Norbornane Mimics of Distorted β-D-Glucopyranosides – Inhibitors of β-D-Glucopyranosidases?

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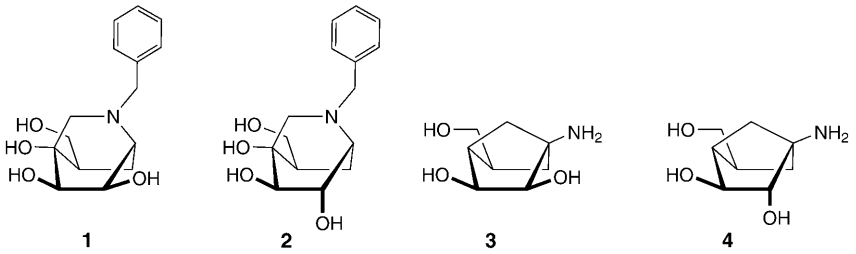
The racemic *gluco*-configured norbornanes **4** and **16** were prepared and tested as inhibitors of β-glucosidases. The known alcohol **5** was deprotected to provide the triol **6**. Silylation (→ **7**), monobenzoylation (→ **8**/**9**), and oxidation provided the regioisomeric ketones **10** and **11**. Reduction of **10** gave the desired *endo*-alcohol **13**, albeit in low yield, while reduction of the isomeric ketone **11** provided mostly the *altro*-configured *endo*-alcohol **12**. The alcohol **13** was desilylated to **14**. Debenzoylation to **15** followed by hydrogenolytic deprotection gave the amino triol **4** that was reductively aminated to the benzylamine **16**. The amino triols **4** and **16** proved weak inhibitors of the β-glucosidase from *Caldocellum saccharolyticum* (**4**: $IC_{50}=5.6$ mM; **16**: $IC_{50}=3.3$ mM) and from sweet almonds (**16**: $IC_{50}=5.5$ mM). A comparison of **4** with the *manno*-configured norbornane **3** shows that **3** is a better inhibitor of snail β-mannosidase than **4** is of β-glucosidases, in keeping with earlier results suggesting that these β-glycosidases enforce a different conformational itinerary.

Introduction. – β-Glycosidases impose a conformational change on their substrates to comply with the stereoelectronic control of glycoside cleavage [1–4]. Several conformations allow the required coplanar arrangement of a non-bonding, doubly occupied orbital of the ring O-atom and the σ* orbital of the scissile bond [5], and it was considered likely that all of them are enforced by different glycosidases [6]. Stable mimics of the individual acceptable conformers may act as selective inhibitors and contribute in assessing the conformational itinerary enforced by a specific glycosidase. It was claimed that inhibitors mimicking such distorted conformers should be inherently more selective than mimics of an intermediate oxocarbenium cation and be more sensitive to small geometric changes [7][8]. In keeping with these considerations, the *manno*-configured isoquinuclidine **1** [9] mimicking a $^{1,4}B/^{1}S_3$ conformation inhibits snail β-mannosidase rather strongly ($K_i=1$ μM at pH 4.5), while the *gluco*-configured diastereoisomer **2** is almost inactive against β-glucosidases, and the (racemic) *manno*-configured norbornane **3** is only a modest β-mannosidase inhibitor ($K_i\approx 50$ μM; extrapolated to the D-*manno*-enantiomer). The difference between the strength of the inhibition by **1** and **3** can be traced back to geometric differences. A superposition[1]) of the isoquinuclidine **1** with C(1) to C(5) and O–C(5) of a β-D-GlcNAc substrate[2]) in the $^{1,4}B$-conformation (root-mean-square deviation (r.m.s.) 0.09 Å) reveals that the atomic distance between

[1]) Calculation and superposition were carried out using Macromodel V. 6 [10]. The structures were minimized with the PRCG algorithm using the MM3* force field.

[2]) The structure was taken from the X-ray crystal structure of the chitinase B from *Serratia marcescens* complexed with this substrate [11].

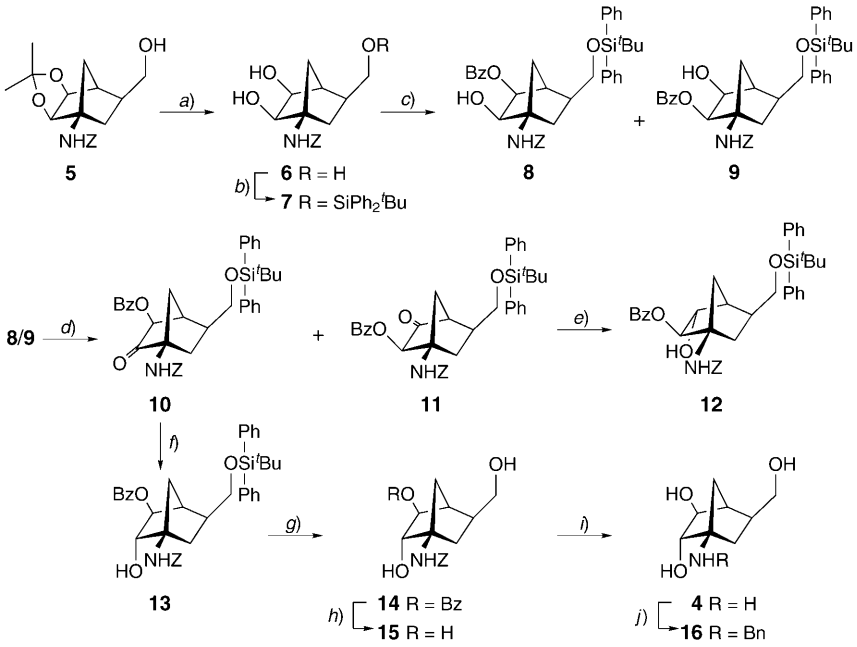
the N-atom of **1** (which is to interact with the catalytic acid residue of the glycosidase) and the glycosidic O-atom of the substrate amounts to 0.6 Å, while the corresponding distance for the norbornane **3** and the substrate in a $^{1,4}B$-conformation (r.m.s. 0.19 Å) is 1.3 Å. Minimization of the distance between the N-atom of **3** and the glycosidic O-atom of the substrate by varying the conformation of the substrate from $^{1,4}B$ towards $^{4}H_3$ shows that at the point of the least distance ($d = 0.14$ Å), the substrate possesses a $^{4}H_3$ conformation with a dihedral angle C(5)O–C(1)–C(2)–C(3) of 0.8°. This suggests that the isoquinuclidine **1** and the norbornane **3** could mimic a different conformation of the reactive substrate, although they both comprise a biased cyclohexane boat mimicking the tetrahydropyran ring of the reactive conformer of the glycoside. Could the different location and orientation of the N-atom of **3** qualify the *gluco*-configured norbornane **4** as inhibitor of β-glucosidases, in contradistinction to the isoquinuclidine **2**? To answer this question, we decided to prepare the *gluco*-norbornane **4** and its *N*-benzylated analogue **16**.



