Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *89* (10) S299–S337 (2024)
Supplementary material

SUPPLEMENTARY MATERIAL TO

N-2 Alkylated analogues of aza-galactofagomine as potential inhibitors of *β*-glucosidase

FILIP ĐURKOVIĆ[1], MARIO ZLATOVIĆ[1], DUŠAN SLADIĆ[1], IRENA NOVAKOVIĆ[2], FILIP BIHELOVIĆ[1]* and ZORANA FERJANČIĆ[1]**

[1]*University of Belgrade – Faculty of Chemistry, Studenstki trg 12–16, Belgrade, Serbia and* [2]*University of Belgrade – Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, Belgrade, Serbia*

J. Serb. Chem. Soc. *89* (10) (2024) 1255–1267

TABLE OF CONTENTS:

GENERAL EXPERIMENTAL S300

EXPERIMENTAL PROCEDURES S301

β-GLUCOSIDASE INHIBITION ASSAY FOR COMPOUNDS 12, 13, 14, 22, AGF AND CONDURITOL *β*-EPOXIDE S314

LIGAND INTERACTIONS SCHEMES FOR COMPOUNDS 12, 13, 22 AND AGF S317

COPIES OF NMR SPECTRA S321

* Corresponding authors. E-mail: (*)filip@chem.bg.ac.rs; (**)zferjan@chem.bg.ac.rs

GENERAL EXPERIMENTAL

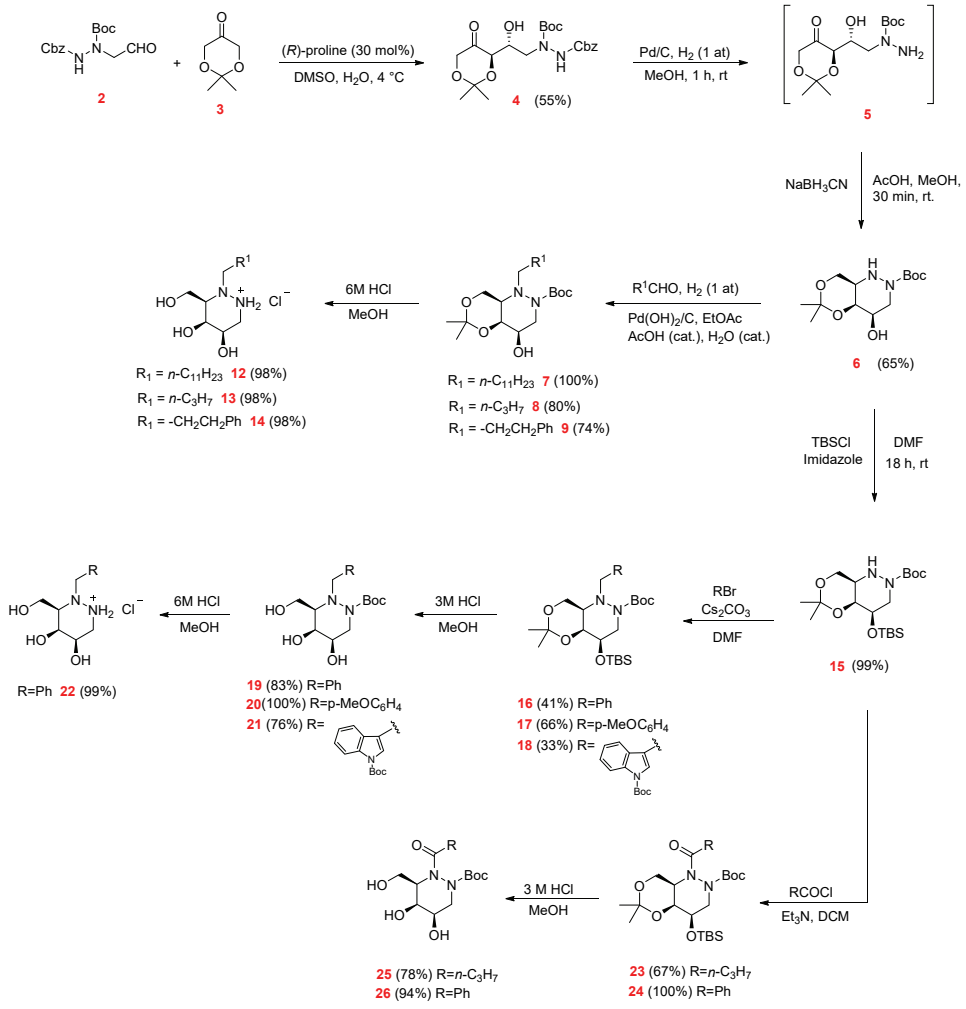
All chromatographic separations* were performed on Silica gel 60 (0.063-0.2 mm), Merck. Standard techniques were used for the purification of reagents and solvents.† NMR spectra were recorded on Varian/Agilent 400 (^{1}H NMR at 400 MHz, ^{13}C NMR at 100 MHz) and on Bruker Avance III 500 (^{1}H NMR at 500 MHz, ^{13}C NMR at 125 MHz), in deuterated chloroform, if not otherwise stated. Chemical shifts are expressed in ppm (δ) using tetramethylsilane as internal standard, coupling constants (J) are in Hz. IR spectra were recorded on Thermo Scientific Nicolet Summit FT-IR instrument, and are expressed in cm^{-1}. Mass spectra were obtained on Orbitrap Exploris 240 spectrometer. Melting point was determined on LLG-uniMELT 2 melting point apparatus, and it is uncorrected. Optical rotation was measured on Rudolph Research Analytical AUTOPOL IV Automatic Polarimeter.

* For description of the technique of dry-flash chromatography, *see*: a) L. M. Harwood, *Aldrichimica Acta,* **1985**, *18*, 25; b) *Vogel's Textbook of Practical Organic Chemistry*, Longman Scientific & Technical, 5th edition, London, 1989, p. 220; c) For some improvements of the separation technique, *see*: D. S. Pedersen, C. Rosenbohm, *Synthesis,* **2001**, 2431-2434.

† D. D. Perrin, W. L. F. Armarego, *Purification of Laboratory Chemicals*, 3rd edition, Pergamon Press, **1988**.

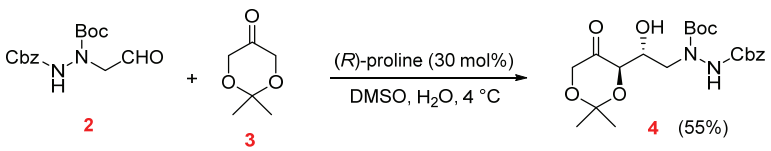
EXPERIMENTAL PROCEDURES

The experimental procedures for the new AGF analogues are arranged in a natural order and follows the Scheme S1.



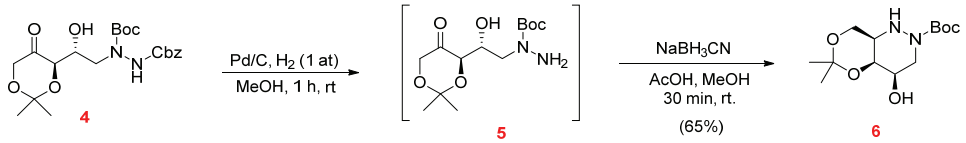
Scheme *S-1*

*2-Benzyl 1-(tert-butyl) 1-((R)-2-((R)-2,2-dimethyl-5-oxo-1,3-dioxan-4-yl)-2-hydroxyethyl)hydrazine-1,2-dicarboxylate (**4**)*



The freshly prepared aldehyde **2** (2.84 g; 9.22 mmol) was dissolved in DMSO (32 mL). The dioxanone **3** (3.20 g; 24.61 mmol; 2.67 eq) and (*R*)-proline (318 mg; 2.77 mmol; 30 mol%) were added. The mixture was stirred until homogeneous solution was formed. Water was added subsequently. The mixture was transferred to refrigerator and stirred for 3 days at 4 °C. The reaction mixture was diluted with EtOAc (100 mL), washed with water (4 x 50 mL) and brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by dry-flash chromatography (benzene/ethyl acetate = 85:15), to afford 2.49 g (61%) of the pure aldol **4** as viscous mass. All spectral data are in accordance with the literature (J. Marjanovic Trajkovic, Z. Ferjancic, R. N. Saicic, *Tetrahedron* **73** (2017) 2629).

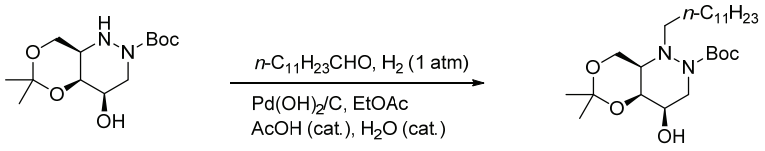
*tert-Butyl (4R,4aS,8aR)-4-hydroxy-6,6-dimethyltetrahydro-1H-[1,3]dioxino[5,4-c]pyridazine-2(3H)-carboxylate (**6**)*



4 5 6

A mixture of **4** (500 mg; 0.114 mmol) and 10% Pd/C (100 mg; 0.094 mmol; 82 mol%) in methanol (30 mL) was stirred for 1 hour under a hydrogen atmosphere (1 atm). The mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The residue was dissolved in MeOH (2 mL) and AcOH (8 mL) was added to the solution. The resulting mixture was stirred for 2 minutes and $NaBH_3CN$ (215 mg; 0.342 mmol; 3 eq) was added. Stirring was continued for 30 minutes at room temperature. The reaction mixture was diluted with DCM (50 mL) and neutralized with saturated $NaHCO_{3(aq)}$. Water layer was extracted two more times with DCM (2 x 50 mL). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated on rotovap. The product was purified by dry-flash chromatography (ethyl acetate/petroleum ether/methanol = 76:20:4), to afford 213 mg (65%) of **6**, as a white solid. All spectral data are in accordance with the literture (J. Marjanovic Trajkovic, Z. Ferjancic, R. N. Saicic, *Tetrahedron* **73** (2017) 2629).

tert-Butyl (4R,4aS,8aR)-1-dodecyl-4-hydroxy-6,6-dimethyltetrahydro-1H-[1,3]dioxino[5,4-c]pyridazine-2(3H)-carboxylate (7)

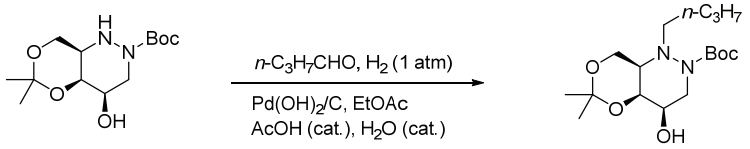


6 **7** (100%)

Compound **6** (10 mg; 0.035 mmol) and dodecyl aldehyde (33 mg; 0.179 mmol; 5.1 eq) were dissolved in EtOAc (500 µL) and water (6 µL) with catalytic amount of AcOH was added under argon atmosphere. 10% $Pd(OH)_2$ (10 mg; 0.007 mmol; 20 mol%) was added to the resulting solution, and the mixture was stirred under hydrogen atmosphere (1 atm) for 6 hours. The mixture was filtered through a pad of celite and the filtrate was concentrated under reduced pressure. The product was purified by column chromatography (petroleum ether/ethyl acetate = 1:1), to afford 16 mg (100%) of **7**, as a colorless film. Peaks in ^1H and ^{13}C NMR spectrum are broad and split, due to the presence of a *N*-Boc rotamers. ^1H NMR (400 MHz,

$CDCl_3$, δ): 4.13–3.60 (*m*, 4H), 3.19–2.60 (*m*, 3.5H), 2.34 (*t*, 0.5H, *J* = 7.6), 1.66–1.57 (*m*, 1H), 1.47, 1.45 and 1. 41 (3 x *bs*, 15H), 1.26 (*bs*, 20H), 0.88 (*t*, 3H, *J* = 6.7). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 99.16, 80.10, 66.81, 65.74, 61.48, 54.17, 50.20, 31.89, 31.87, 29.64, 29.60, 29.56, 29.41, 29.32, 29.29, 29.22, 29.07, 28.31, 27.96, 27.13, 24.75, 22.65, 18.87, 14.08. Under the recording conditions, signal for one carbon could not be detected. HRMS (ESI-Orbitrap) *m/z*: $[M+H]^+$ calcd. for $C_{25}H_{49}N_2O_5$: 457.3636, found: 457.3629.

