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Design and Evaluation of Dihydroxytetrahydro-1*H*-pyrrolo[2,1-*c*]-[1,4]benzothiazines as Conformationally Restricted Transition-State Inhibitors of *β*-Ribosidases

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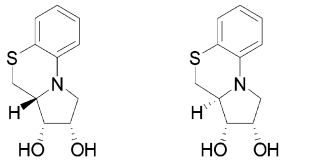
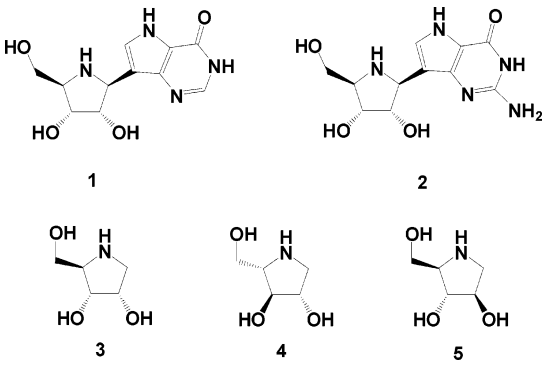


The preparation of three new chiral thiazines from ribose is described. Two of these are dihydroxytetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzothiazines with iminopentitol substructures corresponding to the L-lyxo and D-ribo configurations. They were designed to present a favorable transition-state mimic for the inhibition of ribosidases. This new thiazine class opens the way to the development of new inhibitors to carbohydrate processing enzymes of therapeutic importance such as nucleoside hydrolases and purine nucleoside phosphorylases.

Stereochemical considerations in the design of inhibitors of glycosidases are important elements. Glycosides are invariably acetals or aminals in the case of *N*-glycosides such as nucleosides. Nucleoside hydrolases are especially important because they are found in several pathogens such as protozoa[1] and bacteria[2] but not in mammalian systems and are therefore excellent drug targets. The purine salvage pathway of parasitic protozoa is especially targeted because these organisms cannot synthesize purines de novo. Theoretical[3] and experimental[4−6] findings assert that stereoelectronic control in the displacement of aglycons from glycosides requires that the ligand leaving the tetrahedral center be aligned anti-periplanar to a doubly occupied nonbonding orbital (electron lone pair). N-Glycanases including nucleoside hydrolases,[7−9] hypoxanthine−guanine phosphoribosyltransferases (HGPRTases),[10] and purine nucleoside phosphorylases (PNPs)[11−14] have mechanistic similarities. Iminopentitol analogues that are formally dihydroxytetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzothiazines were designed and tested for their ability to inhibit bacterial ribosidases. These have a cationic site that approximates the oxocarbenium center in the transition state and restricted mobility centered around the conformations favored by the stereoelectronic control models.

Iminopentitols are ground-state analogues of intermediate/transition-state species in the cleavage of glycosidic linkages of ribosides where the oxocarbenium charge is mimicked by the protonated nitrogen. Examples of ribo iminopentitols that have been successfully used as inhibitors are immucillin-H (**1**) and its derivative (**2**).[15,16] Naturally occurring iminopentitols that have the D-ribo (**3**) as well as L- and D-arabino configurations (**4** and **5**, respectively) have been isolated from several sources.[17−20]



The stereoelectronic control of *β*-D-ribosidase hydrolysis or aglycon displacement requires an unfavorable alignment of the hydroxymethyl group with the departing alkoxy group at C1 (Figure 1A). In the case of nucleoside

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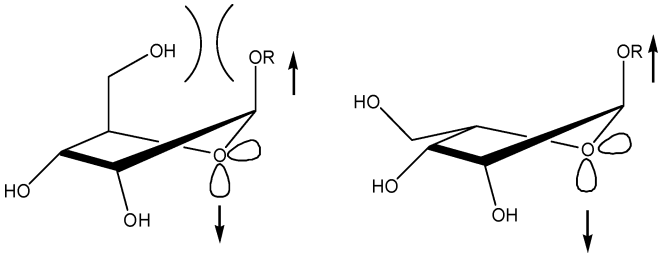


FIGURE 1. Favorable conformations for transition-state structures of β-D-ribofuranosides (A) and α-L-lyxofuranosides (B) in which the aglycon fragment is anti-periplanar with one doubly occupied nonbonding orbital.

