Identification of a Novel Oxazolidinone (U-100480) with Potent Antimycobacterial Activity

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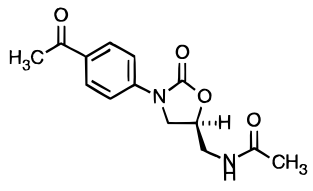
During the course of our investigations in the oxazolidinone antibacterial agent area, we have identified a subclass with especially potent *in vitro* activity against mycobacteria. The salient structural feature of these oxazolidinone analogues, **6** (U-100480), **7** (U-101603), and **8** (U-101244), is their appended thiomorpholine moiety. The rational design, synthesis, and evaluation of the *in vitro* antimycobacterial activity of these analogues is described. Potent activity against a screening strain of *Mycobacterium tuberculosis* was demonstrated by **6** and **7** (minimum inhibitory concentrations or MIC's ≤0.125 μg/mL). Oxazolidinones **6** and **8** exhibit MIC_{90} values of 0.50 μg/mL or less against a panel of organisms consisting of five drug-sensitive and five multidrug-resistant strains of *M. tuberculosis*, with **6** being the most active congener. Potent *in vitro* activity against other mycobacterial species was also demonstrated by **6**. For example, **6** exhibited excellent *in vitro* activity against multiple clinical isolates of *Mycobacterium avium* complex (MIC's = 0.5−4 μg/mL). Orally administered **6** displays *in vivo* efficacy against *M. tuberculosis* and *M. avium* similar to that of clinical comparators isoniazid and azithromycin, respectively. Consideration of these factors, along with a favorable pharmacokinetic and chronic toxicity profile in rats, suggests that **6** (U-100480) is a promising antimycobacterial agent.

Introduction

Mycobacteria are ubiquitous organisms that are becoming increasingly important pathogens. The resurgence of reported cases of tuberculosis, along with the recent emergence of multidrug-resistant strains of *Mycobacterium tuberculosis*, has refocused attention on this disease.[1] Perhaps the most important factor in the reemergence of tuberculosis is the human immunodeficiency virus (HIV) pandemic. Tuberculosis prevalence is high among patients infected with HIV.[1] Disseminated *Mycobacterium avium* complex infections are also closely associated with HIV-infected individuals.[2] Such infections contribute substantially to morbidity and mortality and are difficult to control with current treatment regimens. Therefore, an urgent need exists for the development of new antimycobacterial agents with a unique mechanism of action.

The oxazolidinones, exemplified by DuP 721 (**1**), are a relatively new class of orally active, totally synthetic antibacterial agents discovered by workers at DuPont.[3] Preliminary inquiries into the mechanism of action of the oxazolidinones have revealed that they are bacterial protein synthesis inhibitors, with inhibition uniquely occurring at an early event in the initiation phase of protein synthesis.[4] Their spectrum of activity encompasses anaerobic organisms and Gram-positive aerobic bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* (MRSE), as well as the enterococci.[5] DuP 721 has also been reported to exhibit good activity against *M. tuberculosis*.[6] However, in drug safety studies conducted at The Upjohn Co., it was shown that (±)-DuP 721 exhibited lethal toxicity in rats when dosed orally at 100 mg/kg b.i.d. for 30 days.[7] Therefore, the preparation of novel oxazolidinones with potent antimycobacterial activity, but without the liability of toxicity, remained a viable goal.



1

Intensive studies at The Upjohn Co. have resulted in novel and potent oxazolidinone antibacterial agents incorporating a substituted piperazine moiety (see generic structure **2**).[8] In contemplating alternative analogues of **2**, we were attracted to congeners containing various piperazine surrogates. One facet of this structure−activity relationship effort focused on thiomorpholine-derived compounds of generic structure **3** as potential targets. In this paper we describe the preparation of selected examples of **3** and report the remarkable *in vitro* activity manifested by these compounds against *M. tuberculosis*, the causative agent of tuberculosis, along with *M. avium* and other mycobacterial species.

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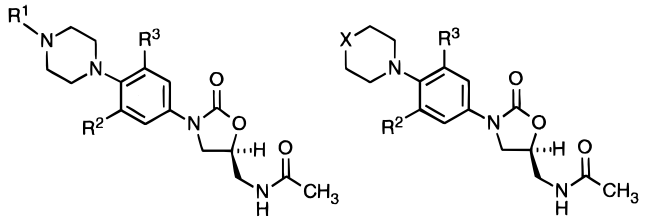


2 **3**

(R^1 = alkyl, acyl, etc.; R^2, R^3 = H, F) (X = S, SO, SO_2; R^2, R^3 = H, F)

