Potent, Orally Active GPIIb/IIIa Antagonists Containing a Nipecotic Acid Subunit. Structure−Activity Studies Leading to the Discovery of RWJ-53308

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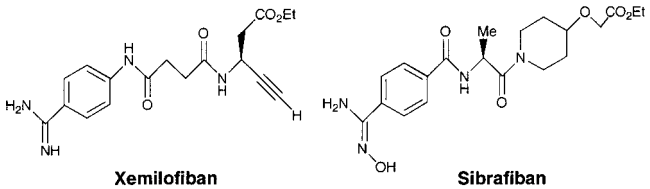
Although intravenously administered antiplatelet fibrinogen receptor (GPIIb/IIIa) antagonists have become established in the acute-care clinical setting for the prevention of thrombosis, orally administered drugs for chronic use are still under development. Herein, we present details from our exploration of structure-activity surrounding the prototype fibrinogen receptor antagonist RWJ-50042 (racemate of **1**), which was derived from a unique approach involving the γ-chain of fibrinogen (Hoekstra et al. *J. Med. Chem.* **1995**, *38*, 1582). Our analogue studies culminated in the discovery of RWJ-53308 (**2**), a potent, orally active GPIIb/IIIa antagonist. To progress from RWJ-50042 to a suitable candidate for clinical development, we conducted a series of optimization cycles that employed solid-phase parallel synthesis for the rapid, efficient preparation of nearly 250 analogues, which were assayed for fibrinogen receptor affinity and inhibition of platelet aggregation induced by four different activators. This strategy produced several promising analogues for advanced study, including 3-(3,4-methylenedioxybenzene)-β-amino acid analogue **3** (significant improved in vivo potency) and 3-(3-pyridyl)-β-amino acid **2** (significantly improved potency, oral absorption, and duration of action). In dogs, **2** displayed significant ex vivo antiplatelet activity on oral administration at 1.0 mg/kg, 16% systemic oral bioavailability, minimal metabolic transformation, and an excellent safety profile. Additionally, **2** was found to be efficacious in three in vivo thrombosis models: canine arteriovenous (AV) shunt (0.01−0.1 mg/kg, iv), guinea pig photoactivation-induced injury (0.3−3 mg/kg, iv), and guinea pig ferric chloride-induced injury (0.3−1 mg/kg, iv). On the basis of its noteworthy preclinical data, RWJ-53308 (**2**) was selected for clinical evaluation.

Introduction

The serious cardiovascular disorders of myocardial infarction and unstable angina are associated with the activation and subsequent aggregation of platelets. Adhesion of platelets to damaged blood vessel walls, or following rupture of atherosclerotic plaque, triggers a constellation of events that promote thrombus formation and arterial thrombosis. The final step in platelet aggregation, which is common to diverse activating agents such as ADP, collagen, and serotonin, involves the adhesive protein fibrinogen.[1] This large molecule binds to the activated, membrane-bound glycoprotein (GP) IIb/IIIa complex to cross-link platelets as part of a growing thrombus. Because of the central role played by this integrin, compounds that compete effectively with fibrinogen at its receptor can serve as potent antithrombotic agents.[2] Indeed, the prevention of thrombosis by such "fibrinogen receptor antagonists" has been clinically established and three intravenous drugs have reached the marketplace: the monoclonal antibody abciximab,[3] the cyclic peptide eptifibatide,[4] and the nonpeptide tirofiban.[5] Although these drugs are efficacious in an acute-care setting, it would also be desirable to have drugs for oral administration in a chronic regimen. Many fibrinogen receptor antagonists have been designed on the basis of the integrin tripeptide recognition motif Arg-Gly-Asp (RGD), which is present in the Aα-chain of fibrinogen. Since this has entailed nonpeptide molecular scaffolds bearing basic and acidic moieties to represent the Arg and Asp side chains, respectively, the resulting zwitterionic species have not been especially noted for oral bioavailability. This problem has led researchers to a focus on prodrug entities, such as xemilofiban[6] and sibrafiban,[7] which have reached advanced stages of clinical development.



Because the RGD sequence is present in a variety of adhesive proteins, we originally set out on an alternative approach involving the KQAGD sequence in the γ-chain of fibrinogen, which is known to be important in the binding of fibrinogen to GPIIb/IIIa.[8] By means of NMR studies on the C-terminal γ-chain peptide from positions 385−411, we ascertained the presence of a turn geometry in the key KQAGD domain (γ406−410).[9−13] We

