Synthesis of Cis and Trans Isomers of an Isoxazoline Ring-Hydroxylated Metabolite of Roxifiban, a Platelet Glycoprotein IIb/IIIa Receptor Antagonist

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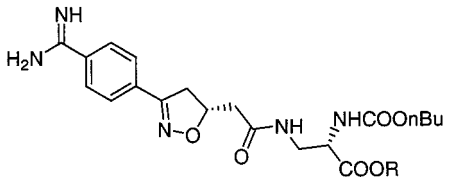
Introduction

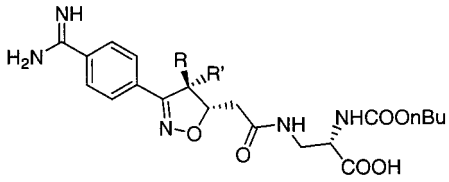
Roxifiban (**1**) is a potent and selective antagonist of the platelet glycoprotein IIb/IIIa receptor, the major receptor for fibrinogen on the surface of platelets.[1,2] Since the final step in platelet adhesion is the binding of fibrinogen to GPIIb/IIIa, an antagonist of this receptor is expected to provide benefit in a number of cardiovascular disorders which involve inappropriate platelet adhesion.[3] Roxifiban is an ester prodrug of the active GPIIb/IIIa antagonist XV459 (**2**), which is unusual among reported GPIIb/IIIa antagonists in that it binds with equal affinity to both the resting and activated forms of GPIIb/IIIa.[1,2] This could offer advantages over other such agents in providing a greater degree of antiplatelet activity as well as a better pharmacokinetic profile arising from the reservoir of drug bound to unactivated platelets.

Following dosing of **1** in rats, dogs, or humans, the major metabolite was **2**, as expected. In rats, intravenous infusion of either **1** or **2** yielded several hydroxylated metabolites of **2**, which were identified using LC/MS, LC/NMR, and high-field NMR.[4] One interesting metabolite was the ring 4-hydroxylated compound **3**. NMR studies were unable to firmly establish the stereochemistry of the ring hydroxylation, although based on steric considerations the trans isomer **3a** would be expected to result from enzymatic oxidation of **2**.

Since the relative stereochemistry of the 4-hydroxy group with respect to the 5-substituent of **3** was unclear, both the cis and trans isomers were synthesized for comparison with the isolated material. The relative stereochemistry of the synthetic standards was firmly established in each case through creation of the isoxazoline ring via [3 + 2] dipolar cycloaddition reactions, since the known stereochemistry of the olefin dipolarophile would be retained in the product.[5]



1 (R = Me)
2 (R = H)



3a (R = OH, R' = H)
3c (R = H, R' = OH)

Figure 1.

The approach to *trans*-4-hydroxy-5-substituted isoxazolines reported by Wallace[6] was used to prepare **3a**, as shown in Scheme 1. The trans-olefinic boronate **5** was prepared in 63% yield by hydroboration of 3-butyn-1-ol, protected as the tetrahydropyran ether **4**, using zirconium catalysis.[7] The resulting trans boronate underwent cycloaddition with the nitrile oxide derived from **6**,[8] with concomitant oxidation of the boronate to the alcohol, in the presence of sodium percarbonate.[9] The crude product was acetylated, and the THP protecting group was removed to provide **7** in 70% yield from **5**. Oxidation of the primary alcohol provided the acid **8** in 76% yield, which was coupled with the amine **9**,[10] derived from (*S*)-asparagine, to provide **10** in 84% yield. While the relative stereochemistry of the ring substituents was fixed as trans, the intermediate **8** was racemic, so coupling with the optically active **9** provided the acetate of **10** as a mixture of diastereomers. These were separated by preparative HPLC using a chiral stationary phase; the acetate was hydrolyzed to the alcohol during this separation. Roxifiban, having the (*R*) stereochemistry shown in **1**, is levorotatory,[11] so we tentatively assigned the absolute configuration of the (−) isomer of **10** as (4*S*,5*S*) (**10a**), which corresponds to that of Roxifiban. However, both diastereomers were carried through the remainder of the synthesis separately.

Conversion of the nitriles **10** to the amidines **11** was achieved in 23% yield by treatment with excess hydroxylamine, followed by selective acetylation of the amidoxime and hydrogenolysis to the amidine.[12] Attempted hydroly-

