C-3- and C-4-Alkylated Polyhydroxypyrrolidines: Enantiospecific Syntheses and Glycosidase Inhibitory Activity

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Short, efficient, and stereoselective syntheses of enantiomerically pure C-3- and C-4-alkylated analogues of (2*R*,3*S*,4*R*)-3,4-dihydroxy-2-(hydroxymethyl)pyrrolidine, a potent α-galactosidase inhibitor, from 4-hydroxy-L-proline are presented. Grignard addition or enolate alkylation of a *N*-(9-phenylfluoren-9-yl)-4-oxo-3-[(methoxymethyl)oxy]proline and epoxidation or hydroboration of a 4-methylene-3-[(methoxymethyl)oxy]proline proceeded with complete stereoselection and in excellent yields. The inhibitory activities of the synthesized pyrrolidines were measured and showed that the fit of *A. niger* α-galactosidase and the jack bean α-mannosidase around C-3 of the pyrrolidine ring (α face) must be very tight, while the fit around C-4 (α face) is much looser. Positioning a methylene group between the hydroxyl at C-4 and the pyrrolidine ring completely abolishes the inhibitory activity (see analogue **5**).

Introduction

Glycoproteins located in the outer envelope of cells are key players in a variety of biological recognition events, such as cell−cell communication and cell growth, as well as in some stages of the bacterial and viral infection cycles. These glycoproteins are synthesized by attaching a common oligosaccharide unit to the nascent protein chain; this oligosaccharide conjugate then undergoes a series of hydrolysis and glycosilation reactions to provide the final, mature form of the protein. Among all the types of enzymes that process glycoproteins, glycosidases play a very important role: that of trimming monosaccharide units from the oligosaccharide already bound to the protein chain.[1] Inhibition of this pruning process has led to interesting biological responses, namely, antiviral, anti-HIV, anticancer, antifeedant, and immunoregulatory activities.[2]

Polyhydroxylated pyrrolidine, piperidine, and indolizidine alkaloids have attracted considerable attention recently due to their ability to inhibit glycosidases. A myriad of analogues of these compounds have been synthesized in search of better inhibitors or more promising pharmacological agents.[3] Since detailed information about the structures of the target oligosaccharide-processing enzymes is lacking, most analogues have been designed to bear resemblance to the proposed reaction intermediates: the glycosyl cations.[3] The most recurrent themes in the structural variations chosen to prepare these analogues have been the following: (1) to permute the configurations of the hydroxyl-bearing carbons[3a,b,d] and (2) to flatten the heterocyclic ring to mimic the halfchair conformation of the glycosyl cation.[3c] While these approaches have provided several highly potent inhibitors and have helped to delineate the enzymic structural requirements around the glycosidic center, progress in our knowledge of the detailed interactions of the enzymic active sites with the inhibitors have not ensued. This state of affairs has prompted us to undertake a program of synthesis and evaluation of analogues of polyhydroxylated pyrrolidines and indolizidines in which the steric parameters of specific parts of the inhibitors are systematically varied by incorporation of an extra methylene group into the molecules.[5] We have chosen the potent α-galactosidase inhibitor **1** as our first model compound,[6] since it is one of the simplest glycosidase inhibitors known, and have designed compounds **2**−**5** (Figure 1) as probes to find out how tight the inhibitor−enzyme interaction is around carbons 3 and 4 of the pyrrolidine ring.

We have recently reported an efficient approach to the synthesis of polyhydroxylated pyrrolidines and indolizidines;[6] this methodology, conveniently modified, should allow the introduction of carbon appendages at C-3 and C-4 of the pyrrolidine ring of **1**. Our plans to synthesize the enantiomerically pure compounds **2**-**5** rest on the functionalization of the versatile ketol **7**, prepared in four steps from *trans*-4-hydroxy-L-proline (**6**, 72% overall yield).[6] Attack of carbon nucleophiles on the carbonyl group at C-4 should yield precursors of **2**, **3**, and **5**, while alkylation of the enolate of an O-protected derivative of

(1) Warren, L. *Bound Carbohydrates in Nature*; Cambridge University Press: Cambridge, 1994.

(2) For reviews of glycosidase inhibitors see: (a) Elbein, A. D. *Am. Rev. Biochem.* **1987**, *56*, 497. (b) Elbein, A. D. *FASEB J.* **1991**, *5*, 3055. (c) Winchester, B.; Fleet, G. W. J. *Glycobiology* **1992**, *2*, 199.

(3) (a) Winkler, D. A. *J. Med. Chem.* **1996**, *39*, 4332. (b) Asano, N.; Oseki, K.; Kizu, H.; Matsui, K. *J. Med. Chem.* **1994**, *37*, 3701. (c) Papandreou, G.; Tong, M. K.; Ganem, B. *J. Am. Chem. Soc.* **1993**, *115*, 11682. (d) Winchester, B.; Al Daher, S.; Carpenter, N. C.; Cenci di Bello, I.; Choi, S. S.; Fairbanks, A. J.; Fleet, G. W. J. *Biochem. J.* **1993**, *290*, 743, and references therein.

(4) (a) Fleet, G. W. J.; Nicholas, S. J.; Smith, P. W.; Evans, S. V.; Fellows, L. E.; Nash, R. J. *Tetrahedron Lett.* **1985**, *26*, 3127. (b) Austin, G. N.; Baird, P. D.; Fleet, G. W. J.; Peach, J. M.; Smith, P. W.; Watkin, D. J. *Tetrahedron* **1987**, *43*, 3095.

(5) Only a handful of analogues bearing extra methylene groups have been prepared. Pyrrolidines: (a) Bols, M.; Persson, M. P.; Butt, W. M.; Jørgensen, M.; Christensen, P.; Hansen, L. T. *Tetrahedron Lett.* **1996**, *37*, 2097. (b) Burley, I.; Hewson, A. T. *Tetrahedron Lett.* **1994**, *35*, 7099. Piperidines: (c) Khanna, I. K.; Weier, R. M.; Julien, J.; Mueller, R. A.; Lankin, D. C.; Swenton, L. *Tetrahedron Lett.* **1996**, *37*, 1355. (d) Hansen, A.; Tagmose, T. M.; Bols, M. *Tetrahedron* **1997**, *53*, 697. (e) Reference 4a. Indolizidines: (f) Pearson, W. H.; Hembre, E. J. *J. Org. Chem.* **1996**, *61*, 5546.