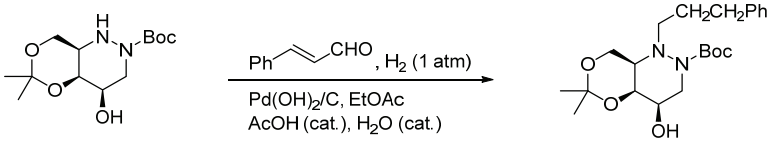
*tert-Butyl (4R,4aS,8aR)-1-butyl-4-hydroxy-6,6-dimethyltetrahydro-1H-[1,3]dioxino[5,4-c]pyridazine-2(3H)-carboxylate (**8**)*



6 **8** (80%)

Compound **6** (10 mg; 0.035 mmol) and butyl aldehyde (31 μL; 0.347 mmol; 10 eq) were dissolved in EtOAc (500 μL) and water (6 μL) with catalytic amount of AcOH was added under argon atmosphere. To the resulting solution, 10% $Pd(OH)_2$ (10 mg; 0.007 mmol; 20 mol%) was added and the mixture was stirred under hydrogen atmosphere (1 atm) for 18 hours. The mixture was filtered through a pad of celite and the filtrate was concentrated under reduced pressure. The product was purified by column chromatography (petroleum ether/ethyl acetate = 1:1), to afford 10 mg (80%) of **8**, as a colourless film. Peaks in ^{1}H and ^{13}C NMR spectrum are broad and split, due to the presence of a *N*-Boc rotamers. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 4.09–3.83 (*m*, 3H), 3.70–3.59 (*m*, 1H), 3.21–2.68 (*m*, 4H), 2.53–2.42 (*m*, 1H), 1.47, 1.44 and 1. 40 (3 x *bs*, 17H), 1.35–1.28 (*m*, 2H), 0.90 (*t*, 3H, *J* = 6.8). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 99.29, 80.22, 67.00, 66.16, 61.72, 54.47, 50.01, 39.03, 30.18, 29.60, 28.33, 20.39, 19.05, 14.24. Under the recording conditions, signal for one carbon could not be detected. HRMS (ESI-Orbitrap) *m/z*: $[M+H]^+$ calcd. for $C_{17}H_{33}N_2O_5$: 345.2384, found: 345.2378.

*tert-Butyl (4R,4aS,8aR)-4-hydroxy-6,6-dimethyl-1-(3-phenylpropyl)tetrahydro-1H-[1,3]dioxino[5,4-c]pyridazine-2(3H)-carboxylate (**9**)*

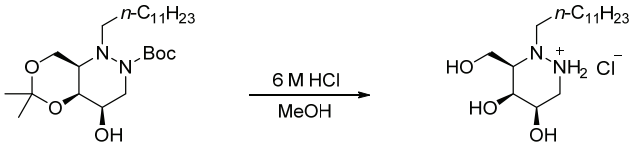


6 **9** (74%)

Compound **6** (20 mg; 0.069 mmol) and cinnamaldehyde (131 μL; 1.040 mmol; 15 eq) were dissolved in EtOAc (1000 μL) and water (12 μL) with catalytic amount of AcOH was added under argon atmosphere. To the resulting solution, 10% $Pd(OH)_2$ (30 mg; 0.021 mmol; 30 mol%) was added and the mixture was stirred under hydrogen atmosphere (1 atm) for 36 hours. The mixture was filtered through a pad of celite and the filtrate was concentrated under reduced pressure. The product was purified by column chromatography (petroleum ether/ethyl acetate = 1:1), to afford 21 mg (74%) of **9**, as a colorless film. Peaks in ^{1}H and ^{13}C NMR spectrum are broad and split, due to the presence of a *N*-Boc rotamers. ^{1}H NMR (400 MHz,

$CDCl_3$, δ): 7.28–7.18 (*m*, 4H), 7.15 (*t*, 1H, *J* = 7.0), 4.10–3.79 (*m*, 3H), 3.70–3.58 (*m*, 1H), 3.14 (*bs*, 2H), 3.03–2.81 (*m*, 3H), 2.68–2.58 (*m*, 1H), 2.51 (*d*, 1H, *J* = 8.6), 1.80–1.72 (*m*, 1H), 1.69–1.58 (*m*, 1H), 1.44 and 1. 37 (2 x *bs*, 15H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 142.71, 128.62, 128.30, 125.66, 99.34, 80.41, 67.01, 65.99, 61.59, 54.52, 49.55, 33.42, 33.35, 29.58, 29.53, 28.49, 18.98. Under the recording conditions, signal for one the quaternary carbon from Boc group could not be detected. HRMS (ESI-Orbitrap) *m/z*: $[M+H]^+$ calcd. for $C_{22}H_{35}N_2O_5$: 407.2540, found: 407.2534.

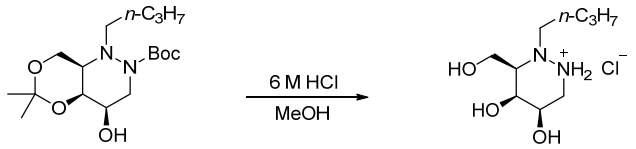
*(3R,4S,5R)-2-Dodecyl-4,5-dihydroxy-3-(hydroxymethyl)hexahydropyridazin-1-ium chloride (**12**)*



7 **12** (98%)

Compound **7** (12.5 mg; 0.027 mmol) was dissolved in MeOH (200 μL), and 6 M $HCl_{(aq)}$ (200 μL) was added. The resulting solution was stirred overnight at room temperature. The volatiles were removed on rotovap, to afford 9.5 mg (98%) of a pure hydrochloride salt **12** as a colourless film. ^{1}H NMR (400 MHz, D_2O, δ): 4.26–4.23 (*m*, 1H), 3.99–3.93 (*m*, 3H), 3.54–3.49 (*m*, 1H), 3.48–3.40 (*m*, 1H), 3.31–3.21 (*m*, 2H), 3.16 (*dd*, 1H, J_1 = 13.4, J_2 = 4.7), 1.84–1.73 (*m*, 2H), 1.35 and 1. 29 (2 x *bs*, 18H), 0.87 (*t*, 3H, *J* = 6.6). ^{13}C NMR (100 MHz, D_2O, δ): 66.94, 66.52, 64.92, 59.29, 53.77, 44.70, 31.78, 29.67, 29.60, 29.54, 29.47, 29.30, 29.06, 26.41, 22.99, 22.47, 13.70. HRMS (ESI-Orbitrap) *m/z*: $[M+H]^+$ calcd. for $C_{17}H_{37}N_2O_3$: 317.2799, found: 317.2795.

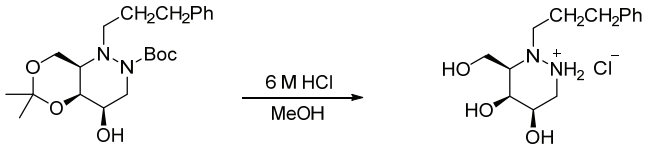
*(3R,4S,5R)-2-Butyl-4,5-dihydroxy-3-(hydroxymethyl)hexahydropyridazin-1-ium chloride (**13**)*



8 **13** (98%)

Compound **8** (9.5 mg; 0.029 mmol) was dissolved in MeOH (200 μL), and 6 M $HCl_{(aq)}$ (200 μL) was added. The resulting solution was stirred overnight at room temperature. The volatiles were removed on rotovap, to afford 6.8 mg (98%) of a pure hydrochloride salt **13** as a colorless film. ^{1}H NMR (400 MHz, D_2O, δ): 4. 26 (*bs*, 1H), 4.00–3.96 (*m*, 2H), 3.93 (*ddd*, 1H, J_1 = 10.4, J_2 = 5.0, J_3 = 2.9), 3.53–3.48 (*m*, 1H), 3.48–3.40 (*m*, 1H), 3.29 (*dd*, 1H, J_1 = 13.6, J_2 = 10.4), 3.30–3.21 (*m*, 1H), 3.18 (*dd*, 1H, J_1 = 13.6, J_2 = 5.0), 1.83–1.65 (*m*, 2H), 1.39 (*sx*, 2H, *J* = 7.4), 0.94 (*t*, 3H, *J* = 7.4). ^{13}C NMR (100 MHz, D_2O, δ): 67.08, 66.21, 65.02, 59.25, 53.79, 44.48, 24.25, 19.15, 12.69. HRMS (ESI-Orbitrap) *m/z*: $[M+H]^+$ calcd. for $C_9H_{21}N_2O_3$: 205.1547, found: 205.1545.

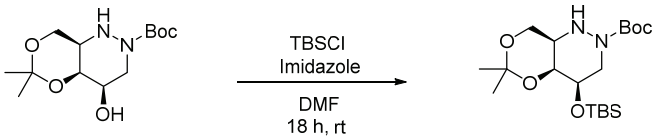
*(3R,4S,5R)-4,5-Dihydroxy-3-(hydroxymethyl)-2-(3-phenylpropyl)hexahydropyridazin-1-ium chloride (**14**)*



9 **14** (98%)

Compound **9** (20 mg; 0.049 mmol) was dissolved in MeOH (500 μL), and 6 M $HCl_{(aq)}$ (500 μL) was added. The resulting solution was stirred overnight at room temperature. The volatiles were removed on rotovap, to afford 14.6 mg (98%) of a pure hydrochloride salt **14** as a colorless film. ^{1}H NMR (400 MHz, D_2O, δ): 7.41–7.35 (*m*, 2H), 7.31–7.26 (*m*, 3H), 4.19 (bs, 1H), 3.92–3.84 (*m*, 3H), 3.45–3.35 (*m*, 2H), 3.27–3.19 (*m*, 2H), 3.15 (*dd*, 1H, J_1 = 13.6, J_2 = 5.0), 2.78–2.65 (*m*, 2H), 2.14–2.00 (*m*, 2H). ^{13}C NMR (100 MHz, D_2O, δ): 140.68, 128.67, 128.44, 126.36, 66.97, 65.93, 64.88, 59.17, 53.15, 44.59, 31.65, 24.06. HRMS (ESI-Orbitrap) *m/z*: $[M+H]^+$ calcd. for $C_{14}H_{23}N_2O_3$: 267.1703, found: 267.1699.