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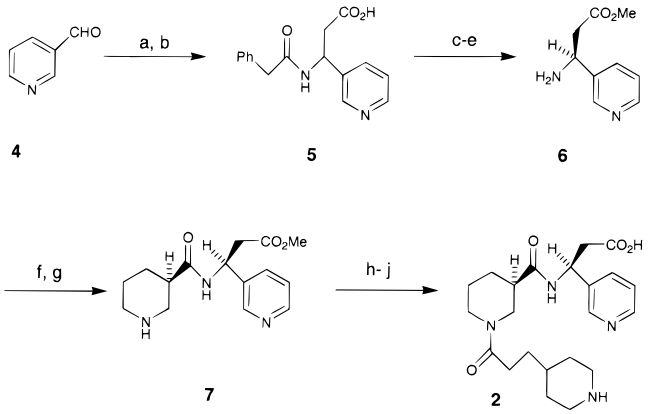
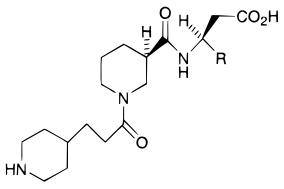
Scheme 1. Synthesis of **2**[a]



[a] (a) Malonic acid, NH_4OAc, EtOH, reflux. (b) $PhCH_2COCl$, Et_3N, aq acetone. (c) Penicillin amidase, pH 7.5. (d) 6 N HCl (aq). (e) 4 N HCl/1,4-dioxane/MeOH. (f) *N*-Boc-(*R*)-nipecotic acid, HBTU, HOBT, NMM, MeCN. (i) LiOH, aq THF. (j) 4 N HCl/1,4-dioxane.

then used nipecotic acid as a scaffold and appended the appropriate Lys and Asp moieties to arrive at a novel series of compounds, represented by **1** (*R*-isomer of RWJ-50042).[14] Although nipecotamide **1** exhibited oral activity, it had modest potency and a short duration of action in vivo (ex vivo inhibition of platelet aggregation following oral administration to dogs at 10 mg/kg). A well-focused optimization program that relied heavily on the rapid, solid-phase parallel synthesis of analogues[15] solved this problem quickly and delivered a potent clinical candidate, RWJ-53308 (**2**). Significantly,



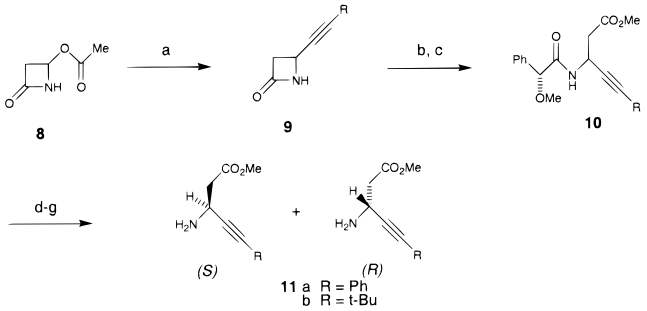
1 R = H
2 R = 3-pyridyl
3 R = 3,4-methylenedioxyphenyl

this compound can be dosed both intravenously and orally, has a good duration of action, and demonstrates excellent safety characteristics. Herein, we report details of the optimization process, solid-phase synthesis, structure−activity relationships, and enantioselective scale-up synthesis connected with our novel nipecotamide class of fibrinogen receptor antagonists.

Results and Discussion

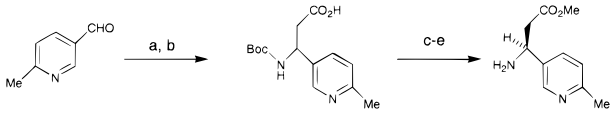
Synthesis of Analogues of RWJ-50042. Given the competitive environment surrounding the GPIIb/IIIa area of antithrombotic therapy,[2] we had pursued synthetic methodology to expedite the rapid preparation of analogues of **1**: namely, solid-supported parallel organic synthesis.[16,17] In our preliminary report, we used a synthetic procedure involving N-terminal attachment of the substrate to a 2-chlorotrityl resin and found that substitution on the β-amino acid carbon of **1** with an arene is important for potency improvement.[15] Compound **3** represented early success from the parallel synthesis arrays and sufficient quantities were then obtained by enantioselective synthesis.

Scheme 2. Synthesis of 3-Ethynyl-β-amino Esters[a]



[a] (a) R-CC-Li, THF, −78 °C. (b) 4 N HCl/1,4-dioxane/MeOH. (c) (*R*)-PhCH(OMe)COCl, NMM. (d) Silical gel chromatography. (e) Boc_2O, DMAP. (f) *N*,*N*,*N*′,*N*′-Tetramethylguanidine, MeOH. (g) 4 N HCl/1,4-dioxane.

Scheme 3. Ephedrine Salt Resolution of a β-Amino Acid[a]

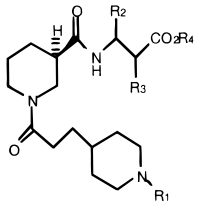


[a] Malonic acid, NH_4OAc, EtOH, reflux. (b) BOC_2-O, Et_3N, aq 1,4-dioxane. (c) (1*R*,2*S*)-ephedrine, EtOAc, crystallization. (d) 10% citric acid/$CHCl_3$. (e) 4 N HCl/1,4-dioxane/MeOH.