(6) (a) Blanco, M. J.; Sardina, F. J. *Tetrahedron Lett.* **1994**, *35*, 8493. (b) Blanco, M. J.; Sardina, F. J. *J. Org. Chem.* **1996**, *61*, 4748.

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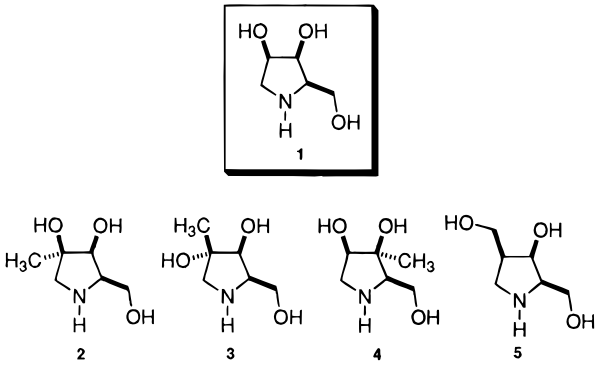
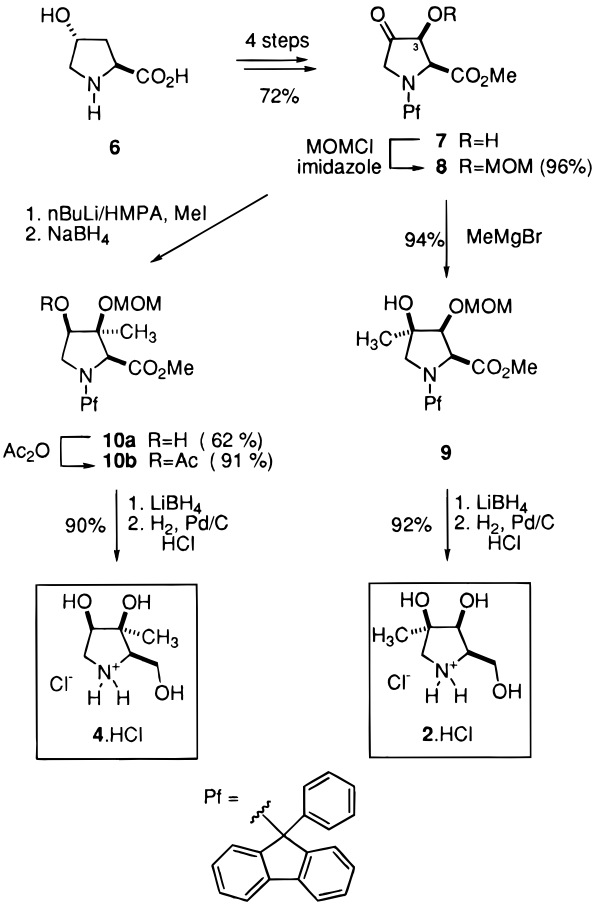


Figure 1.

Scheme 1



7 should lead to a precursor of **4**. A key question to be addressed during the syntheses will be the provision of regio- and stereocontrol.

Results and Discussion

We chose 4-methylpyrrolidine **2** as our initial target (Scheme 1). Since all of the C−C bond-forming reactions that we planned to use required strong basic media, we proceeded to protect the hydroxyl group of **7** with MOMCl and imidazole (500 mol % each) in DMF at room temperature to secure the key intermediate, ketone **8**, in 96% yield. When bases stronger than imidazole (*i*-Pr_2NEt or *N*-methylmorpholine) were employed for this protection reaction, small amounts of the C-3 epimer of the ketone **8** were detected in the crude product along with the desired **8**. The ease of abstraction of H-3 was even more evident when we attempted to alkylate ketone **8** with MeLi (THF, −78 °C) to introduce the desired methyl group at C-4: the alkylation product (**9**) was obtained in a modest 50% yield, the material balance being starting ketone **8**. When this methylation reaction was quenched with D_2O, the recovered **8** showed a high deuterium incorporation at C-3, showing that the MeLi was acting partly as a base. Surprisingly, no products coming from epimerization at C-3 could be detected in this reaction. Aparently, the enolate of **8** had formed regioselectively and had been protonated stereoselectively during the quench. We carried out Molecular Mechanics (MM3) and semiempirical (AM1 and PM3) calculations on 3,4-disubstituted *N*-Pf-$\Delta^{3,4}$-dehydroproline model systems (analogous to the enolate intermediate) to account for these results. Our calculations showed that the CO_2Me group in these molecules is locked in a pseudoaxial position due to the interaction with the Pf and the OMOM groups, thus blocking the β face of the pyrrolidine ring. The fluorenyl ring shields the hydrogens at C-5 from abstraction by the base.[7]

This facile regioselective enolization−stereoselective reprotonation boded well for the preparation of the 3-methyl analogue **4**, but it cast serious doubts on the viability of our approach to the C-4-alkylated analogues (**2** and **5**). After some experimentation, we found two solutions for this enolization problem. Thus, addition of $LiClO_4$ (a Lewis acid, to activate the carbonyl group, 500 mol %) to a THF solution of ketone **8**, prior to addition of MeLi (−78 °C), afforded **9** in 80% yield. A better procedure simply involved alkylation of **8** with the less basic MeMgBr (120 mol %, THF, −78 to −25 °C, 2 h), which provided the desired tertiary alcohol **9** in 94% yield. It is likely that the success of the addition of MeMgBr to ketone **8** also depends on chelation of the nucleophile with the carbonyl and MOM groups.[8]

The configuration at C-4 was established by NOE experiments that showed that H-2, H-3, and the methyl group at C-4 were all syn to each other. The reaction was completely stereoselective; we could not detect the C-4 epimer of **9** in the crude reaction mixture. MM and semiempirical calculations on 3-substituted *N*-Pf-4-oxoprolines showed that these molecules adopt two almost isoenergetic, minimum energy conformations, in which two substituents (one of them being always the Pf group) are pseudoequatorial and the remaining (pseudoaxial) group (OMOM or CO_2Me) effectively blocks the β face of the pyrrolidine ring, thus explaining the observed stereochemistry at C-4 in **9**.