Synthesis. – Deprotection of the known acetal **5** [8] in EtSH/EtOH provided the triol **6** (88%; *Scheme*) that was silylated with $^{t}BuPh_2SiCl$ [12] to yield 83% of the diol **7**. Treatment of **7** with benzoyl chloride (BzCl) afforded the regioisomeric monobenzoates **8**/**9** 82:18 (91%) that could not be separated on account of a facile Bz-group migration. The mixture **8**/**9** was, therefore, oxidised by *Dess–Martin*'s periodinane [13]. The resulting ketones were readily separated by chromatography to afford **10** (71%) and **11** (13%). Reduction of **10** with bulky reducing agents afforded only a mixture **8**/**9**, while reduction with $NaBH_4$ in MeOH provided the desired *endo*-alcohol **13** (29%) together with **8**/**9** 82:18 (65%). This somewhat disappointing result can be explained by the steric hindrance of an *exo*-approach by the [(benzyloxy)carbonyl]-amino group, perhaps forming a non-productive complex with the reducing agent, as proposed by *Martinez et al.* [14]. The role played by the bridgehead substituent is corroborated by the reduction of the ketone **11** which provided mostly (49%) the *endo*-alcohol **12** resulting form the expected *exo*-attack of the reducing agent, besides 29% of **8**/**9** 82:18. The still rather high amount of the undesired mixture **8**/**9** suggests that the vicinal benzoyloxy group also contributes to shielding of the *exo*-side. Desilylation of **13** with NH_4F [15] provided the diol **14** (89%), which was debenzoylated with MeONa to afford the triol **15** (98%). Hydrogenolysis of **15** provided the amino triol **4** (93%). Reductamination of **4** with PhCHO and $NaBH_4$ yielded 75% of the benzylamine **16**.

The ketone **10** shows a strong NOE (12.3%) between H–C(3) and H–C(5) (*Fig.*). The unambiguous assignment of the ^{1}H-NMR signals is based on a DQFCOSY and a

Scheme



a) EtSH, H_2SO_4, EtOH; 88%. *b*) ^{t}BuPh$_2$SiCl, 1*H*-imidazole, DMF; 83%. *c*) BzCl, 4-(dimethylamino)-pyridine (DMAP), toluene; **8**/**9** 82:18 (91%). *d*) *Dess–Martin*'s periodinane, CH_2Cl_2; **10** (71%) and **11** (13%). *e*) $NaBH_4$, MeOH; **12** (49%) and **8**/**9** 82:18 (29%). *f*) $NaBH_4$, MeOH; **13** (29%) and **8**/**9** 82:18 (65%). *g*) NH_4F, MeOH; 89%. *h*) MeONa, MeOH; 98%. *i*) Pd/C, H_2, EtOH; 93%. *j*) PhCHO, $MgSO_4$, MeOH; then $NaBH_4$, MeOH; 75% from **15**.

HSQC spectrum. Due to signal overlap, the interpretation of the NOEs in **11** was not reliable. The *endo*-alcohol **13** shows a NOE of 8.8% between H–C(3) and H–C(5), whereas the interpretation of the NOEs of **12** was again hampered by signal overlap. However, a vicinal coupling of *ca.* 3.3 Hz between H–C(3) and H–C(4) evidences the *exo*-orientation of H–C(3) of **12** (*cf.* [16]).

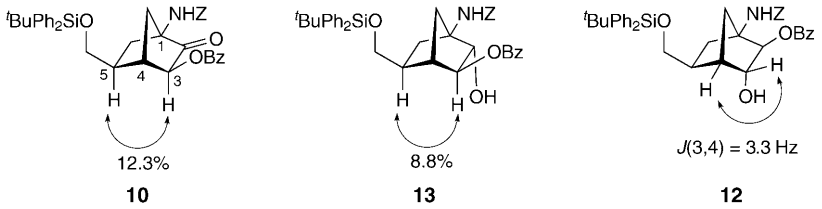


Figure. *NOEs Observed between H–C(3) and H–C(5) of* **10** *and* **13**, *and* J*(3,4) of* **12**

Inhibition Studies. – The results of the inhibition of *Caldocellum saccharolyticum* β-glucosidase and sweet almonds β-glucosidases by the racemic norbornanes **4** and **16** are summarized in the *Table*. Neither **4** nor **16** are inhibitors, as shown by the lowest IC_{50}

value of 3.3 mM (inhibition of the *C. saccharolyticum* β-glucosidase by **16**) at pH 6.8 where a significant fraction of the inhibitor is unprotonated[3]). A comparison of the inhibition constants with those obtained for snail β-mannosidase has to account for the different pH of the assays. The pK_{HA} value suggests that, at pH 4.5, only one in 10000 molecules of **3** ($pK_{HA} = 8.6$ [8]) is not protonated. Snail β-mannosidase is inhibited by **3** 5.5 times more strongly at pH 5.5 than at pH 4.5 [17]. Thus, an inhibitor geometrically corresponding to **3**, but with a hypothetical pK_{HA} of 4.5 should inhibit *ca.* 55000 times more strongly than **3**, and display an inhibition constant of 50–100 nM. Analogous considerations for **4** (hypothetical analogue possessing a pK_{HA} of 6.8) suggest an inhibition constant of *ca.* 5 μM[4]).