Chemistry

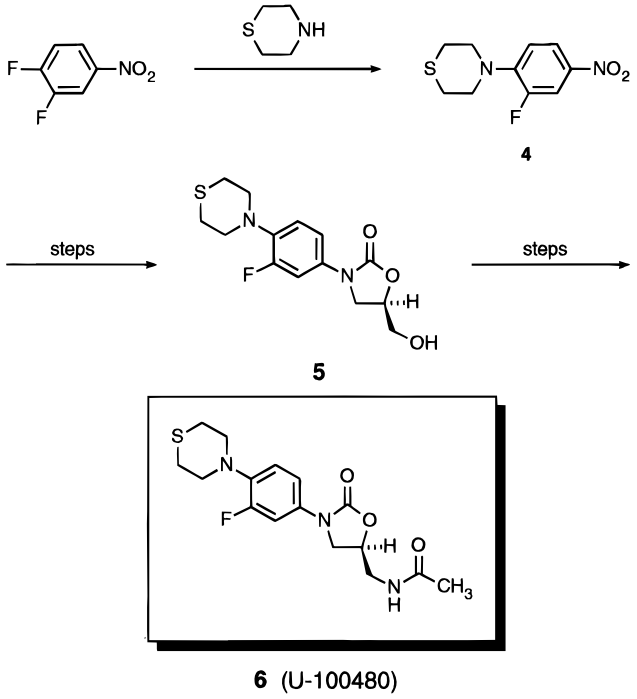
At the outset, our initial focus was on examples of **3** wherein at least one of the R^2 and R^3 groups was fluorine. The often remarkable potentiating effect of one or two fluorine atoms flanking the *p*-phenyl substituent has been described previously.[8−10] Much of the methodology used to prepare these compounds closely parallels that utilized for their piperazine- and morpholine-substituted progenitors (see Scheme 1).[11] To this end, thiomorpholine and 3,4-difluoronitrobenzene were reacted to generate the adduct **4**. Intermediate **4** was then converted in three steps to the (*R*)-5-(hydroxymethyl)-oxazolidinone **5** in 82% overall yield. Elaboration of the C-5 hydroxymethyl moiety of **5** to the corresponding acetamidomethyl subunit was accomplished as described in the preceding paper, with the exception that the intermediate C-5 azidomethyl group was reduced by a reaction sequence involving treatment with triphenylphosphine (THF) and then hydrolysis of the resultant intermediate iminophosphorane.[12] The product amine was generally not purified but rather acetylated and the acetamide purified by recrystallization or trituration to give the targeted oxazolidinone analogue **6** (U-100480) in 77−80% yield from **5**.

Thioether linkages are amenable to further elaboration. We wanted to prepare the sulfur oxidation products of **6** to probe the antibacterial activity of such derivatives and also to have potential metabolites available. The metabolic oxidation of sulfides to sulfoxides is a facile and generally reversible process. In contrast, the sulfone oxidation state appears to be a metabolic dead end.[13] The chemical oxidation of sulfides to sulfoxides and sulfones under a variety of conditions is well precedented.[14] As shown in Scheme 2, we were pleased to find that **6** could be readily oxidized to the corresponding sulfoxide **7** (U-101603) in 95% yield, employing sodium metaperiodate under conditions described by Leonard and Johnson.[15] The corresponding sulfone **8** (U-101244) was prepared in 85% isolated yield by treating **6** with catalytic osmium tetraoxide in the presence of *N*-methylmorpholine *N*-oxide.[16]

Results and Discussion

Oxazolidinones **6** and **7** were submitted for a preliminary evaluation of their *in vitro* activity against *M. tuberculosis*[17] (Table 1). As shown in the table, both analogues were exceptionally active against a screening strain of *M. tuberculosis*, being comparable to or more active than the clinical comparator isoniazid. Compounds **6** and **8** were tested against a panel of 10 *M. tuberculosis* isolates (Table 2). Test isolates included five "drug-sensitive" and five "drug-resistant" strains. The resistant isolates' phenotypes covered resistance to the common antitubercular drugs isoniazid, streptomycin, rifampin, ethambutol, and ethionamide (see Table 3 for antibiograms of resistant *M. tuberculosis* isolates). Oxazolidinone **6** exhibited a MIC range of 0.03−0.50 μg/

