Cyclic guanidine containing amino acids that promote glucocerebrosidase

Cyclic guanidine containing amino acids that promote glucocerebrosidase

Zahraa S. Al-Taie,[a] Barbara Bartholomew,[b] Simon J. Coles,[c] Daniel M Evans,[d] Jackie Hollinshead,[b] Leigh F. Jones,[e] Rolf Kraehenbuehl,[f] Patrick J. Murphy,[d*] Robert J. Nash,[b*] Yana B. Penkova,[b] and Graham J. Tizzard.[c]

a) Department of Chemistry, College of Science, Al-Nahrain, Baghdad, Iraq.
b) PhytoQuest Limited, Plas Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, UK
c) UK National Crystallography Service, Chemistry, Faculty of Engineering and Physical Sciences, University of Southampton, SO17 1BJ. UK.
d) School of Chemistry, Bangor University, Gwynedd, LL57 2UW, UK.
e) School of Biological, Physical and Forensic Sciences, The University of Wolverhampton, Wulfruna Street, Wolverhampton. WV1 1LY. UK.
f) Centre for Environmental Biotechnology, Bangor University, Deiniol Rd, Bangor, LL57 2UW, UK.

Abstract: Novel cyclic guanidine containing amino acids (*S*)-**1**, **2** and **3** were prepared from L-serine **5**. (*S*)-2-Imino-3-methylimidazolidine-4-carboxylic acid hydrochloride salt **2**·HCl and (3S,6S)-3,6-bis(hydroxymethyl)-1,4-dimethylpiperazine-2,5-dione **15** showed in vitro promotion of the enzyme glucocerebrosidase.

Keywords: Cyclic guanidines; glycosidase inhibitors; glycosidase promoters; *N*-methylation;

1. Introduction

Cyclic guanidines are of considerable interest to both synthetic and medicinal chemists due to a wide range of biological activities and significant synthetic challenges.[1] Cyclic guanidine containing amino acids are of particular interest and several naturally occurring examples are known including L-enduracididine (I),[2] L-β-hydroxyenduracididine (II),[3] and capreomycidine (III),[4] (figure 1). These compounds, isolated in their free form or as sub-units of cyclic peptides[3,5] have been the subject of considerable synthetic effort.[6]

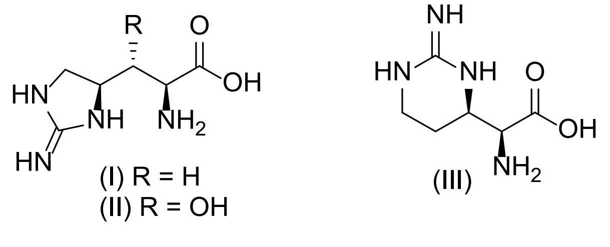


Figure 1. Naturally occurring cyclic guanidine amino acids.

We were interested in the preparation of the cyclic amino acid **1** and the selectively methylated derivates **2** and **3** as part of our studies in the preparation of cyclic guanidines,[7] (Figure 2).

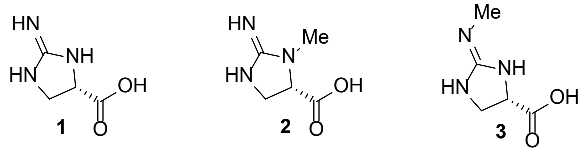
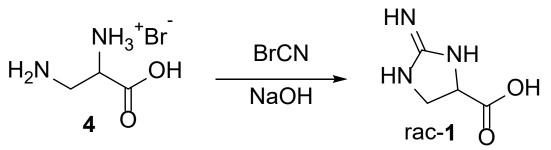


Figure 2. Synthetic cyclic guanidine amino acids.

2. Results and discussion

On investigation of the literature for the synthesis of similar structures we found a report of the synthesis of rac-**1** from the reaction of the diamine **4** with cyanogen bromide.[8] (Scheme 1) A homochiral version of this synthesis might be possible if the diamine could be made in enantiomerically pure form but it was felt that this might present a significant synthetic challenge.



Scheme 1. Reported synthesis of Racemic **1**.[8.]

The carbon backbones of **1-3** resemble the amino acid L-serine **5** and for this reason, this was selected as the starting material for an enantioselective multi-step synthesis. Our initial proposed synthesis was to convert L-serine **5** into the cyclic guanidine **7** via esterification, guanidinylation with **6** and cyclisation. The corresponding synthesis of the *N*-methylated intermediate **9** should similarly be realized by the guanidinylation of the known[9] intermediate with **6** followed by cyclisation, (figure 3).

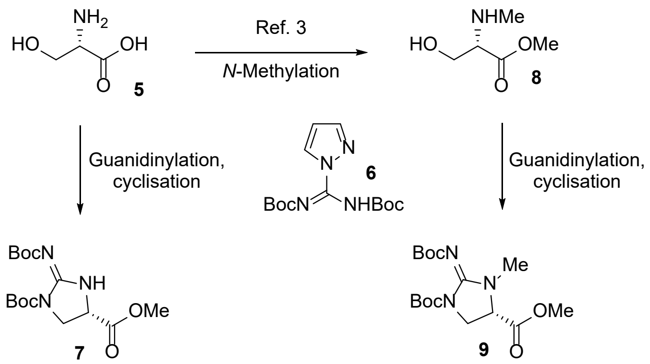
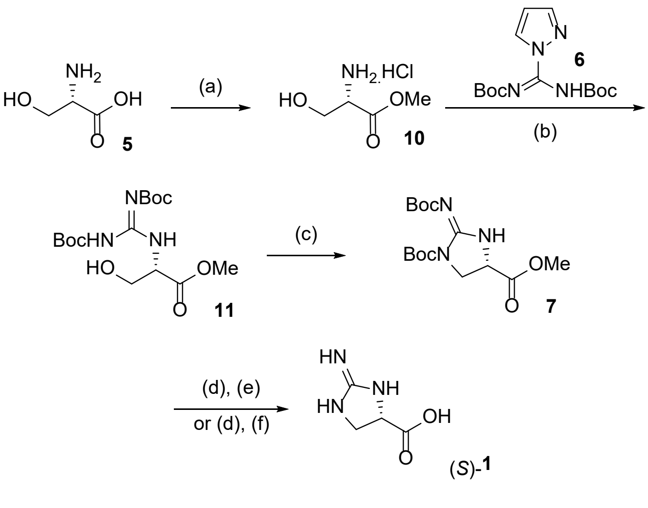


Figure 3. Proposed synthetic routes.

