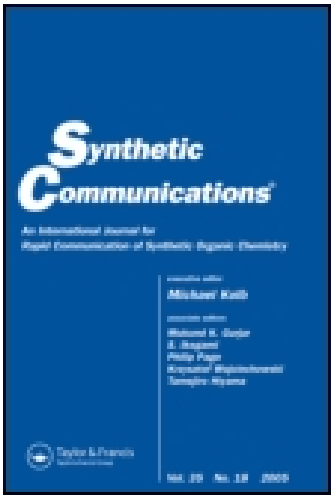

Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

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Published online: 10 Dec 2007.

To cite this article: Ashok K. Gangopadhyay , Gopal V. Gole , Ravindra D. Jadhav & Bansi Lal (2007) Novel Chiral Auxiliary for Attempted Resolution of Key Roxifiban Intermediate: A Simple Diastereoselective Coupling Approach for the Synthesis of Roxifiban, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 37:23, 4157-4171, DOI: 10.1080/00397910701575012

To link to this article: http://dx.doi.org/10.1080/00397910701575012

Synthetic Communications®, 37: 4157–4171, 2007

ISSN 0039-7911 print/1532-2432 online
DOI: 10.1080/00397910701575012

Novel Chiral Auxiliary for Attempted Resolution of Key Roxifiban Intermediate: A Simple Diastereoselective Coupling Approach for the Synthesis of Roxifiban

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Abstract: This article describes a simple method for the synthesis of roxifiban, a potent glycoprotein GP IIb-IIIa receptor antagonist, by a diastereoselective coupling approach to give >99.9% optical purity. We have also described an attempt to resolve the key synthetic intermediate by diastereomeric ester formation. Although we have been able to separate two diastereomeric esters, the removal of the chiral auxiliary led to partial racemization.

Keywords: diastereoselective coupling, 1,9-dideoxy forskolin, dynamic kinetic resolution, glycoprotein IIb-IIIa receptor, roxifiban

INTRODUCTION

Development of glycoprotein GP IIb-IIIa (GP IIb-IIIa) (integrin αIIb-β3) receptor antagonists has been the focus for antithrombotic research for the past several years.[1] Several orally active development candidates earlier failed in clinical trials, possibly due to poor to moderate bioavailability and/or short half life.[2] An orally active low-molecular-weight GP IIb-IIIa receptor antagonist roxifiban (**1**) was under clinical development with promising pharmacokinetic properties.[3] In this article, we describe the synthesis of roxifiban with >99.9% diastereomeric excess.

Received April 30, 2007
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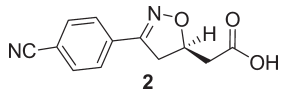


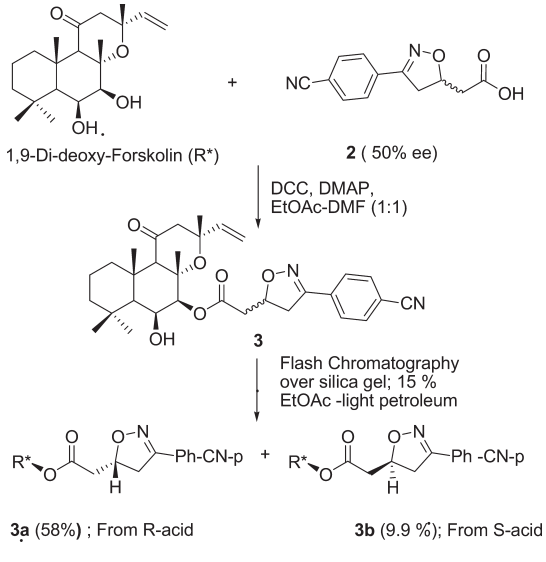
Figure 1. Key chiral intermediate for roxifiban sythesis.

RESULT AND DISCUSSION

We have followed essentially the synthetic protocol described by Xue et al.[4] However the major difficulty was the resolution of the key acid intermediate (**2**) (Fig. 1). These authors reported the resolution of (**2**) by using chinchonidine with 29% yield and 98% ee.[5] Enzymatic hydrolysis of butyl esters with lipase P30 gave 41.5% yield and 98% ee.[4] In our hands, we could not achieve more than 64% ee for resolution after three crystallizations and 42% ee for enzymatic hydrolysis.

We envisaged a new approach for resolution of acid **2**. Thus acid **2** with ee 50% was treated with 1,9-di-deioxy forskolin[6] in the presence of DCC-DMAP[7] to give the required **3a** in 58% yield and the undesired **3b** in 9.9% yield (Scheme 1). Pure **3a** was subjected to removal of the chiral auxiliary (Table 1). The best result was achieved by using iodosilane generated in situ to provide **2** with 66% ee.

While this study was under investigation, Pesti et al.[12] reported the synthesis of the R-acid **2** with 80.4% yield and >99.9% ee after crystallization by following enzymatic hydrolysis. This attracted our attention to the use of thioethyl ester of (±) acid **2**. Thus (±) acid **2** was reacted with ethanethiol



Scheme 1.