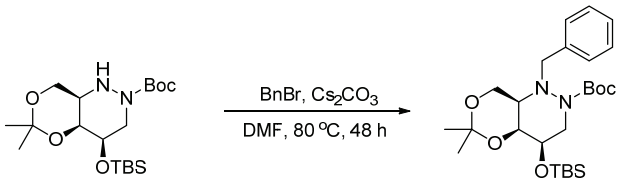
*tert-Butyl (4R,4aS,8aR)-4-((tert-butyldimethylsilyl)oxy)-6,6-dimethyltetrahydro-1H-[1,3]dioxino[5,4-c]pyridazine-2(3H)-carboxylate (**15**)*



6 **15** (99%)

Compound **6** (304 mg; 1.0 mmol) and imidazole (137 mg; 2.0 mmol; 2 eq) were dissolved in DMF (1.5 mL) under argon atmosphere, and TBSCl (228 mg; 1.5 mmol; 1.5 eq) was subsequently added. The mixture was stirred overnight at room temperature. After the reaction completion, the mixture was diluted with EtOAc, washed with water, saturated $NaHCO3_{(aq)}$ and brine. Organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by dry-flash chromatography (benzene/ethyl acetate = 1:1), to yield 415 mg (99%) of **15**, as a white crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 4.05 (*dd*, 1H, J_1 = 12.4, J_2 = 2.6), 4.01 (*d*, 1H, J = 1.9), 3.94 (*dd*, 1H, J_1 = 13.6, J_2 = 6.0), 3.90 (*d*, 1H, J = 13.0), 3.67 (*ddd*, 1H, J_1 = 10.8, J_2 = 4.9, J_3 = 3.1), 3.09 (*t*, 1H, J = 11.8), 2.64 (*s*, 1H), 1.48 (*s*, 9H), 1.45 (*s*, 3H), 1.43 (*s*, 3H), 0.90 (*s*, 9H), 0.10 (*s*, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 155.41, 99.00, 80.57, 68.48, 67.86, 62.36, 52.33, 45.95, 29.73, 28.47, 25.93, 18.51, 18.42, -4.24, -4.41. HRMS (ESI-Orbitrap) *m/z*: $[M+H]^+$ calcd. for $C_{19}H_{39}N_2O_5Si$: 403.2623, found: 403.2620.

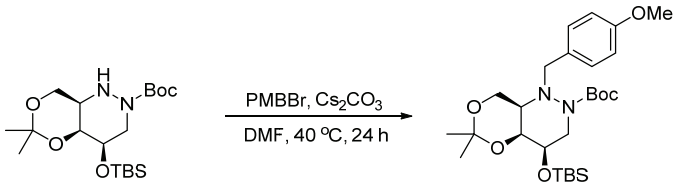
*tert-Butyl (4R,4aS,8aR)-1-benzyl-4-((tert-butyldimethylsilyl)oxy)-6,6-dimethyltetrahydro-1H-[1,3]dioxino[5,4-c]pyridazine-2(3H)-carboxylate (**16**)*



15 **16** (41%)

Compound **15** (25 mg; 0.060 mmol) was dissolved in DMF (1.5 mL) under argon atmosphere. Firstly, the Cs_2CO_3 (136 mg; 0.416 mmol; 7 eq) was added, followed by benzyl bromide (50 µL; 0.416 mmol; 7 eq). The mixture was stirred for 48 hours at 80 °C (full conversion was not achieved, even with prolonged reaction time). The mixture was diluted with Et_2O, washed with water, saturated $NH_4Cl_{(aq)}$ and brine. The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 7:3), to afford 12 mg (41%) of **16**, as a colorless film, and 8.3 mg of the starting material **15**. Peaks in ^{1}H and ^{13}C NMR spectrum are broad and split, due to the presence of a *N*-Boc rotamers. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.44–7.34 (*bs*, 2H), 7.28–7.26 (*bs*, 1H), 7.26–7.23 (*bs*, 1H), 7.22–7.16 (*m*, 1H), 4.54 (*d*, 1H, *J* = 14.5), 4.29–4.02 (*m*, 4H), 3.89–3.53 (*m*, 2H), 3.39–3.23 (*m*, 1H), 3.07–2.74 (*m*, 1H), 1.49 (*s*, 6H), 1.32–1.18 (*m*, 9H), 0.92 (*s*, 9H), 0.12 (*s*, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 161.58, 139.36, 129.60, 127.88, 126.81, 99.22, 79.86, 68.56 (overlap of 2C), 62.01, 55.56, 29.84, 28.22, 26.07, 18.89, 18.54, -4.18, -4.45. Under the recording conditions, signal for two carbons could not be detected. HRMS (ESI-Orbitrap) *m/z*: $[M+Na]^+$ calcd. for $C_{26}H_{45}N_2NaO_5Si$: 515.2912, found: 515.2909.

*tert-Butyl (4R,4aS,8aR)-4-((tert-butyldimethylsilyl)oxy)-1-(4-methoxybenzyl)-6,6-dimethyltetrahydro-1H-[1,3]dioxino[5,4-c]pyridazine-2(3H)-carboxylate (**17**)*

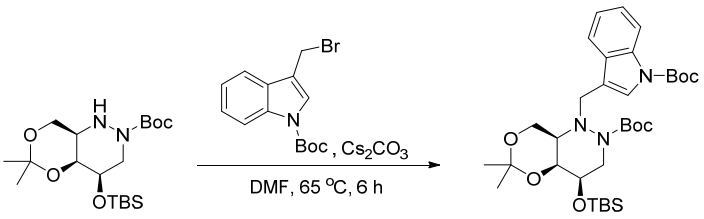


15 **17** (66%)

Substrate **15** (20 mg; 0.050 mmol) was dissolved in DMF (1 mL) under argon atmosphere. Firstly, the Cs_2CO_3 (81 mg; 0.248 mmol; 5 eq) was added, followed by PMB bromide (36 µL; 0.248 mmol; 5 eq). The mixture was stirred for 24 hours at 40 °C (full conversion was not achieved, even with prolonged reaction titme). The mixture was then diluted with Et_2O, washed with water, saturated $NH_4Cl_{(aq)}$ and brine. The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 7:3), to afford 16.5 mg (66%) of **17**, as a colorless film. Peaks in ^{1}H and ^{13}C NMR spectrum are broad and split, due to the presence of a *N*-Boc rotamers. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.34–7.26 (*m*, 2H), 6.80 (*d*, 2H, *J* = 8.0), 4.45 (b*d*, 1H, *J* = 13.8), 4.28–4.01 (*m*, 4H), 3.91–3.78 (*m*, 1H), 3.78 (*s*, 3H), 3.73–3.64 (*m*, 1H), 3.39–3.20 (*m*, 1H), 2.99–2.74 (*m*, 1H), 1.49 (*s*, 6H), 1.33–1.19 (*m*,

9H), 0.91 (*s*, 9H), 0.12 (*s*, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 158.65, 130.36, 114.02, 113.32, 99.17, 77.36, 68.60 (overlap of 2C), 62.02, 55.41, 54.82 (overlap of 2C), 29.80, 28.23, 25.99, 18.81, 18.49, -4.20, -4.45. Under the recording conditions, signal for two carbons could not be detected. HRMS (ESI-Orbitrap) *m/z*: [M+H]+ calcd. for $C_{27}H_{47}N_2O_6Si$: 523.3198, found: 523.3198.

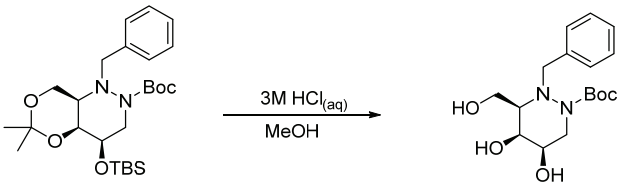
*tert-Butyl (4R,4aS,8aR)-1-((1-(tert-butoxycarbonyl)-1H-indol-3-yl)methyl)-4-((tert-butyldimethylsilyl)oxy)-6,6-dimethyltetrahydro-1H-[1,3]dioxino[5,4-c]pyridazine-2(3H)-carboxylate (**18**)*



15 18 (32%)

Substrate **15** (50 mg; 0.124 mmol) was then dissolved in DMF (2 mL) under argon atmosphere. Firstly, the Cs_2CO_3 (404 mg; 1.242 mmol; 10 eq) was added, followed by *tert*-butyl 3-(bromomethyl)-1H-indole-1-carboxylate (347 mg; 1.118 mmol; 9 eq). The mixture was stirred for 6 hours at 65 °C (full conversion was not achieved, even with prolonged reaction time). The mixture was diluted with Et_2O, washed with water, saturated $NH_4Cl_{(aq)}$ and brine. The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (benzene/ethyl acetate = 9:1), to afford 25 mg (32%) of **18**, as a colourless film. Peaks in ^{1}H and ^{13}C NMR spectrum are broad and split, due to the presence of a *N*-Boc rotamers. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.17–8.09 (*m*, 1H), 7.70–7.64 (*m*, 1H), 7.29–7.24 (*m*, 2H), 7.20 (*t*, 1H, *J* = 7.4), 4.62 (b*d*, 1H, *J* = 14.1), 4.55–4.33 (*m*, 1H), 4.26–4.11 (*m*, 2H), 4.08 (*bs*, 1H), 3.97–3.51 (*m*, 2H), 3.31 (*t*, 1H, *J* = 12.0), 3.06–2.75 (*m*, 1H), 1.67 and 1.64 (2 x *s*, 10H), 1.52 (*bs*, 6H), 1.44–1.24 (*m*, 4H), , 0.91 (*bs*, 13H), 0.12 (*s*, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 155.86, 149.91, 135.81, 124.12, 122.35, 118.24, 115.12, 99.25, 83.27, 68.63 (overlap of 2C), 62.29, 54.93, 46.08, 29.94, 28.34, 28.02, 25.98, 18.92, 18.48, -4.18, -4.46. Under the recording conditions, signals for three carbons could not be detected. Several peaks are missing due to overlap of N-Boc carbons. HRMS (ESI-Orbitrap) *m/z*: $[M+H]^+$ calcd. for $C_{33}H_{54}N_3O_7Si$: 632.3726, found: 632.3724.