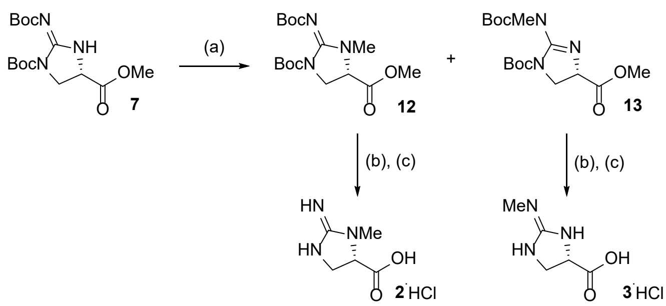
Thus, the esterification of the carboxylic acid group of L-serine **5** with methanol gave ester **10** in 97% yield.[10] Guanidinylation of **10** was easily achieved in 74% yield by treatment with **6** in the presence of triethylamine in methanol to give **11**. Cyclisation **11** was achieved by treatment of dichloromethane solution of **11** sequentially with PPh_3, imidazole and I_2 at -20 °C for three hours to give the key intermediate **7** in 64% yield.[11] We next attempted its deprotection. On treatment of **7** with excess sodium hydroxide in methanol for 24 hours we found that on work up several different products were formed which corresponded to the partial hydrolysis of the Boc protecting groups and the ester. The addition of further equivalents of sodium hydroxide in an attempt to remove the remaining protecting groups led to a complex mixture of compounds (NMR) and this method was abandoned. It was thus decided to remove the Boc protecting groups first, followed by the hydrolysis of the methyl ester. Cleavage of the Boc-protecting groups in **7** was achieved using excess TFA in dichloromethane, which was followed by treatment with ethanolic NaOH solution to cleave the ester group, followed by acidification with HCl (1M). Several problems were associate with the work up of this reaction. Initially it was attempted to remove the NaCl impurity by evaporation of the reaction and trituration of the mixture with absolute ethanol, followed by filtration. However, it was apparent that the compound isolated from this process was contaminated by the corresponding ethyl ester of (*S*)-**1**, which has arisen from re-esterification under the acidic conditions of the work up. This mixture could be re-hydrolised in aqueous NaOH and after acidification, purification by ion exchange chromatography gave (*S*)-**1** as a free base in 15% yield over 2 steps. We repeated the two hydrolysis stages using aqueous NaOH and omitted the ethanol trituration and purified the mixture by dissolving it in a minimum volume of water and precipitated the NaCl impurity by the addition of acetone. This gave (*S*)-**1**·HCl in 63% yield. (Scheme 2)



Scheme 2. a) i) MeOH, $SOCl_2$, 0-5 °C, 24 h, ii) 60 °C, 3 h, 97 %. (b) **6** (1.5 equiv.), NEt_3, MeOH, rt, 36 h, 74 %. (c) PPh_3, I_2, imidazole, CH_2Cl_2, -20 °C, 3h, 64 %. (d) TFA, dry DCM, rt, 24 h (e) i) 1M NaOH, 24 hr, ii) 1M HCl., iii) Ion exchange, 15% (over two steps), (f) i) 1M NaOH, 24 hr, ii) 1M HCl., iii) Precipitation from water with acetone, 63% (over two steps).

We next prepared the *N*-methylated amino acid ester **8**[9] and attempted the same sequence of reaction. Unfortunately, the more sterically hindered secondary amine **8** did not undergo reaction with **6**, even under prolonged forcing conditions. We thus reappraised the synthetic route and proposed the reversal of the two reactions and attempted the *N*-methylation of **7** which we hoped would give **9**, which in turn, should be easily deprotected to give **2**.

The *N*-methylation of the guanidine **7** was now investigated and a literature report[12] of the *N*-methylation of aliphatic amino acids using sodium hydride and methyl iodide in THF/DMF mixtures was of note. As the Boc-protected guanidine NH is likely to be more acidic than the corresponding amino acid we assumed a weaker base might be more effective than NaH and thus treated **7** with excess of MeI in the presence of K_2CO_3 in DMF. (Scheme 2) On work up we found that we obtained a 30:70 mixture of **12** and **13** in 79% yield which were separable by chromatography. The structures of **12** and **13** were confirmed by HMBC correlations to the *N*-methyl group in these and subsequent compounds. Deprotection of **12** was achieved using TFA followed by hydrolysis with aqueous NaOH and final purification by precipitation from water with acetone. This gave **2**·HCl in 41% yield over two steps from **7**. A similar deprotection of **13** using this method gave **3**·HCl in 44% yield. (Scheme 3)



Scheme 3. (a) K_2CO_3 (3 equiv.), MeI (10 equiv.), DMF, 0 °C, 4 h, 30:70 **12**:**13**; 79 %. (b) TFA, dry DCM, rt, 24 h. (c) i) 1M NaOH, 0 °C, 24 h. ii) 1M HCl. iii) Precipitation from water with acetone; **2**·HCl 41% (over 2 steps), **3**·HCl 44% (over 2 steps).

We were able to grow crystals of **2**·HCl from MeOH/Et_2O and it was found that it crystallises in the monoclinic $P2_1$ space group and the asymmetric unit comprises two independent molecules (Z = 4; Z′ = 2), having the same *S*-configuration but different conformations, (figure 4) with respect to their -CO_2H groups. As a result, the individual units in **2**·HCl are arranged in pseudo superimposable rows along the *c* unit cell direction and are connected by the Cl^- counter anions (Cl1 and Cl51, respectively) through H-bonding interactions at distances of (O2(H2)…Cl1) = 3.0580(16) Å and (O52(H52)…Cl51i = 3.0280(15) Å. These individual rows assemble in the brickwork topology to give the space efficient packing motif observed in **2**·HCl, (figure 5). Once again, the counter anions play a pivotal role in locking these units into place through intermolecular H-bonding interactions as shown in Figure 6.

