	Me	Me	H	H
a17	Me	F	H	H	H
a18	H	H	Ph	H	H
a20	H	Cl	H	H	H
a21	Me	H	H	H	H
a22	H	PhO	H	H	H
a23	H	CF_3	H	H	H
a24	H	H	F	H	H
a25	H	H	Cl	H	H
a29	H	H	iPr	H	H
a30	F	H	F	H	H
a31	H	F	Cl	H	H
a33	Cl	H	F	H	H
a35	H	H	COOMe	H	H
a36	H	H	CN	H	H
a39	H	MeO	Me	MeO	H
a41	H	OH	H	H	H
a47	H	H	MeO	H	H
a49	H	H	NO_2	H	H
a50	Me	H	Me	H	Me
a53	OH	H	H	NO_2	H
a54	COOH	H	H	H	H
a57	SO_3Na	H	H	H	H
a59	NO_2	H	H	H	H
a60	H	NO_2	H	H	H
a61	H	MeO	H	H	H
a62	H	H	*n*-C_5H_{11}	H	H
a63	MeO	H	H	H	H
a64	H	I	OH	MeO	H
a69	NO_2	H	NO_2	H	H
a72	CF_3	H	CF_3	H	H
a73	H	I	OH	I	H
a76	NO_2	H	Cl	H	H
a82	COOH	MeO	MeO	H	H
a85	OCH_2COOH	H	H	H	H
a86	CN	H	H	H	H

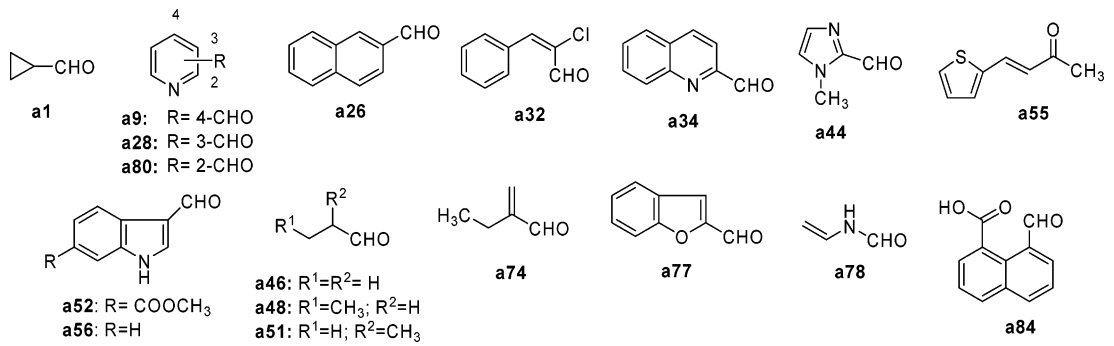
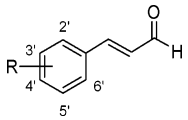


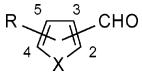
Figure 2. Aldehydes used in reductive amination with scaffolds **A** and **D** (see also Tables 1−3).

Table 2. Substituted Cinnamaldehydes Used in Reductive Amination with Scaffolds **A** and **D** (see also Figure 2 and Tables 1 and 3)



aldehyde	C(2′)	C(3′)	C(4′)	C(5′)	C(6′)
a11	H	H	Et_2N	H	H
a38	H	MeO	H	H	H
a40	H	MeO	MeO	H	H
a37	H	H	NO_2	H	H
a42	NO_2	H	H	H	H
a43	H	H	Me_2N	H	H
a45	H	H	MeO	H	H

Table 3. Heterocyclic Aldehydes: Furane and Thiophene Derivatives Used in Reductive Amination with Scaffolds **A** and **D** (see also Figure 2 and Tables 1 and 2)



aldehyde	X	C(2)	C(3)	C(4)	C(5)
a8	O	H	CHO	He	H
a10	O	CHO	H	H	H
a19	S	H	CHO	H	H
a27	S	CHO	H	H	H
a58	O	CHO	H	H	$MeCOOCH_2$
a65	O	CHO	H	Me	Me
a66	S	CHO	H	H	Me
a67	O	CHO	H	H	4-(Cl)Ph
a68	O	CHO	H	H	Et
a70	S	CHO	Me	H	H
a71	O	CHO	H	H	NO_2
a75	S	CHO	H	Br	H
a79	S	CHO	H	H	Cl
a81	S	CHO	H	H	NO_2
a83	O	CHO	H	H	Me

were shaken for the required time and temperature, and workup processes based on the used of scavengers were optimized in order to reduce further manipulation to simple filtrations. Samples were analyzed by HPLC coupled to a multichannel UV, ELS, and MS detectors (see Experimental Section). It is worth noting that, as a general trend, random ^{1}H NMR analyses showed that UV results were not as reliable as those obtained with ELS since some of the library members had little or no absorption in their UV spectra. For this reason, tandem HPLC−ELS−MS was routinely used as purity criterion.

A library of ureas was obtained as shown in Scheme 2 by condensation of **C** with amines **e1**−**e54** in a 1:1 DCM/DMF solvent system at rt for 24 h. The reaction mixture was quenched with Amberlyst 15 resin to remove excess amine, and purities (HPLC−ELS−MS) were above 80% for 69% of the library members (see Supporting Information).

Libraries of amides, sulfonamides, and carbamates (Schemes 3 and 4) derived from either scaffold **B** or scaffold **E** were synthesized in single plates by reaction with acyl chlorides, sulfonyl chlorides, and chloroformates, respectively. This plate design required the optimization of a general synthetic protocol suitable for the different types of reagents. Interestingly, the use of a slight excess reagent in DCM at rt, followed by quenching with 9:1 MeOH/H_2O and addition of Amberlite IRA-400 resin as acid scavenger was considered optimum in terms of simplicity and overall purities (HPLC−ELS−MS), which were above 80% for 72% amides **B(b)** and 53% amides **E(b)**, 42% sulfonamides **B(c)** and 38% sulfonamides **E(c)**, and 47% carbamates **B(d)** and 35% carbamates **E(d)** (see Supporting Information). In general, scaffold **B** afforded slightly better results than scaffold **E** in these transformations.