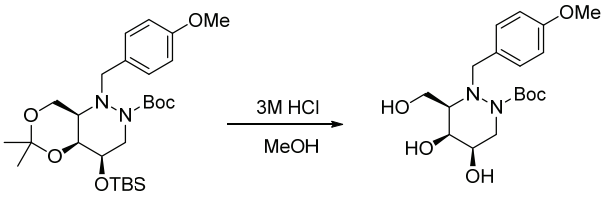
*tert-Butyl (3R,4S,5R)-2-benzyl-4,5-dihydroxy-3-(hydroxymethyl)tetrahydropyridazine-1(2H)-carboxylate (**19**)*



16 19 (83%)

Benzyl derivative **16** (12 mg; 0.025 mmol) was dissolved in MeOH (850 µL), and 3 M $HCl_{(aq)}$ (150 µL) was added. The resulting solution was stirred for 1 hour at room temperature. The mixture was neutralized with saturated $NaHCO_{3(aq)}$ and the volatiles were removed on rotovap. The resulting solid was suspended in EtOAc. The mixture was filtered through a pad of celite and the filtrate was concentrated under reduced pressure. The product was purified by column chromatography (EtOAc/EtOH 9:1), to afford 6.9 mg (83%) of a product **19** as a white foam. Peaks in ^{1}H and ^{13}C NMR spectrum are broad and split, due to the presence of a *N*-Boc rotamers. ^{1}H NMR (400 MHz, CD_3OD, δ): 7.35–7.06 (*m*, 5H), 4.11–3.75 (*m*, 6H), 3.58–3.50 (*m*, 1H), 3.45–3.39 (*m*, 1H), 3.05–2.94 (*m*, 1H), 1.26, 1.17 and 1.14 (3 x *s*, 9H). ^{13}C NMR (126 MHz, CD_3OD, δ) 158.78, 157.75, 138.72, 138.39, 130.96, 130.45, 129.27, 129.15, 128.78, 128.66, 128.50, 81.80, 68.59, 65.87, 64.88, 64.72, 60.07, 60.00, 57.94, 57.81, 45.42, 43.03, 28.44, 28.24.

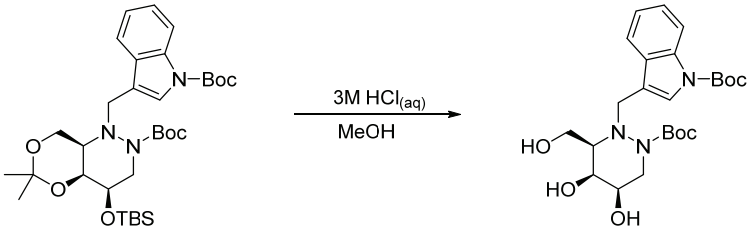
*tert-Butyl (3R,4S,5R)-4,5-dihydroxy-3-(hydroxymethyl)-2-(4-methoxybenzyl)tetrahydropyridazine-1(2H)-carboxylate (**20**)*



17 **20** (100%)

Compound **17** (16.5 mg; 0.032 mmol) was dissolved in MeOH (850 µL), and 3 M $HCl_{(aq)}$ (150 µL) was added. The resulting solution was stirred for 1 hour at room temperature. The mixture was neutralized with saturated $NaHCO_{3(aq)}$ and the volatiles were removed on rotovap. Resulting solid was suspended in EtOAc. The mixture was filtered through a pad of celite and the filtrate was concentrated under reduced pressure. The product was purified by column chromatography (EtOAc/MeOH 9:1), to afford 11.6 mg (100%) of a pure product **20** as a colorless film. Peaks in ^{1}H and ^{13}C NMR spectrum are broad and split, due to the presence of a *N*-Boc rotamers. ^{1}H NMR (400 MHz, CD_3OD, δ): 7.35–7.22 (*m*, 2H), 6.88–6.78 (*m*, 2H), 4.15–3.85 (*m*, 6H), 3.77, 3.76 and 3.75 (3 x *s*, 3H), 3.68–3.59 (*m*, 1H), 3.54–3.47 (*m*, 1H), 3.13–3.02 (*m*, 1H), 1.37, 1.29, 1.26, 1.24 and 1.22 (5 x *s*, 9H). ^{13}C NMR (100 MHz, CD_3OD, δ): 159.28, 156.34, 130.61, 130.21, 129.26, 128.88, 113.21, 113.09, 112.76, 80.30, 67.13, 67.10, 63.98, 63.40, 63.22, 62.89, 58.58, 58.55, 55.73, 55.62, 54.27, 54.21, 43.95, 41.53, 27.02, 26.82. HRMS (ESI-Orbitrap) *m/z*: $[M+H]^+$ calcd. for $C_{18}H_{29}N_2O_6$: 369.2020, found: 369.2018.

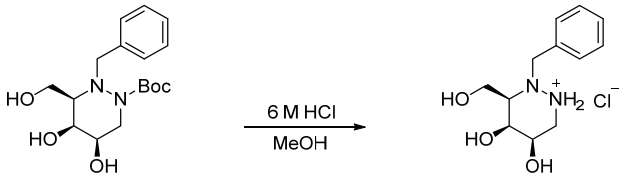
*tert-butyl 3-(((4R,5S,6R)-2-(tert-butoxycarbonyl)-4,5-dihydroxy-6-(hydroxymethyl)tetrahydropyridazin-1(2H)-yl)methyl)-1H-indole-1-carboxylate (**21**)*



18 **21** (76%)

Compound **18** (21 mg; 0.033 mmol) was dissolved in MeOH (850 µL), and 3 M $HCl_{(aq)}$ (150 µL) was added. The resulting solution was stirred for 1 hour at room temperature. The mixture was neutralized with saturated $NaHCO_{3(aq)}$ and the volatiles were removed on rotovap. The resulting solid was suspended in EtOAc. The mixture was filtered through a pad of celite and the filtrate was concentrated under reduced pressure. The product was purified by column chromatography (EtOAc/MeOH 95:5), to afford 12.1 mg (76%) of a pure product **21** as a colorless film. Peaks in ^{1}H and ^{13}C NMR spectrum are broad and split, due to the presence of a *N*-Boc rotamers. ^{1}H NMR (400 MHz, CD_3OD, δ): 8.12–8.05 (*m*, 1H), 7.86 and 7.75 (2 x *d*, 1H, *J* = 7.7), 7.67 and 7.55 (2 x *s*, 1H), 7.33–7.20 (*m*, 2H), 4.35 (*d*, 0.7H, *J* = 13.4), 4.25–4.04 (*m*, 3.3H), 3.96–3.87 (*m*, 2H), 3.69–3.51 (*m*, 2H), 3.27–3.21 (*m*, 0.7H), 3.17–3.09 (*m*, 0.3H), 1.67, 1.66, 1.39 and 1.00 (4 x *s*, 18H). ^{13}C NMR (100 MHz, CD_3OD, δ): 157.93, 150.99, 137.12, 131.86, 126.18, 126.11, 125.54, 125.41, 123.82, 123.66, 121.05, 120.87, 118.45, 118.09, 116.27, 115.91, 84.98, 81.80, 81.74, 68.58, 68.53, 65.89, 64.95, 64.58, 63.91, 60.21, 59.91, 48.08, 45.44, 42.74, 28.46, 28.41, 28.37, 27.91. HRMS (ESI-Orbitrap) *m/z*: $[M+H]^+$ calcd. for $C_{24}H_{36}N_3O_7$: 478.2548, found: 478.2547.

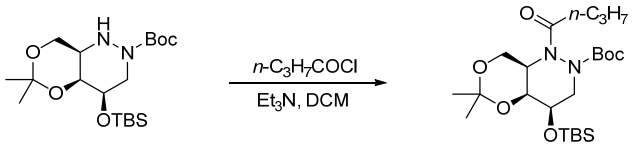
*(3R,4S,5R)-2-Benzyl-4,5-dihydroxy-3-(hydroxymethyl)hexahydropyridazin-1-ium chloride (**22**)*



19 **22** (99%)

Compound **19** (6.9 mg; 0.020 mmol) was dissolved in MeOH (200 µL), and 6 M $HCl_{(aq)}$ (200 µL) was added. The resulting solution was stirred overnight at room temperature. The volatiles were removed on rotovap, to afford 5.5 mg (99%) of a pure hydrochloride salt **22** as a colourless film. ^{1}H NMR (400 MHz, D_2O, δ): 7.53–7.48 (*m*, 5H), 4.62 (*d*, 1H, *J* = 13.9), 4.24 (*t*, 1H, *J* = 2.6), 4.16 (*d*, 1H, *J* = 14.0), 4.11 (*dd*, 1H, J_1 = 12.7, J_2 = 5.6), 4.04 (*dd*, 1H, J_1 = 12.7, J_2 = 4.7), 3.98 (*ddd*, 1H, J_1 = 9.0, J_2 = 5.6, J_3 = 3.0), 3.41–3.35 (*m*, 1H), 3.27–3.17 (*m*, 2H). ^{13}C NMR (100 MHz, D_2O, δ): 131.03, 130.30, 129.50, 129.10, 66.61, 65.17, 64.43, 59.35, 57.19, 44.35. HRMS (ESI-Orbitrap) *m/z*: $[M+H]^+$ calcd. for $C_{12}H_{19}N_2O_3$: 239.1390, found: 239.1387.

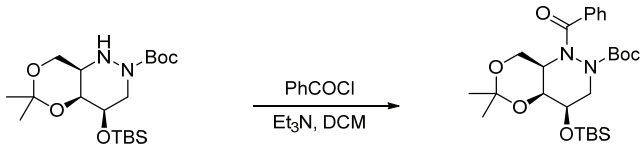
*tert-Butyl (4R,4aS,8aR)-4-((tert-butyldimethylsilyl)oxy)-1-butyryl-6,6-dimethyltetrahydro-1H-[1,3]dioxino[5,4-c]pyridazine-2(3H)-carboxylate (**23**)*



15 **23** (67%)

Substrate **15** (22 mg; 0.055 mmol) was dissolved in DCM (800 µL) and Et_3N (38 µL; 0.275 mmol; 5 eq) was added under argon atmosphere. Butyryl chloride (23 µL; 0.220 mmol; 4 eq) was slowly added to the reaction mixture dropwise, at room temperature. The mixture was stirred for 30 minutes, upon which time the reaction was completed. The mixture was diluted with DCM and washed with saturated $NaHCO_{3(aq)}$, water and brine. The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 75:25), to afford 17 mg (67%) of **23**, as a colorless film. Peaks in ^1H and ^{13}C NMR spectrum are broad and split, due to the presence of a *N*-Boc rotamers. ^1H NMR (400 MHz, $CDCl_3$, δ): 4.66 (*dd*, 0.38H, J_1 = 13.8, J_2 = 7.6), 4.34 (dd, 0.62H, J_1 = 10.0, J_2 = 5.3), 4.17 (*dd*, 0.62H, J_1 = 5.7, J_2 = 1.9), 4.12 (*dd*, 0.62H, J_1 = 5.8, J_2 = 1.8), 4.09–3.92 (*m*, 1.7H), 3.88–3.75 (*m*, 2.3H), 3.55 (*dd*, 0.38H, J_1 = 9.7, J_2 = 6.2), 3.43 (*dd*, 0.62H, J_1 = 11.0, J_2 = 7.2), 2.35–2.19 (*m*, 2H), 1.69–1.60 (*m*, 2H), 1.52 and 1.48 (2 x *s*, 9H), 1.41 and 1.36 (2 x *s*, 6H), 0.94 (*t*, 3H, *J* = 7.2), 0.90 and 0.87 (2 x *s*, 9H), 0.09, 0.07 and 0.06 (3 x *s*, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 175.35, 175.26, 155.01, 154.59, 100.47, 98.95, 82.16, 81.97, 67.42, 66.79, 66.57, 66.49, 60.52, 58.98, 51.87, 49.48, 48.18, 46.81, 33.60, 33.58, 28.38, 27.38, 25.88, 25.79, 24.81, 22.88, 20.44, 18.38, 18.24, 18.15, 17.99, 14.10, 14.05, -4.49, -4.55, -4.58, -4.64. HRMS (ESI-Orbitrap) *m/z*: $[M+Na]^+$ calcd. for $C_{23}H_{44}N_2NaO_6Si$: 495.2861, found: 495.2871.