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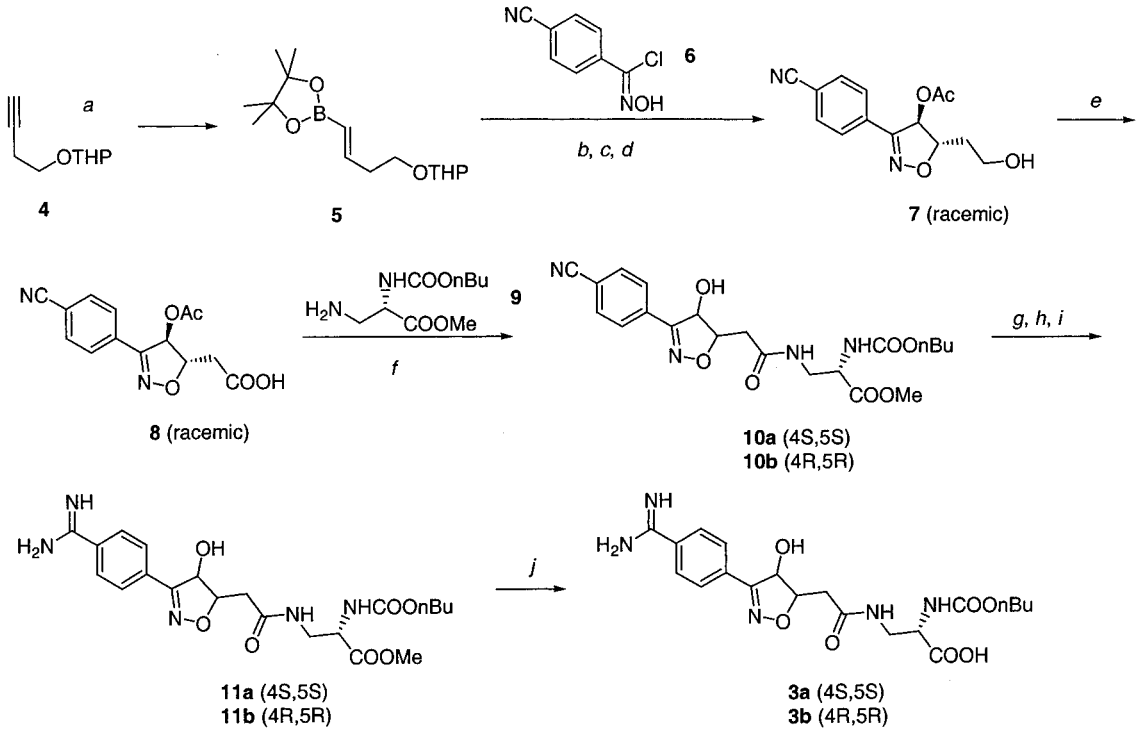
Scheme 1. Synthesis of *trans*-4-Hydroxy Metabolite (3a and 3b)[a]



[a] Reagents: *a*, pinacolborane, Cp_2ZrHCl; *b*, $Na_2CO_3 \cdot H_2O_2$; *c*, acetic anhydride, pyridine; *d*, acetic acid, H_2O; *e*, PDC; *f*, DCC, HOBT, Et_3N; HPLC separation; *g*, $H_2NOH \cdot HCl$, Et_3N; *h*, acetic anhydride; *i*, H_2, Pd/C; *j*, rabbit liver esterase.

sis of the methyl ester under either acidic or basic conditions led to significant dehydration of the ring to the isoxazole, so the ester was removed by treatment with rabbit liver esterase, providing **3a** and **3b** in 95% yield. The direction of optical rotation remained the same for each intermediate, with the (−) isomer again assumed to be that corresponding to Roxifiban and assigned the structure **3a**.

No known examples of *cis*-4-hydroxy-5-substituted 3-phenylisoxazolines have been reported in the literature. Again taking advantage of the stereochemical retention obtained from the [3 + 2] cycloaddition of nitrile oxides,[5] the cis isomer **3c** was prepared as shown in Scheme 2, this time using furan as the dipolarophile.[13] The cycloadduct **12**, obtained in 91% yield, was hydrated to the lactol **13** in 72% yield using a known procedure,[14] followed by Jones oxidation to the lactone **14** in 87% yield. This racemic material could be resolved by HPLC using a chiral stationary phase, providing the pure enantiomers which were carried on separately. The lactone was opened in 67% yield by treatment with **9**, providing **15**. Once again, the levorotatory isomer was assigned the absolute stereochemistry (4*R*,5*S*) (**15c**), corresponding to that of Roxifiban. Amidine elaboration and ester hydrolysis were achieved in 23% yield on the separate diastereomers as described above for the trans case. The diastereomer which again retained the (−) optical rotation was tentatively assigned structure **3c**.

In addition to the theoretically expected relative stereochemistry resulting from concerted cycloaddition, the trans stereochemistry of **7** and the cis stereochemistry of **14** were supported by examination of the vicinal NMR coupling constants observed for the proton at the 4-position of the ring, geminal to the oxygen substituent. In **7**, this signal appeared as a doublet at δ 6.30, with $J = 2.5$ Hz, while in **14** the doublet at δ 6.46 had $J = 6.9$ Hz. Although not completely diagnostic, the trans isomers of 4,5-disubstituted isoxazolines generally have smaller coupling constants than cis isomers.[15] According to the vicinal Karplus correlation,[16] the observed coupling constants represent dihedral angles of approximately 119° and 28° for the trans and cis isomers, respectively. These values are in good agreement with the range of dihedral angles achievable by the trans and cis isomers through ring flexibility, as measured by examination of Dreiding models of the trans isomer (110−140°, giving calculated coupling constants of 1.5−5.9 Hz) and cis isomer (10−30°; 8.5−6.7 Hz). The carbinol methine of the final products **3** also showed similar coupling constants (3.3 Hz for the trans isomers, 7.3 Hz for the cis isomers).