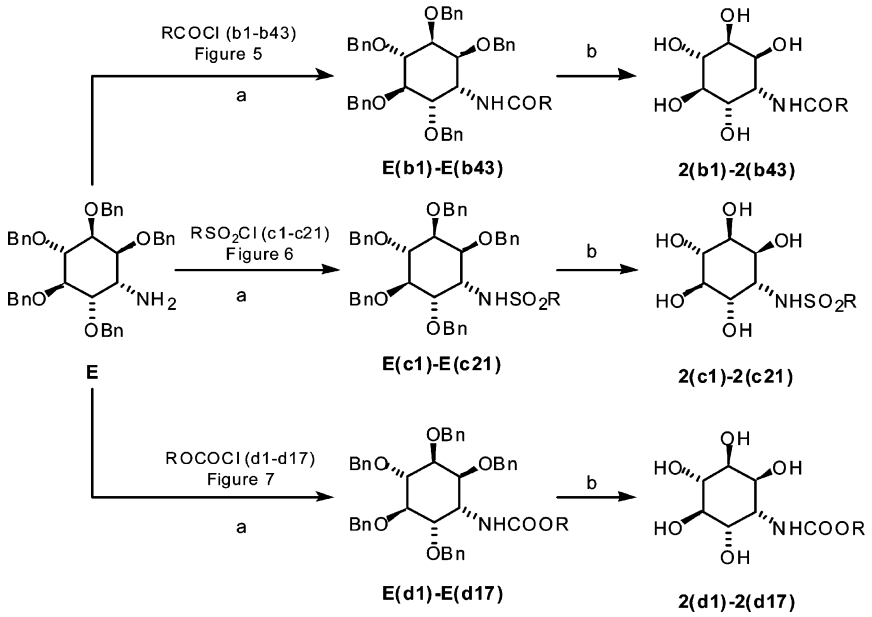
Reductive amination of the selected aldehydes **a1**−**a85** with scaffolds **A** and **D** was carried out using cyanoborohydride on Amberlite IRA-400 resin as reducing agent (Scheme 2). Initial experiments in solution with aliphatic aldehydes under slightly acidic conditions showed the efficiency of this classical protocol.[14−16] Adaptation to our automated system required a previous adjustment of the stoichiometry for good overall conversions. In this context, the use of triethylorthoformate was also required to favor the usually sluggish formation of the intermediate imines.[17] Purities of the resulting libraries according to this protocol were lower than those described above, since only 12% amines **A(a)** and 27% amines **D(a)** showed purities higher than 80% (HPLC−ELS−MS), while purities over 70% were found for 48% amines **A(a)** and 38% amines **D(a)** (see Supporting Information). Data analysis showed that unreacted scaffolds were the major contaminants in most cases.

As stated above, OBn deprotection was required for all library members in order to obtain target aminocyclitols. This step required a careful adjustment of the reaction conditions and experimental setup in order to make the process compatible with the restrictions imposed by the automated system. Thus, despite preliminary "on bench" experiments using catalytic (Pd/C) hydrogenation in the presence of ammonium formate as hydrogen source had proved satisfactory, technical considerations made not possible its application to our automated parallel synthetic protocol.[18] As an alternative, BCl_3 in DCM at −78 °C had also been shown to be very efficient for our purposes since it cleanly afforded the required fully deprotected aminocyclitol hydrochlorides in quantitative yields.[9] However, the use of air-sensitive reagents at low temperatures over long reaction times in the automated library production setup used for the above libraries was precluded. For this reason, we carried out *O*-benzyl deprotections by means of a conventional "on-bench" parallel system. Despite this protocol was applied to all library members, only fully deprotected aminocyclitols arising from O-benzylated precursors of purities higher than 70% (HPLC−ELS−MS) were submitted to enzyme inhibition studies.

Biological Results and Discussion

According to the above purity criteria, selected library members were evaluated as inhibitors of recombinant glucocerebrosidase (Imiglucerase, Cerezyme from Genzyme) at 100 and 200 μM inhibitor concentrations (see Supporting Information for details). For the most active members of each series, IC_{50} and K_i values were calculated. In all cases, active compounds behaved as competitive inhibitors of Imiglucerase (Table 4).

Scheme 2[a]



[a] Reagents and conditions: (a) cyanoborohydride on Amberlite IRA-400 resin, AcOH, 1:1 MeOH/$(MeO)_3C$; (b) 1, amines in DMF; 2, Amberlyst 15; (c) BCl_3 (1M in heptane), DCM, −78 °C.