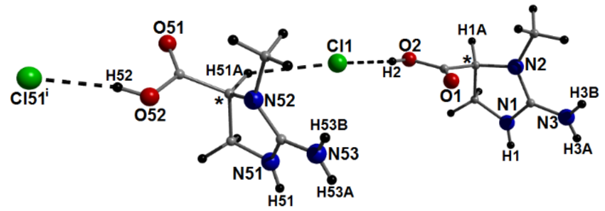


Figure 4. The asymmetric unit in **2**·HCl comprising two independent *S*-configured molecules and highlighting some of the intermolecular electrostatic and H-bonding interactions with their neighbouring Cl^- counter anions, Cl1 and Cl51, respectively. These H-bonded distances (represented as dashed lines) are: O2(H2)…Cl1 = 3.0580(16) Å and (O52(H52)…Cl51i = 3.0280(15) Å. Symmetry code (i) = *x*, *y*, -1 + *z*. The chiral centres are marked with an asterisk. Colour code: Grey (C), Blue (N), Red (O), Green (Cl) and Black (H).

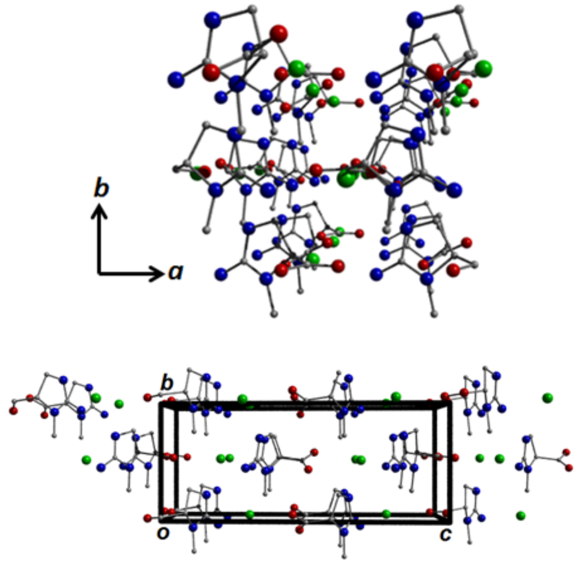


Figure 5. Crystal packing arrangement in **2**·HCl highlighting the pseudo superimposable rows as viewed along the *c* (top) and *a* (bottom) unit cell direction. Hydrogen atoms have been omitted for clarity.

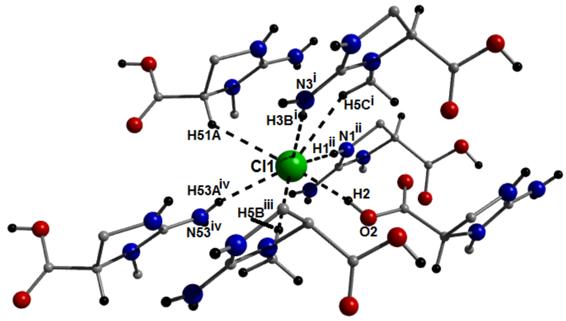
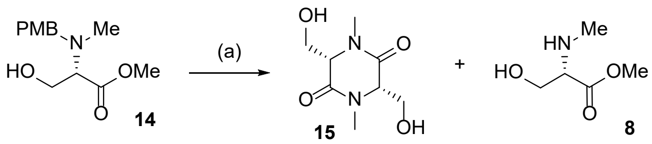


Figure 6. A chloride counter anion (Cl1) H-bonding to six individual **2**·HCl units as represented by dashed lines. H-bonding distances are: O2(H2)…Cl1 = 3.0580(16) Å; N3i(H3Bi)…Cl1 = 3.308(2) Å; N1ii(H1ii)…Cl1 = 3.2572(18) Å and N53iv(H53Aiv)…Cl1 = 3.2495(17) Å. Symmetry codes: (i) = 2-*x*, ½ + *y*, 1-*z*; (ii) = 2-*x*, -½ + *y*, 1-*z*; (iii) = 1-*x*, ½ + *y*, 1-*z*; (iv) = -1 + *x*, *y*, *z*. Some hydrogen atoms have been omitted for clarity.

3. Bioactivity

Compounds (*S*)-**1**·HCl, **2**·HCl and **3**·HCl together with the 1,4-dimethylpiperazine-2,5-dione **15**, (a by-product from the hydrogenation of the PMB-protected serine derivative **14** used to prepare **8** (Scheme 4) were assayed for their glycosidase activity on a panel of enzymes as described previously[12] and glucocerebrosidase was added. Table 1 shows the glycosidase results obtained. There was some weak inhibition of yeast α-glucosidase and almond β-glucosidase (less than 50% at 8mM) but most notable was a stimulation of glucocerebrosidase activity where at 0.8 mM **2**·HCl gave 390%, (*S*)-**1**·HCl gave 5% (28% at 8 mM) and **3**·HCl gave 4% (15% at 8 mM). Interestingly, **15** also gave 330% promotion of glucocerebrosidase at 0.4 mM. **2**·HCl, (*S*)-**1**·HCl and **3**·HCl weakly inhibited yeast α-glucosidase at 8 mM (less than 50%) .



Scheme 4. (a) Pd/C and $Pd(OH)_2/C$ (1:1), H_2 (1 atm.), THF/i-PrOH (3:1), rt, 28h; **15** (10%)**, 8** (57%).

The enzyme glucocerebrosidase is deficient in the lysosomal storage disorder Gaucher's disease and mutations of the glucocerebrosidase gene (GBA) are the most important risk factor yet discovered for Parkinson disease (PD) and this results in reduced enzyme activity.[14] Iminosugars such as Miglustat and Isofagomine have attracted interest as chaperone molecules for glucocerebrosidase when used at sub-inhibitory concentrations but the guanidines described here have the advantage of appearing to increase glucocerebrosidase activity without inhibiting the enzyme at high concentrations.