Table. *Inhibition of β-Glucosidases by the Norbornanes* **4** *and* **16** *at pH 6.8 and the Indicated Temperature*

Compound	β-Glucosidase from *Caldocellum saccharolyticum* at 55°	β-Glucosidase from sweet almonds at 37°
4 (pK_{HA} 8.4)	$IC_{50} = 5.6$ mM	no inhibition
16 (pK_{HA} 7.9)	$IC_{50} = 3.3$ mM	$IC_{50} = 5.5$ mM

These considerations suggest that the geometry of the *manno*-configured norbornane **3** is closer to that of the reactive β-D-mannopyranoside conformer than the geometry of the *gluco*-configured norbornane **4** is to the reactive β-D-glucopyranoside conformer. This conclusion is consistent with earlier results of inhibition studies of the *manno*- and *gluco*-analogue isoquinuclidines **1** and **2** and supports the hypothesis of a different reactive conformation for these glycosidases (*cf.* [5][18][19]).

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Experimental Part

General. See [8]. The β-glucosidases and 4-nitrophenyl β-D-glucopyranoside were purchased from *Sigma*, and used without any further purification.

Benzyl N-*[(1*RS*,2*RS*,3*SR*,4*SR*,5*SR*)-2,3-Dihydroxy-5-(hydroxymethyl)bicyclo[2.2.1]hept-1-yl]carbamate* (**6**). A soln. of **5** (1.4 g, 4.03 mmol), conc. H_2SO_4 (0.05 ml, 0.94 mmol) and EtSH (12.5 g, 202 mmol) in EtOH (30 ml) was heated to reflux, stirred for 5 d, treated with EtSH (10 g, 160 mmol), stirred for 1 d, treated with EtSH (8.4 ml, 135 mmol), stirred for 1 d, cooled to 23°, treated with $NaHCO_3$ (80 mg, 0.95 mmol), and evaporated. FC (AcOEt/MeOH 20:1 → 10:1) gave **6** (1.09 g, 88%). Colourless powder. R_f (AcOEt/MeOH 10:1) 0.48. M.p. 129°. IR (KBr): 3600–2500*s* (br.), 1723*s*, 1587*m*, 1499*s*, 1466*s*. ^{1}H-NMR (400 MHz, CD_3OD): 1.34 (br. *dd, J* = 12.0, 3.5, H_{exo}–C(6)); 1.56 (br. *d*, *J* = 10.0, H–C(7)); 1.61–1.68 (*m*, H–C(5)); 1.68–1.76 (*m*, H_{endo}–C(6), H′–C(7)); 2.02 (br. *s*, H–C(4)); 1.72 (br. *d*, *J* = 7.2, CH_2–C(5)); 3.78 (br. *s*, H–C(2), H–C(3)); 5.04 (*s*, PhCH_2); 7.26–7.53 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, CD_3OD; assignments based on a HSQC spectrum): 32.68 (*t*, C(6)); 34.52 (*t*, C(7)); 40.80 (*d*, C(5)); 44.07 (*d*, C(4)); 63.68 (*t*, CH_2–C(5)); 66.08 (*t*, PhCH_2); 67.19 (*s*, C(1)); 74.10, 75.09

[3]) We have already reported a marked dependence of the inhibition on the pH in the assay [8], corroborating the hypothesis that the inhibitor binds to the glycosidase in the unprotonated form.

[4]) $K_i = IC_{50}/2$.

(2*d*, C(2), C(3)); 128.82 (*d*); 128.96 (4*d*); 138.43 (*s*); 158.09 (*s*, C=O). ESI-MS: 308.1 (35, $[M+H]^+$), 330.1 (100, $[M+Na]^+$). Anal. calc. for $C_{16}H_{21}NO_5$ (307.35): C 62.53, H 6.89, N 4.56; found: C 62.28, H 6.77, N 4.41.