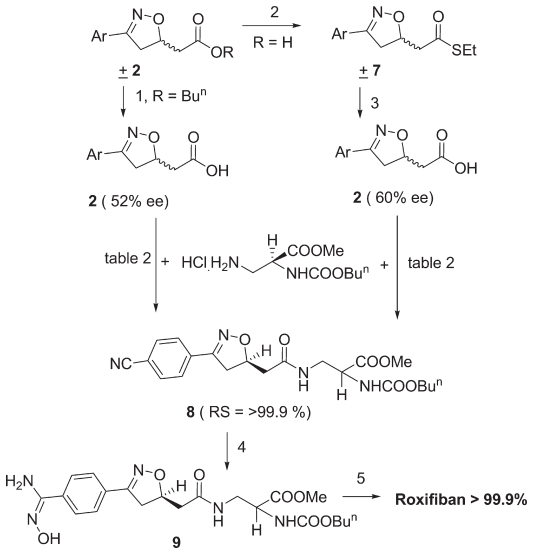
Table 1. Study of different reactions to **3a** to obtain enantiomerically pure **2**

Sr. no.	Reagent	Reaction condition	Yield (%)	$[\alpha]_D^{25}$ in degree	Ee excess (%)	Ref.
1	5% Aqueous. TFA	75–80°C, 1.5 h	56	−61.81	44	
2	6N HCl: dioxane (1:1)	R_t, 72 h	30	−65.22	47	
3	6N HCl: dioxane (1:1)	50°C, 16 h	18	−76.1	54	
4	Me_3SiCl, NaI, CH_3CN	Reflux, 3 h	60	−94.56	66	[8]
5	Me_3SiCl, NaI, Cu_2O, Mol. sieve 4A°, CH_3CN	Reflux, 2 h	60	−76	52	[8][a]
6	Me_3SiCl, NaI, Na_2SO_4, CH_3CN	Reflux, 2 h	30	−65.22	47	[8][a]
7	Me_3SiCl, NaI, $CaCO_3$, Na_2SO4, CH_3CN	Reflux, 48 h	—	—	—	[8][a]
8	$(nBu_3Sn)_2O$, AIBN, ether	9 h at rt 2 h, 50°C	—	—	—	[9]
9	$(nBu_3Sn)_2O$, dry toluene	Reflux, 72 h	12	−43.48	30	[10]
10	nBuSn (O) OH, H_2O, CH_3CN	Reflux, 16 h	—	—	—	[11]
11	nBuSn (O) OH, MeOH[b]	Reflux, 54 h	—	—	—	[11][a]
12	nBuSn (O) OH, MeOH[b]	100°C, 72 h	—	—	—	[11][a]

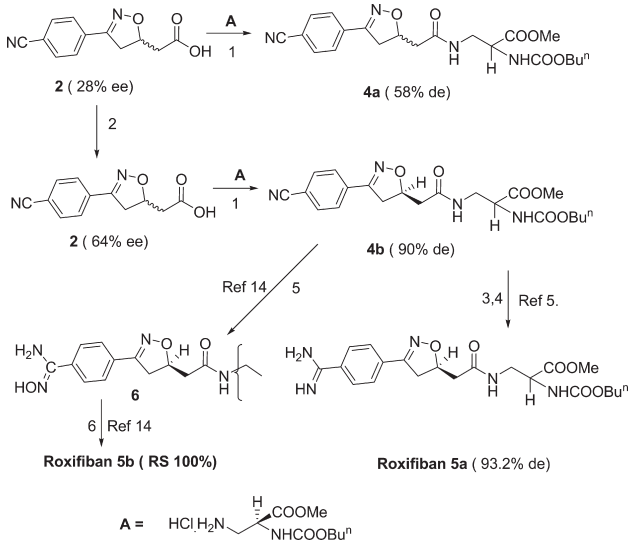
[a]Modification of reported procedure.
[b]Transesterification attempted with an idea to hydrolyze enzymatcally.

in presence of dicyclohexyl carbodiimide (DCC) and 4-NN-dimethyl-amino pyridine (DMAP) to give ester **7** in 91% yield (Scheme 2), which was then subjected to enzymatic hydrolysis according to the protocol described by Pesti et al. to give **2** with 80% yield and 60% ee.

While further studies on the optimization of enzymatic resolution were in progress, we thought of exploring the possibility of diastereoselective coupling of partially resolved acid **2** with S-amine-**A**. As a model experiment, acid **2** (ee 28%) was coupled with amine-**A** using mixed anhydride method to give **4a** in 75% yield as described in Scheme 3. The chiral high pressure liquid chromatography (HPLC) column showed the desired isomer (RS) was obtained in 58%



Scheme 2. 1) Hydrolysis by amano lipase P30;[4] 2) EtSH, DCC, DMAP, EtOAc; 3) amano lipase P30, NaH_2PO_4 buffer, Me_3N, triton X;[12] 4) $NH_2OH \cdot HCl$, MeOH; 5) Ac_2O, AcOH, H_2, 10% Pd-C.



Scheme 3. 1) NMM, Isobutyl chloroformate, Et_3N, DMF, $-20^{\circ}C$, 1 h, acid–amine = 1:1.1 eqv.; 2) Chinchonidine, repeated crystallization from acetone; 3) MeOH, dry HCl; 4) NH_4OAc; 5) $NH_2OH \cdot HCl$, MeOH; 6) Ac_2O, AcOH, H_2, 10% Pd-C.

Table 2. Reaction of acid with amine as described in Schemes 2 and 3 by using NMM, isobutyl chloroformate, and Et_3N in DMF

Sr. no.	Comp. no.	Starting R:S ratio of acid **2**	Ratio of acid and amine used	Yield (%)	Diastereomeric (de) excess (%)[a]
1[b]	**4a**	64:36	1:1	75	58
2[b]	**4b**	82:18	1:1.1	80	90
3[b]	**8**	82:18	1.2:1	66.4	>99.9
4[c]	**8**	76:24	1.25:1	73	>99.9
5[d]	**8**	77:23	1.25:1	70	>99.9

[a]The diastereomeric ratio was determined by chiral HPLC on chiralcel OD column, solvent system; hexane: EtOAc (90:10) + 0.1% diethyl amine, flow rate, 1 mL/min; $R_t = 28.64$ (RS); $R_t = 22.06$ (SS).