Scheme 1

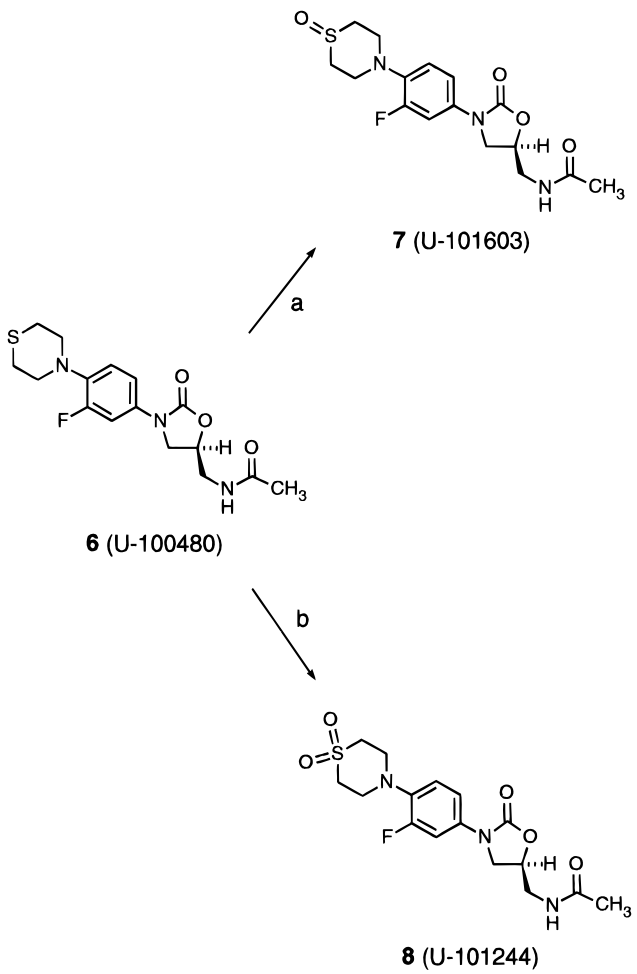


4

5

6 (U-100480)

Scheme 2[a]



7 (U-101603)

6 (U-100480)

8 (U-101244)

[a] Reagents: (a) $NaIO_4$, MeOH, H_2O; (b) catalytic OsO_4, NMO, acetone, H_2O.

Table 1. *In Vitro* Activity of Selected Oxazolidinone Analogues against *M. tuberculosis* H37Rv

	MIC (μg/mL)[a]		
organism	**6**	**7**	isoniazid
M. tuberculosis	≤0.125	≤0.125	0.2

[a] For minimum inhibitory concentration (MIC) determinations in Tables 1−3, the compounds were incorporated into 7H10 agar medium (Difco Laboratories, Detroit, MI) at concentrations of 2.0, 0.50, 0.125, and 0.03 μg/mL. The *M. tuberculosis* test organisms were grown in 7H9 medium (Difco) containing 0.05% Tween 80. After 7 days of incubation at 37 °C, the broths were adjusted to the turbidity of a 1.0 McFarland standard; the organisms were then diluted 10-fold in sterile water containing 0.10% Tween 80. The resultant bacterial suspensions were spotted onto the drug-supplemented 7H10 plates. After a 21 day cultivation at 37 °C, the growth of the organisms was scored. The MIC was defined as the lowest concentration of drug that completely inhibited growth of the organism.

Table 2. *In Vitro* Activity of Selected Oxazolidinone Analogues against Multiple Strains of *M. tuberculosis*

		no. of strains inhibited at concentration (μg/mL)			
compd	organism group[a]	0.03	0.125	0.50	2
6	S	1	2	2	
	R		3	2	
8	S		1	4	1
	R			4	

[a] S: group of five "drug-sensitive" isolates. R: group of five "drug-resistant" isolates.

mL and was the most active oxazolidinone tested. Interestingly, organisms resistant to numerous antitubercular agents were not cross-resistant with **6** and **8**.

Good *in vitro* activity was also observed for **6** against other mycobacterial species (Table 4). Generally, **6** was comparable to or significantly more active than the current clinical benchmark azithromycin. Analogue **6** exhibited potent *in vitro* activity against multiple isolates of *M. avium* complex, with a MIC_{50} of 2 μg/mL and a MIC_{90} of 4 μg/mL (Table 5).

Oxazolidinone **6** has been tested in a *M. tuberculosis* mouse infection model.[18] The degree of efficacy seen for orally administered **6** (mean cfu reduction in spleens and lungs of 3.1 and 4.9 log units, respectively) was comparable to that observed for the clinical standard isoniazid (mean cfu reduction in spleens and lungs of 3.3 and 4.7 log units, respectively), although **6** was dosed at a higher level (100 versus 25 mg/kg, administered orally once daily, 5 days/week for 4 weeks). In a comparative dose−response study in beige mice, **6** was found to have activity against *M. avium* complex similar to that of azithromycin (both drugs administered orally at a level of 100 mg/kg once daily, 5 days/week for 4 weeks).[19]

A preliminary evaluation of the pharmacokinetic behavior and metabolism of **6** in the rat was encouraging.[20] Compound **6** was well absorbed after oral administration, although significant first-pass metabolism to the sulfoxide **7** and, to a much lesser extent, the sulfone **8** was observed. In a gratifying result, the combined plasma concentrations of **6** and/or **7** were found to be quite high and persistent; both **7** and **8** exhibit *in vitro* activity against *M. tuberculosis* similar to that of **6**.

Orally administered **6** had an acceptable safety profile in rats when dosed at 50 mg/kg b.i.d. for 29 days.[7] This dosing regimen was very well tolerated, and drug-related findings were considered to be of minor toxicological relevance.