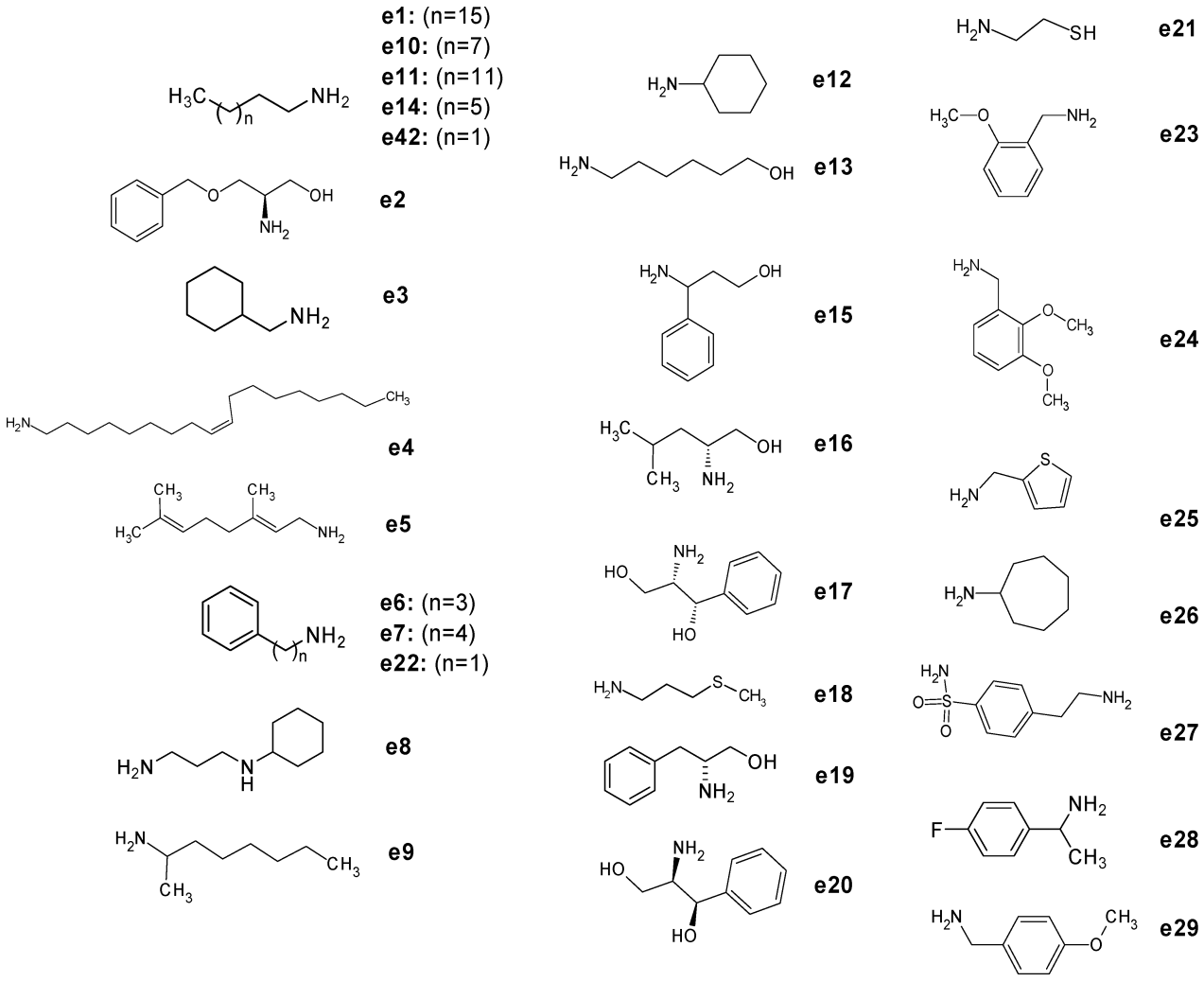


Figure 3. Amines used in the synthesis of ureas by condensation with scaffold **C**.

In a previous work,[10] we tested a limited number of amides, which were mostly inactive. In the present paper, a large variety of amides (62) as well as sulfonamides (24) and carbamates (22) with *scyllo* or *chiro* configuration were assayed.[19] All compounds of the above series were inactive except amides **1(b06)** and **1(b40)** with K_i values around 200

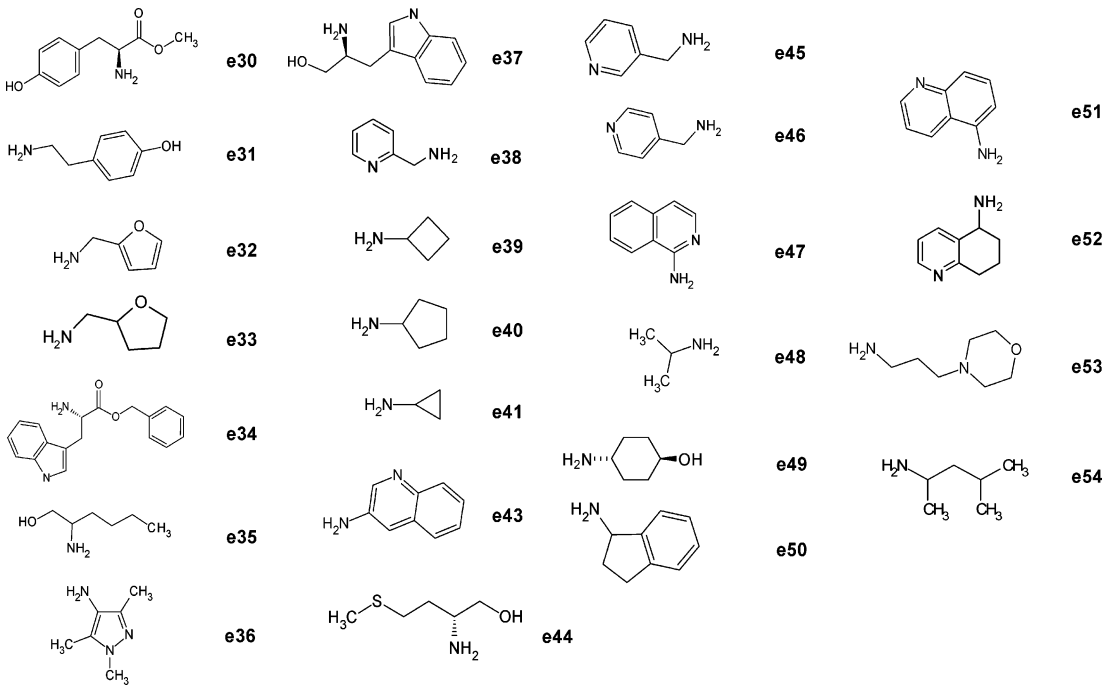


Figure 4. Amines used in the synthesis of ureas by condensation with scaffold **C**.