Benzyl N-*[(1*RS,*2*RS,*3*SR,*4*SR,*5*SR*)-5-{[(*tert-*Butyl)diphenylsilyloxy]methyl}-2,3-dihydroxybicyclo[2.2.1]hept-1-yl]carbamate* (**7**). A soln. of **6** (1.06 g, 3.45 mmol), 1*H*-imidazole (350 mg, 5.1 mmol) and ^{t}BuPh$_2$SiCl (1.04 g, 3.78 mmol) in DMF (30 ml) was stirred at 23° for 3 d, treated with ^{t}BuPh$_2$SiCl (53 mg, 0.19 mmol), stirred for 1 d, treated with ^{t}BuPh$_2$SiCl (53 mg, 0.19 mmol), stirred for 3 d, cooled to 0°, treated with H_2O (30 ml), and extracted with Et_2O (2×30 ml, 2×15 ml). The combined org. layers were dried ($MgSO_4$) and evaporated. FC (cyclohexane/AcOEt 2:1→1:1) gave **7** (1.57 g, 83%). Colourless powder. R_f (cyclohexane/AcOEt 1:1) 0.47. M.p. 120°. IR ($CHCl_3$): 3505*w*, 3423*w*, 2933*m*, 1959*w*, 1898*w*, 1722*s*, 1607*w*, 1587*w*, 1508*s*. ^{1}H-NMR (400 MHz, $CDCl_3$; assignments based on a DQFCOSY and a HSQC spectrum): 1.04 (*s*, *t*-Bu); 1.43 (*dd*, *J*=12.5, 5.8, H$_{exo}$–C(6)); 1.49–1.53 (*m*, H$_{endo}$–C(6)); 1.52 (br. *d*, *J*=10.3, H–C(7)); 1.68–1.74 (*m*, H–C(5)); 1.86 (br. *d*, *J*≈9.6, H′–C(7)); 2.20 (br. *s*, H–C(4)); 3.04 (br. *s*, OH); 3.42–3.52 (*m*, CH$_2$–C(5)); 3.68 (br. *s*, OH); 3.77, 3.82 (2 br. *s*, H–C(2), H–C(3)); 5.06 (*s*, PhC*H*$_2$); 5.28 (*s*, NH); 7.31–7.64 (*m*, 15 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$; assigments based on a HSQC spectrum): 19.24 (*s*, Me$_3$*C*); 26.89 (*q*, *Me*$_3$C); 30.37 (*t*, C(7)); 33.32 (*t*, C(6)); 39.02 (*d*, C(5)); 42.97 (*d*, C(4)); 63.19 (*s*, C(1)); 66.24 (*t*, CH$_2$–C(5)); 66.76 (*t*, Ph*C*H$_2$); 73.90, 74.15 (2*d*, C(2), C(3)); 127.69 (4*d*); 128.04 (2*d*); 128.19 (*d*); 128.56 (2*d*); 129.67 (2*d*); 133.64 (2*s*); 135.57 (4*d*); 136.27 (*s*); 156.44 (*s*, C=O). ESI-MS: 546.0 (6.4, $[M+H]^+$), 567.9 (100, $[M+Na]^+$). Anal. calc. for $C_{32}H_{39}NO_5Si$ (545.75): C 70.43, H 7.20, N 2.57; found: C 70.38, H 7.18, N 2.58.

*(1*RS,*2*RS,*3*SR,*4*SR,*5*SR*)-1-{[(Benzyloxy)carbonyl]amino}-5-{[(*tert-*butyl)diphenylsilyloxy]methyl}-2-hydroxybicyclo[2.2.1]hept-3-yl Benzoate* (**8**) *and (1*RS,*2*RS,*3*SR,*4*SR,*5*SR*)-1-{[(Benzyloxy)carbonyl]amino}-5-{[(*tert-*butyl)diphenylsilyloxy]methyl}-3-hydroxybicyclo[2.2.1]hept-2-yl Benzoate* (**9**). A soln. of **7** (500 mg, 0.92 mmol) and Et_3N (234 mg, 2.3 mmol) in toluene (8.4 ml) was treated with BzCl (135 mg, 0.96 mmol), stirred at 20° for 3 d, treated with DMAP (20 mg, 0.16 mmol), stirred for 2 d, and evaporated. FC (hexane/AcOEt 3:1) yielded **8**/**9** 82:18 (540 mg, 91%). Colourless foam. R_f (cyclohexane/AcOEt 2:1) 0.55. IR ($CHCl_3$): 3432*w*, 3015*s*, 1964*w*, 1899*w*, 1817*w*, 1718*s*, 1602*w*, 1585*w*, 1507*m*, 1552*w*, 1428*w*, 1278*s*. ^{1}H-NMR (300 MHz, $CDCl_3$, **8**/**9** 82:18): data of **8**: 1.07 (*s*, *t*-Bu); 1.52 (br. *t*, *J*≈12, H$_{exo}$–C(6)); 1.62–1.78 (*m*, H$_{endo}$–C(6)); 1.80–1.98 (*m*, H–C(5), 2 H–C(7)); 2.43 (br. *s*, H–C(4)); 2.53 (br. *d*, *J*=6.2, exchange with CD_3OD, OH); 3.56 (*d*, *J*=5.9, CH$_2$–C(5)); 4.02 (br. *dd*, *J*=6.2, addn. of CD_3OD → br. *d*, *J*=5.0, H–C(2)); 4.96 (br. *d*, *J*=5.3, H–C(3)); 5.06 (*s*, PhC*H*$_2$); 5.39 (br. *s*, exchange with CD_3OD, NH); 7.32–8.34 (*m*, 20 arom. H); data of **9**: 2.29 (br. *s*, H–C(4)); 4.17 (br. *t*, *J*=4.9, addn. of CD_3OD → *m*, H–C(3)); 5.00–5.09 (*m*, PhC*H*$_2$, H–C(2)); 5.23 (br. *s*, exchange with CD_3OD, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): data of **8**/**9** 82:18: 19.39 (*s*, Me$_3$*C*); 27.03 (*q*, *Me*$_3$C); 32.51 (*t*, C(6), C(7)); 66.55 (*t*, Ph*C*H$_2$); 77.75 (*d*, C(3)); 127.64 (5*d*); 127.94 (2*d*); 128.01 (*d*); 128.37 (2*d*); 128.43 (2*d*); 129.62 (4*d*); 129.68 (*d*); 135.49 (3*d*); 129.70, 133.16, 133.54, 136.38 (4*s*); 155.74 (*s*, N–C=O); 166.43 (*s*, O–C=O); data of **8**: 39.35, 40.48 (2*d*, C(4), C(5)); 62.99 (*s*, C(1)); 66.10 (*t*, CH$_2$–C(5)); 75.06 (*d*, C(2)); data of **9**: 39.60 (*d*, C(4) or C(5)); 62.27 (*s*, C(1)); 75.39 (*d*, C(3)). HR-ESI-MS: 672.2751 ($[M+Na]^+$, $C_{39}H_{43}NNaO_6Si^+$; calc. 672.2757). Anal. calc. for $C_{39}H_{43}NO_6Si$ (649.86): C 72.08, H 6.67, N 2.16; found: C 72.08, H 6.73, N 2.41.