The NMR spectra of the cis isomers **3c** and **3d** displayed a doubling of the backbone hydrogens of the diaminopropionate moiety which was not observed in **3a** and **3b**. The β-alanine derivative **16**, prepared by the same method as **15** (Scheme 3), showed no peak doubling, but the O-methylated derivative **17** displayed a distinct pair of signals (2:1 ratio, both doublets with $J = 7.3$ Hz) for the isoxazoline C-4 proton and also a pair of singlets for the methoxy protons. Both pairs coalesced reversibly to sharp signals at 90 °C, suggesting that restricted conformational mobility was indeed the cause. Since no such effects were seen in the trans isomers, a barrier to

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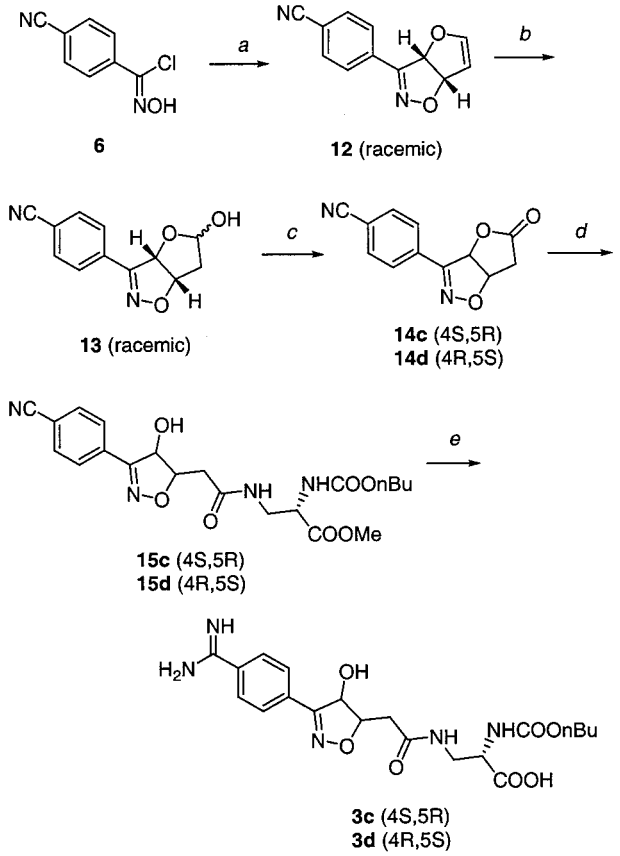
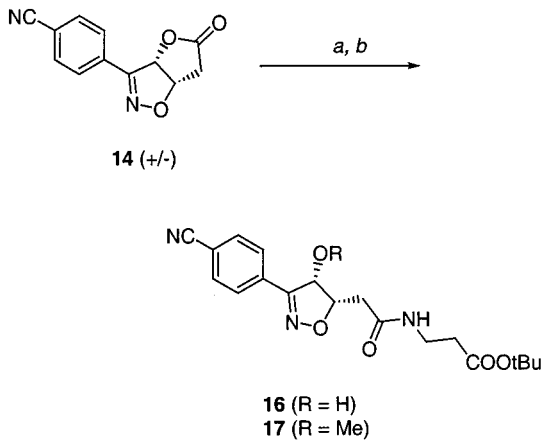
Scheme 2. Synthesis of *cis*-4-Hydroxy Metabolite (3c and 3d)[a]



[a] Reagents: *a*, furan, Et_3N; *b*, LiBr, AG50W−X2; *c*, Jones reagent; *d*, **9**; *e*, see Scheme 1.

Scheme 3. Synthesis of 16 and 17[a]



[a] Reagents: *a*, *β*-alanine *tert*-butyl ester HCl, Et_3N, MeCN; *b*, NaH, DMF, then MeI.

isoxazoline ring flexibility was presumably present in the cis but not the trans isomers. The reason for this difference is unknown, although intramolecular hydrogen bonding between the amide NH and the 4-oxygen substituent (only possible in the cis isomer) is one possibility.

Comparison of the proton NMR spectra and mass spectral fragmentation patterns of **3a**−**3d** with those of the isolated metabolite confirmed that the levorotatory trans isomer corresponded to the isolated ring 4-hydroxylated metabolite[4] and also confirmed that the tentative assignment of this isomer to structure **3a** (ring 4*S*,5*S*) was correct. The latter point was supported by comparing the activities of **3a** and **3b** in the human platelet-rich plasma aggregation assay[17] with that observed for the isolated metabolite (Table 1); the IC_{50} value for **3a** was much closer to that of the isolated material than was that of **3b**.

Table 1. Platelet Aggregation Results

compound	IC_{50}, nM[a]
3a	140 ± 37 (n = 3)
3b	6500 (n = 1)
isolated metabolite	33 (n = 1)

[a] See ref 17 for details.

In conclusion, the known retention of relative stereochemistry inherent in the [3 + 2] dipolar cycloaddition reactions of nitrile oxides has been used to prepare both the trans and cis isomers of a ring-hydroxylated metabolite of the GPIIb/IIIa antagonist XV459, the active drug form of Roxifiban. In the course of this work, the first example of a *cis*-4-hydroxy-5-substituted isoxazoline was prepared, and a possible difference in ring mobility between the cis and trans isomers was observed. Comparison of these synthetic standards with the isolated metabolite demonstrated the trans relative stereochemistry in this material.