[b]0.3 g to <1 g scale.

[c]>1 g to 5 g scale.

[d]>15 g scale.

de. If we compare this value (58% de) with the starting acid (28% ee), it is clear that significant diastereomeric enrichment (~30%) had occurred. We started exploiting this finding to develop an alternative synthesis of roxifiban. We repeated the experiment with the acid **2** (64% ee) and coupling with 1.1 equivalent of amine to get **4b** in 79% yield with 90% de. Compound **4b** was converted into roxifiban **5a** by a reported method[5] to give a chiral purity of 93.2% de.

We designed a number of experiments, the results of which are summarized in Table 2. It is clear from the table depending upon the starting R:S ratio in **2** that it is essential to take excess of acid (the percentage of S acid present and additional 10% of acid) or, in other words, to get 100% chirally pure **8** for n mmol of amine, the requirement of acid (with X% R + Y% S) should be [1.1 n + n × Y/100] mmol. The rest of the process is the same as described by earlier authors.

We also observed that amide **4b** with 90% de was converted into oxime **6** in 58% yield with the same chiral ratio. The same material was hydrogenated over 5% Pd-C after treatment with Ac_2O in AcOH, as described earlier.[14] Roxifiban (**5b**) was obtained in 33% yield with >99.9% chiral purity.

Thus the final synthetic protocol was described in Scheme 2. The racemic acid **2** can be resolved partially by a chinchonidine method or better by dynamic kinetic resolution of thioethyl ester **7** followed by coupling with the amine (Table 1) to get optically pure **8**, which was converted to roxifiban as described earlier with chiral purity >99.9%.

CONCLUSION

We have been able to successfully separate the diastereomers obtained by the reaction of partially resolved **2** with 1,9-di-deoxy forskolin. However, the

removal of the chiral auxiliary led to partial racemization. From a trial experiment, we had identified the preference of R-acid **2** toward S-amine in mixed anhydride coupling procedure as used by us. It can be anticipated that other coupling reagent may have similar selectivity. This finding led us to discover a novel diastereoselective coupling approach that involve use of excess partially resolved acid to get very high optical purity of the desired intermediate. This intermediate was converted to roxifiban with >99.9% chiral purity. We have shown the reproducibility of the process by gradually scaling up the reaction up to 15-g batch. We are currently exploring the application of this method to synthesize other biologically active diastereomers.

EXPERIMENTAL

General Procedures

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. IR spectra were determined with a Perkin-Elmer 157 spectrophotometer as KBr film unless otherwise mentioned. ^{1}H NMR spectra were recorded in $CDCl_3$ unless otherwise mentioned on a Jeol FT-90 spectrometer or Bruker ACP 300 spectrometer with TMS as internal standard, and coupling constant values are expressed in hertz. Light petroleum refers to the fraction of bp 60–80°C. For flash-column chromatography, silica gel (finer than 0.08-mm particle size) was used. For reverse-phase medium pressure liquid chromatography (MPLC) purification, RP-18 (30-μ particle size) was used. Precoated (silica gel 60 F_{254}) thin-layer chromatography (TLC) plates were used for checking purity of compounds. UV light at λ 366 nm and 254 nm as well as spray reagents such as 0.2% ninhydrin in acetone, Draggendroff, and I_2 vapor was used to detect the spots on TLC. All compounds were homogeneous on TLC and gave proper spectral characteristics.

[(R)-3-(4-Cyano-phenyl)-4,5-dihydro-isoxazol-5-yl]-acetic acid 6-Hydroxy-3,4a,7,7,10a-pentamethyl-1-oxo-3-vinyl-dodecahydro-benzo[f]chromen-5-yl ester (3a) and [(S)-3-(4-Cyano-phenyl)-4,5-dihydro-isoxazol-5-yl]-acetic acid 6-hydroxy-3,4a,7,7,10a-pentamethyl-1-oxo-3-vinyl-dodecahydro-benzo[f]chromen-5-yl ester (3b)

In a round-bottomed flask fitted with a guard tube, a solution of 5,6-dihydroxy-3,4a,7,7,10a-pentamethyl-3-vinyl-dodecahydro-benzo[f]chromen-1-one (1,9-dideoxy forskolin) (3.45 g; 10.27 mmol) and DCC (1.86 g; 9.03 mmol) in EtOAc (40 mL) were chilled in an ice box to 0°C. To this, a solution of [3-(4-cyano-phenyl)-4,5-dihydro-isoxazole-5-yl]-acetic acid (52% ee) (1.7 g;

7.39 mmol) in EtOAc (10 mL) and DMF (5 mL) was added under vigorous stirring. After 10 min, DMAP (0.15 g; 1.25 mmol) was added. The reaction mixture was stirred for 1 h at room temperature followed by 16 h at room temperature. The reaction mixture was kept at -10°C for 2 h, and DCU was filtered off and washed with cold EtOAc (2×10 mL). The combined filtrate was washed with 10% $NaHCO_3$ (2×10 mL) followed by water (3×10 mL) and brine (5 mL). The EtOAc layer was dried over anhydrous Na_2SO_4. The solvent was removed in a rotary evaporator and purified by flash chromatography over silica gel with 10 to 20% EtOAc in light petroleum to give **3a** (2.35 g; 58%) and **3b** (0.4 g; 9.9%).