With **9** in hand, we turned our attention to the reduction of the ester group, which proved more difficult than anticipated. $LiEt_3BH$ failed to achieve the desired transformation,[6] but $LiBH_4$ (300 mol %, THF) at room temperature did reduce ester **9** to give an unstable triol that was immediately subjected to hydrogenolysis in an acidic medium [H_2, 52 psi, Pd/C (10%), 30% w/w, MeOH/HCl] to remove all protecting groups. In this way, the

(7) Molecular Mechanics calculations were carried out with the Accumodel 1.0 program (MicroSimulations). Semiempirical calculations were carried out with the MacSpartan Plus 1.0 program (Wavefunction). Several dozens of starting conformations of each molecule were optimized to find the lowest energy conformer (MM). The structures within 3 kcal of the lowest energy conformers were further optimized by semiempirical (AM1 and PM3) calculations.

(8) Chen, X.; Hortelano, E. R.; Eliel, E. L.; Frye, S. V. *J. Am. Chem. Soc.* **1992**, *114*, 1778.

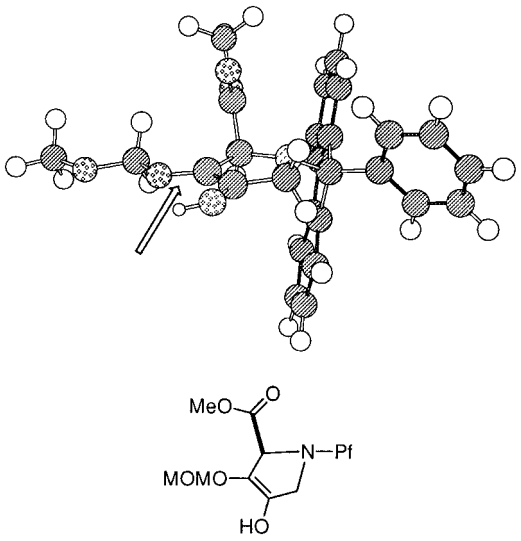


Figure 2.

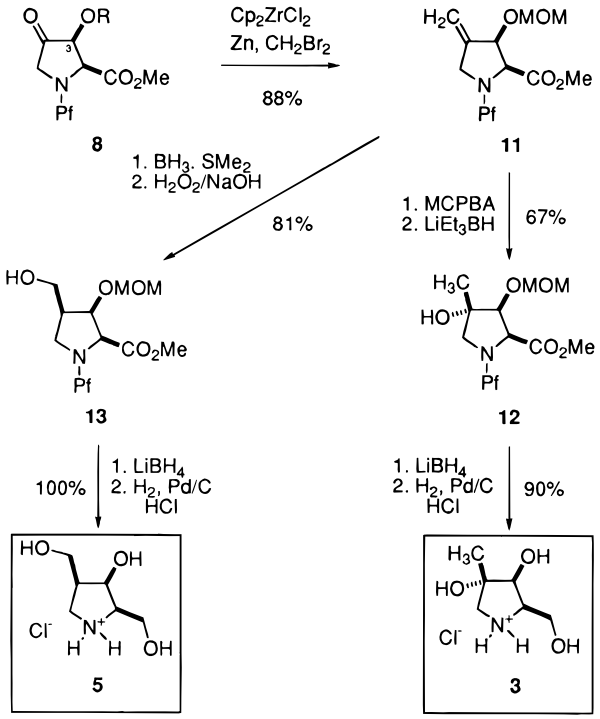
inhibitor analogue **2**·HCl was obtained in 92% combined yield (60% overall yield, eight steps from **6**).

Once the C-4-α-methyl analogue **2** had been secured, our attention turned to the preparation of the C-3-methyl analogue **4**, which required the regiospecific alkylation of ketone **8** at C-3. With regard to this transformation, Lubell and co-workers had shown that 3-alkyl-4-oxoprolines can be regioselectively alkylated at C-3, although the question of the stereoselectivity of the reaction was not addressed.[9] Our own results (vide supra) showed that ketone **8** underwent proton abstraction at C-3 when treated with bases, but despite this fact, attempts to methylate the enolate of **8** generated with LHMDS, NaHMDS, or LDA led only to decomposition products. Fortunately, and somewhat surprisingly, treatment of **8** with *n*-BuLi (110 mol %, THF/HMPA 9/1, −78 °C, 1 h) led to the exclusive formation of an enolate that could be alkylated with MeI (300 mol %, −78 to 0 °C). Due to the instability of the resulting 3-methyl ketone, the crude product was subjected to carbonyl reduction with $NaBH_4$ (250 mol %, MeOH/THF 1/1, −78 °C) to give **10a** (62% combined yield) as the sole reaction product. We did not detect other diastereoisomers in the crude reaction mixture. NOE experiments carried out on the acetate **10b** (Ac_2O, py, 91% yield) allowed us to assign the configurations shown for the newly introduced stereogenic centers. Once again, we attribute the remarkable degree of stereoselection observed in both the alkylation and reduction steps to the conformations induced in the reacting molecules by the Pf group. Figure 2 shows the minimum energy conformation of the enol form of **8** (a model for the corresponding enolate). As can be seen from Figure 2, the carboxy methyl group is pseudoaxial and blocks the upper face of the reacting C3, thus forcing approach of the electrophile toward the α face (marked by an arrow).

Reduction of the methoxycarbonyl group of **10a** ($LiBH_4$, 300 mol %, THF, rt, 24 h) followed by hydrogenolysis in an acidic medium [H_2, 52 psi, Pd/C (10%), 30% w/w, MeOH/HCl], as above, led to the C-3-methyl analogue **4**·HCl in 90% combined yield (38% overall yield, nine steps from **6**).

(9) Sharma, R.; Lubell, W. D. *J. Org. Chem.* **1996**, *61*, 202.

Scheme 2



In view of the fact that the additions to compounds with an sp^2 center at C-4 had proceeded from the α face with complete stereoselection, we decided to explore the utility of alkene **11** as a common intermediate toward analogues **3** and **5**: an epoxidation−reduction sequence should lead to a precursor of **3**, while hydroboration−oxidation−reduction should provide access to **5** (Scheme 2).