Experimental Section

Starting materials, reagents, and solvents were obtained from commercial sources and used as received unless otherwise indicated. All reactions were run under a nitrogen atmosphere. Flash chromatography refers to the medium-pressure column chromatography method described by Still et al.[18] Preparative HPLC was performed using a reverse phase C_{18} column (41.4 × 250 mm), eluting with water−acetonitrile−trifluoroacetic acid at 40 mL/min, using a 30-min linear gradient from 97.5:2.5:0.05 to 20:80:0.05. Compounds were isolated as trifluoroacetate salts following HPLC purification. Melting points (mp) are uncorrected. Proton NMR spectra were measured at 300 mHz in chloroform-*d* ($CDCl_3$), dimethyl sulfoxide-d_6 (DMSO-d_6), methanol-d_4 (MeOH-d_4), or acetone-d_6 and the peaks are reported in parts per million downfield from tetramethylsilane (δ). Mass spectra (MS) and high-resolution mass spectra (HRMS) were measured using positive ion electrospray ionization (ES^+) or ammonia chemical ionization (NH_3−CI). Abbreviations used: DCM, dichloromethane; EtOAc, ethyl acetate; IPA, 2-propanol; MeCN, acetonitrile; MeOH, methanol; TEA, triethylamine.

2-[4-(4,4,5,5-Tetramethyl[1,3,2]dioxaborolan-2-yl)but-3-enyloxy]tetrahydropyran (5). A solution of 2-but-3-ynyloxytetrahydropyran (**4**, 1.54 g, 10 mmol) in DCM (5 mL) was treated with 4,4,5,5-tetramethyl[1,3,2]dioxaborolane (pinacolborane, prepared and purified according to the procedure of Tucker et al.;[19] 1.34 g, 10.5 mmol). This solution was added to a separate flask containing bis(cyclopentadienyl)zirconium chloride hydride (129 mg, 500 μmol) at 0 °C, and the mixture was stirred for 30 min on ice and then at room temperature for 4 d. Ether was added, and the mixture was washed with water, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (hexanes:EtOAc 90:10) to provide **5** (1.78 g, 63%) as a colorless oil: ^1H NMR ($CDCl_3$) δ 6.64 (dt, J = 18.0, 6.6 Hz, 1H), 5.53 (dt, J = 18.0, 1.5 Hz, 1H), 4.60 (t, J = 2.9 Hz, 1H), 3.84 (m, 2H), 3.50 (m, 2H), 2.47 (qd, J = 7.0, 1.5 Hz, 2H), 1.8−1.5 (m, 6H), 1.26 (s, 12H); MS (NH_3−CI) m/z 300 [$(M + NH_4)^+$, 20%], 102 [(2-hydroxytetrahydropyran)$^+$, 100%].

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4-Acetoxy-3-(4-cyanophenyl)-5-(2-hydroxyethyl)-4,5-dihydroisoxazole (7). A solution of **5** (1.65 g, 5.84 mmol) and 4′-cyano-1-chlorobenzaldoximine (**6**)[8] (1.06 g, 5.84 mmol) in THF (25 mL) was treated with sodium percarbonate (1.38 g, 8.76 mmol) and stirred for 48 h at room temperature. The mixture was filtered, and the solid was washed with EtOAc. The filtrate was concentrated, and the residue was partially purified by flash chromatography (toluene:EtOAc 85:15) to provide a gum (1.21 g, 65%). Without further purification, this material was dissolved in pyridine (10 mL) and treated with acetic anhydride (0.4 mL, 4.21 mmol). After 8 h at room temperature, the solution was poured into water and acidified to pH 4.5 by dropwise addition of concentrated HCl. The mixture was extracted with EtOAc. The extracts were washed with water, dried, and concentrated to provide a gum (1.30 g). Without purification, this material was dissolved in acetic acid (20 mL), THF (10 mL), and water (5 mL) and heated at 50−55 °C for 3 h. The cooled solution was diluted with DCM, washed with water and saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography ($CHCl_3$:IPA 97.5:2.5) to provide **7** (398 mg, 41%) and recovered starting material, which was resubjected to the reaction conditions and purified to provide additional **7** (282 mg, 70% total yield) as a white solid: mp 141−143 °C; ^{1}H NMR ($CDCl_3$) δ 7.81 (d, J = 8.8 Hz, 2H), 7.71 (d, J = 8.8 Hz, 2H), 6.30 (d, J = 2.5 Hz, 1H), 4.84 (td, J = 6.9, 2.5 Hz, 1H), 3.88 (q, J = 5.8 Hz, 2H), 2.12 (s, 3H), 1.98 (m, 3H); MS (NH_3−CI) m/z 215.1 [(M + H)$^+$, 100%]. Anal. Calcd for $C_{14}H_{14}N_2O_4$: C, 61.31; H, 5.14; N, 10.21. Found: C, 61.27; H, 5.05; N, 10.16.