Data

3a: mp, 103°C; IR (KBr): 3528 (br), 2926 (br), 2229, 1744, 1719, 1399, 1190 cm^{-1}; mass: ESI^+, 549 $(M^+ + 1)$, ESI–, 547 $(M - 1)^{-1}$, EI, 548, 533, 515, 480, 465, 462 (100%), 391, 378, 360, 347 (100%), 336; ^{1}H NMR (300 MHz): 0.95 (s, 3H), 1.20 (m, 8H), 1.40 (m, 4H), 1.48 (s, 3H), 1.65–1.80 (m, 2H), 1.88 (br, 1H), 2.42 (m, 1H), 2.53 (d, 1H, $J_{gem} = 18$, 2-H^{β}), 2.59 (d, 1H, $J_{gem} = 18$, 2-H^{α}), 2.76 (s, 1H, 10b-H), 2.82 (dd, 1H, $J_{gem} = 15.3$, $J = 6.9$), 3.0 (dd, 1H, $J_{gem} = 15.3$, $J = 6.6$), 3.18 (dd, 1H, $J_{gem} = 16.8$, $J = 7.5$), 3.57 (dd, 1H, $J_{gem} = 16.8$, $J = 10.5$), 4.39 (br, 1H, 6-*H*), 5.07 (d, 1H, $J_{cis} = 10.8$, 2-H_{cis} vinylic), 5.10 (d, $J = 7.4$, 5-*H*), 5.12 (m, 1H, 5-*H*, dihydroisoxazol), 5.26 (d, 1H, $J_{trans} = 17.4$, 2-H_{trans} vinylic), 5.96 (dd, 1H, $J_{trans} = 17.4$, $J_{cis} = 10.8$, 1-*H* vinylic), 7.69 (d, 2H, $J = 8.4$, Ar*H*), 7.76 (d, 2H, $J = 8.4$, Ar*H*); ^{13}C NMR: 16.9, 18.4, 23.8, 24.2, 31.7, 33.1, 34.3, 37.7, 39.7, 40.13, 41.3, 43.6, 50.0, 55.1, 65.4, 69.5, 75.1, 78.2, 78.3, 82.1, 112.8, 113.7, 127.1, 132.5, 133.6, 146.3, 155.4, 169.1, 205.7; ^{13}C DEPT 135: CH_2: 18.4, 39.7, 40.1, 41.2, 43.6, 50.1, 112.9; CH & CH_3: 16.9, 23.8, 24.3, 31.7, 33.2, 55.1, 65.4, 69.55, 78.3, 82.1, 127.2, 132.6, 146.3.

3b: mp, 91–94°C; ^{1}H-NMR (300 MHz): mass: ESI^+, 549 $(M^+ + 1)$, ESI^-, 547 $(M - 1)^{-1}$, EI, 548, 533, 515, 480, 465, 462 (100%), 360, 336; 0.80 (s, 3H), 1.24 (s, 3H), 1.42 (s, 3H), 1.58 (s, 3H), 1.65–1.80 (m, 2H), 1.88 (br, 1H), 2.42 (m, 1H), 2.54 (d, 1H, $J_{gem} = 18$, 2-H^{β}), 2.68 (d, 1H, $J_{gem} = 18$, 2-H^{α}), 2.78 (s, 1H, 10b-H), 2.90 (m, 2H), 3.28 (dd, 1H, $J_{gem} = 16.8$, $J = 7.5$), 3.53 (dd, 1H, $J_{gem} = 16.8$, $J = 10.5$), 4.24 (br, 1H, 6-*H*), 5.07 (d, 1H, $J_{cis} = 10.8$, 2-H_{cis} vinylic), 5.10 (d, $J = 7.4$, 5-*H*), 5.12 (m, 1H, 5-*H*, dihydroisoxazol), 5.26 (d, 1H, $J_{trans} = 17.4$, 2-H_{trans} vinylic), 5.96 (dd, 1H, $J_{trans} = 17.4$, $J_{cis} = 10.8$, 1-*H* vinylic), 7.69 (d, 2H, $J = 8.4$, Ar*H*), 7.76 (d, 2H, $J = 8.4$, Ar*H*) (additional peaks appeared in the regions 0.8–2.0 and 2.4–2.5, which is due some DCU contamination).

Removal of Chiral Auxiliary from **3a** to Get **2** (Table 1)

1. A solution of **3a** (0.274 g; 0.5 mmol) in 5% aqueous TFA (2.5 mL) was heated at 70–80°C for 1.5 h. The starting material disappeared. The

solvent was removed. The residue was dissolved in 10% $NaHCO_3$ (5 mL) and extracted with ether (3 × 5 mL). The aqueous layer was acidified with 1N HCl to pH 2; the solid was filtered and dried. It was further purified by flash chromatography over silica gel with 2% MeOH in $CHCl_3$. The pure product **2** was crystallized from hot EtOAc–light petroleum to yield 0.063 g (55%). $[\alpha]_D^{25} = -61.81^\circ$ (C = 0.18 in $CHCl_3$) (44% ee, based on optical rotation).