Table 1: Glycosidase Results Expressed as % Inhibition

Compound	Concentration	α-D-glucosidase	α-D-glucosidase	β-D-glucosidase	glucocerebrosidase (GC)	N-acetyl-β-D-glucosidase
		Yeast	Bacillus	almond	Genzyme	Bovine kidney
(*S*)-1·HCl	8 mM	45	---	35	-28	1
2·HCl	8 mM	44	17	20	-390[(i)]	34
3·HCl	8 mM	30	---	17	15	19
15	0.4 mM	---	---	---	-330	---
Calystegine B2	0.8 mM	18	---	96	100	0
DMDP	0.8 mM	100	---	97	44	-7

(i) For glucocerebrosidase (GC) 0.8 mM

4. Conclusions

In conclusion, we have developed a method for the formation of the guanidine cyclic amino acid skeleton and have prepared (*S*)-**1**·HCl in good overall yield in 5 steps from L-serine. The synthesis of the *N*-methyl analogues **2**·HCl and **3**·HCl were also achieved via a *N*-methylation of a cyclic guanidine followed by a two stage deprotection. Interestingly a publication by Silva et al.[14] detailed the formation of the orthogonally protected five-membered cyclic guanidine **16** from L-serine **5** in 11 steps, (Figure 7). Our work offers an alternate strategy to access selectively alkylated cyclic guanidines in fewer steps, however our method currently lacks regioselectivity in the alkylation step.

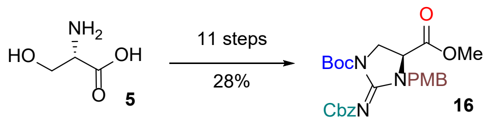


Figure 7. Preparation of orthogonally protected guanidine **16**.[14]

Acknowledgments: The authors would like to thank the Iraqi government (ZSA-T) and acknowledge the support of the Centre for Environmental Biotechnology Project part-funded by the European Regional Development Fund (ERDF) through the Welsh Government. We would like to thank the BEACON II scheme (ERDF; PJM) and the EPSRC National Mass Spectrometry Facility at Swansea.

Supplementary Materials: The following are available online: NMR spectra for compounds (*S*)-**1**, **2**, **3**, **7**, **11-13** and **15**, together with crystallographic data for Compound **2**.HCl, in crystallographic information file (CIF) format. The data were submitted to the Cambridge Structural Database and given the following CCDC deposition number 2162041. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html.

5. Experimental

Column chromatography was carried out on silica gel (particle size 40–63 μm) and TLC was conducted on precoated Kieselgel 60 F_{254} (Art. 5554; Merck) with the eluent (DE, EA, PE, ME, CF, DCM) specified in each case. All anhydrous reactions were conducted in oven-dried apparatus under a static atmosphere of nitrogen. Ether (DE), THF and dichloromethane where dried on a Pure Solv MD-3 solvent purification system. Dry methanol and DMF were purchased from Sigma Aldrich. Chemical shifts are reported in δ values relative to the residual solvent peak as an internal standard. Proton (400 MHz) and carbon (100 MHz) NMR were recorded in $CDCl_3$ on a Bruker AC400 spectrometer unless otherwise stated. Mass spectra data were obtained on a ThermoFisher Scientific LTQ Orbitrap XL at the EPSRC Mass Spectrometry Service Centre at the University of Wales, Swansea or on a Q Exactive Plus (Thermo Scientific) instrument run with positive electrospray ionization (ESI). Infrared spectra were recorded as thin films (oils) or solids on a Bruker Tensor 27 series instrument.

Methyl L-serinate hydrochloride 10.[10] Neat $SOCl_2$ (9.66 mL, 133.2 mmol, 2 equiv.) was added dropwise to a suspension of L-serine **5** (7.00 g, 66.6 mmol) in dry methanol (20 mL) under vigorous stirring whilst keeping the temperature between 0-5 °C. After overnight stirring, the resulting solution was heated at 60 °C for 3 h stirring to remove excess HCl and SO_2 formed during the reaction and then evaporated under vacuum. The reaction was then evaporated in vacuo and the product recrystallized from methanol to give **10** (10.1 g, 64.7

mmol) in 97 % yield as a white solid. Mp. 168-169 °C (Lit.[15] 163-166 °C); $[\alpha]_D^{23}$ 4.2 (c = 2.0 MeOH; Lit.[15] $[\alpha]_D^{26}$ 4.6 (c = 2.0, MeOH)); **δ_H** (D_2O) 4.30 (1H, br t, *J* 4.0 Hz, CH), 4.13 (1H, dd, *J* 12.6, 4.4 Hz, CH_2), 4.02 (1H, dd, *J* 12.6, 3.4 Hz, CH_2), 3.88 (3H, s, OCH_3); **δ_C** (D_2O) 168.9, 59.2, 54.7, 53.7; **ν_{max}** cm^{-1} 3363, 2970, 2901, 1743; **m/z** (ESI) 142.0 (12%, $[M+Na]^+$), 239.1 (100%, $[2M+H]^+$); **HRMS** (ESI) $C_4H_9O_3NNa$ ($[M+Na]^+$), expected 142.0475, found 142.0476; $C_8H_{19}O_6N_2$ ($[2M+H]^+$), expected 239.1238, found 239.1246.