4-Acetoxy-3-(4-cyanophenyl)-4,5-dihydroisoxazol-5-ylacetic Acid (8). A solution of **7** (500 mg, 1.82 mmol) in DMF (10 mL) was treated with PDC (2.74 g, 7.29 mmol) and stirred at room temperature. After 15 h, water was added and the mixture was extracted twice with ether. The aqueous phase was acidified to pH 2 with HCl and then extracted twice more with ether. The combined ether extracts were extracted four times with saturated aqueous $NaHCO_3$. The combined aqueous extracts were acidified to pH 2 with concentrated HCl to form a dense precipitate. This mixture was extracted with ether (three times), and these ether phases were dried and concentrated to provide **8** (397 mg, 76%) as a white solid: mp 165−167 °C; ^{1}H NMR ($CDCl_3$) δ 7.80 (d, J = 8.7 Hz, 2H), 7.72 (d, J = 8.7 Hz, 2H), 6.34 (d, J = 3.7 Hz, 1H), 4.94 (ddd, J = 6.9, 5.5, 3.6 Hz, 1H), 2.95 (dd, J = 16.9, 5.5 Hz, 1H), 2.92 (dd, J = 16.9, 7.0 Hz, 1H), 2.13 (s, 3H); HRMS (ES$^+$) m/z calcd for $C_{14}H_{13}N_2O_5$ [(M + H)$^+$] 289.0824, found 289.0829. Anal. Calcd for $C_{14}H_{12}N_2O_5$: C, 58.33; H, 4.20; N, 9.72. Found: C, 58.22; H, 4.21; N, 9.53.

***trans*-2-Butoxycarbonylamino-3-{2-[3-(4-cyanophenyl)-4-hydroxy-4,5-dihydroisoxazol-5-yl]acetylamino}propionic Acid Methyl Ester (10).** A mixture of **8** (500 mg, 1.81 mmol), 3-amino-*N*-butyloxycarbonyl-(*S*)-alanine methyl ester *p*-toluenesulfonate **9**[10] (709 mg, 1.81 mmol), DCC (373 mg, 1.81 mmol), 1-hydroxybenzotriazole hydrate (245 mg, 1.81 mmol), TEA (504 μL, 3.62 mmol), and DMF (5 mL) was stirred at room temperature overnight. The solvent was removed under vacuum, and the residue was taken up in EtOAc and filtered. The filtrate was washed with water, pH 4 buffer, and saturated aqueous $NaHCO_3$ and then was dried and concentrated. The residue was purified by flash chromatography (hexanes:EtOAc 70:30) to provide the acetate of **10** (725 mg, 84%) as an off-white solid: ^{1}H NMR (MeOH-d_4) δ 7.87 (d, J = 8.8 Hz, 2H), 7.78 (d, J = 8.8 Hz, 2H), 6.45 (m, 1H), 4.94 (m, 1H), 4.30 (bt, J = 5.4 Hz, 1H), 4.02 (m, 2H), 3.82 (m, 1H), 3.70 (s, 3H), 3.44 (dd, J = 13.6, 7.0 Hz, 1H), 2.68 (m, 2H), 2.06 (s, 3H), 1.57 (m, 2H), 1.37 (m, 2H), 0.92 (t, J = 6.6, 3H); MS (ES$^+$) m/z 489.3 [(M + H)$^+$, 2.5%], 425.4 (100%). This mixture of diastereomers was separated by preparative HPLC (CHIRALPAK AD column, 90:10 MeOH:water, 1.0 mL/min), resulting in hydrolysis of the acetate, to give peak 1, assigned structure **10b**: $[\alpha]^{25}_D$ = +198.2° (*c* 0.30, MeOH); ^{1}H NMR (MeOH-d_4) δ 7.97 (d, J = 8.2 Hz, 2H), 7.78 (d, J = 8.2 Hz, 2H), 5.35 (d, J = 3.3 Hz, 1H), 4.79 (m, 1H), 4.31 (bt, J = ca. 6 Hz, 1H), 4.02 (m, 2H), 3.71 (s, 3H), 3.60 (dd, J = 13.8, 5.4 Hz, 1H), 3.49 (dd, J = 13.8, 6.8 Hz, 1H), 2.52 (m, 2H), 1.56 (m, 2H), 1.38 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H). Peak 2 was assigned structure **10a**: $[\alpha]^{25}_D$ = −141.5° (*c* 0.30, MeOH); ^{1}H NMR (MeOH-d_4) δ 7.97 (d, J = 8.2 Hz, 2H), 7.78 (d, J = 8.2 Hz, 2H), 5.35 (d, J = 2.9 Hz, 1H), 4.80 (m, 1H), 4.33 (bt, J = ca. 6 Hz, 1H), 4.00 (m, 2H), 3.72 (s, 3H), 3.67 (dd, J = 13.6, 4.4 Hz, 1H), 3.41 (dd, J = 13.6, 7.5 Hz, 1H), 2.50 (m, 2H), 1.57 (m, 2H), 1.36 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H).