2. A solution of **3a** (0.2 g; 0.37 mmol) was dissolved in dioxane (3 mL) and 6N HCl (3 mL). The resulting reaction mixture was stirred at room temperature for 72 h. The reaction mixture was poured on 10% $NaHCO_3$ (10 mL) and extracted with ether (3 × 5 mL). The aqueous layer was acidified with 1N HCl to pH 2. The solid was filtered and dried. It was crystallized from hot EtOAc–light petroleum to get a pure solid **2**, 0.025 g (30%); $[\alpha]_D^{25} = -65.22^\circ$ (C = 0.184 in $CHCl_3$) (46% ee, based on optical rotation).
3. A solution of **3a** (0.4 g; 0.74 mmol) was dissolved in dioxane (5 mL) and 6N HCl (5 mL). The resulting reaction mixture was heated at 50°C for 16 h. The reaction mixture was poured on 10% $NaHCO_3$ (10 mL) and extracted with ether (3 × 5 mL). The aqueous layer was acidified with 1N HCl to pH 2. The solid was filtered and dried. It was purified by flash chromatography over silica gel with 5% MeOH in $CHCl_3$ followed by flash chromatography over RP-18 with MeOH–water (1:1). It was crystallized from hot EtOAc–light petroleum to get a pure solid **2** (0.030 g; 18%); $[\alpha]_D^{25} = -76.1^\circ$ (C = 0.182 in $CHCl_3$) (54% ee; based on optical rotation).
4. A 25-mL, dry, round-bottomed flask fitted with a condenser with guard tube and dry N_2 inlet, **3a** (0.2 g; 0.36 mmol) was dissolved in dry CH_3CN (1 mL). To this solution, trimethylsilyl chloride (0.139 mL; 1.08 mmol) and NaI (0.165 g; 1.08 mmol) were added under stirring. The resulting reaction mixture was stirred under reflux for 3 h. It was diluted with water (10 mL) and extracted with EtOAc (3 × 5 mL). The organic layer was washed with water and brine. It was dried over anhydrous Na_2SO_4. The solvent was removed, and the residue was purified by flash chromatography with 5% MeOH in $CHCl_3$. The pure product was crystallized from hot EtOAc–light petroleum to give 0.50 g (60%) of **2**. $[\alpha]_D^{25} = -94.56^\circ$ (C = 0.188 in $CHCl_3$) (66% ee; based on optical rotation).
5. A 25-mL, dry, round-bottomed flask fitted with a condenser with guard tube and dry N_2 inlet, **3a** (0.2 g; 0.36 mmol) was dissolved in dry CH_3CN (1 mL). To this solution, trimethylsilyl chloride (0.139 mL; 1.08 mmol), NaI (0.165 g; 1.08 mmol), and Cu_2O (0.02 g; 0.14 mmol) and powdered 4 A° molecular sieve (0.05 g) were added under stirring. The resulting reaction mixture was stirred under reflux for 2 h. It was filtered and washed with EtOAc (3 × 7 mL). The organic layer was washed with water and brine. It was dried over anhydrous Na_2SO_4. The

solvent was removed, and the residue was purified by flash chromatography with 5% MeOH in $CHCl_3$. The pure product was crystallized from hot EtOAc–light petroleum to give 0.50 g (60%) of **1**. $[\alpha]_D^{25} = -76^\circ$ (C = 0.184 in $CHCl_3$); R:S = 77:23 (54% ee; based on optical rotation).

6. A 25-mL, two-necked, dry, round-bottomed flask fitted with a condenser with guard tube and dry N_2 inlet, **2a** (0.2 g; 0.36 mmol) was dissolved in dry CH_3CN (2 mL). To this solution, trimethylsilyl chloride (0.139 mL; 1.08 mmol), NaI (0.165 g; 1.08 mmol), and flame-dried powdered Na_2SO_4 (0.25 g) were added under stirring. The resulting reaction mixture was stirred under reflux for 2 h. It was filtered and washed with EtOAc (3 × 7 mL). The organic layer was washed with water and brine. It was dried over anhydrous Na_2SO_4. The solvent was removed, and the residue was purified by flash chromatography with 5% MeOH in $CHCl_3$. The pure product was crystallized from hot EtOAc–light petroleum to give 0.50 g (60%) of **1**. $[\alpha]_D^{25} = -65.22^\circ$ (C = 0.184 in $CHCl_3$) (based on optical rotation).
7. The reaction in step 6 was repeated with the addition of fused $CaCO_3$ (0.055 g; 0.547 mmol) and refluxed for 48 h without any formation of acid **2**.
8. A 25-mL, two-necked, dry, round-bottomed flask fitted with a condenser with guard tube and dry N_2 inlet was dried with a hot gun under dry N_2 atm. Compound **3a** (0.1 g; 0.18 mmol) was dissolved in ether (10 mL). To the solution, bis (tributyl-tin) oxide (0.22 g; 0.19 mmol) and AIBN (0.01 g) were added. The reaction mixture was stirred at room temperature for 16 h, followed by refluxing for 2 h. No hydrolysis to compound **2** was detected.
9. A 25-mL, two-necked, dry, round-bottomed flask fitted with a condenser with guard tube and dry N_2 inlet was dried with a hot gun under dry N_2 atm. Compound **3a** (0.2 g; 0.36 mmol) was dissolved in toluene (2.6 mL). To the solution, bis (tributyl-tin) oxide (0.44 g; 0.38 mmol) was added. The reaction mixture was refluxed for 72 h. The reaction mixture was cooled to room temperature and extracted with 10% $NaHCO_3$ (2 × 5 mL). The aqueous layer was acidified with citric acid, and the solid separated was extracted with EtOAc (3 × 4 mL). The EtOAc layer was washed with water (3 × 2 mL). It was dried over anhydrous Na_2SO_4. The solvent was removed, and the residue was crystallized from hot EtOAc and light petroleum to give 0.03 g (18%) of **2**. $[\alpha]_D^{25} = -43.48^\circ$ (C = 0.17 in $CHCl_3$) (30% ee; based on optical rotation).
10. A 25-mL, two-necked, dry, round-bottomed flask fitted with a condenser with guard tube and dry N_2 inlet, **3a** (0.2 g; 0.36 mmol) was dissolved in toluene (3 mL). To this solution, MeOH (0.2 mL; 2.3 mmol) and butylstannonic acid (0.019 g; 0.09 mmol) were added. The resulting reaction mixture was stirred under reflux for 24 h; there was a trace of nonpolar product formation. The product was not isolated.