derivatives, the departing alkoxy group is, of course, replaced by a nitrogen functionality that is part of a heterocyclic base. The same antiperiplanar arrangement of leaving group and electron lone pair is still required. Such a conformation is untenable without a large energy penalty for β-D-ribosides but can be readily formed by α-L-lyxosides (Figure 1B). A very potent inhibitor might be one with the α-L-lyxo configuration locked in the appropriate conformation. Such an inhibitor might be as effective as or even more effective than the corresponding D-ribo analogue despite the fact that it is a member of the unnatural L series. A β-D-riboside constrained in the conformation shown in Figure 1A would also be an effective inhibitor but would be more of a synthetic challenge because of the structural constraints.

We describe here the synthesis and preliminary evaluation of the ribosidase inhibition activity of two tricyclic systems containing an iminopentitol moiety with the α-L-lyxo and β-D-ribo configurations. The systems are (2*S*)-3(*R*),4(*S*)-dihydroxy-2,3,3a,4-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzothiazine (**6**) and (2*R*)-3(*R*),4(*S*)-dihydroxy-2,3,3a,4-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzothiazine (**7**). In the α-L-lyxo compound (**6**), the iminopentitol moiety is conformationally restricted so that a would-be leaving group would be close to the antiperiplanar orientation to the ring nitrogen lone pair. The antiperiplanar arrangement can then be met with a slight change of orientation of the aglycon instead of a complete (energetically unfavorable) ring inversion. A similar situation occurs in the case of the β-D-ribo compound **7**, but there is greater flexibility in the sugar ring. We also prepared and evaluated a less conformationally constrained analogue with the α-L-lyxo configuration (**8**) lacking the aromatic ring. Molecular models of compound **6** and **7** showing the near-antiperiplanar arrangement of the C1 hydrogen that occupies the near-axial position and the lone pair on the nitrogen atom are shown in Figure 2.

The strategy we chose for the synthesis is outlined in Scheme 1. It is based on the reaction of an α-aminothiol with a 5-bromo-5-deoxy-4-ulosonic acid with the D-erythro configuration which is prepared by the oxidation of methyl 2,3-di-*O*-acetyl-5-bromo-5-deoxy-β-D-ribofuranoside with chromium trioxide.

Although there is no precedent for the oxidation of bromo glycosides by this method, the oxidation of peracylated β-glycosides is known to give ulosonic acids in

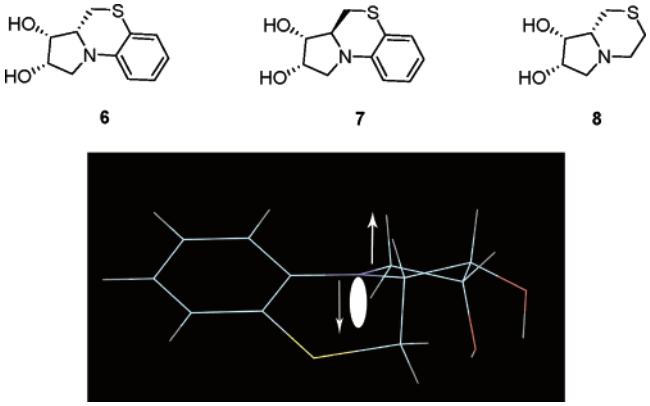
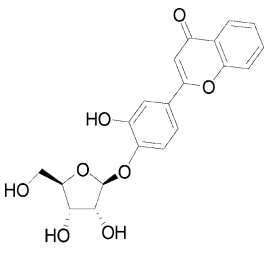


FIGURE 2. 1(*S*),2(*R*),3(*S*)-Dihydroxy-2,3,3a,4-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzothiazine (**6**). The geometry in this molecular model is MM3 optimized. The arrows indicate the orientations of the hydrogen that occupies the position normally occupied by the leaving group and the orientation of the electron lone pair on the nitrogen atom (white ellipsoid).

good yield.[21,22] Displacement of the bromo group of the ulosonic acid ester with the sulfur group of an α-aminothiol followed by reduction of the intermediate imine or aminal formed by reaction of the amino group onto the carbonyl with cyanoborohydride should produce a thiomorpholine ring. The amino group of this should react intramolecularly with the ester group to form a lactam. The desired products can be obtained by reduction of the lactam with borane followed by deacylation.