Attempts to obtain **11** by Wittig reaction on **8** were plagued by low conversions, due to competing ketone enolization; but when the methylenation was performed with the mild reagent developed by Tour and co-workers[10] (Cp_2ZrCl_2, 120 mol %, Zn,[11] 800 mol %, CH_2Br_2 120 mol %, THF, rt, 3 h), the desired alkene was obtained in 88% yield.

Epoxidation of **11** with *m*-CPBA (120 mol %, Na_2CO_3, 240 mol %, $CHCl_3$, 0 °C), followed by treatment of the resulting unstable epoxide with $LiEt_3BH$ (150 mol %, THF, rt), led to tertiary alcohol **12** (67% combined yield) as the sole reaction product. Hydroboration−oxidation of **11** (BH_3·SMe_2, H_2O_2/NaOH) provided the hydroxymethylene compound **13** (81% yield) regio- and stereoselectively. NOE experiments performed on **12** and **13** allowed us to assign the configurations at C-4 as shown. It is remarkable that the electrophilic additions to alkene **11** show the same degree of stereoselection as the nucleophilic additions to ketone **8**, thus attesting to the beneficial conformational effects introduced by the *N*-Pf group on the proline ring.

Finally, reduction of the ester groups of **12** and **13** and removal of the protecting groups were carried out as for alcohol **9** to give the desired analogues **3**·HCl (90% yield;

(10) Tour, J. M.; Bedworth, P. V.; Wu, R. *Tetrahedron Lett.* **1989**, *30*, 3927.

(11) Extreme care in the purification of the Zn used in this reaction is essential for its success: Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*; Wiley: New York, 1967; Vol 1, p 1292.

Table 1. Concentration Requirements for 50% Inhibition (μM)[a]

compd	α-gal	α-man	β-gal	β-glu
1	0.19	17	140	450
2	0.80	26	210	500
3	NI	NI	NI	300
4	12	250	270	NI
5	NI	NI	890	600

[a] NI = less than 50% inhibitioin at 1 mM. α-gal = α-galactosidase (*A. niger*). α-man = α-mannosidase (jack bean). β-gal = β-galactosidase (*A. niger*). β-glu = β-glucosidase (almonds).

36% overall yield, 10 steps from **6**) and **5**·HCl (quant; 49% overall yield, nine steps from **6**).

Glycosidase Inhibition Studies

The four pyrrolidine analogues **2**−**5** were screened for inhibitory activity against a number of common glycosidases that accept *p*-nitrophenyl glycosides as substrates.[12] The results, compared to those of the natural inhibitor **1**, are shown in Table 1.

The most interesting results are those obtained with the α-galactosidase and the α-mannosidase enzymes. As can be easily seen from Table 1, methyl substitution at C-4 (**2**) or C-3 (**4**) adversely affects the inhibitory potency of the analogues, but substitution at C-4 is much more well tolerated than at C-3: **2** retains 25% of the activity of the parent compound against α-galactosidase and 65% against α-mannosidase, while **4** is only 2% as active as the parent compound against α-galactosidase and 7% against α-mannosidase. Positioning the C-4 hydroxyl group further away from the ring (as in **5**) or substitution at C-4 coupled with inversion of stereochemistry (as in **3**) are catastrophic for the inhibitory activity. All the compounds tested are only weakly active against β-glucosidase and β-galactosidase.

To better relate the data of Table 1 to the structures of compunds **2**−**5**, we performed semiempirical calculations to determine if the most stable conformations of **2**−**5** closely matched that of the parent model **1**. The calculations were performed on the protonated forms of the inhibitors (the significant ones at physiological pH) and showed that the lowest energy conformation of **1** is an envelope in which the CH_2OH and the OH at C-3 are pseudoequatorial while the OH at C-4 is pseudoaxial. The most stable conformations of **2**−**4** do not differ significantly from that of **1**, while in **5** both CH_2OH groups occupy pseudoequatorial positions and the OH at C-3 is pseudoaxial (a conformation of **5** that closely matches the most stable conformation of **1** is 1.8 kcal higher in energy than the global minimum).

Conclusion

We have developed short, efficient, and stereoselective syntheses of alkylated analogues of the potent α-galactosidase inhibitor **1**, starting from 4-hydroxyproline. The 3-alkoxy-4-oxoproline **8** was the common, key intermediate in the preparation of pyrrolidines **2**−**5**. Addition of nucleophiles to the ketone group of *N*-Pf-4-oxoprolines and alkylation of the enolate of **8** proceeded with complete stereoselection from the α face of the molecule to provide access to analogues **2** and **4**. Electrophilic additions to the double bond of exomethyleneproline **11** also proceeded stereoselectively from the α face and paved the way to analogues **3** and **5**. The inhibitory activities of **2**−**5** were measured and showed that the fit of *A. niger* α-galactosidase and the jack bean α-mannosidase around C-3 of the pyrrolidine ring (α-face) must be very tight, while the fit around C-4 (α-face) is much looser. Positioning a methylene group between the OH at C-4 and the pyrrolidine ring completely abolishes the inhibitory activity of analogue **5**. The origin of this effect might be conformational in origin since the pseudoaxial−pseudoequatorial dispositions of the OH groups at C-4 and C-3 are inverted in **5** with respect to **1**.

An extension of these studies to other analogues of **1** as well as to analogues of the α-mannosidase inhibitor swainsonine is currently underway and will be reported in due course.

Experimental Section

General Methods. For general methods, see ref 6b. β-Glucosidase (from almonds), α-galactosidase (from *A. niger*), β-galactosidase (from *A. niger*), α-mannosidase (from jack bean), and all *p*-nitrophenyl glycoside substrates were purchased from Sigma Chemical Co.