*tert-Butyl (4R,4aS,8aR)--benzoyl-4-((tert-butyldimethylsilyl)oxy)-6,6-dimethyltetrahydro-1H-[1,3]dioxino[5,4-c]pyridazine-2(3H)-carboxylate (**24**)*

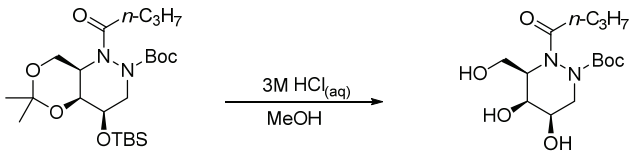


15 **24** (99%)

Compound **15** (40.3 mg; 0.10 mmol) was dissolved in DCM (800 µL) and Et_3N (35 µL; 0.25 mmol; 2.5 eq) was added under argon atmosphere. Benzoyl chloride (23 µL; 0.20 mmol; 2 eq) was slowly added to the reaction mixture dropwise, at room temperature. The mixture was stirred for 30 minutes, upon which time the reaction was completed. The mixture was diluted with DCM and washed with saturated $NaHCO_{3(aq)}$, water and brine. The organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (benzene/ethyl acetate = 85:15), to afford 50 mg (99%) of **24**, as a colorless film. Peaks in ^1H and ^{13}C NMR spectrum are broad and split, due to the presence of a *N*-Boc rotamers. ^1H NMR (400 MHz, $CDCl_3$, δ): 7.51–7.32 (*m*, 5H),

4.74–4.62 (*m*, 0.3H), 4.41–3.99 (*m*, 5.3H), 3.77 (*t*, 0.4H, *J* = 10.3), 3.57–3.43 (*m*, 1H), 1.46, 1.44, 1.38 and 1.36 (4 x *s*, 6H), 1.26 and 1.20 (2 x *s*, 9H), 0.93 and 0.91 (2 x *s*, 9H), 0.14 and 0.13 (2 x *s*, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 171.66, 171.31, 154.37, 153.68, 134.05, 130.80, 130.17, 128.14, 127.84, 127.32, 126.66, 100.59, 99.16, 81.93, 81.85, 67.33, 66.90, 66.86, 66.72, 59.70, 58.86, 53.28, 51.30, 48.35, 47.30, 28.01, 27.87, 26.98, 25.90, 25.81, 24.52, 23.08, 20.77, 18.42, 18.27. HRMS (ESI-Orbitrap) *m/z*: $[M+Na]^+$ calcd. for $C_{26}H_{42}N_2NaO_6Si$: 529.2704, found: 529.2703.

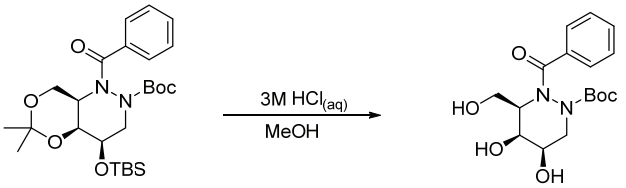
*tert-Butyl (3R,4S,5R)-2-butyryl-4,5-dihydroxy-3-(hydroxymethyl)tetrahydropyridazine-1(2H)-carboxylate (**25**)*



23 **25** (78%)

Compound **23** (15 mg; 0.032 mmol) was dissolved in MeOH (850 μL), and 3 M $HCl_{(aq)}$ (150 μL) was added. The resulting solution was stirred for 1 hour at room temperature. The mixture was neutralized with saturated $NaHCO_{3(aq)}$ and the volatiles were removed on rotovap. The resulting solid was suspended in EtOAc. The mixture was filtered through a pad of celite and the filtrate was concentrated under reduced pressure. The product was purified by column chromatography (EtOAc/MeOH 9:1), to afford 7.9 mg (78%) of a pure product **25** as a white foam. Peaks in ^{1}H and ^{13}C NMR spectrum are broad and split, due to the presence of a *N*-Boc rotamers. ^{1}H NMR (400 MHz, CD_3OD, δ): 4.92–4.87 (*m*, 0.75H), 4.74 (*dd*, 0.25H, J_1 = 11.6, J_2 = 5.7), 4.30 (*dd*, 0.32H, J_1 = 13.8, J_2 = 3.1), 4.22 (*dd*, 0.68H, J_1 = 14.2, J_2 = 2.7), 4.02 (*dd*, 0.74H, J_1 = 12.5, J_2 = 10.0), 3.91 (*d*, 0.5H, *J* = 5.7), 3.84–3.72 (*m*, 2.68H), 3.24 (*dd*, 0.68H, J_1 = 14.2, J_2 = 1.8), 3.17-3.10 (*m*, 0.32H), 2.53–2.23 (*m*, 2H), 1.70–1.57 (*m*, 2H), 1.53, 1.50, 1.49 and 1.44 (4 x *s*, 9H), 1.01–0.91 (*m*, 3H). ^{13}C NMR (400 MHz, CD_3OD, δ): 177.44, 177.35, 158.55, 156.43, 84.04, 83.41, 68.79, 68.67, 68.23, 68.07, 59.57, 59.16, 57.59, 57.50, 52.99, 50.53, 34.85, 34.73, 28.37, 28.33, 18.99, 18.92, 14.26, 14.15. HRMS (ESI-Orbitrap) *m/z*: $[M+Na]^+$ calcd. for $C_{14}H_{26}N_2NaO_6$: 341.1683, found: 341.1682.

*tert-Butyl (3R,4S,5R)-2-benzoyl-4,5-dihydroxy-3-(hydroxymethyl)tetrahydropyridazine-1(2H)-carboxylate (**26**)*



24 **26** (94%)

Compound **24** (50 mg; 0.099 mmol) was dissolved in MeOH (3.4 mL), and 3 M $HCl_{(aq)}$ (600 μL) was added. The resulting solution was stirred for 1 hour at room temperature. The mixture was neutralized with Et_3N (300 μL) and the volatiles were removed on rotovap. The resulting solid was subjected to column chromatography (EtOAc/EtOH 92:8), affording 32.7 mg (94%) of a pure product **26** as a colorless film. Peaks in ^{1}H and ^{13}C NMR spectrum are

broad and split, due to the presence of a *N*-Boc rotamers. ^{1}H NMR (400 MHz, CD_3OD, δ): 7.77–7.35 (*m*, 5H), 5.01–4.92 (*m*, 1H), 4.25 (*dd*, 0.5H, J_1 = 13.8, J_2 = 3.0), 4.18–3.83 (*m*, 4.5H), 3.24–3.13 (*m*, 1H), 1.62–1.24 (*m*, 9H). ^{13}C NMR (400 MHz, CD_3OD, δ): 131.42, 128.98, 128.20, 127.26, 83.71, 68.67, 68.31, 59.70, 59.30, 58.60, 53.25, 47.94, 28.21, 28.07. Under the recording conditions, no signal for two carbons could be detected. HRMS (ESI-Orbitrap) *m/z*: $[M+Na]^+$ calcd. for $C_{17}H_{24}N_2NaO_6$: 375.1527, found: 375.1516.

β-GLUCOSIDASE INHIBITION ASSAY FOR COMPOUNDS 12, 13, 14, 22, AGF AND CONDURITOL *β*-EPOXIDE

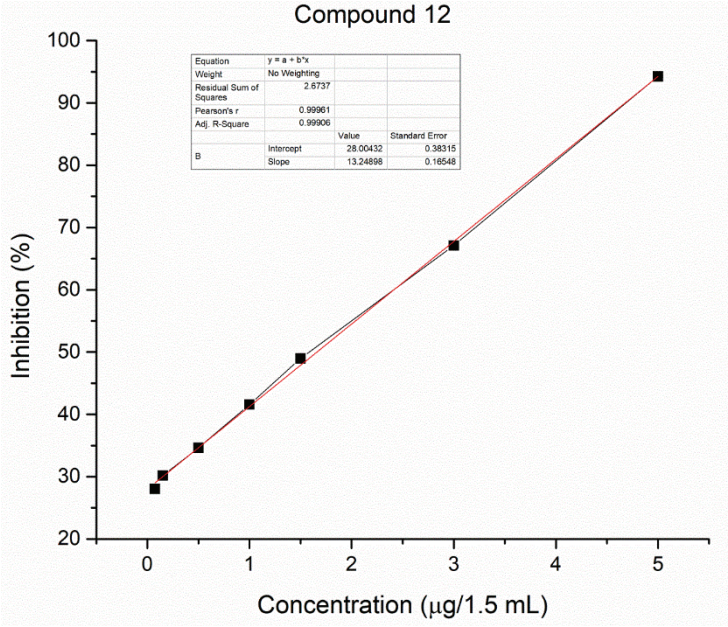


Figure *S-1*. Percentage of inhibition of *β*-glucosidase versus concentration of compound **12.**

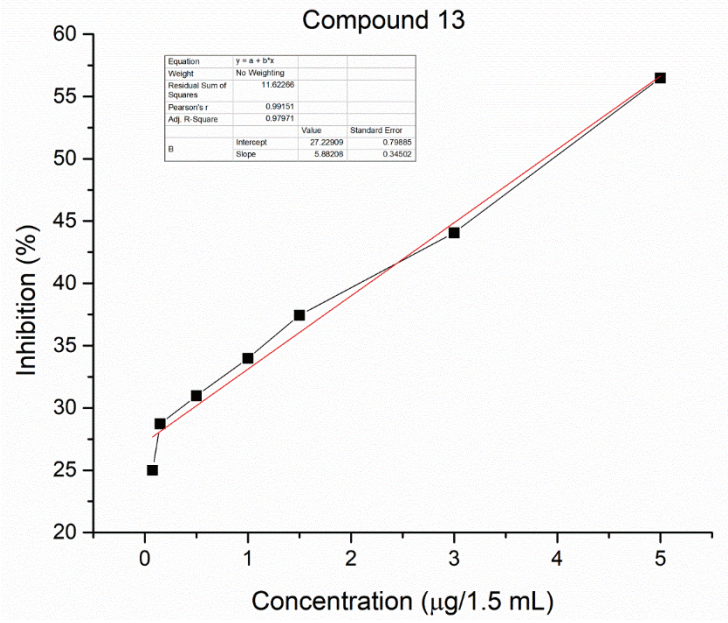


Figure *S-2*. Percentage of inhibition of *β*-glucosidase versus concentration of compound **13.**