Methyl (*N*, *N*'-bis (*tert*-butoxycarbonyl)carbamimidoyl)-L-serinate 11

A solution of methoxy-L-serine hydrochloride **10** (3.0 g, 19.3 mmol) in methanol (20 mL) was added in one portion to a stirred solution of *N*,*N*'-bis-(tert-butoxycarbonyl)-1H-pyrazole-1-carboxamidine **6** (8.98 g, 28.9 mmol, 1.50 equiv.) and NEt_3 (1.95 g, 19.3 mmol, 1.0 equiv., 2.7 mL) methanol (5 mL). After stirring for 36 h, the solvent was removed under reduced pressure and the crude product purified by column chromatography (22-27% EA in PE) to give **11** (5.12 g, 14.17 mmol, 74 %) as a white crystalline solid. Rf 0.18 (30 % EA in PE); $[\alpha]_D^{23}$ 21.5 (c = 1.0, MeOH); **Mp** 110-112 °C; **δ_H** 11.40 (1H, s, NH), 9.26 (1H, d, *J* 5.9 Hz, NH), 4.85 (1H, ddd, *J* 5.9, 5.5, 2.7 Hz, CH), 4.32 (1H, s, OH), 4.03 (1H, dd, *J* 11.2, 2.7 Hz, CH_2), 3.90 (1H, dd, *J* 11.2, 5.5 Hz, CH_2), 3.80 (3H, s, CH_3), 1.50 (9H, s, CH_3), 1.46 (9H, s, CH_3); **δ_C** 170.4, 162.8, 156.4, 152.9, 83.8, 79.8, 64.9, 57.1, 53.1, 28.3, 28.2; **ν_{max}** 3674 (OH), 3322 (NH), 2978, 2901 (C-H, Aliphatic), 1728 (C=O), 1643, 1613, 1568; **m/z (ESI)** 362.2 (100%, $[M+H]^+$), 250.1 (55%); **HRMS (ESI)** $C_{15}H_{27}N_3O_7$ requires 362.1922 found 362.1918.

1-(*tert*-Butyl) 4-methyl (*S*)-2-((*tert*-butoxycarbonyl)imino)imidazolidine-1,4-dicarboxylate 7

Guanidine **11** (2.00 g, 5.53 mmol) and triphenylphosphine (3.34 g, 12.73 mmol, 2.3 equiv.) were dissolved in dry dichloromethane (15 mL) and cooled (-20 °C). Imidazole (1.47 g, 21.58 mmol, 3.9 equiv.) and iodine (2.81 g, 11.07 mmol, 2.00 equiv.) were then added sequentially added in one portion and the resultant solution stirred for 3 h with cooling (-15-20 °C). At this point chloroform (300 mL) was added followed by ammonium chloride solution (aq., sat., 300 mL). The organic layer was separated, and the aqueous layer further extracted with chloroform (3 x 100 mL). The combined organic phases were washed with water (50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography (20-25 % EA in PE) and recrystallization from diethyl ether/hexane to give **7** (1.31 g, 5.53 mmol) in 64% yield as a white solid. Rf 0.13 (50 % EA in PE); $[\alpha]_D^{23}$ 25 (c = 1, MeOH); **Mp** 126-128 °C; **δ_H** 9.60 (1H, br s, NH), 4.56-4.68 (1H, m, CH), 3.85-3.99 (2H, m, CH_2), 3.76 (3H, s, CH_3), 1.51 (9H, s, 3 x CH_3), 1.48 (9H, s, 3 x CH_3); **δ_C** 172.2, 152.1, 149.5, 148.8, 83.9, 81.9, 63.6, 52.6, 47.7, 28.1, 28.1; **ν_{max}** 3313, 2984, 1759, 1707, 1649; **m/z (ESI)** 709.3 (50%, $[2M+Na]^+$), 366.2 (25%, $[M+Na]^+$), 344.2 (100%, $[M+H]^+$); **HRMS (ESI)** $C_{15}H_{26}N_3O_7$ ($[M+H]^+$) requires 344.1816 found 344.1818.

(*S*)-2-Iminoimidazolidine-4-carboxylic acid *S*-1·HCl

Guanidine **7** (23.0 mg, 0.067 mmol) was dissolved in dichloromethane (2 mL) and cooled (0 °C), whereupon trifluoroacetic acid (4 mL) was added and the mixture stirred to rt overnight. The solvent was then evaporated to give crude product as a light brown gum (19 mg) which was used in next step without any further purification. Aqueous NaOH solution (2 mL, 1 M. 2 mmol) was added to a cooled flask (0 °C) containing the crude intermediate (19 mg). The reaction mixture was stirred for 3 h then warmed to rt overnight. After acidification (pH ~2-3) with aqueous HCl (1M, ca 3-4 mL), evaporation under reduced pressure gave the crude product. This material was dissolved in a minimum volume of water (ca. 1 mL) and acetone (ca. 5-10 mL) was added until precipitation of NaCl occurred. After cooling (0 °C) for 30 mins, the supernatant was removed by filtration through a short plug of celite© and the filtrate evaporated to give *S*-**1**·HCl (7 mg, 0.042 mmols) as a gum in 63 % yield over 2 steps. $[\alpha]_D^{18}$ -38 (c = 0.87, H_2O); **δ_H** (D_2O) 4.88 (1H, dd, *J* 10.8, 5.4 Hz, CH), 4.20 (1H, app t, *J* 10.6 Hz, CH), 4.01 (1H, dd, *J* 10.2, 5.4 Hz, CH); **δ_C** (D_2O) 174.5, 159.7, 56.1, 46.3; **IR ν_{max}** 3448, 2884, 2125 (C=O); **m/z (ESI)** 130.1 (78%, $[M+H]^+$), 152.0 (55%, $[M+Na]^+$), 259.1 (60%, $[2M+H]^+$), 410.2 (100%, $[3M+Na]^+$); **HRMS (ESI)** $C_4H_7O_2N_3Na$ ($[M+Na]^+$) requires 152.0430, found 152.0427.