(2*S*,3*S*)-3-(Methoxymethoxy)-4-oxo-*N*-(9′-phenylfluoren-9′-yl)pyrrolidine-2-carboxylic Acid Methyl Ester (8). Imidazole (573 mg, 8.33 mmol, 500 mol %) and ClMOM (0.63 mL, 8.33 mmol, 500 mol %) were added to a solution of the keto alcohol **7** (665 mg, 1.67 mmol) in DMF (7 mL). The resulting solution was stirred at room temperature for 18 h to give a red-brown mixture that was diluted with ether (5 mL), cooled at 0 °C, and treated with aqueous saturated $NaHCO_3$ (2 mL). The reaction mixture was partitioned between aqueous saturated $NaHCO_3$ (10 mL) and ether (15 mL); the organic phase was washed with H_2O (10 mL), H_3PO_4 (10%, 10 mL), and H_2O (10 mL). The aqueous phase was reextracted with ether (10 mL), and the combined organic layers were dried and concentrated to give a residue that was purified by column chromatography (EtOAc/hexanes 1:3) to give 709 mg (96%) of **8** as a white foam: $[\alpha]^{23}_D = -158.2°$ (*c* 1.1, $CHCl_3$); IR (NaCl) 2933, 1773, 1750, 1454 cm^{-1}; ^1H NMR (CD_2Cl_2) δ 3.06 (s, 3H), 3.24 (s, 3H), 3.57 (d, J = 17.2 Hz, 1H), 3.86 (d, J = 17.3 Hz, 1H), 3.93 (d, J = 7.7 Hz, 1H), 4.47 (d, J = 7.8 Hz, 1H), 4.52 (d, J = 6.7 Hz, 1H), 7.24−7.48 (m, 13H); ^{13}C NMR (CD_2Cl_2) δ 51.5, 52.6, 56.2, 61.7, 75.6, 78.3, 97.1, 120.7, 120.8, 125.9, 127.3, 127.4, 128.1, 128.4, 128.6, 128.4, 128.6, 129.2, 129.5, 129.6, 140.5, 141.9, 145.6, 147.3, 171.1, 209.3. Anal. Calcd for $C_{27}H_{25}O_5N$: C, 73.1; H, 5.7; N, 3.2. Found: C, 73.0; H, 5.7; N, 3.1.

(2*S*,3*S*,4*R*)-4-Hydroxy-4-methyl-3-(methoxymethoxy)-*N*-(9′-phenylfluoren-9′-yl)pyrrolidine-2-carboxylic Acid Methyl Ester (9). Methylmagnesium bromide (0.122 mL, 0.366 mmol, 120 mol %, 3.0 M in ether) was added dropwise to a solution of **8** (135 mg, 0.305 mmol) in THF (3 mL) at −78 °C. The resulting mixture was stirred for 2.5 h from −78 to −25 °C. The reaction was quenched at −25 °C with H_3PO_4 (10%, 0.25 mL) and then partitioned between H_2O (45 mL) and CH_2Cl_2 (3 × 50 mL). The organic phase was washed with brine, dried, and concentrated. The residue was purified by column chromatography (EtOAc/hexane 1:1.5) to afford 132 mg (94%) of **9** as a white foam: $[\alpha]^{28}_D = 242.6°$ (*c* 1.7, $CHCl_3$); IR (NaCl) 3510, 1745, 1448 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.16 (s, 3H), 2.86 (d, J = 9.4 Hz, 1H), 3.18 (d, J = 9.5 Hz, 1H), 3.19 (d, J = 8.5 Hz, 1H), 3.21 (s, 3H), 3.31 (s, 3H), 3.65 (d, J = 9.5 Hz, 1H), 4.37 (d, J = 6.8 Hz, 1H), 4.40 (d, J = 6.8 Hz, 1H), 4.63 (bs, 1H), 7.12−7.81 (m, 13H); ^{13}C NMR ($CDCl_3$) δ 20.9, 52.1, 56.0, 59.0, 62.3, 75.9, 76.1, 80.3, 96.4, 120.4, 120.7, 127.3, 127.4, 127.7, 128.0, 128.2, 128.9, 129.0, 129.4, 139.8, 141.7, 142.4, 145.0, 148.2, 175.9. Anal. Calcd for $C_{28}H_{29}O_5N$: C, 73.2; H, 6.4; N, 3.1. Found: C, 73.0; H, 6.3; N, 3.0.

(2*R*,3*S*,4*R*)-3,4-Dihydroxy-2-(hydroxymethyl)-4-methylpyrrolidine Hydrochloride (2·HCl). $LiBH_4$ (13 mg, 0.561

(12) Tropea, J. E.; Molyneaux, R. J.; Kaushal, G. P.; Pan, Y. T.; Mitchell, M.; Elbein, A. D. *Biochemistry* **1989**, *28*, 2027.

mmol, 300 mol %) was added to a solution of **9** (86 mg, 0.187 mmol) in THF (1.5 mL) at room temperature. After 24 h, the reaction mixture was diluted with ether (2 mL), and H_3PO_4 (10%, 0.75 mL) was added dropwise. The reaction mixture was partitioned between H_2O (25 mL) and ether (30 mL). The organic phase was washed with brine, dried, and evaporated. The crude product was dissolved in deoxygenated MeOH (2 mL), and Pd/C (24 mg, 30 wt %, 10%) and HCl (c) (57 μL, 0.564 mmol, 300 mol %) were added. The flask was purged with argon and then evacuated (water aspirator) and pressurized (H_2) three times. The reaction mixture was mechanically shaken under 52 psi of H_2 for 20 h. The catalyst was removed by filtration through a pad of Celite, and the filtrate was evaporated. The residue was washed with toluene to give a clear yellow oil that was recrystallized from methanol/EtOAc to afford 31 mg of **2**·HCl (92%, two steps) as a white solid: mp 140−143 °C; $[\alpha]^{20}_D = 23.1°$ (*c* 0.75, MeOH); IR (KBr) 3390, 3100, 2935, 2800, 2450, 1550 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.21 (s, 3H), 3.01 (d, J = 12.2 Hz, 1H), 3.18 (d, J = 12.2 Hz, 1H), 3.73 (m, 3H), 3.98 (d, J = 4.1 Hz, 1H); ^{13}C NMR (CD_3OD) δ 22.9, 54.1, 59.8, 64.1, 75.6, 77.2. Anal. Calcd for $C_6H_{14}O_3$·NCl: C, 42.5; H, 8.3; N, 8.3. Found: C, 42.2; H, 8.5; N, 8.2.