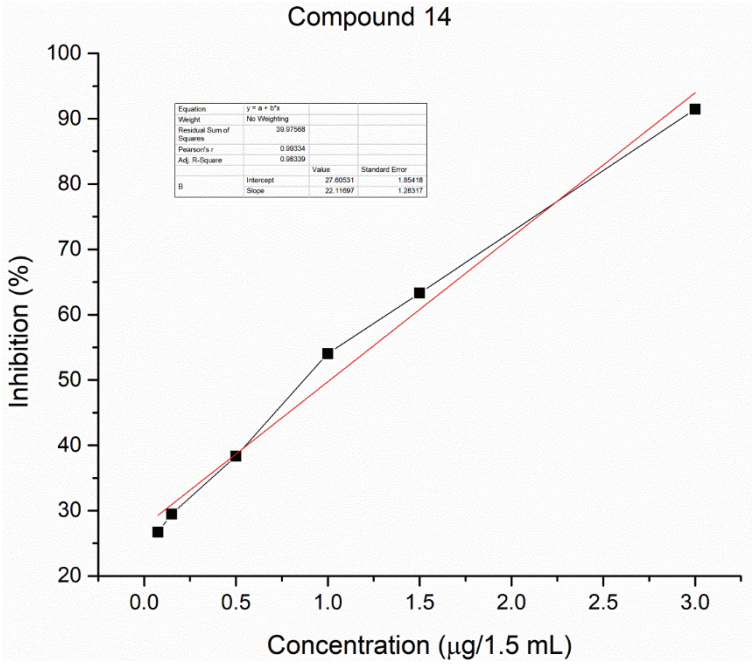


Figure *S-3*. Percentage of inhibition of *β*-glucosidase versus concentration of compound **14.**

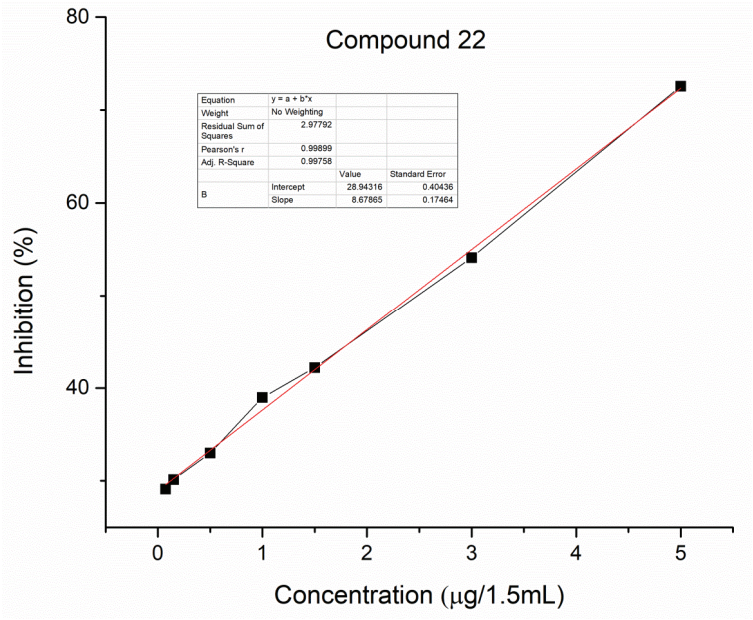


Figure *S-4*. Percentage of inhibition of *β*-glucosidase versus concentration of compound **22.**

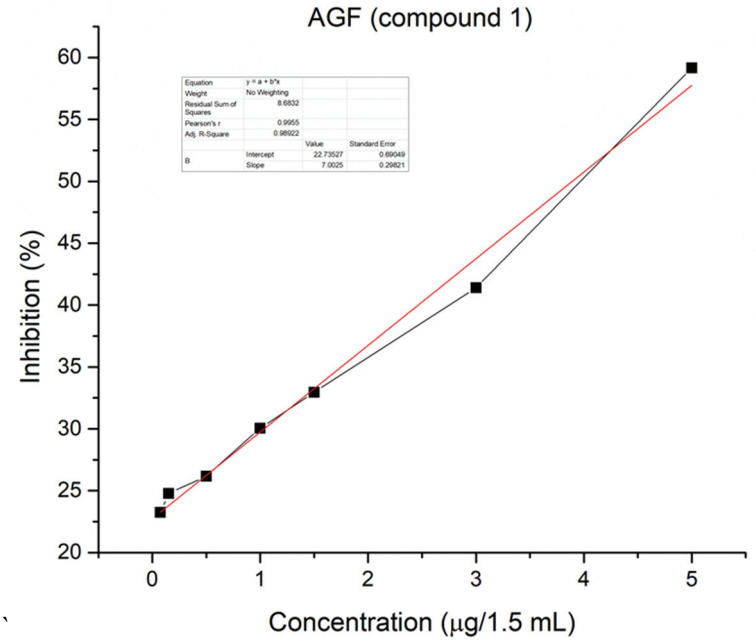


Figure *S-5*. Percentage of inhibition of *β*-glucosidase versus concentration of compound **AGF.**

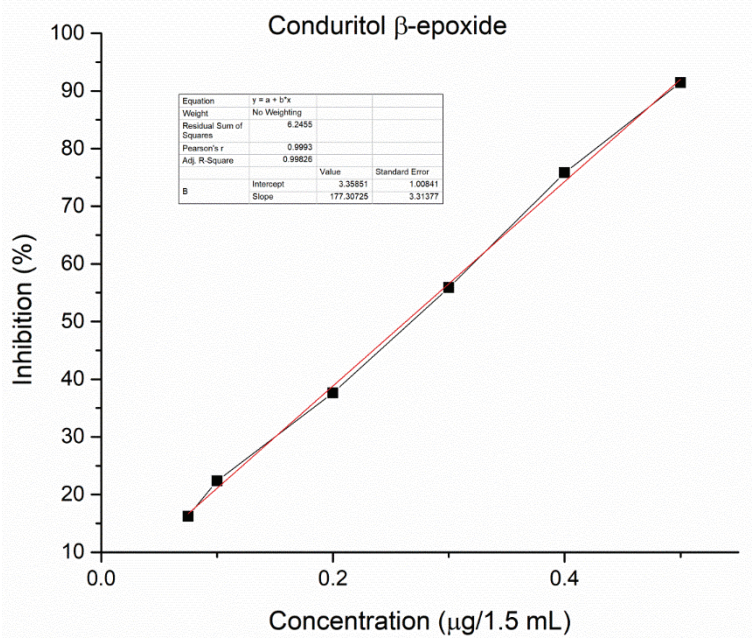


Figure *S-6*. Percentage of inhibition of *β*-glucosidase versus concentration of compound Conduritol *β*-epoxide.

LIGAND INTERACTIONS SCHEMES FOR COMPOUNDS 12, 13, 22 AND AGF

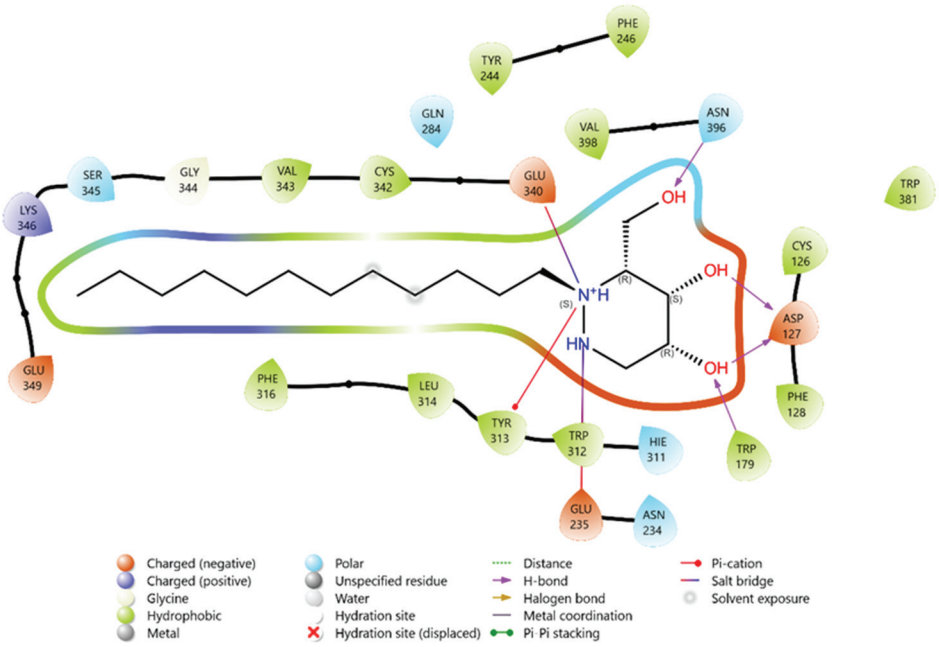


Figure ***S-7***. Ligand interactions for molecule **12** binded in *β*-glucosidase.

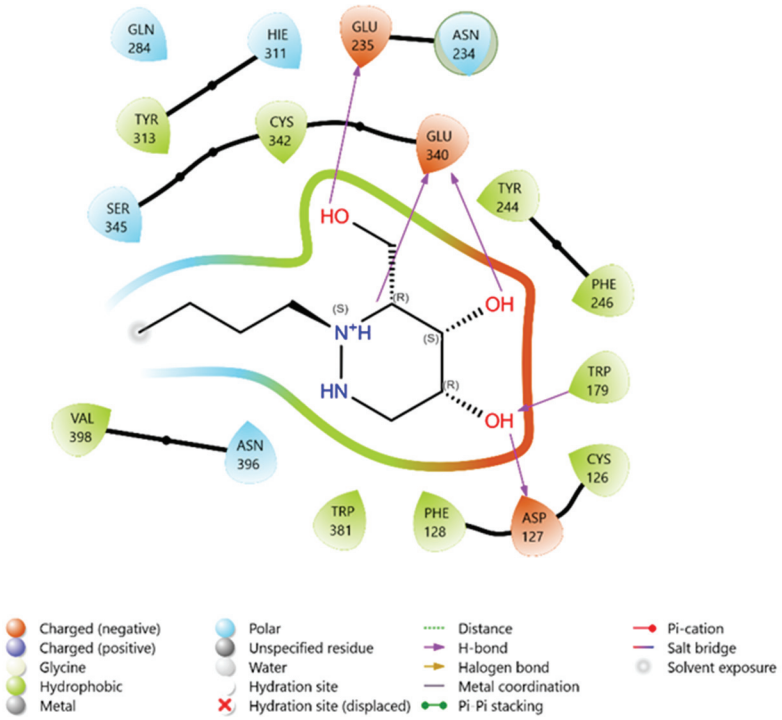


Figure *S-8*. Ligand interactions for molecule **13** binded in *β*-glucosidase.