Oxidation of **8**/**9**. A soln. of **8**/**9** 82:18 (150 mg, 0.231 mmol) in CH_2Cl_2 (7.5 ml) was cooled to 0°, treated with 15% *Dess–Martin* periodinane in CH_2Cl_2 (0.6 ml) during 50 min, stirred for 120 min, and treated with 10% aq. $Na_2S_2O_3$ soln. (10 ml) and $NaHCO_3$ (250 mg). The org. layers were separated, and the aq. layer was extracted with CH_2Cl_2 (4×10 ml). The combined org. layers were dried (Na_2SO_4) and evaporated. FC (hexane/AcOEt 5:1) afforded **10** (106 mg, 71%) and **11** (21 mg, 13%).

*(1*RS,*3*SR,*4*SR,*5*SR*)-1-{[(Benzyloxy)carbonyl]amino}-5-{[(*tert-*butyl)diphenylsilyloxy]methyl}-2-oxobicyclo[2.2.1]hept-3-yl Benzoate* (**10**). R_f (cyclohexane/AcOEt 3:1) 0.61. IR ($CHCl_3$): 3409*w*, 3012*m*, 1769*m*, 1724*s*, 1602*w*, 1512*m*, 1452*w*, 1428*w*, 1264*s*, 1112*s*. ^{1}H-NMR (500 MHz, $CDCl_3$; assignments based on a DQFCOSY spectrum): 1.06 (*s*, *t*-Bu); 1.54–1.58 (*m*, irrad. at 2.16 → NOE of 6.8%, H$_{exo}$–C(6)); 2.03–2.09 (*m*, H$_{endo}$–C(6), H–C(7)); 2.12–2.20 (*m*, irrad. at 5.16 → NOE of 8.5%, irrad. at 2.73 → NOE of 5.8%, H–C(5)); 2.62 (br. *d*, *J*=10.9, H′–C(7)); 2.73 (br. *s*, irrad. at 5.16 → NOE of 3.2%, irrad. at 2.16 → NOE of 2.0%, H–C(4)); 3.69–3.71 (*m*, irrad. at 2.73 → NOE of 1.7%, irrad. at 2.16 → NOE of 3.9%, CH$_2$–C(5)); 5.08 (*s*, PhC*H*$_2$); 5.16 (br. *s*, irrad. at 2.73 → NOE of 4.0%, irrad. at

2.16 → NOE of 12.3%, H–C(3)); 5.49 (br. *s*, NH); 7.30–8.20 (20 arom. H). ^{13}C-NMR (125 MHz, $CDCl_3$): 19.23 (*s*, Me_3C); 26.88 (*q*, Me_3C); 34.36, 39.30 (2*t*, C(6), C(7)); 39.36, 41.17 (2*d*, C(4), C(5)); 65.60 (*t*, CH_2–C(5)); 66.70 (*s*, C(1)); 67.00 (*t*, PhCH_2); 75.32 (*d*, C(3)); 127.80 (4*d*); 128.06 (2*d*); 128.23 (*d*); 128.46 (2*d*); 128.57 (2*d*); 129.79 (*d*); 129.82 (*d*); 129.92 (2*d*); 133.47 (*d*); 135.57 (4*d*); 129.24 (*s*); 133.40 (2*s*); 136.17 (*s*); 154.61 (*s*, N–C=O); 165.36 (*s*, O–C=O); 207.91 (*s*, C=O). ESI-MS: 669.9 (100, $[M+Na]^+$). Anal. calc. for $C_{39}H_{41}NO_6Si$ (647.84): C 72.31, H 6.38, N 2.16; found: C 72.26, H 6.35, N 2.18.

*(1*RS*,2*RS*,4*SR*,5*SR*)-1-{[(Benzyloxy)carbonyl]amino}-5-{[(*tert*-butyl)diphenylsilyloxy]methyl}-3-oxobicyclo[2.2.1]hept-2-yl Benzoate* (**11**). R_f (cyclohexane/AcOEt 3:1) 0.59. IR ($CHCl_3$): 3440*w*, 3012*w*, 1767*m*, 1728*s*, 1602*w*, 1510*m*, 1452*w*, 1418*w*, 1266*s*, 1110*s*. ^{1}H-NMR (500 MHz, $CDCl_3$; assignments based on a DQFCOSY spectrum): 1.06 (*s*, *t*-Bu); 1.55–1.59 (*m*, H_{exo}–C(6)); 1.80–1.89 (*m*, H–C(7)); 2.08–2.18 (*m*, H–C(5)), H′–C(7)); 2.31–2.43 (*m*, H_{endo}–C(6)); 2.81 (br. *s*, H–C(4)); 3.56–3.76 (*m*, CH_2–C(5)); 4.92 (*d*, *J* =11.9), 5.03 (*d*, *J*=12.1) (PhCH_2); 5.19–5.26 (br. *s*, NH); 5.25 (*d*, *J*=2.9, H–C(2)); 7.34–8.06 (*m*, 20 arom. H). ^{13}C-NMR (125 MHz, $CDCl_3$): 19.22 (*s*, Me_3C); 26.86 (*q*, Me_3C); 37.46 (*t*, C(6), C(7)); 49.73 (*d*, C(4), C(5)); 58.50 (*s*, C(1)); 65.39 (*t*, CH_2–C(5)); 66.59 (*t*, PhCH_2); 75.15 (*d*, C(2)); 127.81 (4*d*); 127.82 (4*d*); 128.48 (4*d*); 129.86 (*d*); 130.05 (*d*); 133.20 (*d*); 135.57 (5*d*); 128.93 (*s*); 133.60 (2*s*); 135.64 (*s*); 155.00 (*s*, N–C=O); 165.77 (*s*, O–C=O); 207.57 (*s*, C=O). ESI-MS: 670.0 (100, $[M+Na]^+$). Anal. calc. for $C_{39}H_{41}NO_6Si$ (647.84): C 72.31, H 6.38, N 2.16; found: C 72.18, H 6.56, N 2.05.