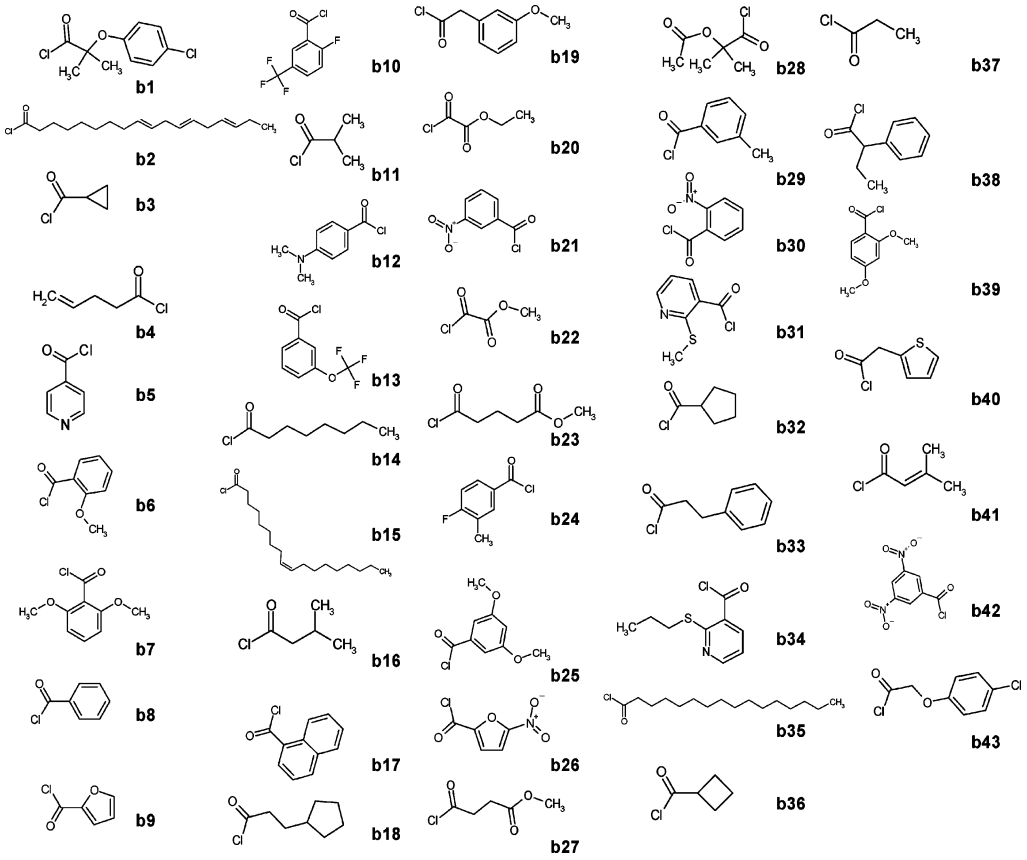


Figure 5. Acyl chlorides used in the synthesis of amides from scaffolds **B** and **E**.

μM and sulfonamide **1(c14)** with a K_i value of 47 μM (Table 4, see also Figures 5 and 6 for chemical structures). On the other hand, among the 42 ureas tested,[19] 14 exhibited low activity at 200 μM (see Supporting Information) whereas 25 showed inhibitory enzymatic activity with IC_{50} values in the range of 108−218 μM. Interestingly, ureas **1(e13)**, **1-(e14)**, and **1(e29)** exhibited K_i values of 49.0, 55.2, and 31.5 μM, respectively (Table 4, see also Figures 3 and 4). In general, long chain aliphatic and para-substituted benzyl ureas were the most potent ones in our assay.

We had also reported[10] that inosamines of general structure **1** (*scyllo*) were better inhibitors of recombinant glucocer-

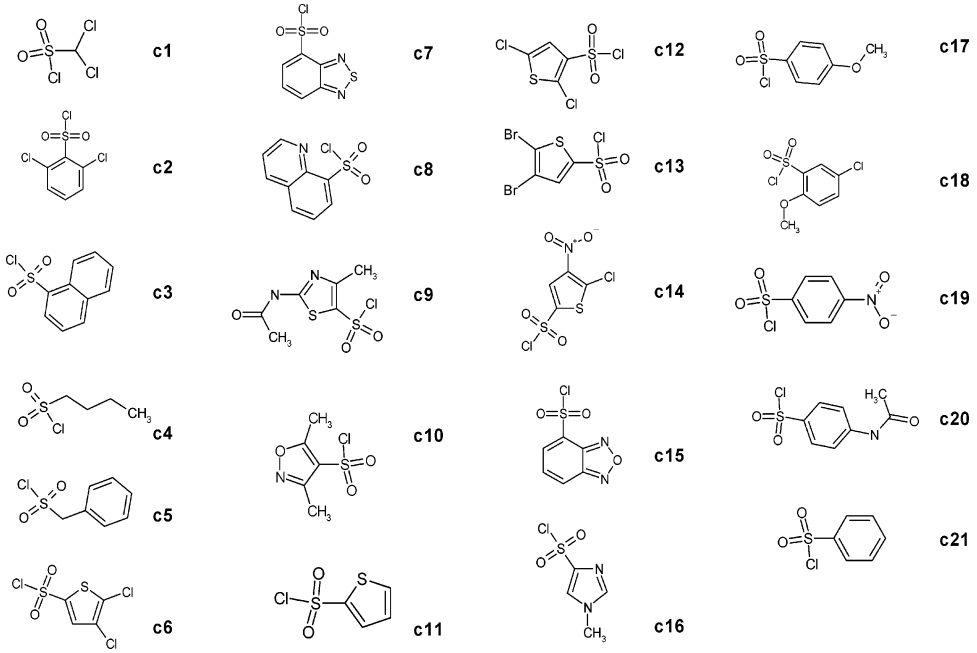


Figure 6. Sulfonyl chlorides used in the synthesis of sulfonamides from scaffolds **B** and **E**.