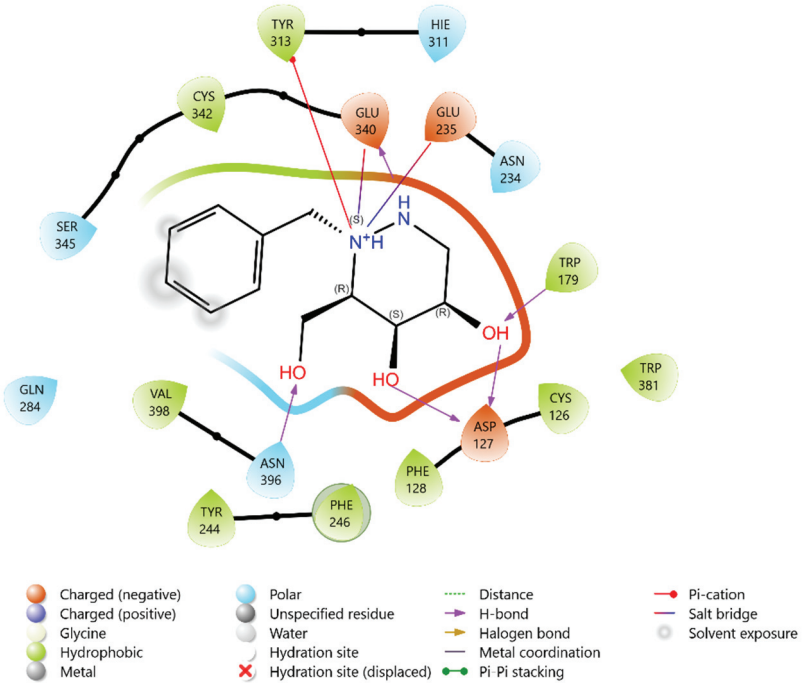


Figure *S-9*. Ligand interactions for molecule **22** binded in *β*-glucosidase.

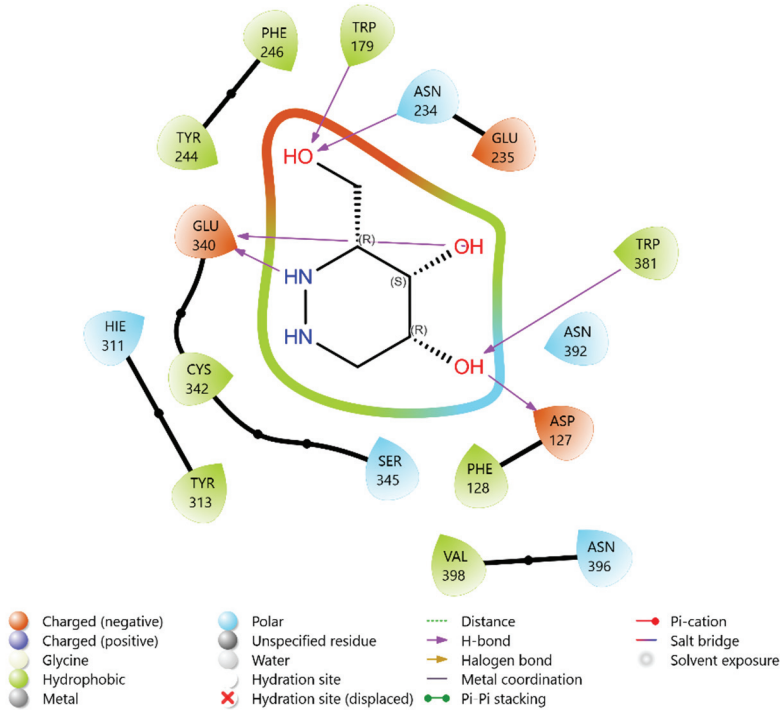
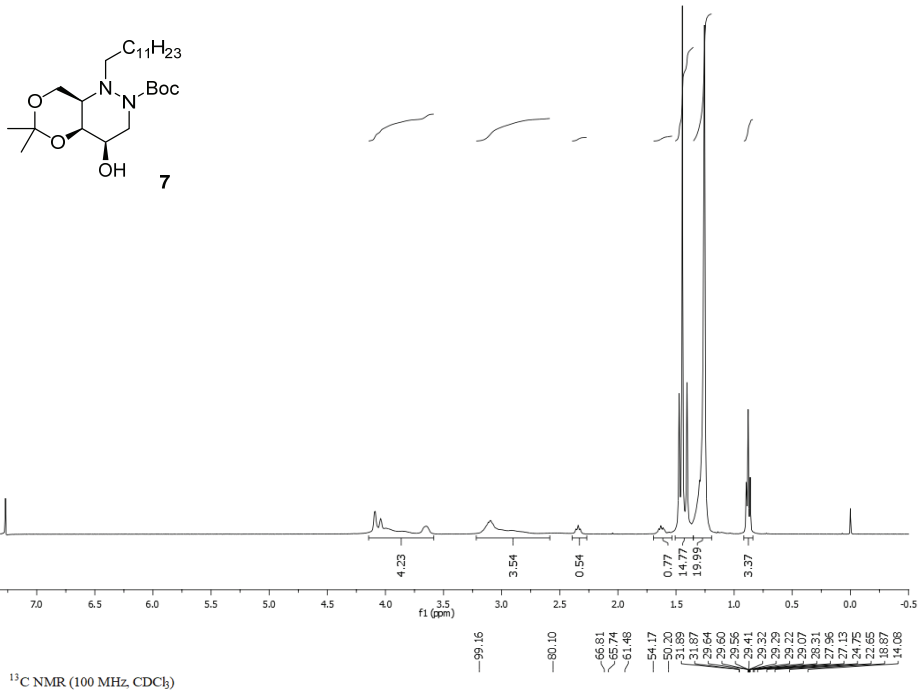
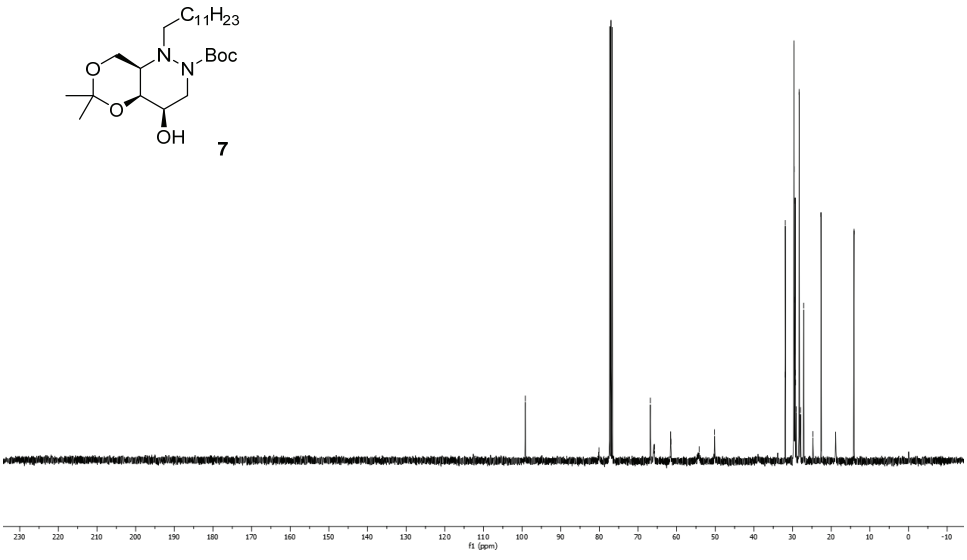


Figure *S-10*. Ligand interactions for molecule **AGF** binded in *β*-glucosidase.

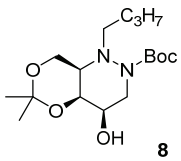
COPIES OF NMR SPECTRA



^{13}C NMR (100 MHz, $CDCl_3$)

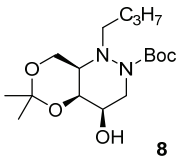


^{1}H NMR (400 MHz, $CDCl_3$)



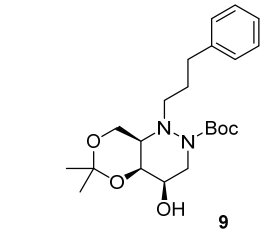
8

^{13}C NMR (100 MHz, $CDCl_3$)



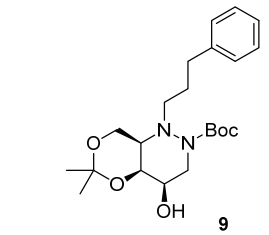
8

^{1}H NMR (400 MHz, $CDCl_3$)



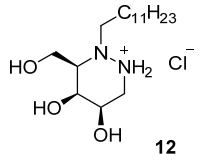
9

^{13}C NMR (100 MHz, $CDCl_3$)



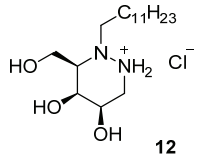
9

^{1}H NMR (400 MHz, D_2O)



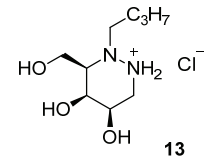
12

^{13}C NMR (100 MHz, D_2O)



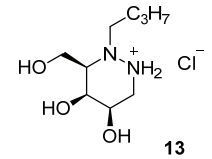
12

^{1}H NMR (400 MHz, D_2O)



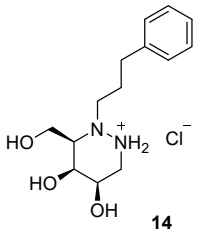
13

^{13}C NMR (100 MHz, D_2O)



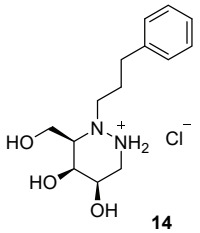
13

^{1}H NMR (400 MHz, D_2O)



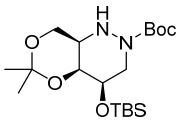
14

^{13}C NMR (100 MHz, D_2O)



14

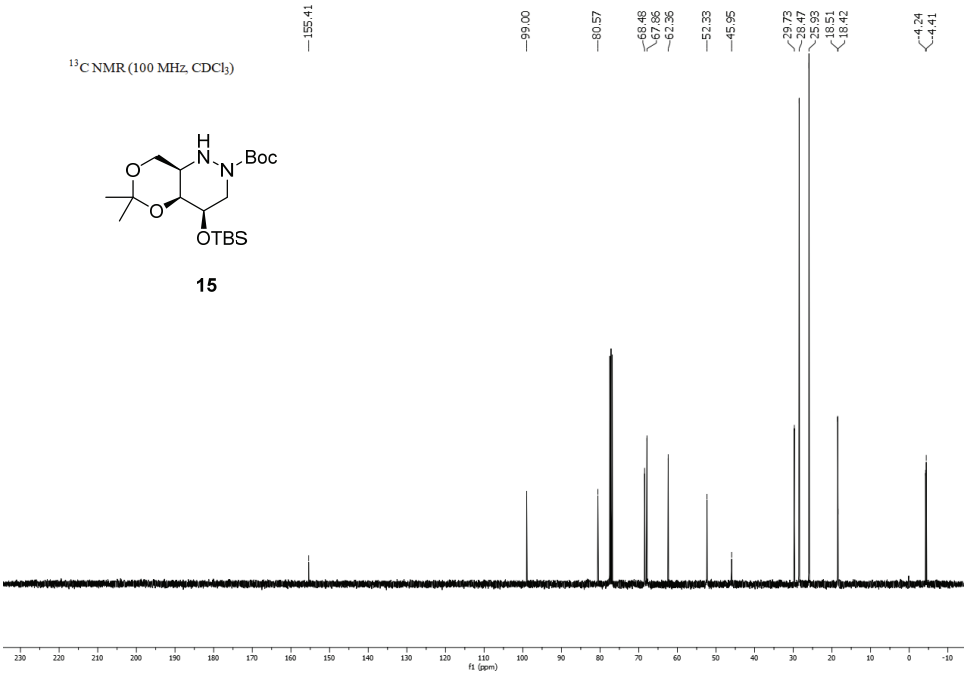
^{1}H NMR (400 MHz, $CDCl_3$)