*(1*RS*,2*SR*,3*SR*,4*SR*,5*SR*)-1-{[(Benzyloxy)carbonyl]amino}-6-{[(*tert*-butyl)diphenylsilyloxy]methyl}-2-hydroxybicyclo[2.2.1]hept-3-yl Benzoate* (**13**). A soln. of **10** (165 mg, 0.255 mmol) in MeOH (15 ml) was treated with $NaBH_4$ (20 mg, 0.53 mmol), stirred vigorously for 15 min at 23°, treated with 20% NaH_2PO_4 soln. (5 ml) and $CHCl_3$ (5 ml), stirred for 2 h, and treated with $CHCl_3$ (5 ml) and H_2O (5 ml). The layers were separated, and the aq. layer was extracted with $CHCl_3$ (3×5 ml). The combined org. layers were dried ($MgSO_4$) and evaporated. FC (hexane/AcOEt 5:1) gave **13** (48 mg, 29%) and a 82:18 mixture of **8**/**9** (108 mg, 65%). Colourless foam. R_f (cyclohexane/AcOEt 2:1) 0.60. IR ($CHCl_3$): 3435*w*, 3011*w*, 1964*w*, 1899*w*, 1824*w*, 1714*s*, 1602*w*, 1512*s*, 1452*w*, 1428*w*, 1276*s*, 1112*s*. ^{1}H-NMR (400 MHz, $CDCl_3$; assignments based on DQFCOSY and HSQC spectra): 1.03–1.06 (*m*, H_{exo}–C(6)); 1.06 (*s*, *t*-Bu); 1.77 (br. *d*, *J*=10.3, H–C(7)); 1.98 (br. *d*, *J*=10.2, irrad. at 4.20→NOE of 4.0%, H′–C(7)); 2.10 (br. *t*, *J*=6.4, irrad. at 4.60 → NOE of 8.8%, H–C(5)); 2.40 (br. *s*, irrad. at 4.60 → NOE of 4.3%, H–C(4)); 2.45 (br. *t*, *J*=10.1, H_{endo}–C(6)); 3.58 (*d*, *J*=6.3, irrad. at 2.10 → NOE of 2.6%, CH_2–C(5)); 4.20 (br. *s*, H–C(2)); 4.31 (br. *s*, exchange with CD_3OD, OH); 4.60 (br. *s*, irrad. at 2.10 → NOE of 11.3%, H–C(3)); 5.03, 5.09 (2*d*, *J*=12.2, PhCH_2); 5.21 (br. *s*, exchange with CD_3OD, NH); 7.33–8.04 (*m*, 20 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$; assignments based on a HSQC spectrum): 19.27 (*s*, Me_3C); 26.91 (*q*, Me_3C); 28.95 (*t*, C(6)); 36.55 (*t*, C(7)); 39.89 (*d,* C(5)); 41.72 (*d*, C(4)); 64.42 (*s*, C(1)); 66.12 (*t*, CH_2–C(5)); 66.98 (*t*, PhCH_2); 81.84 (*d*, C(2)); 85.13 (*d*, C(3)); 127.72 (4*d*); 128.14 (*d*); 128.29 (*d*); 128.37 (3*d*); 128.60 (*d*); 129.66 (3*d*); 129.69 (*d*); 129.71 (*d*); 133.07 (*d*); 135.61 (4*d*); 130.18 (*s*); 133.63 (2*s*); 136.05 (*s*); 156.68 (*s*, N–C=O); 166.38 (*s*, O–C=O). HR-ESI-MS: 672.2745 ($[M+Na]^+$, $C_{39}H_{43}NNaO_6Si^+$; calc. 672.2757). Anal. calc. for $C_{39}H_{43}NO_6Si$ (649.86): C 72.08, H 6.67, N 2.16; found: C 72.22, H 6.83, N 2.29.

*(1*RS*,2*RS*,3*RS*,4*SR*,5*SR*)-1-{[(Benzyloxy)carbonyl]amino}-5-{[(*tert*-butyl)diphenylsilyloxy]methyl}-3-hydroxybicyclo[2.2.1]hept-2-yl Benzoate* (**12**). A soln. of **11** (69 mg, 0.11 mmol) in MeOH (5.5 ml) was treated with $NaBH_4$ (8 mg, 0.21 mmol), stirred for 15 min at 23°, treated with 20% aq. NaH_2PO_4 soln. (5 ml) and $CHCl_3$ (5 ml), and stirred for 20 min. The layers were separated, and the aq. layer extracted with $CHCl_3$ (3×5 ml). The combined org. layers were dried ($MgSO_4$) and evaporated. FC (hexane/AcOEt 5:1) gave **12** (34 mg, 49%) and a 82:18 mixture of **8**/**9** (29 mg, 29%). Colourless foam. R_f (cyclohexane/AcOEt 2:1) 0.61. IR ($CHCl_3$): 3446*w*, 3011*m*, 1967*w*, 1899*w*, 1815*w*, 1712*s*, 1602*w*, 1587*w*, 1506*m*, 1452*m*, 1427*m*, 1320*m*, 1275*s*, 1110*s*. ^{1}H-NMR (400 MHz, $CDCl_3$; assignments based on a DQFCOSY spectrum): 1.00 (*s*, *t*-Bu); 1.55–1.65 (*m*, irrad. at 3.91 → NOE of 3.1%, H_{exo}–C(6), H–C(7)); 1.82–1.89 (*m,* H′–C(7)); 2.01 (br. *t*, *J*≈10.5, irrad. at 2.60 → NOE of 4.0%, H_{endo}–C(6)); 2.29 (br. *d*, *J*≈3, irrad. at 3.91 → NOE of 7.3%, irrad. at 2.60 → NOE of 2.3%, H–C(4)); 2.60 (br. *t*, *J*≈7, irrad. at 2.29 → NOE of 4.4%, H–C(5)); 3.11 (br. *s*, exchange with CD_3OD, OH); 3.55 (*d*, *J*=6.9, irrad. at 2.29 → NOE of 1.2%, irrad. at 2.60 → NOE of 2.6%, CH_2–C(5)); 3.91 (br. *s*, irrad. at 2.29 → NOE of 7.1%, H–C(3)); 4.43 (br. *d*, H–C(2)); 4.96 (br. *d*, *J*≈12.5), 5.03 (*d*, *J*=11.8) (PhCH_2); 5.13