11. A 25-mL, two-necked, dry, round-bottomed flask fitted with a condenser with guard tube and dry N_2 inlet, **3a** (0.2 g; 0.36 mmol) was dissolved in toluene (3 mL). To this solution, benzyl alcohol (0.23 mL; 2.3 mmol) and butylstannonic acid (0.019 g; 0.09 mmol) were added. The resulting reaction mixture was stirred under reflux for 24 h; there was a small amount of nonpolar product formation with lot of decomposed material. The product was not isolated.

(S)-2-Butoxycarbonylamino-3-{2-[3-(4-cyano-phenyl)-4,5-dihydro-isoxazol-5-yl]-acetylamino}-propionic acid methyl ester (4a)

A solution of **2** (28% ee; 0.301 g; 1.3 mmol) was dissolved in DMF (4 mL) and chilled to -20°C in a cold bath. To this solution, NMM (0.144 mL; 1.3 mmol) and isobutylchloroformate (0.175 mL; 1.3 mmol) were added successively under vigorous stirring. After 2 min, (S)-3-amino-2-butoxycarbonylamino-propionic acid methyl ester hydrochloride (0.33 g; 1.3 mmol) was dissolved in DMF (3 mL), neutralized with Et_3N (0.18 mL; 1.3 mmol), and added to the reaction mixture under stirring. The resulting reaction mixture was stirred at -20°C for 1 h and kept in the freezer at -5°C for 16 h. The solvent was removed under reduced pressure, and the residue was taken in EtOAc (15 mL). It was washed successively with 1N HCl (2×5 mL), water (3×3 mL), 1N $NaHCO_3$ (2×3 mL), and water (3×3 mL). The EtOAc layer was dried over anhydrous Na_2SO_4. The solvent was removed. The residue was purified by flash chromatography with 2% MeOH in $CHCl_3$ and finally crystallized from EtOAc to give 0.424 g (75%; 58% de) of **4a**; chiracel OD; solvent system: hexane–EtOH–diethyl amine = 10:90:0.1; flow rate 1.7 mL/min; R_t of RS = 28.64; R_t of SS = 22.06; mp 145–147°C; IR (KBr): 3314 (br), 2958, 2227 (CN), 1745, 1734, 1690, 1654, 1552 (br), 1437, 1275; ^{1}H NMR: 0.9 (9t, 3H, $J = 7.2$, CH_2CH_3), 1.35 (m, 2H, CH_2), 1.60 (m, 2H, CH_2), 2.56, 2.70 ($2 \times$ dd, 2H, $J_{gem} = 15.0$, $J = 6.0$ & 7.5, CH_2CONH), 3.21, 3.50 ($2 \times$ dd, 2H, $J_{gem} = 15.2$, $J = 7.3$ & 10, 4-CH_2), 3.65 (m, 2H, NHCH_2CH), 3.78 (s, 3H, OCH_3), 4.05 (t, 2H, $J = 7.4$, OCH_2CH$_2$), 4.42 (m, 1H, $C^{\alpha}H$), 5.15 (m, 1H, 6-H), 5.69 (br, 1H, NH), 6.35 (br, 1H, NH), 7.68, 7.76 ($2 \times$ d, 4H, J = 8.5, ArH).

1. The acid **2** (64% ee) was treated with (S)-3-amino-2-butoxycarbonylamino-propionic acid methyl ester hydrochloride in the ratio of 1:1.1 molar equivalent (2-g scale) by the same procedure as described for the synthesis of **4a**. The product **4b** was obtained in 79% yield (90% de); chiracel OD; solvent system: hexane–EtOH–diethyl amine = 10:90:0.1; flow rate 1.7 mL/min; R_t of RS = 28.64; R_t of SS = 22.06; mp 150–152°C; mass (ES^+): 431.56 ($M^+ + 1$), 458.56 ($M + Na^+$); IR (KBr): 3314 (br), 2958, 2227 (CN), 1745, 1734, 1690, 1654, 1552 (br), 1437, 1275; ^{1}H NMR: 0.9 (9t, 3H, $J = 7.2$, CH_2CH_3), 1.35 (m, 2H,

CH_2), 1.60 (m, 2H, CH_2), 2.56, 2.70 (2 × dd, 2H, $J_{gem} = 15.0$, $J = 6.0$ & 7.5, CH_2CONH), 3.21, 3.50 (2 × dd, 2H, $J_{gem} = 15.2$, $J = 7.3$ & 10, 4-CH_2), 3.65 (m, 2H, NHCH_2CH), 3.78 (s, 3H, OCH_3), 4.05 (t, 2H, $J = 7.4$, OCH_2CH$_2$), 4.42 (m, 1H, $C^{\alpha}H$), 5.15 (m, 1H, 6-*H*), 5.69 (br, 1H, *NH*), 6.35 (br, 1H, *NH*), 7.68, 7.76 (2 × d, 4H, $J = 8.5$, Ar*H*).