Analogues of **3** were synthesized from resolved β-amino esters (e.g., **6**) by the general route shown for pyridine **2** in Scheme 1. The secondary amide was formed by HBTU (2-[1*H*-benzotriazol-1-yl]-1,1,3,3-tetramethyluronium hexafluorophosphate) activation of *N*-Boc-(*R*)-nipecotic acid and addition of β-amino ester **6** at 5 °C; Boc removal with hydrochloric acid then gave secondary amine **7**. The tertiary amide was formed by coupling with HBTU-activated *N*-Boc-4-piperidinepropanoic acid and purified by flash column chromatography. Typically, the HBTU couplings were effected in 65−95% yields. The purified ester was saponified with lithium hydroxide and the Boc group was removed with hydrochloric acid to give the product for in vivo study.

Since the literature is replete with enantioselective syntheses of β-amino acids, suitable methods for any particular resolved β-amino ester (e.g., **6**) are available. Generally, we used a modified Knoevenagel condensation[18] of aldehyde **4** with malonic acid/ammonia to afford the racemic β-amino acid, then separated enantiomers by classical resolution of diastereomeric salts. Alternatively, asymmetric synthesis could be employed. For instance, the synthesis of the 3-(3,4-methylenedioxybenzene)-β-amino ester for **3** was accomplished in high diastereomeric excess by using an asymmetric Michael addition of lithiated α-methylbenzylamine to the requisite cinnamate.[15,19] For pyridyl-containing **6**, however, removal of the α-methylbenzyl chiral auxiliary group resulted in poor yields. A better method here was resolution of phenylacetamide **5** with penicillin amidase (Scheme 1).[20] 3-Ethynyl-β-amino esters (**11**) were prepared from 4-acetoxyazetidinone (**8**) as reported (Scheme 2).[6b] For large-scale preparation of resolved esters, ephedrine salt resolution of the *N*-Boc-β-amino acid (**13**) was employed, as exemplified in Scheme 3.[6b] For the preparation of antagonists **37**−**39**, the resolved *tert*-butyl β-amino esters were purchased from Oxford Asymmetry (Table 1).

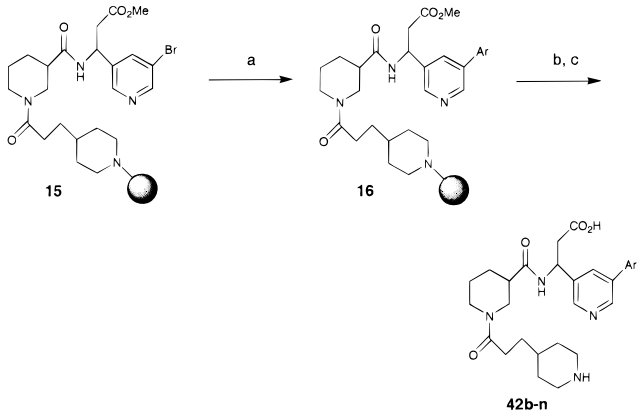
Table 1. Biological Data for Variant β-Amino Acid GPIIb/IIIa Antagonists



compd	R_1	R_2	R_3	R_4	human GFP[a] IC_{50} (μM)	Fg binding[b] IC_{50} (nM)	oral duration,[c] min (3 mg/kg)
1	H	H	H	H	0.27 ± 0.08	4.8 ± 2.0	120
2	H	(*S*)-3-pyridyl	H	H	0.06 ± 0.01	0.36 ± 0.27	360
3	H	(*S*)-3,4-methylenedioxy-Ph	H	H	0.02 ± 0.01	0.50 ± 0.20	180
27	Me	(*S*)-3,4-methylenedioxy-Ph	H	H	0.84 ± 0.67	0.24 ± 0.14	120[d]
28	H	(*S*)-3,4-methylenedioxy-Ph	H	Et	36 ± 4	48 ± 28	<30
29	H	(*R*)-C≡CPh	H	H	3.5 ± 0.5	0.29 ± 0.06	ND[e]
30	H	(*S*)-C≡CPh	H	H	0.08 ± 0.08	0.22 ± 0.13	<30[f]
31	H	(*R*)-C≡C-*t*-Bu	H	H	31 ± 18	5.6 ± 1.7	ND
32	H	(*S*)-C≡C-*t*-Bu	H	H	0.08 ± 0.02	42 ± 10	ND
33	H	(*S*)-3-quinolinyl	H	H	0.02 ± 0.03	0.18 ± 0.05	150[f]
34[g]	H	(*S*)-3-quinolinyl	H	H	1.2 ± 0.40	0.37 ± 0.11	ND
35	H	(*S*)-2-thienyl	H	H	0.09 ± 0.02	0.10 ± 0.03	ND
36	H	(*S*)-5-Cl-2-thienyl	H	H	0.04 ± 0.00	0.10 ± 0.03	ND
37	H	(*R*)-Me	H	H	8.0	13.5 ± 8.5	ND
38[h]	H	(*R*)-CH_2Ph	H	H	0.97 ± 0.33	0.37 ± 0.24	ND
39	H	(*R*)-CH_2Ph	(*S*)-OH	H	0.21 ± 0.01	17.9 ± 5.1	ND
40	H	H	(*R*,*S*)-OH	H	1.6 ± 0.35	3.5 ± 1.5	ND
41	H	(*S*)-3-Cl-5-CF_3-2-pyridyl	H	H	11.8 ± 2.7	66.8 ± 7.5	30
Xemilofiban (SC-54684)					0.30 ± 0.10	2.0 ± 1.0	>180