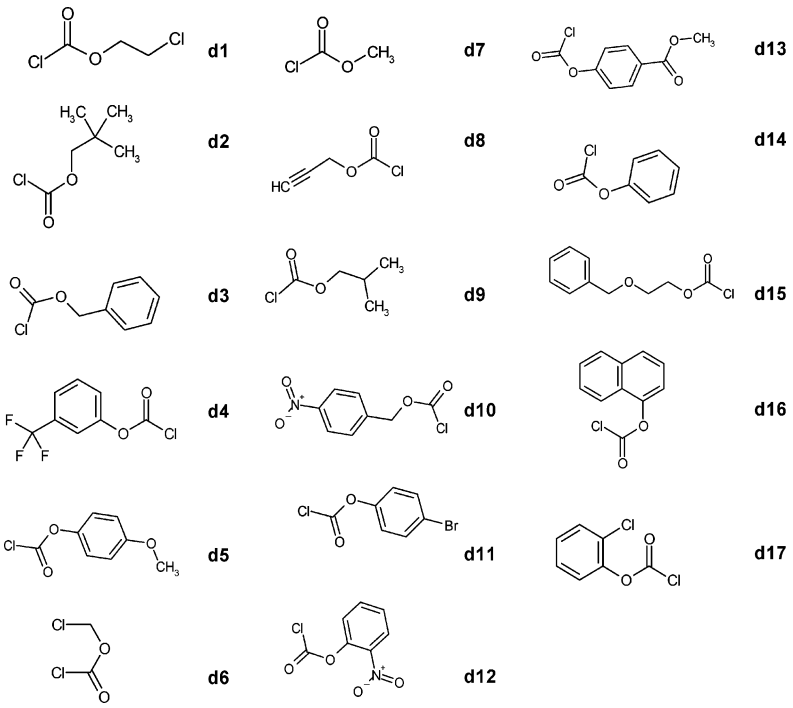
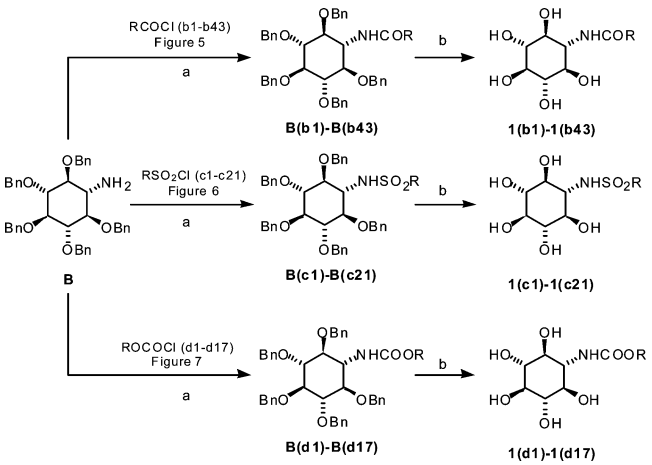


Figure 7. Chloroformates used in the synthesis of carbamates from scaffolds **B** and **E**.

ebrosidase than diastereomeric aminocyclitols **2** (*chiro*) (see Scheme 2). This finding was reasoned by considering the structural similarities between inosamines **1** and the enzyme substrate glucosyl ceramide. In the present work, a library of 41 inosamines **1(a)** and 32 insosamines **2(a)**,[19] mostly showing substituted *N*-benzyl groups, were tested. Among inosamines **1(a)** (*scyllo* configuration) the highly lipophilic *N*-(4-phenyl)benzyl **1(a18)** and *N*-(3-phenoxy)benzyl **1(a22)** derivatives were the best inhibitors (Table 4). *N*-(2-Methoxy)-benzyl inosamine **1(a63)** was also a good inhibitor with a K_i value of 28 μM. Except for compounds **1(a7)** and **1(a64)**, the remaining halogenated benzyl inosamines [**1(a17)**, **1-(a20)**, **1(a23)**, **1(a25)**, **1(a30)**, **1(a31)**, **1(a33)**, and **1(a76)**, see Table 1] were active, especially those having the electron-withdrawing *m*-F group. As a general trend, halogenated *N*-benzyl derivatives derived from *chiro* inosamines **2(a)** exhibited lower inhibitory activities than their *scyllo* counterparts **1(a)**.

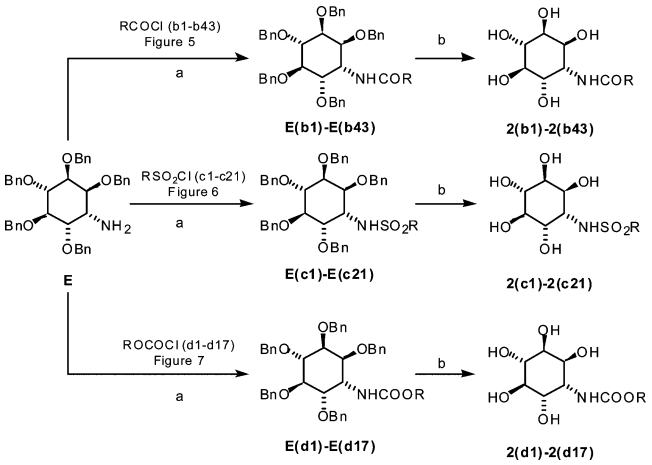
Interestingly, some *chiro* inosamines **2(a)** showed a weak but significant activity as well as a differential inhibition profile with respect to their *scyllo* counterparts **1(a)**. Thus, inosamines **2(a33)** and **2(a63)** (see Table 3) were among the best inhibitors tested, with K_i values of 8.9 and 7.2 μM, respectively, whereas their corresponding *scyllo* analogues

Scheme 3[a]



[a] Reagents and conditions: (a) reagent, DCM, rt, 20 h; (b) BCl_3 (1M in heptane), DCM, −78 °C.

Scheme 4[a]



[a] Reagents and conditions: (a) reagent, DCM, rt, 20 h; (b) BCl_3 (1M in heptane), DCM, −78 °C.