1-(*tert*-Butyl) 4-methyl (*S*)-2-imino-3-methylimidazolidine-1,4-dicarboxylate 12 and 1-(*tert*-butyl) 4-methyl (*S*)-2-((*tert*-butoxycarbonyl)(methyl)amino)-4,5-dihydro-1H-imidazole-1,4-dicarboxylate 13

Finely powdered K_2CO_3 (1.15 g, 8.3 mmol, 3.0 equiv.) was added to a cooled (0 °C) and stirred solution of **7** (951 mg, 2.77 mmol) dissolved in dry DMF (20 mL). After 30 min methyl iodide (3.92 g, 27.7 mmol, 1.82 mL, 10.0 equiv.) was added and the mixture was stirred for 5 h at 0 °C. The reaction was filtered, the filter pad washed with chloroform and then the filtrate freeze-dried. The resulting mixture was triturated with chloroform and the filtered triturates evaporated to dryness. Purification by silica gel chromatography (25-65 % EA in PE) gave **13** (559 mg, 56 %, **Rf** 0.21 in 50 % EA in PE) as an oil, followed by **12** (233 mg, 23 %, **Rf** 0.21 in 60 % EA in PE) as a solid. ***Data for 12***: $[\alpha]_D^{26}$ -6.0 (c 1.0, $CHCl_3$); **δ_H** 4.51 (1H, s, NH), 4.08 (1H, dd, *J* 5.3, 10.0 Hz, CH), 3.96 (1H, dd, *J* 10.0, 11.0 Hz, CH_2), 3.81 (3H, s, Me), 3.80-3.83 (1H, m, CH), 2.90 (3H, s, Me), 1.52 (9H, s, 3 x Me), 1.45 (9H, s, 3 x Me); **δ_C** 170.5, 153.8, 150.4, 82.9, 55.6, 53.0, 44.0, 30.1, 28.2; **ν_{max}** 2977, 2932, 1746, 1683, 1623, 1477, 1453, 1438, 1367, 1305, 1246, 1217, 1140, 1072, 1010, 952, 849, 809, 751, 722, 696, 665, 542; **m/z (ESI)** 358.2

(100%, [M+H]$^+$); **HRMS (ESI)** $C_{16}H_{28}O_6N_3^+$ ([M+H]$^+$) requires 358.1973, found 358.1971. ***Data for 13:*** $[\alpha]_D^{27}$ 79.0 (c 1.0, $CHCl_3$); **δ_H** 4.58 (1H, t, *J* 9.0 Hz, CH), 3.98-4.19 (2H, br m, CH_2), 3.80 (3H, s, CH_3), 3.18 (3H, s, CH_3), 1.49 (9H, s, 3 x CH_3), 1.44 (9H, s, 3 x CH_3); **δ_C** 171.4 (C=O), 154.1 (C=N), 152.9 (C=O), 149.3 (C=O), 82.5 (C), 81.9 (C), 62.6 (CH), 52.8 (CH_3), 49.3 (CH_2), 35.6 (CH_3), 28.3 (3 x CH_3), 28.2 (3 x CH_3); **ν_{max}** 2978, 2925, 2851, 1738, 1732, 1630; **m/z (ESI)** 358.2 (100%, [M+H]$^+$), 280.2 (25%, [M+Na]$^+$); **HRMS (ESI)** $C_{16}H_{28}O_6N_3^+$ ([M+H]$^+$) requires 358.1973, found 358.1975.

(*S*)-2-Imino-3-methylimidazolidine-4-carboxylic acid hydrochloride salt 2·HCl

Guanidine **12** (166.0 mg, 0.464 mmol) was deprotected under identical conditions to *S*-**1**·HCl to give **2**·HCl (34 mg, 0.190 mmol, 41 %) as a gum. A sample was recrystallised by dissolving in methanol (ca. 1 mL), which was diluted with DE to the cloud point then placed in a freezer overnight to give **2**·HCl as white crystals. **Mp** 218-220 °C; $[\alpha]_D^{26}$ -94 (MeOH, c = 1.0); **δ_H** (D_2O) 4.55 (1H, dd, *J* 10.5, 5.2 Hz, CH), 3.89 (1H, dd (apparent t), *J* 10.5, 10.7 Hz, CH), 3.58 (1H, dd, *J* 10.7, 5.2 Hz, CH), 2.90 (3H, s, Me); **δ_C** (D_2O) 175.0, 164.6, 59.7, 42.0, 29.7; **ν_{max}** 3333, 2910, 1713, 1652; **m/z (ESI)** 144.1 (100%, [M+H]$^+$); **HRMS (ESI)** $C_5H_{10}N_3O_2$ ([M+H]$^+$) requires 144.0768, found 144.0767.

(*S*)-2-(Methylamino)-4,5-dihydro-1H-imidazole-4-carboxylic acid hydrochloride salt 3·HCl

Guanidine **13** (147 mg, 0.411 mmol) was deprotected under identical conditions to *S*-**3**·HCl to give **3**·HCl (33 mg, 0.184 mmol, 44 %) as a clear gum. $[\alpha]_D^{23}$ -38 (c = 0.25 in H_2O); **δ_H** (CD_3OD) δ 4.18 (1H, dd, *J* 5.6, 10.3 Hz, CH), 3.88 (1H, dd, *J* 10.3, 10.5 Hz, CH), 3.71 (1H, dd, *J* 5.6, 10.5 Hz, CH), 2.82 (3H, s, CH_3); **δ_C** (CD_3OD) 179.1, 163.3, 60.6, 52.4, 29.2; **δ_H** (D_2O) 4.61-4.71 (1H, m, CH), 4.00 (1H, apparent t, *J* 10.3 Hz, CH), 3.76-3.84 (1H, m, CH), 2.87 (3H, s, Me); **δ_C** (D_2O) 174.4, 159.9, 55.9, 46.1, 28.4; **IR ν_{max}** 3296, 3158, 1599, 1441, 1427, 1405; **m/z (ESI)** 144.1 (15%, [M+H]$^+$); **HRMS (ESI)** $C_5H_{10}N_3O_2$ requires 144.0768, found 144.0764.