[a] Thrombin-induced gel-filtered platelet aggregation ($n = 2$). [b] Inhibition of biotinylated fibrinogen binding to immobilized GPIIb/IIIa ($n = 2$). [c] Oral duration of at least 50% inhibition of collagen-induced platelet aggregation (ex vivo). [d] 10 mg/kg oral dose. [e] No data. [f] 1 mg/kg oral dose. [g] (*S*)-Nipecotic acid. [h] Racemic nipecotic acid.

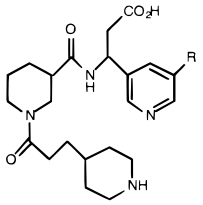
Scheme 4. Solid-Phase Synthesis of 5-Substituted Pyridines by Suzuki Cross-Coupling[a]



[a] (a) $ArB(OH)_2$, $(Ph_3P)_4Pd$, Na_2CO_3, DME. (b) KOTMS, THF. (c) TFA, CH_2Cl_2.

To elaborate further the structure−activity relationships around the pyridine ring of **2**, *N*-terminal 2-chlorotrityl resin attachment of 5-bromopyridine **15** provided a key Suzuki cross-coupling intermediate (Scheme 4). Boronic acids were cross-coupled with **15** using methodology reported by Ellman in good purity (>90%, Table 2).[21] Construction of **15** starting from the 2-chlorotrityl chloride resin and allyl 3-(4-piperidine)propanoate was carried out as reported.[15,22] Intermediate Suzuki cross-coupling products **16** were then saponified on the resin with potassium trimethylsilanolate in THF and cleaved from the resin with trifluoroacetic acid (TFA) to afford **42b**−**n**. Solution-phase Suzuki methodology was employed to prepare thiophene **46** from a 6-chloropyridine precursor (Table 3).[23] Enantioselective

Table 2. GPIIb/IIIa Binding Data for 5-Substituted Pyridines Isolated Following Resin-Bound Suzuki Couplings



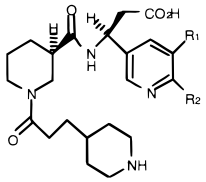
42

compd[a]	R	Fg binding[b] IC_{50} (nM)	FAB-MS *m/e* (MH^+)	yield (%)
a	Br	3.0 ± 0.6	495,497	36
b	3-thienyl	2.0 ± 0.0	499	41
c	2-benzofuranyl	36 ± 4	533	50
d	5-Cl-2-thienyl	57 ± 41	533	52
e	3-NH_2-Ph	3.6 ± 0.5	508	99
f	3-OMe-Ph	11 ± 5.6	523	49
g	4-OMe-Ph	11 ± 4.7	523	53
h	1-naphthyl	26 ± 13	543	50
i	2-naphthyl	57 ± 38	543	52
j	2-OMe-Ph	12 ± 1.8	523	49
k	3-NO_2-Ph	14 ± 7.9	538	43
l	Ph	1.8 ± 0.0	493	17
m	4-Me-Ph	24 ± 1.5	507	39
n	2-thienyl	1.5 ± 0.1	499	40
o	H	3.0 ± 0.0	417	55

[a] Compounds are racemic with respect to both stereogenic centers. [b] Inhibition of biotinylated fibrinogen binding to immobilized GPIIb/IIIa ($n = 2$).

follow-up syntheses of desirable 5-substituted pyridine intermediates **19a**−**c** were accomplished starting from 5-bromonicotinate **17** (Scheme 5). Palladium(0)-mediated alkyne coupling followed by lithium aluminum

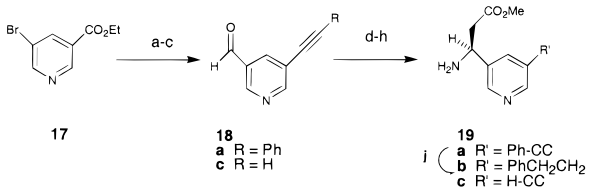
Table 3. Biological Data for Resolved Pyridyl GPIIb/IIIa Antagonists



compd	R_1	R_2	human GFP[a] IC_{50} (μM)	Fg binding[b] IC_{50} (nM)	oral duration,[c] min (3 mg/kg)
43	H	Me	0.03 ± 0.01	0.13 ± 0.04	210
44	Br	H	0.02 ± 0.00	0.21 ± 0.07	180
45	H	Cl	0.02 ± 0.00	0.14 ± 0.01	210
46	H	3-thienyl	0.20 ± 0.05	0.37 ± 0.07	150
47	Cl	Cl	0.22 ± 0.08	0.34 ± 0.11	150
48	Cl	OMe	0.090	3.8 ± 2.9	30
49	Ph	H	0.07 ± 0.01	0.52 ± 0.02	ND
50	PhC≡C	H	0.04 ± 0.00	0.85 ± 0.05	<30[d]
51	$PhCH_2CH_2$	H	0.03 ± 0.00	0.66 ± 0.22	180
52	HC≡C	H	0.03 ± 0.00	0.24 ± 0.06	ND