1(a33) and **1(a63)** were around 4 times less active. Similarly, inosamines **2(a50)**, **2(a57)**, **2(a65)**, and **2(a66)**, showing a heterocyclic moiety, were more active than their corresponding *scyllo* analogues **1(a)**, suggesting the operation of specific interactions with the aromatic residues of the enzyme active center.[20] These interactions could overcome the postulated structural dissimilarity of *chiro* inosamines **2(a)** with the enzyme substrate.[10] In all cases, Lineweaver−Burk plots of assays performed with Imiglucerase at different substrate and inhibitor concentrations showed that these compounds behave as noncompetitive inhibitors, indicating that substrate and inhibitor bind reversibly and independently at different enzyme sites. Replots of slopes versus the inhibitor concentration indicated K_i values in the low micromolar range for the most potent library members, as illustrated in Figure 8 for **1(a22)** and **2(a33)**.

Experimental Section

For general synthetic methods, see Supporting Information.

Synthesis of Scaffolds. Scaffolds **4**, **5**, **6**, **A**, **B**, and **D** were obtained from epoxide **(±)-3** following our previously described protocols.[9,10] The remaining scaffolds and precursors were obtained as follows:

Table 4. IC_{50} (μM) Values of Selected Compounds[a,b]

compd	IC_{50} (K_i)[c]	compd	IC_{50} (K_i)[c]	compd	IC_{50} (K_i)[c]
1(a02)	122	**1(a83)**	205	**1(e36)**	155
1(a13)	180	**1(a85)**	203	**1(e39)**	140
1(a14)	175	**1(b06)**	203 (218)	**1(e40)**	108
1(a17)	76 (31.2)	**1(b40)**	220 (250)	**1(e41)**	139
1(a18)	63 (16.8)	**1(c14)**	158 (47)	**1(e42)**	159
1(a20)	145	**1(e07)**	160	**1(e46)**	184
1(a21)	105	**1(e09)**	170	**1(e47)**	218
1(a22)	35 (4.0)	**1(e13)**	78 (49.0)	**1(e48)**	165
1(a23)	98	**1(e14)**	83 (55.2)	**1(e49)**	168
1(a25)	112	**1(e15)**	184	**2(a33)**	28 (8.9)
1(a26)	162	**1(e16)**	187	**2(a25)**	79 (45.3)
1(a30)	134	**1(e18)**	200	**2(a18)**	190
1(a31)	102	**1(e19)**	188	**2(a22)**	150
1(a32)	140	**1(e22)**	157	**2(a26)**	200
1(a33)	200	**1(e23)**	198	**2(a47)**	200
1(a57)	198	**1(e24)**	177	**2(a50)**	150
1(a61)	187	**1(e25)**	188	**2(a56)**	200
1(a62)	180	**1(e26)**	210	**2(a57)**	81 (48.1)
1(a63)	86 (27.8)	**1(e27)**	195	**2(a63)**	35 (7.2)
1(a76)	220	**1(e28)**	125	**2(a65)**	100
1(a77)	196	**1(e29)**	41 (31.5)	**2(a66)**	170
1(a83)	205	**1(e31)**	160		
		1(e34)	170		

[a] See Schemes 2−4, Figures 2−6, and Tables 1−3 for compound identification. [b] Only library members of purities higher than 70% were screened. [c] K_i values (μM) are shown in parentheses.

(1*RS*,2*SR*,3*RS*,4*SR*,5*SR*,6*SR*)-1-Azido-2,3,4,5,6-pentakis-benzyloxycyclohexane (7). A solution of azido alcohol **6**[9] (1.4 g, 2.48 mmol) in anh THF (20 mL) was dropwise added over an ice-cooled suspension of NaH (60% dispersion in mineral oil, 270 mg, 11.25 mmol), previously washed with hexane (3 × 10 mL) in THF (25 mL) under argon. Stirring was continued until complete gas evolution, and benzyl chloride (0.45 mL, 3.79 mmol) was added next. The reaction mixture was stirred at rt for 24 h, quenched with water (10 mL) and extracted with Et_2O (3 × 25 mL). The combined organic layers were washed with brine (20 mL), dried, and evaporated. The resulting oil was purified by flash chromatography using a mixture of hexane/EtOAc (4:1) as eluent to afford azide **7**.

IR (KBr, cm^{-1}): 2920, 2103. ^1H NMR (500 MHz, $CDCl_3$): 7.50−7.30 (m, 25H), 4.93−4.85 (m, 5H), 4.77 (d, 1H, J = 9.5 Hz), 4.75 (d, 1H, J = 9.5 Hz), 4.72 (d, 1H, J = 12 Hz), 4.62 (dd, 1H, J = 12 Hz, J' = 4.5 Hz), 4.47 (d, 1H, J = 12 Hz), 4.0 (dd, 1H, J = 9.5 Hz, J' = 4 Hz), 3.91 (t, 1H), 3.84−3.77 (m, 2H), 3.71−3.68 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 138.8, 138.7, 138.4, 138.0, 137.9, 128.4−127.5, 81.9, 81.6, 79.4 (2×), 76.0, 75.8, 75.3, 73.6, 73.4, 73.3. 60.2, 29.6. EIMS $[M + H]^+$: 657.