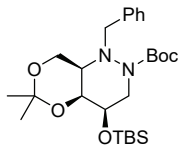
15

^{13}C NMR (100 MHz, $CDCl_3$)

15

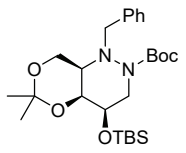


^{1}H NMR (400 MHz, $CDCl_3$)



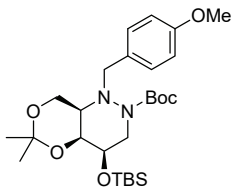
16

^{13}C NMR (100 MHz, $CDCl_3$)



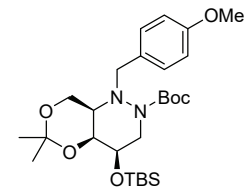
16

^{1}H NMR (400 MHz, $CDCl_3$)



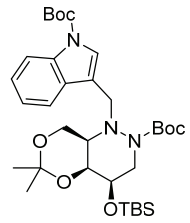
17

^{13}C NMR (100 MHz, $CDCl_3$)



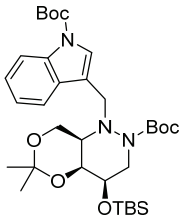
17

^{1}H NMR (400 MHz, $CDCl_3$)



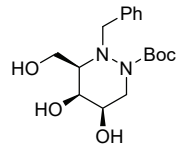
18

^{13}C NMR (100 MHz, $CDCl_3$)



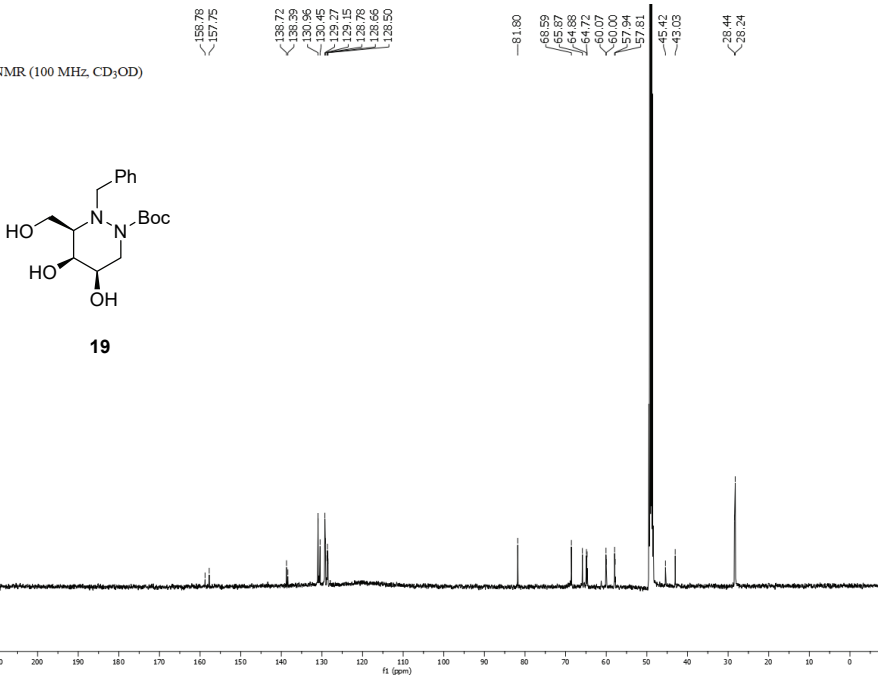
18

^{1}H NMR (400 MHz, CD_3OD)



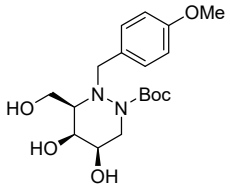
19

^{13}C NMR (100 MHz, CD_3OD)



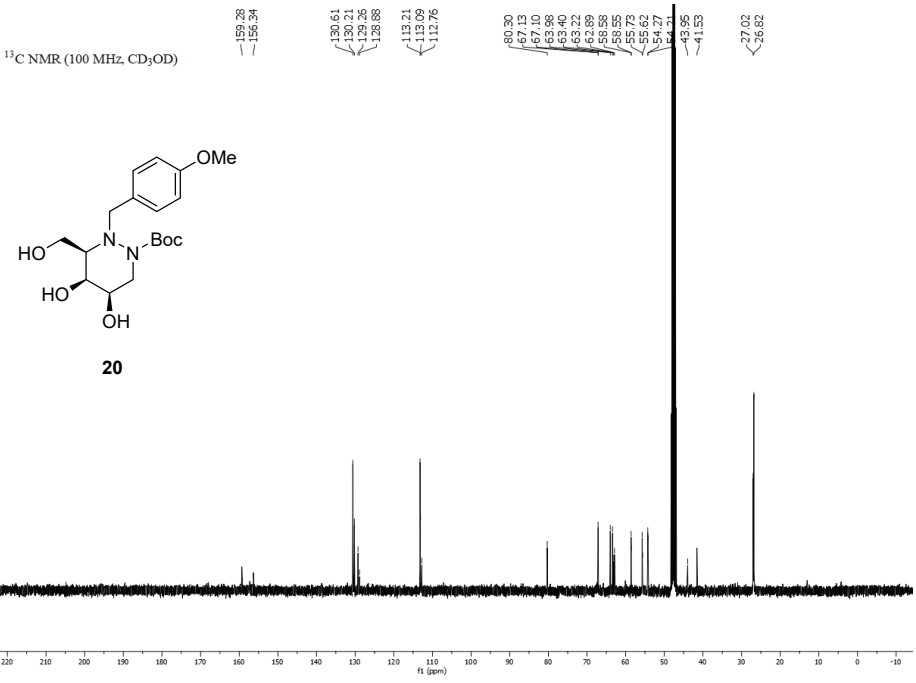
19

^{1}H NMR (400 MHz, CD_3OD)



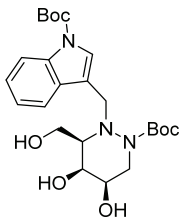
20

^{13}C NMR (100 MHz, CD_3OD)



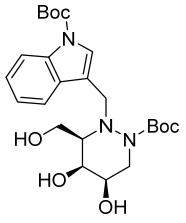
20

^{1}H NMR (400 MHz, CD_3OD)



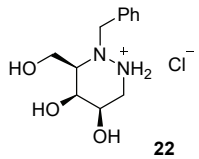
21

^{13}C NMR (100 MHz, CD_3OD)



21

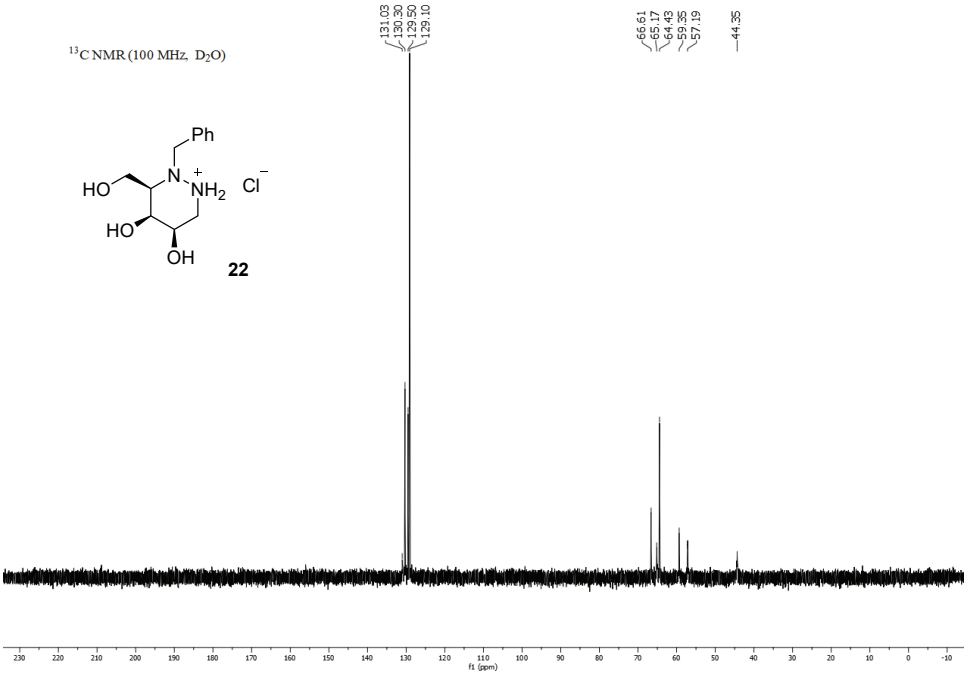
^{1}H NMR (400 MHz, D_2O)



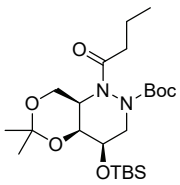
22

^{13}C NMR (100 MHz, D_2O)

22

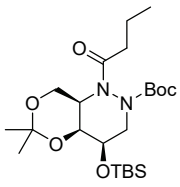


^{1}H NMR (400 MHz, $CDCl_3$)



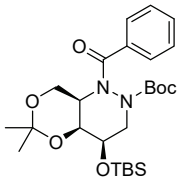
23

^{13}C NMR (100 MHz, $CDCl_3$)



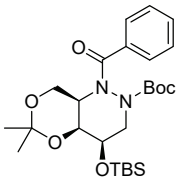
23

^{13}H NMR (400 MHz, $CDCl_3$)



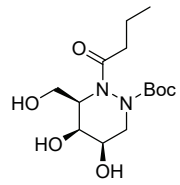
24

^{13}C NMR (100 MHz, $CDCl_3$)



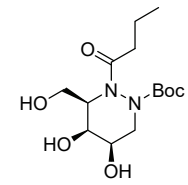
24

^{1}H NMR (400 MHz, CD_3OD)



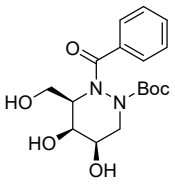
25

^{13}C NMR (100 MHz, CD_3OD)



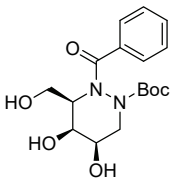
25

^{1}H NMR (400 MHz, CD_3OD)



26

^{13}C NMR (100 MHz, CD_3OD)



26