[a] Thrombin-induced gel-filtered platelet aggregation ($n = 2$). [b] Inhibition of biotinylated fibrinogen binding to immobilized GPIIb/IIIa ($n = 2$). [c] Oral duration of at least 50% inhibition of ADP-induced platelet aggregation (ex vivo). [d] 1 mg/kg oral dose.

Scheme 5. Synthesis of Resolved 3-(5-Alkynyl-3-pyridyl)-β-amino Esters[a]



[a] (a) Ph-CC-H or TMS-CC-H, $(Ph_3P)_2PdCl_2$, Et_3N, CuI, EtOAc. (b) $LiAlH_4$, THF. (c) PDC, CH_2Cl_2. (d) Malonic acid, NH_4OAc, EtOH, reflux. (e) Boc_2O, Et_3N, aq 1,4-dioxanel. (f) (1*R*,2*R*)-Ephedrine, EtOAc, crystallization. (g) 10% citric acid/$CHCl_3$. (h) 4 N HCl/1,4-dioxane/MeOH. (i) K_2CO_3, EtOH. (j) H_2, 10% Pd-C, MeOH.

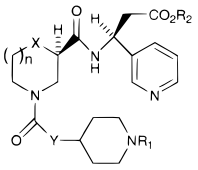
hydride reduction to the alcohol and oxidation of the alcohol gave the pyridine-3-carboxaldehydes **18**. Modified Knoevenagel condensation,[18] *N*-Boc protection, ephedrine salt resolution by crystallization, and protecting group manipulation afforded **19a**−**c** for use in syntheses exemplified by Scheme 1. Amidine **54** was prepared by acylation of secondary amine **53** with ethyl formimidate hydrochloride (Table 4).[24] For alkene **56**, intermediate *N*-Boc-3-(4-piperidine)prop-2-enoic acid (**21**) was prepared by Wittig reaction of ethyl 2-(triphenylphosphoranylidene)acetate with the aldehyde derivative of **20** (Scheme 6). Tris-protected piperazine scaffold **24** was prepared as a precursor to **57** starting from 2,3-diaminopropionate **22**, as reported (Scheme 7).[25] Racemic methyl pyrrolidine-3-carboxylate (**26**) was prepared in two steps from commercially available lactam **25** for target **58** (Scheme 8). Bispiperidine urea **59** was prepared by acylation of secondary amine **7** with 4-nitrophenyl chloroformate, amination with *N*-Boc-4,4′-bipiperidine, ester saponification, and HCl-mediated Boc removal (Scheme 9).

Biological Activity. Nipecotamide analogues of **1** with varied β-substituents on the 3-aminopropionic acid unit were evaluated in a binding assay involving biotinylated fibrinogen and GPIIb/IIIa, and a functional assay of human gel-filtered platelet aggregation (nipecotamides **2**, **3**, **27**−**41**, Table 1). Potent inhibition of both processes was desired. Relatively optimal potencies against human gel-filtered platelet aggregation were realized with bulky, *S*-configured β-aryl and β-heteroaryl groups such as 3,4-methylenedioxybenzene (**3**; IC_{50} = 20 nM), 3-quinoline (**33**; IC_{50} = 20 nM), and 5-chloro-2-thiophene (**36**; IC_{50} = 40 nM). Consistent with these results, **3**, **33**, and **36** showed remarkable receptor affinities (IC_{50} values of 0.50, 0.18, and 0.10 nM, respectively). The 3-pyridyl (**2**) and 2-thienyl (**35**) analogues also showed very respectable potency in platelet aggregation (IC_{50} values of 60 and 90 nM, respectively). The 2-alkynyl analogues (**30**, **32**) were quite potent against platelet aggregation (IC_{50} values of 80 nM), although **32** showed less potency in GPIIb/IIIa binding (IC_{50} = 42 nM).

The pharmacology of selected antagonists was studied in canine ex vivo platelet aggregation inhibition experiments (Table 1). Dogs ($n = 3$) were orally administered an aqueous solution of 3 mg/kg compound, and blood samples were drawn at 30-min intervals. The blood sample was treated with ADP (20 μM) to measure platelet aggregation. To assess the pharmacokinetics of the antagonists, dogs were dosed intravenously as well, at one-third or one-tenth of the oral dose (data shown for **2**). While the 3,4-methylenedioxybenzene analogue **3** produced at least 50% inhibition for a 180-min duration, its more hydrophobic *N*-methylpiperidine (**27**) and ethyl ester (**28**) analogues each inhibited aggregation at least 50% for only 120 min. Similarly, quinoline **33** exhibited a duration of action of 150 min. A pharmacokinetics study of **3** in dogs revealed an oral systemic availability of 14 ± 2% (AUC method), with intravenous and oral plasma half-lives of 21 ± 2 min and 108 ± 36 min, respectively (based on plasma levels of **3**). While **3** possessed adequate in vivo potency, its overall pharmacodynamic profile was too deficient to be considered for preclinical development.