2. In a two-necked, round-bottomed flask fitted with a guard tube and passing tube, a solution of **4b** (1 g; 2.32 mmol) in dry MeOH (30 mL) was prepared. The solution was chilled to 0°C in an icebox, and dry HCl gas was passed through the solution for 1 h. The resulting solution was stirred at 0°C for 5 h. The solvent was removed under reduced pressure at 40°C in a rotary evaporator. The residue was dried in vacuum desiccators over KOH for 3 h. The crude material was dissolved in dry MeOH (15 mL), and dried ammonium acetate (0.89 g; 11.6 mmol) was added under stirring at room temperature. The reaction mixture was stirred for 16 h at room temperature. The solvent was removed, and the residue was purified by flash chromatography over RP-18 with 0.2% AcOH–MeOH = 70:30. The pure fractions were pulled together and evaporated to dryness under reduced pressure in a rotary evaporator. The residue thus obtained was crystallized from dry MeOH and ether to give 0.3 g (25.5%) roxifiban **5a**; mp 181–183°C; mass (ES^+): 448.67 ($M^+ + 1$); IR (KBr): 3339 (br), 2958, 2932, 1733, 1686 (br), 1649, 1539 (br), 1412, 1276, 1242; ^{1}H NMR (DMSO-D_6): 0.86 (t, 3H, $J = 7.2$, CH_2CH_3), 1.29 (m, 2H, $CH_2CH_2CH_3$), 1.48 (m, 2H, CH_2CH_3), 1.69 (s, 3H, CH_3COO), 2.99 (m, 2H), 3.22 (m, 2H), 3.55 (m, 2H), 3.61 (s, 3H, OCH_3), 3.92 (t, 2H, $J = 6.3$, OCH_2CH$_2$), 4.14 (br, 1H, $C^{\alpha}H$ of A_2Pr), 5.0 (m, 1H, 5-*H*), 7.5 (br, 1H, *NH*), 7.80, 7.84 (2 × d, 4H, $J = 8.4$, Ar*H*), 8.23 (br, 1H, NH); HPLC, $R_t = 15.18$ (3.4%), $R_t = 19.87$ (96.6%); chiral OD column; solvent system EtOH–hexane–TFA = 15:85:0.1, flow rate 1.2 mL/min.

(S)-2-Butoxycarbonylamino-3-(2-{3-[4-(N-hydroxycarbamimidoyl)-phenyl]-4,5-dihydro-isoxazol-5-yl}acetylamino)-propionic acid methyl ester (6)

In a 25-mL, round-bottomed flask fitted with a guard tube **4b** (0.121 g; 0.281 mmol) was dissolved in dry MeOH (5 mL). To the solution, $NH_2OH \cdot HCl$ (0.049 g; 0.701 mmol) was added, followed by Et_3N (0.098 mL; 0.701 mmol) at room temperature under vigorous stirring. The stirring was continued for 16 h. The solvent was removed. The crude thus obtained was purified by flash chromatography over silica gel with 5% MeOH in $CHCl_3$. Crystallization from hot EtOH–light petroleum gave 0.116 g (90%) of **6**; mp 194–196°C; mass (ES^+): 464.57 ($M^+ + 1$), 486.27 ($M + Na^+$); ^{1}H NMR (DMSO-D_6): 0.95 (t, 3H, $J = 7.3$, CH_2CH_3), 1.3 (m, 2H, CH_2CH_3), 1.53 (m, 2H, $CH_2CH_2CH_3$), 2.35, 2.55 (2 × m, 2H,

CH$_2$CONH), 3.15–3.35 (m, 2H, 4-*CH*$_2$), 3.55 (m, 2H, NH*CH*$_2$CH), 3.65 (s, 3H, O*CH*$_3$), 3.98 (t, 3H, $J = 7.1$, O*CH*$_2$CH$_2$), 4.20 (m, 1H, $C^{\alpha}H$), 4.95 (m, 1H, 5-*H*), 5.86 (s, 2H, *NH*$_2$), 7.50 (d, 1H, $J = 7.5$, *NH*COO), 7.67, 7.84 (2 × d, 4H, $J = 8.5$, Ar*H*), 8.18 (br, 1H, *NH*), 9.98 (s, 1H, NH*OH*).

The compound **6** (0.075 g; 0.162 mmol) was dissolved in glacial AcOH (10 mL) in a small hydrogenation bottle, and Ac_2O (0.017 mL; 0.178 mmol) was added. The reaction mixture was stirred for 1 h under a dry N_2 blanket. To the solution, 5% Pd-C was added and hydrogenated at 15 psi for 30 min. The catalyst was filtered off. The filtrate was evaporated under reduced pressure. The crude material was purified as described for the synthesis of **5a** to give 0.07 g (85%) Roxifiban **5b**; mp 200°C (d)–210°C; HPLC, $R_t = 19.89$ (>99.9%); chiral OD column; solvent system EtOH–hexane–TFA = 15:85:0.1, flow rate 1.2 mL/min.

[3-(4-Cyano-phenyl)-4,5-dihydro-isoxazol-5-yl]-thioacetic acid ethyl ester[13] (7)

To a solution of racemic [3-(4-cyano-phenyl)-4,5-dihydro-isoxazol-5-yl]-acetic acid (21.2 g; 92 mmol) and ethanethiol (10.2 mL; 137 mmol) in EtOAc (200 mL) and DMF (5 mL), a solution of DCC (20.86 g; 101 mmol) in EtOAc (50 mL) was added at 0°C. After 10 min, DMAP (1.59 g; 13 mmol) was added. The reaction mixture was stirred at 0°C for 30 min, and then the ice bath was removed. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was kept in the freezer at −5°C for 1 h. The DCU was filtered off. The filtrate was washed with water (2 × 50 mL). It was dried over anhydrous Na_2SO_4. Solvent was removed. The residue was purified by flash chromatography with 10–20% EtOAc in light petroleum. It was crystallized from EtOAc and light petroleum to give 23 g (91.2%); mp 92–94°C. All other physicochemical data agreed with reported values.