Scaffold C: (1′*RS*,2′*RS*,2′*RS*,3′*SR*,4′*SR*,5′*RS*,6′*SR*)-*N*-(2,3,4,5,6-Pentakis-benzyloxycyclohexyl)isocyanate. In a well-ventilated hood (*caution: phsosgene is volatile and toxic*), a solution of phosgene in toluene (0.6 mL of a 2 M solution in toluene) is added dropwise to an ice-cooled solution of scaffold **B**[10] (500 mg, 0.79 mmol) in DCM (30 mL). The reaction mixture is stirred for 1 h while the temperature is allowed to rise to rt over this period. The resulting cloudy reaction mixture was treated with 0.1 N NaOH (20 mL), phases were separated, and the aqueous phase was washed with 20 mL of DCM. The combined organic extracts were dried and evaporated to afford scaffold

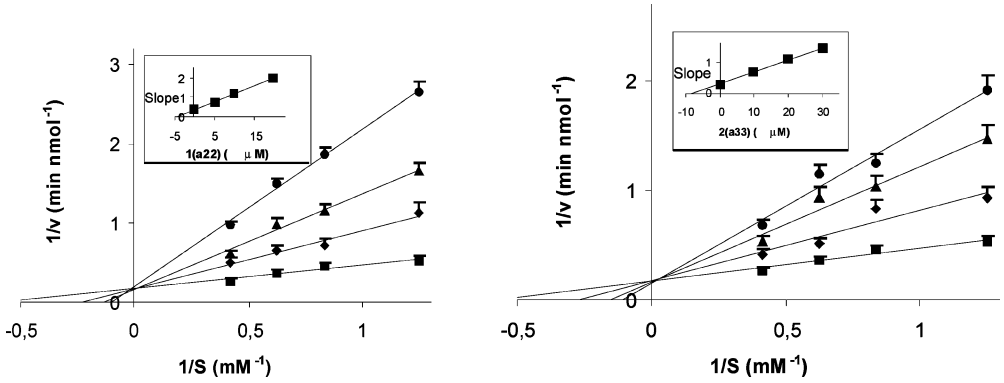


Figure 8. Lineweaver−Burk plot for **1(a22)** and **2(a33)** with Imiglucerase.

C (490 mg, 95%), which was used for library production without further purification.

IR (KBr, cm^{-1}): 2200. ^1H NMR (300 MHz, $CDCl_3$): 7.39−7.26 (m, 25H), 4.96−4.86 (m, 10H), 3.74−3.58 (m, 5H), 3.47−3.44 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, 300 MHz): 138.1, 137.5, 128.4−127.6, 82.6, 82.5, 80.9, 76.5−75.9, 60.1, 39.7. EIMS $[M + H]^+$: 656.

Scaffold E: (1*RS*,2*SR*,3*RS*,4*SR*,5*SR*,6*SR*)-1-Amino-2,3,4,5,6-pentakis-benzyolxycyclohexane. A solution of the azide **7** (1.3 g, 2.0 mmol) in anh THF (30 mL) was added to a cooled solution (0 °C) of $LiAlH_4$ (45 mg, 1.2 mmol) in THF (30 mL) under argon. The reaction mixture was stirred for 30 min at rt and next quenched by slow addition of EtOAc (20 mL) and H_2O (20 mL). The mixture was extracted with Et_2O (3 × 50 mL), and the combined organic layers were washed with brine (50 mL), dried, and concentrated under reduced pressure. The crude product was purified by flash chromatography on deactivated silica gel (3% Et_3N) using DCM/MeOH (12:1) as eluent to afford the final compound.

IR (KBr, cm^{-1}): 3100, 2900, 1650−1490. ^1H NMR (500 MHz, $CDCl_3$): 7.40−7.25 (m, 25H), 4.90−4.50 (m, 10H), 4.20−3.80 (m, 2H), 3.80−3.70 (m, 2H), 3.67 (m, 1H), 3.40 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, 300 MHz): 139.3, 139.2, 139.0, 138.9, 138.8, 128.6−127.6, 82.4, 82.0, 80.1, 80.0, 78.2, 76.0, 75.9, 73.5, 73.2, 73.1, 50.7. EIMS $[M + H]^+$: 630.

Automated Library Production. Reactions were carried out in a 2 mL polypropylene 96-well plate format, which was held in Charybdis frames. After the reaction, plate evaporation was achieved with a HT12-series II system from Genevac. All liquid handling for reactants and reagents was performed using a four-needle MultiProbe HT from Perkin-Elmer using specifically designed racks to hold the reagent tubes and the reaction plates. An eight-needle MultiProbe HT from Perkin-Elmer was use for all plate replication. After the addition of the reactants, the plates were shaken at 800 rpm for the required time and temperature. Reaction workups were optimized for the use of scavengers and/or simple filtrations. In all cases, transfers were carried out by means of an eight-needle robot, solvents were evaporated, and each plate was portioned into different stock plates for analytical purposes and biological screening.

All HPLC−ELS−MS analytical spectra for libraries **A-E** (benzylated compounds) were recorded for each sample using the following conditions: MS: Micromass MUX 9 way, ESP+ve, CV 25V, 100−1000 amu (PT1-3 Fractionlynx, ESP±, ELS + DAD available); HPLC: Luna C18 (2), 50 × 4.6 mm i.d., 3 μm; A, acetonitrile + 0.1% formic Acid (v/v); B, water + 0.1% formic acid (v/v) 10% A (1 min) to 95% A in 4 min, hold 1 min, rt 6.0 min, 2.0 mL/min; UV-260 mn; sample solvent: DMSO. Analytical data were processed with MassLynx diversity software (Waters Corporation). HRMS were obtained with a Waters Micromass LCT Premier apparatus equipped with a dual electrospray (ESI) LockSpray ion source and data were acquired in positive ESI. Compounds were analyzed by flow inject analysis using acetonitrile/water (75:25) as mobile phase

Plate 1: Synthesis of Ureas by Reaction of Scaffold C with Amines e51−e54. Scaffold **C** (400 μL, 35 μmol, from a DMF stock solution) was added to each well plate by means of an automatic sampler. Amines **e51−e54** (38.5 μmol in 400 μL from DCM stock solutions) were next added likewise. Reaction plates were shaken at 800 rpm for 20h at rt, and solvent was next evaporated by means of a centrifugal evaporator. Each well was reconstituted with MeOH (1 mL), and activated Amberlyst 15 resin (around 80 mg) was added. Plates were next shaken at 800 rpm for 3h at rt, well contents were filtered to a second plate, and resins were washed with additional MeOH (300 μL). Aliquots (2 μL) were taken for analytical purposes (HPLC−ELS−MS, see Supporting Information), and plates were finally evaporated to dryness.