In summary, oxazolidinone **6** and its metabolites exhibit potent *in vitro* activity against *M. tuberculosis*; strains resistant to conventional antituberculosis drugs were not cross-resistant to these compounds.[17] The *in vitro* activity of **6** extends to other mycobacterial species, including *M. avium* complex, an opportunistic pathogen associated with the acquired immune deficiency syndrome (AIDS). In general, oxazolidinone **6** was comparable to or significantly more active *in vitro* than the clinical comparator azithromycin against nontuberculosis species of mycobacteria. In mouse models, orally administered **6** has shown efficacy similar to that of isoniazid and azithromycin against *M. tuberculosis* and *M. avium*, respectively.[18,19] Oxazolidinone **6** exhibits favorable pharmacokinetics in rats after oral dosing[20] and was well tolerated in a chronic toxicity study.[7] Consideration of the above findings suggests that further studies probing the utility of **6** (U-100480) as a general antimycobacterial agent are warranted.

Experimental Section

General. For general experimental techniques utilized, see the preceding paper.[11]

4-(2-Fluoro-4-nitrophenyl)thiomorpholine (4). A solution of 3,4-difluoronitrobenzene (68.703 g, 47.81 mL, 0.432 mol) and *N*,*N*-diisopropylethylamine (83.724 g, 112.84 mL, 0.648 mol) in dry CH_3CN (900 mL) was treated with thiomorpholine (53.475 g, 0.518 mol) at ambient temperature and the reaction mixture heated to gentle reflux for 24 h. At this point, the reaction was determined to be complete by TLC (15% EtOAc/hexane, short wave UV). The reaction mixture was cooled to ambient temperature and concentrated by rotary evaporation to a dark orange-red liquid which was diluted with EtOAc and transferred to a separatory funnel. The organic layer was washed with 1 N HCl until the washings were acidic and then washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give 104.290 g (99%) of the title compound as an orange crystalline solid: mp 59.5−60.5 °C; IR (mull) 1604, 1494, 1334, 1325, 1255, 1229, 1193, 1078, 806 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.98 (ddd, 1H, J = 1.0, 2.6, 9.0 Hz), 7.91 (dd, 1H, J = 2.6, 12.9 Hz), 6.93 (t, 1H, J = 8.8 Hz), 3.60−3.56 (m, 4H), 2.82−2.79 (m, 4H); MS m/z (rel intensity) 242 (M^+, 92), 227 (16), 195 (30), 168 (100), 138 (19), 121 (16), 95 (13),

Table 3. Antibiograms of Resistant *M. tuberculosis* Isolates

		antibiotic and test concentration (μg/mL)							
CDC[a] isolate no.	organism group no.	SM[b] 2.0	SM 10.0	INH[c] 0.2	INH 1.0	INH 5.0	RIF[d] 1.0	THA[e] 10.0	EMB[f] 5.0
91-2218	R[g]	R	R	R	R	S[h]	S	R	S
91-2219	R	S	S	R	R	S	R	S	S
91-2225	R	R	R	R	R	R	R	S	S
91-2227	R	R	S	R	R	R	R	S	R
91-2230	R	R	R	R	R	S	R	S	S

[a] CDC: Centers for Disease Control. [b] SM: streptomycin. [c] INH: isoniazid. [d] RIF: rifampin. [e] THA: ethionamide. [f] EMB: ethambutol. [g] R: resistant. [h] S: susceptible.

Table 4. *In Vitro* Activity of Oxazolidinone **6** (U-100480) against Selected Mycobacteria

organism	MIC (μg/mL)[a]	
	6	azithromycin
M. avium complex (MAC) ATCC 49601[b]	4	4
MAC 101[c]	0.5	4
M. simiae	2	4
M. xenopi	2	1
M. malmoense	2	16
M. fortuitum	8	>64

[a] The minimum inhibitory concentrations (MICs) in Tables 4 and 5 were determined by broth dilution method using 7H11 broth, pH 6.6, supplemented with 10% oleic acid−albumin−dextrose−catalase enrichment (Difco Laboratories, Detroit, MI). [b] MAC ATCC 49601 was obtained as a clinical isolate from a patient with AIDS at the State University of New York Health Science Center, Syracuse. [c] MAC 101 (serotype 1) was kindly provided by Lowell S. Young, Kuzell Institute for Arthritis and Infectious Diseases, Pacific Presbyterian Medical Center, San Francisco, CA.

Table 5. *In Vitro* Activity of Oxazolidinone **6** (U-100480) against Multiple Clinical Isolates of *M. avium* Complex

species (no. of isolates)[a]	MIC (μg/mL)[b]		
	range	50%	90%
M. avium complex (20)	0.5−4	2	4

[a] MAC isolates 1408 and 3404 were kindly provided by Leonid B. Heifets, Mycobacteriology Clinical Reference Laboratory, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO. The remaining MAC isolates were obtained from patients with AIDS and disseminated MAC infections at the State University of New York Health Science Center, Syracuse, NY. [b] 50% and 90%, MICs at which 50% and 90% of the isolates are inhibited, respectively.

46 (17); HRMS calcd for $C_{10}H_{11}N_2O_2FS$ 242.0522, found 242.0525. Anal. ($C_{10}H_{11}N_2O_2FS$) C, H, N.