(br. *s*, exchanges with CD_3OD, NH); 7.30–7.92 (*m*, 20 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 19.30 (*s*, Me_3C); 26.92 (*q*, Me_3C); 35.00, 35.41 (2*t*, C(6), C(7)); 40.75 (*d*, C(4), C(5)); 62.98 (*s*, C(1)); 66.54 (*t*, PhCH_2, CH_2–C(5)); 80.32 (*d*, C(3)); 84.67 (*d*, C(2)); 127.67 (4*d*); 128.01 (3*d*); 128.52 (4*d*); 129.63 (*s* and 4*d*); 133.39 (*d*); 133.72 (2*s*); 135.65 (*s* and 4*d*); 167.55 (*s*, O–C=O). HR-ESI-MS: 672.2761 ($[M+Na]^+$, $C_{39}H_{43}NNaO_6Si^+$; calc. 672.2757).

*(1*RS*,2*SR*,3*SR*,4*SR*,5*SR*)-1-{[(Benzyloxy)carbonyl]amino}-2-hydroxy-5-(hydroxymethyl)bicyclo-[2.2.1]hept-3-yl Benzoate* (**14**). A soln. of **13** (64 mg, 0.099 mmol) and NH_4F (91 mg, 2.5 mmol) in MeOH (20 ml) was stirred for 2.5 h at 50°, treated with NH_4F (100 mg, 2.7 mmol), stirred for 20 h, and evaporated. The residue was triturated with AcOEt and filtered, and the filtrate evaporated. FC (hexane/AcOEt 1:2) yielded **14** (36 mg, 89%). Colourless foam. R_f (cyclohexane/AcOEt 1:2) 0.29. IR ($CHCl_3$): 3435*w*, 1709*s*. 1602*w*, 1513*m*, 1300*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.04 (*dd*, *J* = 12.1, 5.0, H_{exo}–C(6)); 1.81 (*d*, *J* = 10.0, H–C(7)); 1.90 (br. *s*, exchange with D_2O, OH); 2.00 (*d*, *J* = 9.7, H′–C(7)); 2.03–2.08 (*m*, H–C(5)); 2.38 (br. *s*, H–C(4)); 2.50 (br. *t*, *J* ≈ 10, H_{endo}–C(6)); 3.47–3.60 (*m*, CH_2–C(6)); 4.22 (br. *s*, H–C(2)); 4.47 (br. *s*, exchange with D_2O, OH); 4.60 (br. *s*, H–C(2)); 5.02, 5.03 (2*d*, *J* = 12.2, PhCH_2); 5.43 (br. *s*, exchange with D_2O, NH); 7.28–8.02 (*m*, 10 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 29.08 (*t*, C(6)); 36.41 (*t*, C(7)); 40.12 (*d*, C(5)); 41.53 (*d*, C(4)); 64.31 (*s*, C(1)); 65.27 (*t*, CH_2–C(5)); 66.96 (*t*, PhCH_2); 81.66 (*d*, C(2)); 84.95 (*d*, C(3)); 128.00 (2*d*); 128.16 (*d*); 128.27 (2*d*); 128.47 (2*d*); 129.50 (2*d*); 129.81 (*s*); 133.04 (*s*); 135.89 (*d*); 156.59 (*s*, N–C=O); 166.30 (*s*, O–C=O). HR-ESI-MS: 434.1580 ($[M+Na]^+$, $C_{23}H_{25}NNaO_6^+$; calc. 434.1580). Anal. calc. for $C_{23}H_{25}NO_6$ (411.45): C 67.14, H 6.12, N 3.40; found: C 67.00, H 6.23, N 3.42.