The ex vivo results for pyridine analogue **2** were particularly interesting (Figure 1). Although it is less potent than **3** functionally in vitro, **2** provided a favorable duration of action of 360 min at 3 mg/kg and ca. 300 min at 1 mg/kg, indicative of improved oral absorption and/or binding to unactivated GPIIb/IIIa (Figure 1). Intravenous administration of **2** (0.3 mg/kg) produced a duration of action of 300 min, as well. Overall, **2**

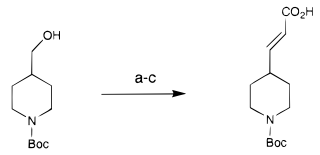
Table 4. Biological Data for Structurally Diverse Pyridyl GPIIb/IIIa Antagonists



compd	X	Y	*n*	R_1	R_2	human GFP[a] IC_{50} (μM)	Fg binding[b] IC_{50} (nM)	oral duration,[c] min (3 mg/kg)
53	CH_2	CH_2CH_2	1	H	Me	41	7.2 ± 4.8	60
54	CH_2	CH_2CH_2	1	CH=NH	H	0.35 ± 0.05	0.75 ± 0.54	90
55	CH_2	CH_2CH_2	1	H	$(CH_2)_2$-*c*-C_6H_{11}	2.2 ± 1.0	3.8 ± 0.2	120
56	CH_2	(*E*)-CH=CH	1	H	H	0.16 ± 0.04	0.81 ± 0.15	210
57	NH	CH_2CH_2	1	H	H	0.60 ± 0.24	1.7 ± 0.3	ND
58[d]	CH_2	(*E*)-CH=CH	0	H	H	0.14 ± 0.07	0.58 ± 0.03	360
59	CH_2	4-piperidine	1	H	H	0.45	0.65 ± 0.55	180

[a] Thrombin-induced gel-filtered platelet aggregation ($n = 2$). [b] Inhibition of biotinylated fibrinogen binding to immobilized GPIIb/IIIa ($n = 2$). [c] Oral duration of at least 50% inhibition of ADP-induced platelet aggregation (ex vivo). [d] Racemic pyrrolidine-3-carboxylic acid.

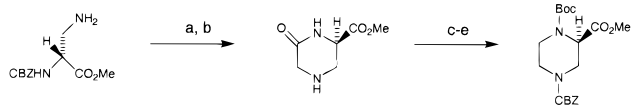
Scheme 6. Synthesis of the Intermediate *N*-Boc-3-(4-piperidine)prop-2-enoic Acid[a]



20 **21**

[a] (a) Oxalyl-Cl, Et_3N, DMSO. (b) Ph_3PCHCO_2Et, THF. (c) 1 N NaOH.

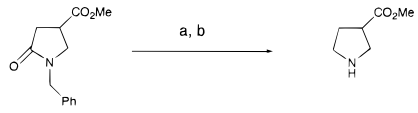
Scheme 7. Synthesis of Bis-Protected Methyl Piperazine Carboxylate Scaffold[a]



22 **23** **24**

[a] (a) $BrCH_2CO_2Me$, THF. (b) H_2, Pd-C, EtOH. (c) CBZ-Cl, iPr_2NEt, DCM. (d) BH_3, THF. (e) Boc_2O, iPr_2NEt, $CHCl_3$.

Scheme 8. Synthesis of Methyl Pyrrolidine-3-carboxylate[a]



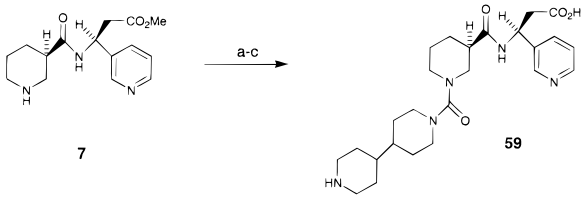
25 **26**

[a] (a) BH_3, THF. (b) H_2, Pd-C, MeOH.

exhibited a favorable pharmacokinetic and pharmacodynamic profile with an oral systemic availability of 16 ± 8% (AUC method), and intravenous and oral plasma half-lives of 85 ± 24 min and 114 ± 15 min, respectively. Furthermore, this compound was well tolerated in that platelet count, heart rate, and blood pressure remained normal throughout the experiments.