4-[2-Fluoro-4-[(benzyloxycarbonyl)amino]phenyl]-thiomorpholine. A solution of 4-(2-fluoro-4-nitrophenyl)-thiomorpholine (**4**; 1.89 g, 7.81 mmol) in 20% H_2O/THF (75 mL) was treated with W-2 Raney nickel (400 mg). The reaction mixture was thoroughly purged with N_2 and then shaken under 40 psi of H_2. After 2 h, the reduction was complete by TLC analysis (30% EtOAc/hexane). The pale yellow reaction solution was decanted from the catalyst, and the catalyst was rinsed several times with solvent. The combined washings were immediately placed under an atmosphere of N_2, cooled with an ice bath, and then treated with $NaHCO_3$ (2.62 g, 31.2 mmol, 4 equiv) followed by benzyl chloroformate (1.23 mL, 8.59 mmol, 1.10 equiv). After 0.5 h, the reaction was concentrated under reduced pressure to a dark tan solid. The crude material was dissolved in CH_2Cl_2, washed with H_2O and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to a dark tan solid. Purification by silica gel chromatography (100 g of silica gel, eluted with 5−9% EtOAc/hexane) gave 2.42 g (90%) of the title compound as a tan solid. Recrystallization of this lot from 30% EtOAc/hexane (three crops) gave 2.24 g of the title product as an off-white solid: mp 141−142 °C; IR (mull) 3308, 1696, 1535, 1529, 1423, 1310, 1257, 1200, 1066, 893, 744, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.42−7.30 (m, 5H), 6.96 (apparent d, J = 8.7 Hz, 1H), 6.93−6.87 (m, 1H), 6.90 (dd, J = 8.7 Hz, 1H), 6.59 (br s, 1H), 5.20 (s, 2H), 3.30−3.25 (m, 4H), 2.83−2.79 (m, 4H); MS m/z (rel intensity) 346 (M^+, 49), 211 (73), 183 (13), 91 (100); HRMS calcd for $C_{18}H_{19}N_2O_2FS$ 346.1151, found 346.1157. Anal. ($C_{18}H_{19}N_2O_2FS$) C, H, N.

***(R)*-[3-[3-Fluoro-4-(4-thiomorpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methanol (5).** A solution of 4-[2-fluoro-4-[(benzyloxycarbonyl)amino]phenyl]thiomorpholine (20.322 g, 58.66 mmol) in dry THF (250 mL) was cooled to −78 °C (dry ice/acetone bath) under N_2. *n*-Butyllithium (37.40 mL of a 1.6 M solution in hexanes, 59.83 mmol) was added to the reaction mixture over 10 min. The resultant light yellow solution was stirred at −78 °C for 30 min and then treated with (*R*)-(−)-glycidyl butyrate (8.626 g, 8.474 mL, 59.83 mmol) dropwise over 2 min. The reaction mixture was stirred for an additional 30 min at −78 °C, and then the cooling bath was removed. The reaction mixture was allowed to warm to ambient temperature overnight. Saturated aqueous NH_4Cl (50 mL) was added to the reaction mixture. The reaction mixture was transferred with EtOAc washings to a separatory funnel containing additional saturated aqueous NH_4Cl and extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated by rotary evaporation until a precipitate was noted. Hexane was added at this point, and the solids were filtered off (hexane wash of filter cake) and dried *in vacuo* to give 16.725 g (91%) of the title oxazolidinone as a white solid: mp 136−137 °C; $[\alpha]_D$ −50° (*c* 1.0, EtOAc); IR (mull) 1731, 1707, 1517, 1431, 1423, 1324, 1228, 1218, 1198, 1154, 1098, 804 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.43 (dd, 1H, J = 2.6, 14.0 Hz), 7.13 (dd, 1H, J = 1.6, 7.8 Hz), 6.95 (dd, 1H, J = 9.1, 9.1 Hz), 4.78−4.70 (m, 1H), 4.03−3.92 (m, 3H), 3.76 (dd, 1H, J = 3.9, 12.6 Hz), 3.31−3.28 (m, 4H), 2.83−2.80 (m, 4H); MS m/z (rel intensity) 312 (M^+, 100), 238 (83), 164 (12), 151 (20), 149 (18), 138 (11), 135 (10); HRMS calcd for $C_{14}H_{17}N_2O_3FS$ 312.0944, found 312.0942. Anal. ($C_{14}H_{17}N_2O_3FS$) C, H, N.