The hydrolysis of β-riboside under catalysis by β-ribosidases features an oxocarbenium ion transition state, similar to nucleoside hydrolases, nucleoside phosphorylases, and related enzymes. The β-ribosidase inhibitory properties of **6**−**8** were evaluated using 3′,4′-dihydroxyflavone-4′-β-D-ribofuranoside **16** (DHF-riboside) as the substrate and bacterial lysates or whole cells as the source of β-ribosidase. The hydrolysis of this chromogenic substrate can be catalyzed by β-ribosidases to release 3′,4′-dihydroxyflavone, which forms a highly colored chelate with iron.[23]



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The synthetic strategy for preparing (2*S*)-3(*R*),4(*S*)-dihydroxy-2,3,3a,4-tetrahydro-1*H*-pyrrolo[2,1-*c*]-[1,4]benzothiazine (**6**) and its diasteriomer **7** was successful. Treatment of the 5-bromo-5-deoxy-4-ulosonic acid methyl ester with aminothiophenol, followed by reduction, cyclization, and deprotection, yielded the lactam

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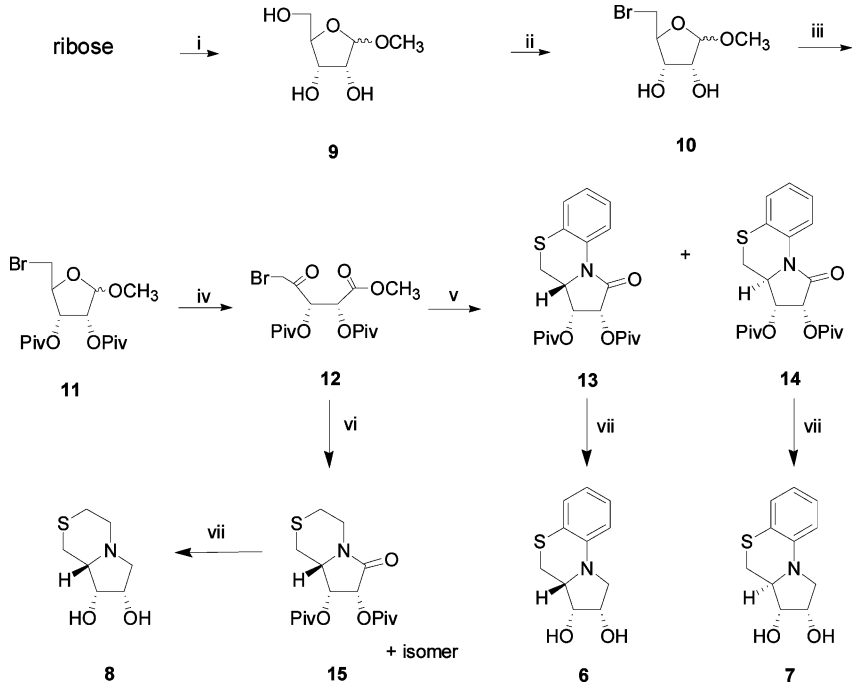
SCHEME 1[a]



[a] Key: (i) HCl/MeOH; (ii) Ph_3P, CBr_4, pyridine, 86.7% for two steps; (iii) PivCl, pyridine, 91.1%; (iv) CrO_3, Ac_2O, HOAc, 96.6%; (v) (1) 2-aminothiophenol, CH_3OH, (2) $NaCNBH_3$, CH_3OH, (3) Na_2CO_3, CH_2Cl_2, 67% for two steps; (vi) (1) $HS(CH_2)_2NH_2$, CH_3OH, (2) $NaCNBH_3$, CF_3COOH, 57% for two steps; (vii) (1) BH_3−THF, (2) $NaOCH_3$, CH_3OH, 91% for two steps for preparation of **6**, 87% for **7**, and 85% for preparation of **8**.