(2*S*,3*S*,4*R*)-4-Hydroxy-3-methyl-3-(methoxymethoxy)-*N*-(9′-phenylfluoren-9′-yl)pyrrolidine-2-carboxylic Acid Methyl Ester (10a). A solution of **8** (66 mg, 0.149 mmol) in THF (2.1 mL) was added dropwise (15 min) to a solution of *n*-BuLi (0.14 mL, 0.16 mmol, 105 mol %, 1.1 M in hexanes) and HMPA (70 μL) in THF (0.7 mL) at −78 °C; the resulting solution was stirred for 1.2 h at −78 °C, and then MeI (46 μL, 0.75 mmol, 500 mol %) was added and the stirring continued for 4.5 h from −78 to 0 °C. The reaction was quenched by addition of H_3PO_4 (10%, 0.25 mL). The resulting suspension was partitioned between H_2O (30 mL) and ether (45 mL). The organic phase was washed with saturated $Na_2S_2O_3$ (30 mL) and then with H_2O (30 mL). The organic phase was dried, filtered, and concentrated. Due to the instability of the resulting tertiary alcohol, the crude mixture was dissolved in THF/MeOH (1/1, 1.2 mL), cooled at −78 °C, and treated with $NaBH_4$ (15 mg, 0.372 mmol, 250 mol %). The resulting suspension was stirred for 4 h at −78 °C. The reaction was quenched with H_3PO_4 (10%, 0.25 mL). The crude mixture was partitioned between H_2O (20 mL) and ether (2 × 30 mL). The organic phase was washed with brine, dried, and concentrated. The residue was purified by column chromatography (EtOAc/hexane 1/4) to give 42 mg (62%, two steps) of **10a** as a white foam: $[\alpha]^{27}_D = 161.7°$ (*c* 0.6, $CHCl_3$); IR (NaCl) 3500, 1744, 1451 cm^{-1}; ^{1}H NMR (CD_2Cl_2) δ 0.83 (s, 3H), 2.74 (s, 1H), 3.21 (s, 3H), 3.30 (s, 3H), 3.40 (d, J = 11.4 Hz, 1H), 3.72 (dd, J = 3.3, 11.7 Hz, 1H), 4.43 (d, J = 11.9 Hz, 1H), 4.51 (d, J = 6.9 Hz, 1H), 4.65 (d, J = 6.9 Hz, 1H), 7.08−7.82 (m, 13 H); ^{13}C NMR (CD_2Cl_2) δ 23.4, 52.0, 55.3, 55.8, 70.1, 75.8, 75.9, 83.6, 93.0, 120.4, 120.8, 126.9, 127.4, 127.6, 128.0, 128.1, 128.2, 128.8, 129.0, 129.2, 129.4, 139.8, 141.7, 142.4, 145.2, 148.5, 176.2. Anal. Calcd for $C_{28}H_{29}O_5N$: C, 73.2; H, 6.4; N, 3.1. Found: C, 73.5; H, 6.4; N, 3.1.

(2*S*,3*S*,4*R*)-4-Acetoxy-3-methyl-3-(methoxymethoxy)-*N*-(9′-phenylfluoren-9′-yl)pyrrolidine-2-carboxylic Acid Methyl Ester (10b). Pyridine (0.2 mL) was added to a solution of **10a** (20 mg, 0.044 mmol) in Ac_2O (0.7 mL). The resulting solution was stirred at room temperature for 20 h, and then aqueous saturated $NaHCO_3$ (0.2 mL) was added. The reaction mixture was partitioned between aqueous saturated $NaHCO_3$ (10 mL) and EtOAc (15 mL). The organic phase was washed with saturated $CuSO_4$ (10 mL) and H_2O (10 mL). The aqueous phase was reextracted with EtOAc (10 mL). The combined organic layers were washed with brine, dried, and evaporated. The residue was purified by column chromatography (EtOAc/hexane 1/4) to afford 20 mg (91%) of **10b** as a white foam: $[\alpha]^{27}_D = 193.1°$ (*c* 0.55, $CHCl_3$); IR (NaCl) 2935, 1746, 1451 cm^{-1}; ^{1}H NMR (C_6D_6) δ 1.10 (s, 3H), 1.72 (s, 3H), 3.14 (s, 1H), 3.17 (s, 3H), 3.26 (s, 3H), 3.59 (dd, J = 6.2, 11.2 Hz, 1H), 3.71 (dd, J = 6.8, 11.2 Hz, 1H), 4.56 (d, J = 7.5 Hz, 1H), 4.79 (d, J = 7.5 Hz, 1H), 4.87 (t, J = 6.5 Hz, 1H), 6.91−7.85 (m, 13H); ^{13}C NMR (C_6D_6) δ 20.3, 21.0, 50.5, 51.9, 55.0, 70.7, 77.3 (2C), 83.0, 92.4, 119.8, 120.2, 126.3, 126.5, 126.7, 127.0, 127.8, 128.5, 128.8, 129.1, 139.6, 142.5, 143.4, 146.2, 148.0, 169.9, 171.4. Anal. Calcd for $C_{30}H_{31}O_6N$: C, 71.8; H, 6.2; N, 2.8. Found: C, 72.0; H, 6.3; N, 2.9.

(2*R*,3*S*,4*R*)-3,4-Dihydroxy-2-(hydroxymethyl)-3-methylpyrrolidine Hydrochloride (4·HCl). By following the same procedure described above for **2**·HCl, 15 mg (90%, two steps) of **4**·HCl were obtained as a white solid: mp 156−158 °C; $[\alpha]^{20}_D = 14.1°$ (*c* 0.8, MeOH); IR (KBr) 3390, 3100, 2930, 2800, 2450, 1550 cm^{-1}; ^{1}H NMR (D_2O) δ 1.56 (s, 3H), 3.00 (dd, J = 12.2, 5 Hz, 1H), 3.39 (m, 2H), 3.60 (m, 1H), 3.79 (dd, J = 4.1, 12.0 Hz, 1H), 3.94 (t, J = 5 Hz, 1H); ^{13}C NMR (D_2O ref dioxane) δ 21.3, 51.1, 59.8, 67.5, 77.6, 79.6. Anal. Calcd for $C_6H_{14}O_3NCl$: C, 42.5; H, 8.3; N, 8.3. Found: C, 42.3; H, 8.5; N, 8.2.