***(R)*-[3-[3-Fluoro-4-(4-thiomorpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl Methanesulfonate.** A solution of (*R*)-[3-[3-fluoro-4-(4-thiomorpholinyl)phenyl]-2-oxo-5-oxazolidinyl]-methanol (**5**; 9.530 g, 30.51 mmol) in dry CH_2Cl_2 (250 mL) was cooled with an ice bath to ca. 5 °C and treated with Et_3N (4.631 g, 6.38 mL) and methanesulfonyl chloride (3.669 g, 2.48 mL). After 2 h at this temperature, TLC analysis (10% MeOH/$CHCl_3$) revealed the reaction to be complete. The reaction mixture was transferred to a separatory funnel (CH_2Cl_2 wash). The organic layer was washed with H_2O, saturated aqueous $NaHCO_3$, and brine. The organic layer was then dried (Na_2SO_4), filtered, and concentrated *in vacuo* (no heating) to a white solid (11.780 g, 99%): mp 152−153 °C; [a]$_D$ −52° (*c* 0.98, $CHCl_3$); IR (mull) 1749, 1522, 1451, 1425, 1421, 1367, 1342, 1335, 1223, 1216, 1201, 1175, 990 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.42 (dd, J = 2.6, 13.9 Hz, 1H), 7.10 (ddd, J = 1.2, 2.6, 8.8 Hz, 1H), 6.96 (dd, J = 9.0, 9.0 Hz, 1H), 4.96−4.87 (m, 1H), 4.50 (dd, J = 3.7, 11.7 Hz, 1H), 4.42 (dd, J = 4.2, 11.7 Hz, 1H), 4.12 (dd, J = 9.1, 9.1 Hz, 1H), 3.92 (dd, J = 6.1, 9.1 Hz, 1H), 3.33−3.27 (m, 4H), 3.10 (s, 3H), 2.84−2.79 (m, 4H); MS m/z (rel intensity) 390 (M^+, 100), 375 (1), 343 (5), 316 (64); HRMS calcd for $C_{15}H_{19}FN_2O_5S_2$ 390.0719, found 390.0721. Anal. ($C_{15}H_{19}FN_2O_5S_2$) C, H, N.

***(R)*-[3-[3-Fluoro-4-(4-thiomorpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl *p*-Toluenesulfonate.** A solution of (*R*)-[3-[3-fluoro-4-(4-thiomorpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methanol (**5**; 1.47 g, 4.69 mmol) in pyridine (15 mL) was cooled to 0 °C (ice bath). *p*-Toluenesulfonyl chloride (1.34 g, 7.04 mmol) was added and the solution allowed to stir at 0 °C under N_2 for 7 h. At this point, the reaction mixture was stoppered and stored in a freezer overnight (16 h). In the morning, the reaction mixture showed only a trace of starting material by TLC (5% MeOH/$CHCl_3$, short wave UV). The product was precipitated by quenching the reaction with ice water (100 mL). The solid was isolated by suction filtration and washed thoroughly with water. The title compound, 2.02 g (92%), was recovered as a white solid: mp 141 °C; $[\alpha]_D$ −95° (*c* 1.00, EtOAc); IR (mull) 1746, 1517, 1447, 1416, 1370, 1360, 1226, 1216, 1193, 1176, 1080, 972, 873 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, 2H, J = 8.3 Hz), 7.42−7.33 (obscured dd, 1H), 7.36 (d, 2H, J = 8.5 Hz), 7.06 (dd, 1H, J = 2.5, 8.8 Hz), 6.98 (dd, 1H, J = 8.8, 8.8 Hz), 4.87−4.78 (m, 1H), 4.27 (dd, 1H, J = 4.0, 11.5 Hz), 4.22 (dd, 1H, J = 4.6, 11.5 Hz), 4.06 (dd, 1H, J = 9.1, 9.1 Hz), 3.86 (dd, 1H, J = 5.9, 9.1 Hz), 3.34−3.28 (m, 4H), 2.87−2.80 (m, 4H), 2.46 (s, 3H); MS m/z (rel intensity) 466 (M^+, 100), 392 (44), 176 (16), 164 (15), 150 (14), 148 (16), 91 (34); HRMS calcd for $C_{21}H_{23}N_2O_5FS_2$ 466.1032, found 466.1036. Anal. ($C_{21}H_{23}N_2O_5FS_2$) C, H, N.

***(R)*-[3-[3-Fluoro-4-(4-thiomorpholinyl)pheny]-2-oxo-5-oxazolidinyl]methyl Azide. Procedure 1:** A solution of (*R*)-[3-[3-fluoro-4-(4-thiomorpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl *p*-toluenesulfonate (2.00 g, 4.29 mmol) in dry DMF (15 mL) was treated with solid NaN_3 (1.67 g, 25.7 mmol)

and heated to 65 °C (external temperature) for 6 h. The reaction mixture was then allowed to cool to ambient temperature, stirring over a weekend (158 h). At this point, the reaction was found to be complete by TLC (6% $CH_3CN/CHCl_3$, short wave UV). The reaction mixture was diluted with EtOAc (300 mL) and then washed with water (3 × 250 mL). The aqueous portions were back-extracted with more EtOAc (2 × 150 mL). The EtOAc portions were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give 1.447 g (100%) of the title compound as an off-white solid: mp 110−111 °C; $[\alpha]_D$ −122° (*c* 1.00, EtOAc); IR (mull) 2101, 1736, 1520, 1449, 1421, 1361, 1241, 1230, 1196, 1126, 1040, 973 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.42 (dd, 1H, J = 2.5, 14.0 Hz), 7.12 (ddd, 1H, J = 1.1, 2.6, 8.8 Hz), 6.96 (dd, 1H, J = 9.0, 9.0 Hz), 4.83−4.74 (m, 1H), 4.05 (dd, 1H, J = 8.9 Hz), 3.82 (dd, 1H, J = 6.2, 8.9 Hz), 3.71 (dd, 1H, J = 4.6, 13.2 Hz), 3.59 (dd, 1H, J = 4.4, 13.2 Hz), 3.32−3.27 (m, 4H), 2.84−2.79 (m, 4H); MS *m*/*z* (rel intensity) 337 (M^+, 100), 263 (8), 224 (6), 191 (77), 164 (20), 150 (40), 135 (12); HRMS calcd for $C_{14}H_{16}N_5O_5FS$ 337.1009, found 337.1009. Anal. ($C_{14}H_{16}N_5O_5FS$) C, H, N.