Benzyl N-*[(1*RS*,2*SR*,3*SR*,4*SR*,5*SR*)-2,3-Dihydroxy-5-(hydroxymethyl)bicyclo[2.2.1]hept-1-yl]carbamate* (**15**). A soln. of **14** (34 mg, 0.083 mmol) in MeOH (5 ml) was treated with MeONa (5.5 mg, 0.10 mmol), stirred for 24 h at 23°, treated with MeONa (3 mg, 0.06 mmol), stirred for 10 h, treated with MeONa (3 mg, 0.06 mmol), treated with AcOH (12.5 mg, 0.21 mmol), and evaporated. FC (AcOEt/MeOH 10:0.5 → 10:1) gave **15** (25 mg, 98%). Colourless powder. R_f (AcOEt/MeOH 10:1) 0.48. IR (CH_2Cl_2): 3603*m*, 3427*m*, 2927*m*, 1701*s*, 1605*m*, 1514*s*, 1454*w*, 1358*w*. ^{1}H-NMR (300 MHz, CD_3OD): 0.94 (*dd*, *J* = 12.1, 4.4, H_{exo}–C(6)); 1.66 (*td*, *J* = 10.6, 2.2, H–C(7)); 1.97–2.03 (*m*, H–C(4), H–C(5), H′–C(7)); 2.21 (br. *t*, *J* ≈ 10, H_{endo}–C(6)); 3.40 (*d*, *J* = 7.5, CH_2–C(5)); 3.46 (br. *s*, H–C(2)); 3.93 (br. *s*, H–C(3)); 4.87 (*s*, PhCH_2); 7.23–7.34 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz, CD_3OD): 29.43 (*t*, C(6)); 35.56 (*t*, C(7)); 40.98 (*d*, C(5)); 44.90 (*d*, C(4)); 64.93 (*s*, C(1)); 65.51 (*t*, CH_2–C(5)); 67.00 (*t*, PhCH_2); 82.31 (*d*, C(2)); 84.10 (*d*, C(3)); 128.36 (2*d*); 128.52 (*d*); 128.99 (2*d*); 137.80 (*s*); 158.21 (*s*, C=O). HR-ESI-MS: 330.1308 ($[M+Na]^+$, $C_{16}H_{21}NNaO_5^+$; calc. 330.1317). Anal. calc. for $C_{16}H_{21}NO_5$ (307.35): C 62.53, H 6.89, N 4.56; found: C 62.37, H 6.78, N 4.60.

*(1*RS*,2*SR*,3*SR*,4*SR*,5*SR*)-1-Amino-5-(hydroxymethyl)bicyclo[2.2.1]heptane-2,3-diol* (**4**). A suspension of **15** (44 mg, 0.143 mmol) and 10% Pd/C (5 mg) in EtOH (5 ml) was hydrogenated for 20 h at 21° under H_2, filtered, and evaporated. Ion-exchange chromatography (*Amberlite CG-120*; H_2O/NH_3 1:0 → 95:5) gave **4** (23 mg, 93%). Colourless solid. pK_{HA} 8.40. IR (KBr): 3600–2400*s* (br.), 1598*m*, 1449*s*, 1332*s*, 1207*m*, 1040*s*. ^{1}H-NMR (300 MHz, D_2O): 1.01 (br. *dd*, *J* ≈ 12.4, 4.0, H_{exo}–C(6)); 1.63 (br. *d*, *J* = 10.4, H–C(7)); 1.71–1.82 (*m*, H–C(4), H–C(5)); 2.00–2.06 (*m*, H_{endo}–C(6), H′–C(7)); 3.36 (*d*, *J* = 7.4, CH_2–C(5)); 3.46 (br. *s*, H–C(2)); 3.79 (br. *s*, H–C(3)). ^{13}C-NMR (75 MHz, D_2O): 26.79 (C(6)); 33.15 (C(7)); 39.02 (C(5)); 43.58 C(4)); 62.53 (C(1)); 63.62 (CH_2–C(5)); 80.44, 80.96 (C(2), C(3)). ESI-MS: 174.1 ($[M+H]^+$); 196.0 ($[M+Na]^+$).

*(1*RS*,2*SR*,3*SR*,4*SR*,5*SR*)-1-(Benzylamino)-5-(hydroxymethyl)bicyclo[2.2.1]heptane-2,3-diol* (**16**). A suspension of **15** (36 mg, 0.117 mmol) and 10% Pd/C (5 mg) in EtOH (5 ml) was hydrogenated for 2.5 h at 21° under H_2, filtered, and evaporated. A soln. of the residue in MeOH (2 ml) was treated with PhCHO (19 mg, 0.18 mmol) and $MgSO_4$ (100 mg), stirred for 22 h at 21°, and filtered (washing with 1 ml of MeOH). The filtrate was treated with $NaBH_4$ (25 mg, 0.661 mmol), stirred for 20 h, acidified with 1.25M HCl in MeOH until pH 2, and evaporated. Ion-exchange chromatography (*Amberlite CG-120*, H_2O/NH_3 1:0 → 95:5) gave **16** (23 mg, 75%). Colourless solid. pK_{HA} 7.90. IR (KBr): 3600–2400*s* (br.), 1952*w*, 1877*w*, 1811*w*, 1774*w*, 1637*w*, 1605*w*, 1453*s*, 1332*m*, 1202*m*, 1030*s*. ^{1}H-NMR (300 MHz, D_2O): 0.97 (*ddd*, *J* = 12.3, 4.6, 2.0, H_{exo}–C(6)); 1.46 (br. *d*, *J* = 10.6), 1.68 (br. *d*, *J* = 10.3) (2 H–C(7)); 1.77–1.93 (*m*, H–C(5), H_{endo}–C(6)); 1.96 (br. *s*, H–C(4)); 3.44 (*d*, *J* = 7.4, CH_2–C(5)); 3.54 (br. *s*, H–

C(2)); 3.74 (*s*, PhCH_2); 3.89 (br. *s*, H–C(3)). ^{13}C-NMR (300 MHz, D_2O): 27.63 (C(6)); 33.06 (C(7)); 39.43 (C(5)); 43.01 (C(4)); 47.94 (Ph*C*H_2); 64.17 (C(1)); 67.68 (CH_2–C(5)); 81.10, 81.78 (C(2), C(3)); 127.04; 128.23 (2 C); 128.46 (2 C); 139.37. HR-ESI-MS: 264.1596 ([M+H]$^+$, $C_{15}H_{22}NO_3^+$; calc. 264.1600).

Determination of the Inhibition Constants. Inhibition constants were determined in the same way as reported in [7] at pH 6.8 using a 0.06M KH_2PO_4/K_2HPO_4 buffer and 4-nitrophenyl β-D-glucopyranoside as the substrate.

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