Synthesis of Amides, Sulfonamides, and Chloroformates by Reaction of Scaffolds B (plate 2) and E (plate 3) with Acyl Chlorides (b1−b43), Sulfonyl Chlorides (c1−c21), and Chloroformates (d1−d17). Scaffolds **B** or **E** (200 μL, 35 μmol, from DCM stock solutions) were added to each well plate by means of an automatic sampler, and a solution of the corresponding reagent (38.5 μmol in 200 μL from DCM stock solutions) was next added. Reaction plates were shaken at 450 rpm for 20 h at rt, and solvent was next evaporated by means of a centrifugal evaporator. Each well was reconstituted with a 9:1 mixture of MeOH/H_2O (1 mL) and treated with activated Amberlite IRA-400 resin (around 80 mg). Plates were next shaken at rt for 20 h at 800 rpm, well contents were filtered to a second plate, and resins were washed with additional MeOH (300 μL). Aliquots (2 μL) were taken for analytical purposes (HPLC−ELS−MS, see Supporting Information) and the combined extracts were evaporated to dryness.

Synthesis of Amines by Reductive Amination of Aldehydes (a1−a85) with Scaffolds A (Plate 4) and D (Plate 5). Scaffolds **A** or **D** (400 μL, 35 μmol, from a 1:1 MeOH/$(MeO)_3C$ stock solution) were added, by means of an automatic sampler, to well plates containing 27 mg (175 μmol) of cyanoborohydride on Amberlite IRA-400 resin. Each well was next sampled with 100 μL of 0.4 N AcOH in MeOH, followed by 200 μL of a stock solution of the required aldehyde in 1:1 MeOH/$(MeO)_3C$. Reaction plates were shaken at 800 rpm for 20h at rt, filtered, and evaporated to dryness. Each plate was reconstituted by addition of MeOH (300 μL), and aliquots (2 μL) were taken for analytical purposes (HPLC−ELS−MS, see Supporting Information) prior final evaporation to dryness.

Parallel Debenzylaton Reactions. Plates 1−5 were reconstituted in DCM (500 μL), cooled to −78 °C, and treated with 350 μL of 1 M BCl_3 in heptane. After shaking for 2h at −78 °C, the plates were allowed to warm to rt and further shook for additional 20 h before quenching at −78 °C with MeOH (500 μL). Centrifugal evaporation led to residues that were reconstituted in EtOAc (800 μL). Subsequently, all plates were centrifuged, and the solvent was removed by pipetting. This process was repeated twice, and the resulting solids were dried by centrifugal evaporation. Only library members arising from benzylated precursors showing purities higher than 70% (HPLC−LS−MS) were analyzed by HRMS and screened against Imiglucerase (see Supporting Information for details).

Enzyme Inhibition Assays. Imiglucerase activity was determined with 2.4 mM 4-methylumbelliferyl-β-D-glucopyranoside in the presence of 0.25% (w/v) sodium taurocholate and 0.1% (v/v) Triton X-100 in McIlvaine buffer (pH 5.2) as previously described.[10] The IC_{50} values were determined by plotting percent activity versus log [I], using at least five different inhibitor concentrations. Type of inhibition and K_i values for more active inhibitors were determined by Lineweaver−Burk or Dixon plots of assays performed with different concentrations of inhibitor and substrate (see Supporting Information for details).

Conclusions

Libraries of *scyllo* N-substituted aminocyclitol derivatives and racemic *chiro* counterparts have been produced by means of parallel solution-phase methodology with the help of robotic technology. Chemical diversity has been introduced by reaction of selected scaffolds with a set of aldehydes, acyl chlorides, sulfonyl chlorides, chloroformates, and amines to afford the corresponding amines, amides, sulfonamides, carbamates and ureas, respectively. The optimized methodology has proven excellent for the production of amides, in terms of overall purities of the resulting libraries. Sulfonamides and carbamates have been obtained in slightly lower efficiency, while amines afforded only modest results. Selected library members have been evaluated as inhibitors of recombinant glucocerebrosidase with K_i values ranging in the low micromolar scale for the most active members. The results here presented give support to our initial findings concerning the structural requirements of N-substituted aminocyclitols as glucocerebrosidase inhibitors. In general, the need for a basic nitrogen atom can be inferred from the weak activity elicited by the libraries of amides, sulfonamides and carbamates tested. As expected, the slightly more basic ureas afforded better, although still weak, inhibitors, while inosamines afforded the most potent compounds. Interestingly, the noticeable inhibitory activity shown by some *N*-benzyl-substituted *scyllo* and *chiro* inosamines has disclosed new hits which will be amenable for further refining by SAR studies. On the other hand, the design of selective competitive inhibitors of glucocerebrosidase can be exploited for the development of chemical chaperones[21] as an alternative therapy for the treatment of Gaucher disease.

Acknowledgment. Partial financial support from Ministerio de Ciencia y Tecnología (Spain) (Projects MCYT BQU2002-03737 and CTQ2005-00175/BQU), Fondos Feder (EU), Ministerio de Sanidad y Consumo, Spain (Project FIS, PIO40767), CSIC (Project 2004-80F026), and Generalitat de Catalunya (Projects 2005SGR01063) is acknowledged. P.S. and M.E.-G. thank Spanish "Ministerio de Educación y Cultura" and CSIC, respectively, for predoctoral fellowships. The authors also thank Genzyme for a generous gift of Imiglucerase (Cerezyme) and Mrs. Mireia Garrido for experimental contributions.

Supporting Information Available. General synthetic methods and resin activation protocols; purities of the libraries (% ELS); NMR of scaffolds **C**, **E**, and **7**; HRMS data of screened compounds; glucocerebrosidase inhibition data; and K_i plots of **1a(22)**, **1e(29)**, and **2a(33)** as representative examples. This material is available free of charge via the Internet at http://pubs.acs.org.

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