Procedure 2: A solution of (*R*)-[3-[3-fluoro-4-(4-thiomorpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl methanesulfonate (23.860 g, 61.11 mmol) in dry DMF (300 mL) under N_2 was treated with solid NaN_3 (19.867 g, 305.55 mmol) at ambient temperature. The stirred slurry was then heated to 65 °C for 5 h, at which time TLC analysis (5% MeOH/$CHCl_3$) revealed the reaction to be complete. After cooling to ambient temperature, the reaction mixture was transferred to a separatory funnel with H_2O (1000 mL) and EtOAc (500 mL). The mixture was extracted with EtOAc. The combined EtOAc extracts were thoroughly washed with H_2O and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give 19.786 g (96%) of the title azide as a pale yellow solid identical in all respects with the compound obtained from the corresponding tosylate.

(*S*)-*N*-[[3-[3-Fluoro-4-(4-thiomorpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamide (6, U-100480). A solution of (*R*)-[3-[3-fluoro-4-(4-thiomorpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl azide (19.662 g, 58.28 mmol) in dry THF (290 mL) was treated with triphenylphosphine (16.815 g, 64.11 mmol) over 10 min. After 2.0 h, TLC analysis (10% MeOH/$CHCl_3$) revealed the conversion to iminophosphorane was complete. H_2O (2.10 mL, 116.56 mmol) was added and the reaction mixture heated to 40 °C (internal temperature) for 5 h and then allowed to cool to ambient temperature overnight. At this point, TLC analysis (10% MeOH/$CHCl_3$) indicated incomplete hydrolysis of the iminophosphorane intermediate. More H_2O (8.40 mL) was added, and the reaction was heated to 40 °C for 5 h. At this time, TLC indicated complete conversion to the 5-(aminomethyl)oxazolidinone intermediate. The reaction mixture was first concentrated by rotary evaporation (benzene was added several times to azeotrope off the H_2O) and then under high vacuum to give the crude amine as an off-white solid. This material was dissolved in CH_2Cl_2 (250 mL), treated with pyridine (46.099 g, 47.10 mL, 582.79 mmol) and acetic anhydride (29.749 g, 27.49 mL, 291.40 mmol), and then stirred overnight at ambient temperature. TLC analysis (10% MeOH/$CHCl_3$) showed complete conversion to **6**. The reaction mixture was diluted with CH_2Cl_2, transferred to a separatory funnel, and then washed with 1 N HCl until the washings were acidic. The organic layer was then washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give crude **6** (U-100480) as a cream-colored solid. The crude product was triturated with hot $CHCl_3$; most but not all of the solids dissolved. After cooling to ambient temperature, the solids were filtered off (cold $CHCl_3$ wash) and dried *in vacuo* to furnish 13.174 g of analytically pure title compound as a white solid. A second crop of 3.478 g, also analytically pure, afforded a combined yield of 81%: mp 186.5−187.0 °C; $[\alpha]_D$ −8° (*c* 1.00, $CHCl_3$); IR (mull) 1749, 1746, 1641, 1656, 1518, 1448, 1419, 1225, 1215, 1158, 1106, 1083, 867 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.42 (dd, 1H, J = 2.6, 14.0 Hz), 7.06 (ddd, 1H, J = 1.0, 2.6, 8.8 Hz), 6.95 (dd, 1H, J = 9.0, 9.0 Hz), 6.61 (br t, 1H, J = 6.0 Hz), 4.81−4.72 (m, 1H), 4.02 (dd, 1H, J = 9.0, 9.0 Hz), 3.75 (dd, 1H, J = 6.7, 9.1 Hz), 3.71−3.55 (m, 2H), 3.32−3.27 (m, 4H), 2.84−2.79 (m, 4H), 2.02 (s, 3H); MS *m*/*z* (rel intensity) 353 (M^+, 100), 309 (31), 279 (5), 250 (17), 235 (14), 225 (20), 212 (7), 176 (19), 138 (18), 42 (28); HRMS calcd for $C_{16}H_{20}N_3O_3FS$ 353.1209, found 353.1200. Anal. ($C_{16}H_{20}N_3O_3FS$) C, H, N.