(2*S*,3*R*)-4-Methylene-3-(methoxymethoxy)-*N*-(9′-phenylfluoren-9′-yl)pyrrolidine-2-carboxylic Acid Methyl Ester (11). CH_2Br_2 (0.10 mL, 1.53 mmol, 220 mol %) was added to a suspension of **8** (307 mg, 0.69 mmol), Zn (362 mg, 5.54 mmol, 800 mol %), and Cp_2ZrCl_2 (243 mg, 0.83 mmol, 120 mol %) in THF (1.7 mL). The resulting gray suspension was stirred at room temperature for 3 h, turning into a yellow suspension. The reaction was quenched by adding H_2O dropwise until gas evolution ceased. The reaction mixture was partitioned between ether (30 mL) and H_2O (20 mL). The aqueous phase was reextracted with ether (20 mL), and the combined organic layers were washed with brine, dried, and evaporated. The residue was purified by column chromatography (EtOAc/hexane 1/4) to give 269 mg (88%) of **11** as a white foam: $[\alpha]^{28}_D = -24.8°$ (*c* 1.0, $CHCl_3$); IR (NaCl) 1741, 1448, 743 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.21 (s, 3H), 3.26 (s, 3H), 3.52 (d, J = 13.7 Hz, 1H), 3.61 (d, J = 8.1 Hz, 1H), 3.94 (d, J = 13.6 Hz, 1H), 4.44 (d, J = 6.8 Hz, 1H), 4.96 (bs, 1H), 5.04 (bs, 1H), 7.15−7.62 (m, 13H); ^{13}C NMR ($CDCl_3$) δ 50.9, 51.0, 55.7, 63.6, 76.1, 76.7, 95.7, 106.6, 119.9, 120.0, 125.9, 126.6, 127.3 (2C), 127.5 (2C), 127.9, 128.4 (2C), 128.5, 128.7, 140.5, 142.6, 146.7, 147.3, 172.4. Anal. Calcd for $C_{28}H_{27}O_4N$: C, 76.2; H, 6.2; N, 3.2. Found: C, 76.0; H, 6.2; N, 3.1.

(2*S*,3*S*,4*S*)-4-Hydroxy-4-methyl-3-(methoxymethoxy)-*N*-(9′-phenylfluoren-9′-yl)pyrrolidine-2-carboxylic Acid Methyl Ester (12). A solution of *m*-CPBA (70%, 54 mg, 0.22 mmol, 120 mol %) in $CHCl_3$ (0.75 mL) was added dropwise to a suspension of **11** (80 mg, 0.18 mmol) and Na_2CO_3 (37 mg, 0.44 mmol, 240 mol %) in $CHCl_3$ (1 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 15 h and then quenched with an aqueous solution of Na_2SO_3 (10%, 0.5 mL). The resulting mixture was partitioned between aqueous saturated $NaHCO_3$ (20 mL) and CH_2Cl_2 (2 × 25 mL). The combined organic layers were washed with brine, dried, and evaporated. Due to the instability of the resulting epoxide, the reaction mixture was used in the next step without further purification. To a solution of the crude epoxide in THF (1.5 mL) at 0 °C was added dropwise $LiEt_3BH$ (0.32 mL, 0.27 mmol, 150 mol %, 0.9 M in THF). After 5 min, the bath was removed, and the resulting mixture was stirred at room temperature for 15 h. The reaction was quenched with H_3PO_4 (10%, 0.2 mL). The suspension was partitioned between H_2O (20 mL) and CH_2Cl_2 (2 × 30 mL). The combined organic layers were washed with brine, dried, and evaporated. The resulting oily residue was purified by short column chromatography (EtOAc/hexane 1:3) to afford 57 mg (67%) of **12**: $[\alpha]^{29}_D = 166.5°$ (*c* 1.3, $CHCl_3$); IR (NaCl) 3520, 1744, 1448 cm^{-1}; ^{1}H NMR (CD_2Cl_2) δ 1.43 (s, 3H), 3.25 (s, 3H), 3.40 (s, 3H), 3.41 (d, J = 8.4 Hz, 2H), 3.87 (d, J = 9.5 Hz, 1H), 4.27 (d, J = 9.5 Hz, 1H), 4.51 (d, J = 6.6 Hz, 1H), 7.13−7.73 (m, 13H); ^{13}C NMR (CD_2Cl_2) δ 25.9, 52.3, 55.9, 61.0, 66.1, 68.2, 76.6, 77.8, 96.1, 120.4, 120.5, 121.1, 125.6, 127.5, 127.6, 127.9, 128.3, 128.9, 129.1, 129.3, 129.7, 130.0, 140.6, 141.1, 147.1, 177.7. Anal. Calcd for $C_{28}H_{29}O_5N$: C, 73.2; H, 6.4; N, 3.1. Found: C, 73.0; H, 6.2; N, 3.0.

(2*S*,3*R*,4*S*)-4-(Hydroxymethyl)-3-(methoxymethoxy)-*N*-(9′-phenylfluoren-9′-yl)pyrrolidine-2-carboxylic Acid Methyl Ester (13). BH_3·SMe_2 (0.18 mL, 0.18 mmol, 37 mol %; a solution of BH_3·SMe_2 1.0 M in THF was prepared beforehand) was added to a solution of **11** (220 mg, 0.50 mmol) in THF (1 mL) at 0 °C. After 5 min at 0 °C, the bath was removed, and the reaction mixture was stirred at room