[3-(4-Cyano-phenyl)-4,5-dihydro-isoxazol-5-yl]-acetic acid (2)[12]

Finely powdered **7** (27.4 g; 100 mmol) was suspended in 0.65 N NaH_2PO_4 (406 mL), trimethyl amine (25 g in 100 mL water), Triton X 100 (2.3 g), and amino lipase PS 30 (2.3 g), and the mixture was stirred at 40°C. A solution of 6N NaOH (200 mL) was added at intervals such that the pH of the mixture was maintained at 8.9 to 9.5. The addition of NaOH solution was completed in 20 h. It was further stirred for 16 h (90% cv. checked by HPLC). The reaction mixture was filtered through a pad of Celite® and was extracted with dichloromethane (2 × 100 mL). The aqueous layer was acidified with 4N HCl to pH 2. The solid was filtered (20.7 g; 90%). The solid was recrystallized from EtOAc–light petroleum to give 18 g (79%) of

compound **2**; $[\alpha]_D^{25} = -85.1$ (60% ee). The rest of the physicochemical data matched with the reported compound.

Diastereoselective Synthesis of (S)-2-Butoxycarbonylamino-3-{2-[3-(4-cyano-phenyl)-4,5-dihydro-isoxazol-(R)-5-yl]-acetylamino}-propionic acid methyl ester (8)

1. Acid **2** (64% ee) and (S)-3-amino-2-butoxycarbonylamino-propionic acid methyl ester hydrochloride were reacted in a 1.2:1 ratio in a 1-g scale by following exactly the same procedure described for the synthesis of **4a** to give **8** in 66.4% yield. Mp 158–160°C; IR (KBr): 3314 (br), 2958, 2227 (CN), 1745, 1734, 1690, 1654, 1552 (br), 1437, 1275; HPLC >99.9% chiracel OD; solvent system: hexane–EtOH–diethyl amine = 10:90:0.1; flow rate 1.7 mL/min; R_t of RS = 29.7.
2. Acid **2** (R:S = 75:25) and (S)-3-amino-2-butoxycarbonylamino-propionic acid methyl ester hydrochloride were reacted in a 1.25:1 ratio in a 2-g scale by following exactly the same procedure described for the synthesis of **4a** to give **8** in 73% yield. Mp 158–160°C; HPLC >99.9% chiracel OD; solvent system: hexane–EtOH–diethyl amine = 10:90:0.1; flow rate 1.7 mL/min; R_t of RS = 28.54.
3. Acid **2** (R:S = 80:20) obtained from dynamic kinetic resolution of thioethyl ester **7** and (S)-3-amino-2-butoxycarbonylamino-propionic acid methyl ester hydrochloride were reacted in a 1.25:1 ratio in a 20-g scale by following exactly the same procedure described for the synthesis of **4a** to give **8** in 80% yield. Mp 159–160°C; HPLC >99.9% chiracel OD; solvent system: hexane–EtOH–diethyl amine = 10:90:0.1; flow rate 1.7 mL/min; R_t of RS = 28.34.

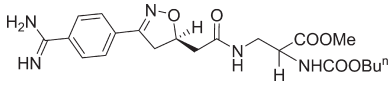
(S)-2-Butoxycarbonylamino-3-(2-{3-[4-(N-hydroxycarbamimidoyl)-phenyl]-4,5-dihydro-isoxazol-(R)-5-yl}acetylamino)-propionic acid methyl ester (9)

This compound was prepared from **8** following the same procedure as described for the synthesis of **6** in 88% yield using a batch size of more than 15 g. Mp 205–206°C; IR (KBr): 3496 (br), 3390, 3362, 2958, 1721, 1690, 1670, 1533 (br), 1347, 1277, 1218 cm^{-1}.

Roxifiban

The compound **8** was converted to roxifiban following the procedure described for the synthesis of **5b** in 89% yield using a batch size of more

than 10 g of **9**. Mp 200 (d)–210 (m), IR (KBr): 3339 (br), 2958, 2932, 1733, 1686 (br), 1649, 1539 (br), 1412, 1276, 1242; mass (ES^+): 448.58 ($M^+ + 1$); ^{1}H NMR (DMSO-D_6): 0.86 (t, 3H, $J = 7.2$, CH_2CH_3), 1.29 (m, 2H, $CH_2CH_2CH_3$), 1.48 (m, 2H, CH_2CH_3), 1.69 (s, 3H, CH_3COO), 2.99 (m, 2H), 3.22 (m, 2H), 3.55 (m, 2H), 3.61 (s, 3H, OCH_3), 3.92 (t, 2H, $J = 6.3$, OCH_2CH_2), 4.14 (br, 1H, $C^{\alpha}H$ of A_2Pr), 5.0 (m, 1H, 5-*H*), 7.5 (br, 1H, *NH*), 7.80, 7.84 (2 × d, 4H, $J = 8.4$, Ar*H*), 8.23 (br, 1H, NH); ^{13}C NMR: 14.1, 19.0, 25.1, 31.1, 41.1, 52.5, 54.1, 64.4, 78.9, 127.2, 128.5, 131.5, 133.9, 156.6 (2 × C), 165.7, 169.6, 171.6, 176.9; ^{13}C-135-DEPT (DMSO-D_6): 14.1, 19.0 (CH_2), 25.1, 31.1 (CH_2), 39.4 (CH_2), 40.2 (CH_2), 41.1 (CH_2), 52.5, 54.1, 64.4 (CH_2), 78.9, 127.2, 128.5. HPLC, $R_t = 23.93$ (>99.9%); chiral OD column; solvent system EtOH–hexane–TFA = 10:85:0.1, flow rate 1.2 mL/min.



ACKNOWLEDGMENTS

We gratefully acknowledge Swati A. Piramal for her interest and encouragement and Swati Bal-Tambe and her group for providing analytical data. Finally we acknowledge A. Ranade for providing useful literature input and fruitful discussion.

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