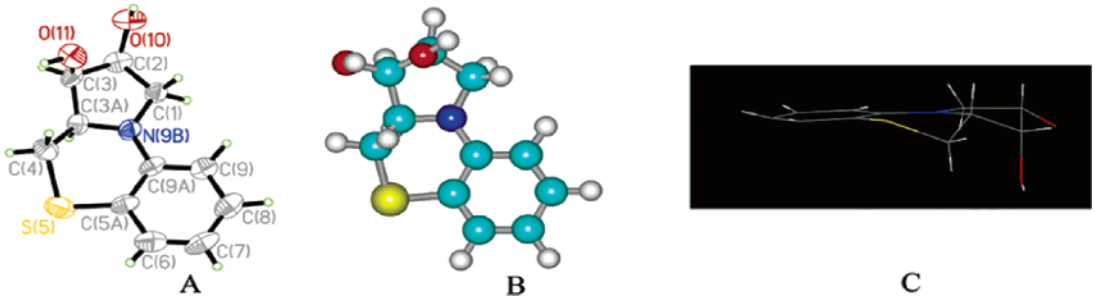


FIGURE 3. (A) ORTEP drawing of the X-ray structure of the tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzothiazine (**6**) compared to the MM3 geometry optimized calculated structure (B). The hydroxyl groups which are free to rotate are in slightly different orientations but the ring geometries are the same. The angles at the ring junctions are practically identical. (C) Another orientation of the X-ray structure of **6**. The X-ray structure of **8** is shown in Figure 4.

product with the L-*lyxo* configuration (**6**) in an overall yield (51%) that was 5 times greater than that of the corresponding D-ribo isomer (**7**). The isomers could be readily identified by the coupling constants of the signals for the 3-protons in NMR spectra. The L-lyxo compound was characterized by a small (~3 Hz) coupling constant between the H2 and H3 signals of the tetrahydropyrrole ring. The identification of the L-lyxo isomer was confirmed by X-ray analyses of the reduced products.

The same sequence using aminoethanethiol instead of aminothiophenol yielded (7*S*)-8(*R*),9(*S*)-dihydroxyhexahydro-1*H*-pyrrolo[1,4]thiazine (**8**) in 85% yield from the corresponding (L-lyxo) lactam which was obtained in 57% yield. The isomeric lactam, some uncyclized amino ester, and an intermediate imine were the other products obtained in the formation of the lactam. The L-lyxo product was obtained in a yield that was 3 times that of the isomeric D-ribo product.

The calculated conformation of the tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzothiazine (**6**) agreed very well with the calculated structure. A comparison of the two structures is shown in (Figure 3). The conformation of the more simple bicyclic system **8** was expected to be similar to that of **6** except that greater freedom in the conformation that the thiomorpholine nucleus could take is expected since it is no longer fused to a rigid aromatic ring.

Compounds **6**−**8** were evaluated for their ability to inhibit the action of β-ribosidases produced by Gram-negative bacteria. There are no commercially available β-ribosidases, and it is the usual course to use intrinsic ribosidase activities from actual bacteria or bacterial cell lysates. Ribosidase inhibition assays were performed using *Salmonella* sp. 35664 to produce β-ribosidase enzymes. Both whole cells and lysed bacteria were tested. Ribosidase activity is indicated by a purple color formed

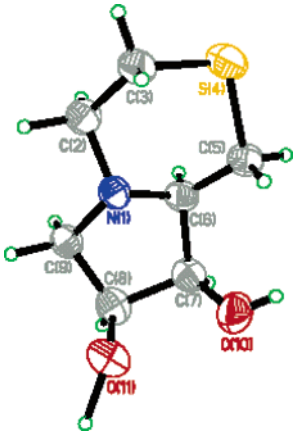


FIGURE 4. ORTEP drawing of the X-ray structure of the hexahydro-1*H*-pyrrolo[1,4]thiazine (**8**)

by complexation of the freed phenol with ferric ions. The results show that the L-lyxo analogue has good inhibitory activity against β-ribosidases produced from this strain. About 70% inhibition at concentration 15 μg/μL (67 mM) at 24 h for both whole cell and lysate was observed. Fifty percent inhibition was observed at a concentration 7.5 μg/μL. The inhibition activity was comparable to that of the D-ribo analogue (**7**). The simpler bicyclic system **8** showed no significant inhibition for the β-ribosidase(s) produced by *Salmonella* sp. 35664. It is important to note that there may be more than one ribosidase activity in *Salmonella* sp. 35664 and that an even better inhibitory activity might be obtained for any one specific purified enzyme. Some abrupt changes in the slope of the inhibition curve suggest the presence of more than one activity.