***trans*-2-Butoxycarbonylamino-3-{2-[3-(4-carbamimidoylphenyl)-4-hydroxy-4,5-dihydroisoxazol-5-yl]acetylamino}propionic Acid Methyl Ester (11a, 11b).** A solution of **10a** (100 mg, 210 μmol), H_2NOH·HCl (36 mg, 520 μmol), and TEA (72 μL, 520 μmol) in methanol (2 mL) was stirred overnight at room temperature. The mixture was concentrated, and the residue was dissolved in DCM, washed with water, dried (Na_2SO_4), and concentrated to provide a sticky solid (66 mg). This was dissolved in acetic acid (2 mL) and treated with acetic anhydride (16 μL, 170 mmol). After 90 min at room temperature, Pd on charcoal (5%, 3 mg) was added and the mixture was stirred overnight under an atmosphere of H_2. The catalyst was removed by filtration, and the residue was purified by preparative HPLC to provide **11a** (31 mg, 23%) as a white solid: ^{1}H NMR (MeOH-d_4) δ 8.03 (d, J = 8.8 Hz, 2H), 7.86 (d, J = 8.8 Hz, 2H), 5.41 (d, J = 3.3 Hz, 1H), 4.81 (m, 1H), 4.33 (bt, J = ca. 6 Hz, 1H), 4.00 (m, 2H), 3.72 (s, 3H), 3.66 (dd, J = 13.9, 4.8 Hz, 1H), 3.44 (dd, J = 13.9, 7.3 Hz, 1H), 2.54 (m, 2H), 1.57 (m, 2H), 1.36 (m, 2H), 0.90 (t, J = 7.3 Hz, 3H); MS (ES$^+$) m/z 464.3 [(M + H)$^+$, 100%]; $[\alpha]^{25}_D$ = −57.6° (*c* 0.30, MeOH).

Likewise, **10b** was converted into **11b** as a white solid: ^{1}H NMR (MeOH-d_4) δ 8.03 (d, J = 8.8 Hz, 2H), 7.85 (d, J = 8.8 Hz, 2H), 5.40 (d, J = 3.3 Hz, 1H), 4.80 (dt, J = 6.8, 3.3 Hz, 1H), 4.31 (bt, J = ca. 6 Hz, 1H), 4.02 (m, 2H), 3.72 (s, 3H), 3.60 (m, 1H), 3.48 (dd, J = 13.9, 6.8 Hz, 1H), 2.53 (m, 2H), 1.59 (m, 2H), 1.38 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H); MS (ES$^+$) m/z 464.3 [(M + H)$^+$, 100%]; $[\alpha]^{25}_D$ = +43.4° (*c* 0.30, MeOH).

***trans*-2-Butoxycarbonylamino-3-{2-[3-(4-carbamimidoylphenyl)-4-hydroxy-4,5-dihydroisoxazol-5-yl]acetylamino}propionic Acid, Trifluoroacetic Acid Salt (3a, 3b).** Compound **11a** (30 mg, 52 μmol) was combined with rabbit liver esterase (suspension in HEPES buffer; 0.3 mL, 80 units) in HEPES buffer (pH 7.1, 0.3 N; 3 mL) and allowed to stand at room temperature for 20 h. The mixture was filtered through a 10 kD cutoff membrane, and the filtrate was concentrated and purified by preparative HPLC to provide **3a** (30 mg, 95%) as an amorphous solid: HPLC t_R 10.49 min; ^{1}H NMR (MeOH-d_4) δ 8.03 (d, J = 8.8 Hz, 2H), 7.85 (d, J = 8.8 Hz, 2H), 5.41 (d, J = 3.3 Hz, 1H), 4.81 (m, 1H), 4.31 (m, 1H), 4.01 (m, 2H), 3.73 (m, 1H), 3.48 (m, 1H), 2.53 (m, 2H), 1.58 (m, 2H), 1.39 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H); HRMS (ES$^+$) m/z calcd for $C_{20}H_{28}N_5O_7$ [(M + H)$^+$] 450.1989, found 450.1974; $[\alpha]^{25}_D$ = −35.2° (*c* 0.14, MeOH). Anal. Calcd for $C_{20}H_{27}N_5O_7$·1.3TFA: C, 45.42; H, 4.77; N, 11.72. Found: C, 45.18; H, 4.92; N, 12.03.

Likewise, **11b** was converted into **3b** as an amorphous solid: HPLC t_R 10.17 min; ^{1}H NMR (MeOH-d_4) δ 8.04 (d, J = 8.8 Hz, 2H), 7.84 (d, J = 8.8 Hz, 2H), 5.42 (d, J = 3.3 Hz, 1H), 4.80 (m, 1H), 4.31 (m, 1H), 4.03 (m, 2H), 3.67 (dd, J = 13.6, 4.8 Hz, 1H), 3.45 (dd, J = 13.6, 7.7 Hz, 1H), 2.54 (m, 2H), 1.58 (m, 2H), 1.38 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H); $[\alpha]^{25}_D$ = +40.7° (*c* 0.20, MeOH).

***cis*-4-(3a,6a-Dihydrofuro[2,3-*d*]isoxazol-3-yl)benzonitrile (12).** A suspension of **6** (4.5 g, 25 mmol) in freshly distilled furan (1000 mL) was cooled to −25 °C under N_2 and treated with a solution of TEA (5 g, 50 mmol) in freshly distilled furan (100 mL) dropwise over 1 h. After 4 h of stirring at −25 °C and then 16 h at room temperature, the mixture was concentrated. Water was added, and the aqueous phase was extracted with EtOAc. The combined organic phases were filtered through Celite, washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated. Flash chromatography (hexanes:EtOAc 80:20) afforded **12** as a white solid: mp 129−131 °C (4.8 g, 91%); ^{1}H NMR (DMSO-d_6) δ 7.93 (s, 4H), 6.87 (d, J = 2.6 Hz, 1H), 6.54 (d, J = 8.8 Hz, 1H), 6.08 (dd, J = 8.8, 2.6 Hz, 1H), 5.43 (t, J = 2.6 Hz, 1H); HRMS (ES$^+$) m/z calcd. for $C_{12}H_8N_2O_2$ ([M + H]$^+$) 213.0664, found 213.0654. Anal. Calcd for $C_{12}H_8N_2O_2$: C, 67.92; H, 3.80; N, 13.20. Found: C, 67.80; H, 3.91; N, 12.90.