Intravenous dosing of **2** in three thrombosis models afforded dose-dependent antithrombotic effects.[27] At cumulative doses of 0.01, 0.03, and 0.1 mg/kg in a canine arteriovenous shunt model, **2** decreased thrombus weight from a control of 79 ± 4 mg to 60 ± 8, 31 ± 7, and 12 ± 1 mg, respectively. Compound **2** caused a dose-dependent prolongation of time to occlusion of photoactivation-injured guinea pig carotid artery at 0.3, 1, and 3 mg/kg, iv. In this assay, while vehicle-treated arteries occluded in 6−12 min, none of the four animals occluded over the 30-min observation period when dosed with 3 mg/kg **2**. In the ferric chloride arterial injury model, vehicle-treated guinea pigs occluded in a 12−35 min period. Intravenous dosing of **2** at 0.3 and 1 mg/kg inhibited thrombosis dose dependently as well; e.g., four out of seven animals remained patent at the 60-min observation point (1 mg/kg).[27]

Scheme 9. Synthesis of Bipiperidine **59**[a]



7 **59**

[a] (a) 4-NO_2PhOCOCl, NMM, MeCN, *N*-Boc-4,4′-bipiperidine. (b) LiOH, aq THF. (c) 4 N HCl/dioxane.

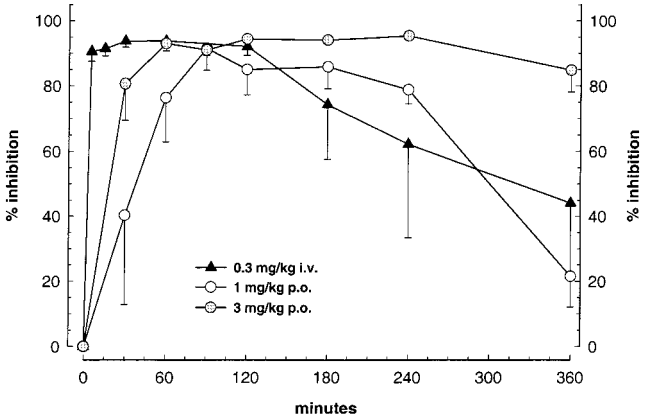


Figure 1. Duration study of inhibition of ADP-induced platelet aggregation ex vivo following intravenous and oral dosing of **2** in conscious dogs ($n = 3$).

Since relatively optimal pharmacodynamics could be attributed to the pyridine substituent of **2**, substituted pyridines were prepared by solid-phase parallel synthesis to probe the pyridine-based structure−activity relationships (Table 2). Since the bulky quinoline β-amino acid of **33** delivered excellent potency, Suzuki cross-couplings at 5-bromopyridine **15** with arylboronic acids were conducted to prepare **42b**−**n**. Some improved

potencies were observed over the parent compound (**42o**) at that position. Racemic bromo (**42a**), 3-thienyl (**42b**), 3-aminophenyl (**42e**), phenyl (**42l**), and 2-thienyl (**42n**) substituents displayed binding IC_{50} values of 4 nM or better. Potency was maintained in the *S*-enantiomer of the 5-phenylpyridine analogue (**49**), and improved with those with the 5-bromo (**44**), 5-phenylethynyl (**50**), 5-phenethyl (**51**), and 5-ethynyl (**52**) groups (Table 3). Oral administration (3 mg/kg) of some of the resolved pyridines produced good duration against canine ex vivo platelet aggregation: **44** (210 min) and **51** (180 min). 6-Methylpyridine **43**, 6-chloropyridine **45**, and 6-(3-thienyl)pyridine **46** showed comparable potency and duration as well. Compound **43** exhibited the best receptor affinity in the pyridine series ($IC_{50} = 0.13$ nM), along with excellent functional activity ($IC_{50} = 30$ nM).

Because **2** displayed the best pharmacodynamic profile of the antagonists synthesized to this point, the 3*S*-(3-pyridyl)-*β*-amino acid was incorporated into molecules with other sites of structural diversity as shown in Table 4. For example, methyl (**53**) and cyclohexylethyl (**55**) esters were prepared, albeit with inferior biological results. *N*-Formamidinopiperidine **54** lost human antiplatelet potency ($IC_{50} = 350$ nM) and showed inferior duration of action ex vivo (90 min) compared to **2**. Olefin-linked *N*-terminal piperidines maintained good duration of action ex vivo in both six- (**56**) and five-membered (**58**) scaffold systems, with the latter antagonist showing duration equal to **2** (360 min). Bipiperidinyl urea **59** gave satisfactory ex vivo results as well, although human antiplatelet potency was diminished ($IC_{50} = 450$ nM). In summary, the relatively robust activity contributed by the 3*S*-(3-pyridyl)-*β*-amino acid pharmacophore of **2** was observed in other examples as well (Table 4).