Although few nucleoside hydrolase inhibitors are known, it is generally accepted that substituted aromatic groups attached to the ring nitrogen of iminopentitols enhance their inhibitory activity.[24] It should be noted that the crystal structure of one class of nucleoside hydrolases reveals an "aromatic stacking network" in the active site.[9] The development of compounds of the new thiazine class described here opens the way to the development of new inhibitors to enzymes of therapeutic importance such as nucleoside hydrolases and purine nucleoside phosphorylases (PNP).

Experimental Section

(2*S*)-3(*R*),4(*S*)-Dihydroxy-2,3,3a,4-tetrahydro-1*H*-pyrrolo-[2,1-*c*][1,4]benzothia-zine (6). A solution of lactam **13** (0.40 g, 1.0 mmol) and BH_3−THF (3.3 mL, 5.0 mmol) in anhydrous THF (10 mL) was refluxed for 4 h, and the TLC and NMR showed the completion of the reduction. The solvent was removed, and methanol was added and concentrated 3 times. The residue was dissolved in methanol (20 mL), followed by addition of NaOMe (0.20 g, 3.8 mmol). The reaction was stirred for 8 h and concentrated. The residue was purified by column chromatography (15:1 CH_2Cl_2/MeOH) to afford a white crystalline solid (0.2 g, 90.9%): mp 127−128 °C; IR ($CHCl_3$) ν_{max} 3113.94, 1124.86 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 6.93 (2H, d, J = 7.5 Hz), 6.48 (1H, td, J = 7.5, 1 Hz), 6.41 (1H, d, J = 8 Hz), 4.37 (1H, m), 4.11 (1H, t, J = 3.8 Hz), 3.80 (1H, dt, J = 10.5, 3 Hz), 3.51 (1H, t, J = 8.5 Hz), 3.15 (1H, t, J = 8.5 Hz), 2.96 (1H, m), 2.85 (1H, dd, J = 12.5, 3 Hz); ^{13}C NMR (125.5 MHz, CD_3OD) δ 146.6, 130.9, 129.8, 119.7, 119.2, 115.1, 77.1, 74.3, 65.7, 55.8, 28.0 ppm; HRFABMA (M + H$^+$) calcd 224.0745, found 224.0745.

(2*R*)-3(*R*),4(*S*)-Dihydroxy-2,3,3a,4-tetrahydro-1*H*-pyrrolo-[2,1-*c*][1,4]benzothiazine (7) was obtained the same way as compound *6* from lactam **14** (87%): ^{1}H NMR (500 MHz, CD_3OD) δ 6.99−6.93 (2H, m), 6.51−6.46 (2H, m), 4.18 (1H, d, J = 4.5 Hz), 4.07 (1H, d, J = 2.5 Hz), 4.01 (1H, m), 3.61 (1H, dd, J = 10.5, 5.0 Hz), 3.22 (1H, d, J = 10.5 Hz), 2.90 (2H, m); ^{13}C NMR (125.5 MHz, CD_3OD) δ 143.0, 127.0, 126.0, 115.5, 115.1, 110.9, 77.1, 73.8, 60.7, 54.0, 23.6 ppm; HRFABMA (M + H$^+$) calcd 224.0745, found 224.0746.

7(*S*)-Hexahydro-8(*R*),9(*S*)-bis-trimethylacetoxy-1*H*-pyrrolo[1,4]thiazin-6-one (15) and Its Isomer 7(*S*)-Hexahydro-8(*R*),9(*R*)-bis-trimethylacetoxy-1*H*-pyrrolo[1,4]thiazin-6-one. A solution of **12** (0.8 g, 2.0 mmol), $HS(CH_2)_2NH_2$ (0.18 g, 2.3 mmol), and $NaCNBH_3$ (0.18 g, 2.9 mmol) in methanol (50 mL) was stirred at room temperature for 24 h and then concentrated. The resulting residue was dissolved in dichloromethane, washed with brine, dried (Na_2SO_4), and concentrated. Compound **15** and its isomer were isolated by column chromatography (4:1 hexanes/ethyl acetate).