***cis*-4-(5-Hydroxy-3a,5,6,6a-tetrahydrofuro[2,3-*d*]isoxazol-3-yl)benzonitrile (13).** A solution of **12** (4.5 g, 21 mmol) and LiBr (4.5 g, 52 mmol) in MeCN (135 mL) was stirred at room temperature and treated with AG50W−X (2.7 g, H^+ form, dried) and then with water (5.4 g). After 1 h of stirring at room temperature, the mixture was filtered to remove the resin and concentrated to afford a white solid. The solid was extracted with IPA:$CHCl_3$ (1:3), and the organic phase was washed with

saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed, and the white solid was triturated with ether. The white solid was collected by filtration, washed with ether and dried to provide **13** as a mixture of diastereomers (ca. 7:3) (3.5 g, 72%): ^{1}H NMR ($CDCl_3$) δ 7.95 (m, 2H), 7.72 (m, 2H), 5.89 (d, J = 7.3 Hz, 0.3H), 5.81 (d, J = 7.3 Hz, 0.7H), 5.72 (d, J = 5.1 Hz, 0.7H), 5.65 (m, 0.3H), 5.47 (m, 0.3H), 5.36 (t, J = 7.0 Hz, 0.7H), 2.40 (m, 2H); HRMS (ES^+) m/z calcd for $C_{12}H_{10}N_2O_3$ ([M + H]$^+$) 230.0691, found 230.0691.

***cis*-4-(5-Oxo-3a,5,6,6a-tetrahydrofuro[2,3-*d*]isoxazol-3-yl)-benzonitrile (14).** A solution of **13** (2.8 g, 12 mmol) in acetone (140 mL) was stirred at 0 °C and treated with Jones reagent (56 mL) dropwise over 20 min. The orange solution was stirred at 0 °C for 30 min and then was treated with IPA (20 mL), and the green solid was removed by filtration. The filtrate was concentrated, and the residue was triturated with ether (50 mL). Filtration, washing with ether, and drying provided **14** as a white solid (2.4 g, 87%) which appeared somewhat unstable to storage: mp 204−206 °C; ^{1}H NMR (acetone-d_6) δ 8.01 (d, J = 8.5 Hz, 2H), 7.93 (d, J = 8.8 Hz, 2H), 6.46 (d, J = 7.0 Hz, 1H), 5.59 (t, J = 7.0 Hz, 1H), 3.31 (dd, J = 19.0, 7.0 Hz, 1H), 2.95 (d, J = 19.0 Hz, 1H); HRMS (ES^+) m/z calcd for $C_{12}H_8N_2O_3$ ([M + H]$^+$) 228.0535, found 230.0549. Anal. Calcd. for $C_{12}H_8N_2O_3$: C, 63.16; H, 3.53; N, 12.28. Found: C, 62.78; H, 3.66; N, 11.58. The enantiomers were separated by preparative HPLC (CHIRALPAK AD column, MeCN, 8.0 mL/min) to afford isomer **14c** with $[\alpha]^{25}_D$ = −166.1° (*c* 0.10, MeOH) and isomer **14d** with $[\alpha]^{25}_D$ = +162.6° (*c* 0.13, MeOH).

***cis*-2-Butoxycarbonylamino-3-{2-[3-(4-carbamimidoylphenyl)-4-hydroxy-4,5-dihydroisoxazol-5-yl]acetylamino}-propionic Acid, Trifluoroacetic Acid Salt (3c, 3d).** A solution of **14c** (208 mg, 1 mmol), **9** (390 mg, 1 mmol) and *N*,*N*-diisopropylethylamine (129 mg, 1 mmol) in MeCN (8 mL) was stirred at reflux for 24 h and cooled to room temperature. Concentration and flash chromatography (hexanes:EtOAc:ethanol 50:41:9) afforded **15c** as a white solid (300 mg, 67%): ^{1}H NMR (MeOH-d_4) δ 7.97 (d, J = 8.8 Hz, 2H), 7.78 (d, J = 8.4 Hz), 5.41 (d, J = 7.3 Hz, 1H), 4.69 (q, J = 7.0 Hz, 1H), 4.34 (t, J = 5.5 Hz, 1H), 4.03 (t, J = 6.4 Hz, 2H), 3.73 (s, 3H), 3.6 (m, 2H), 2.78 (t, J = 6.8 Hz, 2H), 1.59 (m, 2H), 1.38 (m, 2H), 0.92 (t, J = 7.33 Hz, 3H); HRMS (ES^+) m/z calcd for $C_{21}H_{26}N_4O_7$ ([M + H]$^+$) 228.0519, found 447.1881. Using the procedures given for the conversion of **11a** to **3a**, **15c** was converted to **3c** as an amorphous solid (50 mg, 23%): ^{1}H NMR (CD_3OD) δ 8.05 (d, J = 8.8 Hz, 2H), 7.87 (d, J = 8.8 Hz, 2H), 5.45 (d, J = 7.3 Hz, 1H), 4.72 (q, J = 7.0, 1H), 4.37 (dd, J = 7.7, 4.8 Hz, 0.7H), 4.29 (dd, J = 7.7, 4.8 Hz, 0.3H), 4.05 (m, 2H), 3.74 (dd, J = 13.9, 4.8 Hz, 0.7H), 3.66 (dd, J = 13.9, 4.8 Hz, 0.3H), 3.49 (dd, J = 13.9, 7.7 Hz, 0.7 H), 3.44 (dd, J = 13.9, 7.7 Hz, 0.3H), 2.83 (m, 2H), 1.60 (m, 2H), 1.39 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H); HRMS (ES^+) m/z calcd for $C_{20}H_{28}N_5O_7$ [(M + H)$^+$] 450.1989, found 450.1984; $[\alpha]^{25}_D$ = −61.8° (*c* 0.09, MeOH).