***N*-Methyl methyl-L-serinate 8[9] and (3*S*,6*S*)-3,6-bis(hydroxymethyl)-1,4-dimethylpiperazine-2,5-dione 15.**

Methyl *N*-(4-methoxybenzyl)-*N*-methyl-L-serinate[9] (261.8 mg, 1.03 mmol) was dissolved in THF/isopropanol (3:1, 8 mL) and Pd/C (10 %, 250 mg) and $Pd(OH)_2$/C (10 %, 250.0 mg) were added. The mixture was evacuated under vacuum and the atmosphere saturated with hydrogen gas (balloon). The mixture was stirred for 28 h then filtered through a Celite© pad, which was washed with THF/ isopropanol (excess) and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatograph on silica gel (0-3% MeOH in EtOAc) to give **8** (78.7 mg, 0.59 mmol, 57%) as a waxy solid and **15** (10.0 mg, 0.05 mmol, 10%) as a gum. *Data for* ***5***.[9] $[\alpha]_D^{26}$ -3.8 (c = 0.015, MeOH) (lit $[\alpha]_D^{23}$ -3.6 (c = 0.016, MeOH); Rf 0.24 (20% MeOH in EtOAc); **δ_H** 3.84 (1H, dd, *J* 11.1, 4.2 Hz, CH_2), 3.77 (3H, s, CH_3), 3.67 (1H, dd, *J* 11.1, 5.9 Hz, CH_2), 3.32 (1H, dd, *J* 4.2, 5.9, CH), 2.87 (2H, br s, OH, NH), 2.46 (3H, s, CH_3); **δ_C** 173.7, 64.4, 62.1, 52.4, 34.7; **m/z (ESI)** 134.1 (100%, [M+H]$^+$); **HRMS** (ESI) $C_5H_{12}NO_3$ requires 134.0812, found 134.0812. *Data for* ***15***: Rf 0.18 (20% MeOH in EA); **δ_H** (CD_2OD 4.06 (2H, t, *J* 3.5 Hz, 2 x CH), 3.95 (4H, d, *J* 3.5 Hz, 2 x CH_2), 3.02 (6H, s, 2 x CH_3); **δ_C** 167.4, (2 x C=O), 65.6 (2 x $\underline{CH}$), 62.9 (2 x $\underline{CH_2}$), 33.0 (2 x $\underline{CH_3}$); **m/z (ESI)** 203.1 (100 %, [M+H]$^+$), 225.1 (25 %, [M+Na]$^+$), 427.2 (15 %, [2M+Na]$^+$); **HRMS (ESI)** $C_8H_{15}N_2O_4$ ([M+H]$^+$) requires 203.1026 found 203.1025; $C_8H_{14}NaN_2O_4$ ([M+Na]$^+$) requires 225.0846 found 225.0846.

Glycosidase Assays

The main panel of glycosidases were assayed as described previously.[13b] Assays on the Human recombinant β-glucocerebrosidase (Genzyme, Europe) were carried at 27 °C in 0.1 M citric acid/0.2 M disodium hydrogen phosphate buffer at pH 5.9. The incubation mixture consisted of 10 μL of enzyme solution, 10 μL of aqueous solution of extract or compound at the required concentration and 50 μL of 5 mM *para*-nitrophenyl (PNP) β-glucopyranoside (Sigma-Aldrich) made up in buffer at pH 5.9. The reactions were stopped by addition of 70 μL 0.4 M glycine (pH 10.4) during the exponential phase of the reaction, which had been determined at the beginning using uninhibited assays in which water replaced inhibitor. Final absorbances were read at 405 nm using a Versamax microplate reader (Molecular Devices). Assays were carried out in triplicate, and the values given are means of the three replicates per assay. A negative value indicates enzyme activation. The iminosugars DMDP and calystegine B_2 were included in assays as control inhibitors of glucosidases and glucocerebrosidase. Table 1 shows the results for the synthetic compounds products at indicated concentrations.

References

1 (a) D. L. Perry, Jr; B. F. Roberts, G. Debevec, H. A. Michaels, D. Chakrabarti, A. Nefzi *Molecules*, 24 (2019), 1100. (b) M. G. Donahue, *Progress in Heterocyclic Chemistry*; 26 (2014), 1-28. (c) Y. Zong; F. Fang; K. J. Meyer, L. Wang, Z. Ni, H. Gao, K. Lewis, J Zhang, Y, Rao *Nature Communications* 10 (2019), 3268. (d) I. B. Seiple, S. Su, I. S. Young, A. Nakamura, J. Yamaguchi, L. Jørgensen, R. A. Rodriguez, D. P. O'Malley, T. Gaich, M. S Köck, P. S. Baran. *J. Am. Chem. Soc.* 133 (2011), 14710-14726.

2 (a) S. Hori, S.; Kameda, Y. *J. Antibiot.* 21 (1968), 665-667. (b) Higashide, E.; Hatano, K.; Shibata, M.; Nakazawa, K. *J. Antibiot.* 21 (1968), 126-137. (c) Asai, M.; Muroi, M.; Sugita, N.; Kawashima, H.; Mizuno, K.; Miyake, A. *J. Antibiot.* 21 (1968), 138-146.