7(*S*)-Hexahydro-8(*R*),9(*S*)-bis-trimethylacetoxy-1*H*-pyrrolo[1,4]thiazin-6-one (15) was obtained as a white solid (0.40 g, 57%): mp 118−220 °C; IR ($CHCl_3$) ν_{max} 1739.61, 1705.31, 1141.03 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.50 (1H, t, J = 5 Hz), 5.43 (1H, dd, J = 6, 1.5 Hz), 4.43 (1H, dt, J = 13.5, 2.8 Hz), 3.88 (1H, m), 2.97 (1H, m), 2.72−2.64 (2H, m), 2.52 (1H, m), 2.40 (1H, dt, J = 13.5, 2.5 Hz), 1.21 (9H, s), 1.20 (9H, s); ^{13}C NMR (125.5 MHz, $CDCl_3$) δ 177.0, 176.9, 167.7, 68.8, 67.5, 57.7, 42.2, 39.1, 38.8, 27.3, 27.2, 27.1, 27.0 ppm; HRFABMA (M + H$^+$) calcd 358.1689, found 358.1685.

7(*R*)-Hexahydro-8(*R*),9(*R*)-bis-trimethylacetoxy-1*H*-pyrrolo[1,4]thiazin-6-one was obtained as a white solid (0.12 g, 20%): mp 138−139 °C; IR ($CHCl_3$) ν_{max} 1739.70, 1717.38, 1160.52, 1136.98 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.34 (1H, d, J = 7 Hz), 5.09 (1H, dd, J = 7, 2.3 Hz), 4.44 (1H, dt, J = 13.5, 2.8 Hz), 3.60 (1H, dt, J = 11, 2.5 Hz), 3.02 (1H, m), 2.75 (1H, d, J = 13 Hz), 2.62−2.51 (2H, m), 2.42 (1H, m), 1.20 (9H, s), 1.16 (9H, s); ^{13}C NMR (125.5 MHz, $CDCl_3$) δ 177.4, 177.1, 166.6, 70.1, 68.2, 62.3, 42.2, 38.8, 38.8, 30.1, 27.1, 27.0, 26.4 ppm; HRFABMA (M + H$^+$) calcd 358.1689, found 358.1688.

(7*S*)-8(*R*),9(*S*)-Dihydroxyhexahydro-1*H*-pyrrolo[1,4]-thiazine (8). A solution of lactam **15** (0.24 g, 0.67 mmol) and BH_3−THF (2.2 mL, 3.4 mmol) in anhydrous THF (10 mL) was refluxed for 3 h, and the TLC and NMR showed the completion of the reduction. The solvent was removed, and methanol was added and concentrated 3 times. The residue was dissolved in methanol (20 mL), followed by addition of NaOMe (0.10 g, 1.9 mmol). The reaction was stirred for 8 h and concentrated. The residue was applied to an ion-exchange column (Dowex 50WX8-400, 5 g), which was washed with water (30 mL) and eluted with 2 N NH_4OH (30 mL). The eluent was concentrated and yielded a white crystalline solid (0.1 g, 85.0%): mp 92−93 °C; ^{1}H NMR (500 MHz, D_2O) δ 4.23 (1H, m), 4.07 (1H, m), 3.22 (1H, dt, J = 12, 2.5 Hz), 2.86−2.74 (3H, m), 2.52 (1H, dt, J = 13.5, 3 Hz), 2.49 (1H, m), 2.44 (1H, m), 2.33−2.25 (2H, m); ^{13}C NMR (125.5 MHz, D_2O) δ 71.9, 67.4, 67.0, 60.9, 53.9, 26.4, 26.3 ppm; HRFABMA (M + H$^+$) calcd 176.0745, found 176.0745.

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Supporting Information Available: Proton and ^{13}C spectra of compounds **6**, **7**, **8**, **13,** and **14**; experimental details for all numbered compounds; X-ray structures of **6** and **8** (CIF); inhibition data and assay methods; structure schemes. This material is available free of charge via the Internet at http://pubs.acs.org.

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