(*S*)-*N*-[[3-[3-Fluoro-4-(1-oxothiomorpholin-4-yl)phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamide (7). A solution of sodium metaperiodate (0.635 g, 2.97 mmol) in H_2O (7 mL) was cooled to 0 °C (ice bath). Solid (*S*)-*N*-[[3-[3-fluoro-4-(4-thiomorpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamide (**6**; 1.000 g, 2.83 mmol) was added and then MeOH (10 mL) to increase solubility. The reaction mixture was stirred at 0 °C for 3 h and then stoppered and placed in a refrigerator at 5 °C for several days. The reaction mixture was then filtered through a medium-porosity sintered glass funnel (copious $CHCl_3$ wash of the filter cake). The filtrate was transferred to a separatory funnel, and additional H_2O was added. The mixture was extracted with $CHCl_3$. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to give a foamy solid. Radial chromatography (silica gel, 400 μm thickness), eluting with $CHCl_3$, and then a gradient of 2−5% MeOH in $CHCl_3$ afforded, after concentration of appropriate fractions, 0.995 g (95%) of the title compound as a white solid: mp 159−161 °C; $[\alpha]_D$ −10° (*c* 0.68, $CHCl_3$); IR (mull) 3283, 1750, 1727, 1652, 1559, 1520, 1451, 1432, 1337, 1234, 1222, 1196, 1051, 752 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.50 (dd, 1H, J = 2.4, 14.0 Hz), 7.09 (dd, 1H, J = 2.4, 8.9 Hz), 7.05 (dd, 1H, J = 8.8, 8.8 Hz), 6.09 (br t, 1H), 4.82−4.74 (m, 1H), 4.03 (dd, 1H, J = 8.9, 8.9 Hz), 3.80−3.68 (m, 4H), 3.62 (dt, 1H, J = 6.0, 14.7 Hz), 3.27 (dt, 2H, J = 3.5, 13.3 Hz), 3.02−2.97 (m, 4H), 2.03 (s, 3H); MS *m*/*z* (rel intensity) 369 (M^+, 27), 325 (100), 308 (36), 306 (24), 262 (38), 234 (31), 165 (22), 151 (23); HRMS calcd for $C_{16}H_{20}N_3O_4FS$ 369.1158, found 369.1182. Karl-Fischer titration: 2.0% H_2O. Anal. ($C_{20}H_{16}N_3O_4FS \cdot 0.4H_2O$) C, H, N, S.

(*S*)-*N*-[[3-[3-Fluoro-4-(1,1-dioxothiomorpholin-4-yl)-phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamide (8). A slurry of (*S*)-*N*-[[3-[3-fluoro-4-(4-thiomorpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamide (**6**; 280 mg, 0.792 mmol) in 25% water/acetone solution (12 mL) was treated with solid 4-methylmorpholine *N*-oxide (278 mg, 2.38 mmol). Next, osmium tetraoxide (0.037 mL of a 2.5 wt % solution in 2-methyl-2-propanol, 15 mol %) was added dropwise. The reaction mixture was allowed to stir for 16 h under N_2 at room temperature. At this time, the reaction was determined to be complete by TLC (10% MeOH/$CHCl_3$, short wave UV). The reaction was quenched with saturated sodium bisulfite (15 mL) and the mixture extracted into CH_2Cl_2 (4 × 25 mL). The combined organic layers were then washed again with saturated sodium bisulfite (100 mL) and once with brine (100 mL). These aqueous portions were back-extracted with more CH_2Cl_2 (2 × 50 mL). All of the organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and then concentrated under reduced pressure to yield an off-white solid. This solid was adsorbed onto silica gel (2.5 g) and chromatographed on more silica gel (30 g, packed with 10% $CH_3CN/CHCl_3$), eluting with a gradient of 1−5% MeOH in 10% $CH_3CN/CHCl_3$, to give 224 mg (73%) of the title compound as a white solid: mp 202−203 °C; $[\alpha]_D$ −20° (*c* 1.04, DMSO); IR (mull) 1744, 1652, 1644, 1519, 1442, 1424, 1322, 1231, 1219, 1193, 1128, 1048, 809, 749 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.25 (br t, 1H, J = 5.5 Hz), 7.51 (dd, 1H, J = 2.3, 15.7 Hz), 7.22 (dd, 1H, J = 8.9, 8.9 Hz), 7.18 (dd, 1H, J = 2.4, 8.9 Hz), 4.76−4.66 (m, 1H), 4.09 (dd, 1H, J = 9.0, 9.0 Hz), 3.70 (dd, 1H, J = 6.4, 9.0 Hz), 3.48−3.43 (m, 4H), 3.40 ("t", 2H, J = 5.5 Hz), 3.29−3.24 (m, 4H), 1.83 (s, 3H); MS *m*/*z* (rel intensity) 385 (M^+, 74), 341 (87), 282 (32), 257 (100), 163 (27), 85 (80), 56 (45), 42 (46); HRMS calcd for $C_{16}H_{20}N_3O_5FS$ 385.1108, found 385.1102. Anal. ($C_{16}H_{20}N_3O_5FS \cdot 0.4H_2O$) C, H, N.

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