3 H. He, R. T. Williamson, B. Shen, E. I. Graziani, H. Y. Yang, S. M. Sakya, P. J. Petersen, G. T. Carter, *J. Am. Chem. Soc*. 124 (2002), 9729-9736.
4 E. B. Herr, M. E. Haney, G. E. Pittenger, C. E. Higgins, C. E. *Proc. Ind. Acad. Sci.* 69 (1960), 134.
5 (a) L. L. Ling, T. Schneider, A. J. Peoples, A. L. Spoering, I Engels, B. P. Conlon, A. Mueller, T. F. Schäberle, D. E. Hughes, S. Epstein, M. Jones, L. Lazarides, V. A. Steadman, D. R. Cohen, C. R. Felix, K. A. Fetterman, W. P. Millett, A. G. Nitti, A. M. Zullo, C. Chen, K. Lewis, *Nature (London, U.K.)* 517 (2015), 455-459. (b) S. Nomoto, T. Teshima, T. Wakamiya, T. Shiba, *Tetrahedron* 34 (1978), 921-927. (c) H. Yoshioka, T. Aoki, H. Goko, K. Nakatsu, T. Noda, H. Sakakibara, T. Take, A. Nagata, J. Abe, *Tetrahedron Lett.* 23 (1971), 2043-2046.
6 (a) W. Su, *Synth. Commun.* 1996, 26, 407-413. (b) C. J. Schwörer, M. Oberthür, *Eur. J. Org. Chem.* 2009, 6129-6139. (c) K. S. Olivier, M. S. Van Nieuwenhze, *Org. Lett.* 12 (2010) 1680-1683. (d) S. N. Fischer, C. J. Schwörer, M. Oberthür, *Synthesis* 46 (2014), 2234-2240. (e) S. Fuse, H. Koinuma, A. Kimbara, M. Izumikawa, Y. Mifune, H. He, K. Shin-Ya, T. Takahashi, T. Doi, *J. Am. Chem. Soc.* 136 (2014), 12011-12017. (f) V. D. Möeschwitzer, B. M. Kariuki, J. E. Redman, *Tetrahedron Lett.* 54 (2013), 4526-28. (g) W. Craig, J. Chen, D. Richardson, R. Thorpe, Y. Yuan, *Org. Lett.* 17 (2015), 4620-4623. (h) A. M. Giltrap, L. J. Dowman, G. Nagalingam, J. L. Ochoa, R. G. Linington, W. J. Britton, R. J. Payne, *Org. Lett.* 18 (2016), 2788-2791. (i) B. Wang, Y. Liu, R. Jiao, Y. Feng, Q. Li, C. Chen, L. Liu, G. He, G. Chen, *J. Am. Chem. Soc.* 138 (2016), 3926-3932. (j) D. E. DeMong, R. M. Williams, *Tetrahedron Lett.* 42 (2001), 3529-3532. (k) D. E. DeMong, R. M. Williams, *J. Am. Chem. Soc.* 125 (2003), 8561-6565. (l) L. Sanière, L. Leman, J.-J. Bourguignon, P. Dauban, R. H. Dodd, *Tetrahedron* 60 (2004), 5889-5893. (m) M. Benohoud, L. Leman, S. H. Cardoso, P. Retailleau, P. Dauban, J. Thierry, R. H. Dodd, *J. Org. Chem.* **2009**, 74, 5331-5336.
7 (a) M. Dennis, L. M. Hall, P. J. Murphy, A.J. Thornhill, R. Nash, A. L. Winters, M. B. Hursthouse, M. E. Light P. Horton *Tetrahedron Lett*. 44 **(2003)**, 3075-3080. (b) C. Albrecht, S. Barnes, H. Böckemeier, D. Davies, M. Dennis, D. M. Evans, M. D. Fletcher, I. Jones, V. Leitmann, P. J. Murphy, R. Rowles, R. Nash, R. A. Stephenson, P. N. Horton, M. B. Hursthouse, *Tetrahedron Lett*. 49 (2008), 185-188. (c) D. Davies, M. Fletcher, H. Franken, J. Hollinshead, K. Kähm, P. J. Murphy, R. Nash, D. Potter, *Tetrahedron Lett*., 51 (2010), 6825–6829. (d) Z. Al Shuhaib, D. H. Davies, M. Dennis, D. M. Evans, M. D. Fletcher, H. Franken, P. Hancock, J. Hollinshead, I. Jones, K. Kähm, P. J. Murphy, R. Nash, D. Potter, and R. Rowles. *Tetrahedron,* 70 (2104), 4412-4419. (e) Z. Al Shuhaib, M. Arndt, M. Dennis, D. M. Evans, I. Jones, V. Leitmann, P. J. Murphy, D. Roberts, R. Rowles, Y. K. Sadaghiani, A. J. Thornhill, R. J. Nash, J. Hollinshead, B. Bartholomew, G. J. Tizzard, S. J. Coles, *Tetrahedron*, 73 (2017), 845-852.
8 T. Sato, *Bull. Chem. Soc. Jpn*., 35 (1962), 1531–1536.
9 K. N. White, J. P. Konopelski *Org. Lett*., 7 (2005), 4111-4112.
10 C. Carboni, P. J. Quaedflieg, Q. B. Broxterman, P. Linda, L. Gardossi *Tetrahedron Lett.,* 45 (2004), 9649-9652.
11 (a) T. Hirose, T. Sunazuka, S. Tsuchiya, T. Tanaka, Y. Kojima, R. Mori, M. Iwatsuki S. Ōmura, *Chem. Eur. J*., 13 (2008), 8220-8238. (b) P. J. Murphy, D. M. Evans, P. N. Horton, M. B. Hursthouse, *RSC Advances* 4 (2014), 20744-20751. (c) D. M. Evans, P. J. Murphy, *Chem. Commun*. 47 (2012), 3225-3226.
12 J. R. Coggins, N. L. Benoiton, *Can. J. Chem*., 49 (1971), 1968-1971. (b) G. Fráter, U. Müller and W. Günther, *Tetrahedron*, 40 (1984), 1269-1277. (c) P. J. Klein, J. A. Christiaans, A. Metaxas, R. C. Schuit, A. A. Lammertsma, B. N. van Berckel, A. D. Windhorst, *Bioorg. Med. Chem*., 23 (2015), 1189-1206.
13 (a) Z. J. R. Ashworth, B. Bartholomew, D. M. Evans, J. Forde-Thomas, K. F. Hoffmann, R. Murdoch, R. J. Nash, H. Sharp, H. Whiteland, P. J. Murphy, *Heterocycles* 100 (2020) 609–621. (b) A. A. Watson, R. J. Nash, M. R. Wormald, D. J. Harvey, S. Dealler, E. Lees, N. Asano, H. Kizu, A. Kato, R. C. Griffiths, A. J. Cairns G. W. J. Fleet *Phytochemistry* 46 (1997), 255–259.
14 (a) A. H. V. Schapira, *Mol Cell Neurosci*. 66 (2015, Pt A), 37-42. (b) T. U. Han, R. Sam and E. Sidransky *Front. Cell Dev. Biol*. 8 (2020), 271.
15 D. R. C. Silva, E. J. Maria, R. M. S. Ordóñez, J. Thierry, K. Cariou, R. H. Dodd, *Synlett* 28 (2017), 815-818.
16 R. G. Vaswani, A. R. Chamberlin *J. Org. Chem.* 73 (2008), 1661-1681.
17 R. J Nash, A. Kato, C-Y Yu, G. W. J. Fleet *Future Med. Chem*. 3 (2011), 1513-21.