Likewise, **14d** was converted to **3d** as an amorphous solid: ^{1}H NMR (CD_3OD) δ 8.05 (d, J = 8.8 Hz, 2H), 7.86 (d, J = 8.8 Hz, 2H), 5.46 (d, J = 7.3 Hz, 1H), 4.73 (m, 1H), 4.38−4.25 (2m, 1H), 4.05 (m, 2H), 3.78−3.62 (2m, 1H), 3.48 (m, 1H), 2.83 (m, 2H), 1.61 (m, 2H), 1.40 (m, 2H), 0.93 (t, J = 7.3 Hz, 3H); HRMS (ES^+) m/z calcd for $C_{20}H_{28}N_5O_7$ [(M + H)$^+$] 450.1989, found 450.1997; $[\alpha]^{25}_D$ = +41.1° (*c* 0.12, MeOH).

***cis*-3-{2-[3-(4-Cyanophenyl)-4-hydroxy-4,5-dihydroisoxazol-5-yl]acetylamino}propionic Acid *tert*-Butyl Ester (16).** A solution of **14** (2.40 g, 20.5 mmol) in MeCN (100 mL) was treated with β-alanine *tert*-butyl ester HCl (2.20 g, 12.1 mmol) and Et_3N (1.30 g, 12.8 mmol). The mixture was heated at reflux for 24 h and cooled to room temperature. The mixture was concentrated and partitioned between water and EtOAc. An insoluble material was isolated by filtration and dried to provide **16** (1.60 g, 41%) as a white powder: ^{1}H NMR ($CDCl_3$) δ 8.02 (d, J = 8.1 Hz, 2H), 7.70 (d, J = 8.1 Hz, 2H), 6.61 (bs, 1H), 6.06 (bd, J = ca. 8 Hz, 1H), 5.52 (bt, J = ca. 8 Hz, 1H), 4.80 (bm, 1H), 3.50 (m, 2H), 2.94 (m, 2H), 2.46 (bt, J = 5.5 Hz, 2H), 1.47 (s, 9H); MS (ES^+) m/z 374.3 [(M + H)$^+$, 100%].

***cis*-3-{2-[3-(4-Cyanophenyl)-4-methoxy-4,5-dihydroisoxazol-5-yl]acetylamino}propionic Acid *tert*-Butyl Ester (17).** A solution of **16** (40 mg, 110 μmol) in DMF (1.0 mL) was treated with NaH (60% in mineral oil; 6.0 mg, 150 μmol) and stirred until gas evolution was no longer observed. Iodomethane (22 mg, 150 μmol) was added, and the mixture was stirred at room temperature for 2 h. The mixture was partitioned between EtOAc and water, and the organic phase was dried (Na_2SO_4) to provide a colorless oil. This was purified by preparative TLC to provide **17** as a colorless oil (30 mg, 77%): ^{1}H NMR ($CDCl_3$) δ 7.85 (d, J = 8.8 Hz, 2H), 7.72 (d, J = 8.8 Hz, 2H), 5.16 (d, J = 7.3 Hz, 0.33H), 5.15 (d, J = 7.3 Hz, 0.67H), 4.83 (m, 1H), 3.65 (m, 2H), 3.42 (s, 2H), 3.41 (s, 1H), 2.98 (m, 2H), 2.52 (m, 2H), 1.46 (s, 9H); MS (ES^+) m/z 388.4 [(M + H)$^+$, 100%]. Variable-temperature ^{1}H NMR experiments (400 MHz, DMSO-d_6) showed that the two doublets at δ 5.30 and 5.29 (ratio 1:2, J = 7.1 Hz) reversably coalesced at 60 °C, forming a sharp doublet at δ 5.27 (J = 7.1 Hz) at 90 °C, as did the two singlets at δ 3.33 amd 3.32 (ratio 2:1).

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Supporting Information Available: Characterization data (^{1}H NMR) of **3b**, **3c**, **3d**, **5**, **10a**, **10b**, **11a**, **11b**, **12**−**14**, **16** and **17**; HPLC analysis for **3a** and **3b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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