temperature for 3 h. The reaction was quenched by adding H_2O dropwise until H_2 ceased to be given off. The resulting mixture was cooled to 0 °C, and NaOH (2 M, 0.13 mL, 0.25 mmol, 50 mol %) and H_2O_2 (30%, 55 μL, 0.54 mmol, 110 mol %) were added dropwise (the internal temperature should be kept below 40 °C). After 5 min of stirring at 0 °C, the bath was removed and the resulting mixture was heated at 50 °C for 1 h. The reaction mixture was poured into ice and washed with ether (40 mL). The organic phase was washed with H_2O (2 × 30 mL); the aqueous phase was reextracted with ether (2 × 30 mL), and the combined organic layers were washed with brine, dried, and evaporated to give a residue that was purified by column chromatography (EtOAc/hexane 1:1.5), providing 186 mg (81%) of **13** as a white foam: $[\alpha]^{28}_D = 252.2°$ (*c* 1.7, $CHCl_3$); IR (NaCl) 3519, 1753, 1448 cm^{-1}; ^1H NMR (CD_2Cl_2) δ 2.24 (m, 1H), 3.14 (m, 3H), 3.21 (s, 3H), 3.26 (s, 3H), 3.76 (m, 2H), 4.12 (t, J = 7.5 Hz, 1H), 4.33 (d, J = 6.7 Hz, 1H), 4.41 (d, J = 6.7 Hz, 1H), 7.00−7.69 (m, 13H); ^{13}C NMR (CD_2Cl_2) δ 44.3, 50.1, 51.2, 56.0, 60.2, 64.7, 76.8, 80.0, 97.0, 119.6, 120.1, 126.4, 127.0, 127.3, 127.4, 127.8, 127.9, 128.3, 128.4, 128.8, 139.1, 142.2, 142.5, 145.0, 147.5, 173.2. Anal. Calcd for $C_{28}H_{29}O_5N$: C, 73.2; H, 6.4; N, 3.1. Found: C, 72.8; H, 6.5; N, 3.0.

(2*R*,3*S*,4*S*)-3,4-Dihydroxy-2-(hydroxymethyl)-4-methylpyrrolidine Hydrochloride (3·HCl). By following the same procedure as **2**·HCl, **12** afforded 25 mg (90%, two steps) of **3**·HCl as a white solid: mp 162−164 °C; $[\alpha]^{20}_D = 34.1°$ (*c* 0.8, MeOH); IR (KBr) 3390, 3110, 2930, 2800, 2450, 1550 cm^{-1}; ^1H NMR (CD_3OD) δ 1.41 (s, 3H), 3.01 (d, J = 12.1 Hz, 1H), 3.12 (d, J = 12.0 Hz, 1H), 3.69 (m, 3H), 3.89 (d, J = 4.1 Hz, 1H); ^{13}C NMR (CD_3OD) δ 22.8, 52.3, 59.8, 64.3, 75.5, 77.0. Anal. Calcd for $C_6H_{14}O_3NCl$: C, 42.5; H, 8.3; N, 8.3. Found: C, 42.3; H, 8.5; N, 8.2.

(2*R*,3*R*,4*S*)-2,4-Bis(hydroxymethyl)-3-hydroxypyrrolidine Hydrochloride (5·HCl). By following the same procedure as used for **2**·HCl, a solution of **13** (80 mg, 0.17 mmol) in THF (2 mL) was treated with $LiBH_4$ (12 mg, 0.52 mmol, 300 mol %). The crude product was purified by short column chromatography (EtOAc/hexane 1/1) to afford 73 mg (97%) of **(2*R*,3*R*,4*S*)-2,4-bis(hydroxymethyl)-3-(methoxymethoxy)-*N*-(9′-phenylfluoren-9′-yl)pyrrolidine** as a white foam: $[\alpha]^{22}_D = 268.0°$ (*c* 0.7, $CHCl_3$); IR (NaCl) 3604, 1451 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.24 (m, 1H), 2.71 (m, 1H), 2.84 (dd, J = 3.7, 10.8 Hz, 1H), 3.17 (m, 3H), 3.38 (s, 3H), 3.60 (m, 2H), 4.02 (t, J = 5.1 Hz, 1H), 4.64 (d, J = 6.3 Hz, 1H), 4.68 (d, J = 6.5 Hz, 1H), 7.14−7.73 (m, 13H); ^{13}C NMR ($CDCl_3$) δ 44.5, 52.6, 56.2, 60.3, 61.4, 64.5, 77.5, 83.0, 98.8, 119.8, 120.0, 125.8, 126.5, 127.1, 127.3, 127.8, 128.0, 128.4, 128.5, 128.8, 139.0, 142.0, 144.3, 146.9, 148.8. Anal. Calcd for $C_{27}H_{29}O_4N$: C, 75.2; H, 6.8; N, 3.3. Found: C, 75.0; H, 6.7; N, 3.2. A solution of the above compound (20 mg, 0.05 mmol) in deoxygenated MeOH (0.5 mL) was hydrogenated in an acidic medium following the same procedure as used for **2**·HCl. In this way, 8 mg (100%) of **5**·HCl was obtained as a white solid: mp 161−163 °C; $[\alpha]^{20}_D = 22.3°$ (*c* 0.8, MeOH); IR (KBr) 3390, 2940, 1548, 1451 cm^{-1}; ^1H NMR (CD_3OD) δ 3.18 (t, J = 11.1 Hz, 1H), 3.46 (dd, J = 11.1, 7.5 Hz, 1H), 3.62 (m, 1H), 3.71 (dd, J = 10.9, 6.7 Hz, 1H), 3.83 (dd, J = 10.9, 6.8 Hz, 1H), 3.94 (m, 3H), 4.40 (t, J = 2.9 Hz, 1H); ^{13}C NMR (CD_3OD) δ 46.8, 47.4, 59.3, 60.2, 68.2, 71.2. Anal. Calcd for $C_6H_{14}O_3NCl$: C, 39.2; H, 7.7; N, 7.6. Found: C, 38.9; H, 7.9; N, 7.6.

Enzymatic Assays. Enzyme inhibition was assayed colorimetrically by monitoring the release of *p*-nitrophenol from the appropriate *p*-nitrophenyl glycoside substrate according to the procedure described in ref 12. All reaction mixtures contained 20 μmol of sodium acetate buffer (pH = 5), 2 μmol of *p*-nitrophenyl glycoside, and the appropriate enzyme in a final volume of 0.4 mL. Incubations were performed at 37 °C for 15 min, and the reactions were stopped by the addition of 2.5 mL of 0.4 M glycine (pH = 10.4). The *p*-nitrophenol liberated in the reaction was determined by measuring the UV absorbance of the mixture at 410 nm.

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