Conclusion

The synthesis and biological evaluation of novel 3-(3-pyridyl)-*β*-alanine fibrinogen receptor antagonists represent an improvement on the pharmacological profile of nipecotamides related to 3-(3,4-methylendioxybenzene)-*β*-alanine **3**. Pyridine **2** is a highly potent, orally active antiplatelet agent devoid of untoward side effects in our canine studies. The structure−activity relationships of the nipecotic acid series reported herein bear some resemblance to the structures related to the 2-piperidone scaffolded L-734217.[28] Both series contain C-terminal *β*-amino acid and *N*-terminal piperidine pharmacophores. However, while a *β*-methyl substituent on the *β*-amino acid was relatively impotent in the nipecotic acid series,[15] a *β*-methyl in the 2-piperidone series afforded good potency and a candidate for clinical development.[28] Similarly, Searle has employed a *β*-ethynyl substituent on the *β*-amino acid, yet found that a linear central constraint (succinamide) and an *N*-terminal guanidine surrogate (benzamidine) lent good potency, as in xemilofiban.[6b] Interestingly, an ethyl ester of a 3-(3-pyridyl)-*β*-alanine antagonist from Searle's ABAS (aminobenzamidinosuccinyl) series exhibited thrombocytopenia on oral administration to dogs at 3 mg/kg—a side effect not exhibited by non-prodrug **2** in dogs.[6b] In contrast to *β*-substituted *β*-amino acid pharmacophores, the discovery of an isoxazoline-scaffolded series, which led to the development of roxifiban, placed a butylcarbamate group on the α-position of a *β*-amino acid.[29] This type of pharmacophore is present in the intravenously administered tirofiban as well.[5] Our studies in this domain of the nipecotamides will be the subject of a subsequent paper.

Oral administration of **2** at 1 or 3 mg/kg in dogs resulted in good duration of inhibition of ex vivo platelet aggregation (5−6 h) without observable platelet count reduction. Due to the zwitterionic nature of GPIIb/IIIa antagonists, limited oral absorption has been addressed by using prodrugs such as xemilofiban and sibrafiban. To avoid the potential disadvantages of a prodrug such as metabolic variability, a preliminary objective with our nipecotamide series was to identify viable, orally active carboxylic acids. Compound **2**, which exhibits good oral absorption and duration of action at 1 mg/kg, represents the achievement of that important goal. Lotrafiban (SB-214857), another non-prodrug GPIIb/IIIa antagonist, has recently completed phase 2 studies in humans.[30] On the basis of our studies of non-prodrug nipecotamides,[31] pyridine **2** was selected for clinical evaluation as an antithrombotic agent for both oral and intravenous administration. RWJ-53308 (**2**) has now progressed successfully through human phase 2 clinical trials. Sequential intravenous and oral administration of this novel, potent GPIIb/IIIa antagonist could provide a useful regimen for both acute and chronic antiplatelet therapy in humans.

Experimental Section

In Vitro Procedures. The in vitro biotinylated fibrinogen/solid-phase purified GPIIb/IIIa binding assay and the in vitro inhibition of gel-filtered platelet aggregation assay were performed as published.[14]

Ex Vivo Study in Dogs. Mongrel dogs weighing 8−22 kg (Haycock Farms, PA) and fasted overnight were allowed to acclimate in isolation cages. A pre-dose blood sample was drawn from the cephalic vein for platelet aggregation measurements. Compounds, dissolved in 3 mL of saline, were infused intravenously into the contralateral cephalic vein at 0.3 mg/kg, or administered orally by stomach tube at 1 or 3 mg/kg in 10 mL of water. Blood samples were drawn into syringes preloaded with sodium citrate at 5, 15, 30, 60, 120, 180, 240, and 360 min after intravenous dosing and at 30, 60, 90, 120, 180, 240, and 360 min after oral dosing. PRP was collected by centrifugation of whole blood. Platelet count was determined with a Sysmex K-1000 differential cell counter (Baxter Laboratories, McGraw, IL) and adjusted to 300 000/*μ*L by dilution with platelet poor plasma (PPP). A count-adjusted PRP sample was warmed to 37 °C for 2 min and then placed in the test well. Aggregation, induced by 20 *μ*M ADP, was monitored with a Bio-Data platelet aggregometer Model PAP-4 (Bio-Data Corporation, Horsham, PA). Platelet aggregation was recorded as the increase in light transmission. The percentage inhibition of aggregation was calculated on the basis of the difference in percent aggregation in the presence of drug or vehicle.

Pharmacokinetic Studies. Following overnight fast, dogs in the oral group were administered a single dose of compound in water (10 mL) by oral gavage followed by water flush (10 mL). Dogs in the intravenous group received a single intravenous dose in saline (3 mL) over 30 s. All blood samples (ca. 3 mL) were collected from the jugular vein into heparinized Vacutainer tubes. Blood samples from the oral group were collected at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 4.0 h post-dose. Blood samples from the intravenous group were collected at 0, 0.08, 0.25, 0.5, 1.0, 2.0, 3.0, and 4.0 h post-dose. Plasma samples were analyzed for plasma concentration of compound using a validated LCMS assay. Pharmacokinetic analysis was performed on the plasma concentration data using WinNonlin

(Standard Version 1.1, Scientific Consulting, Apex, NC). The maximum plasma concentration (C_{max}, oral), concentration at time 0 (C_0, intravenous), area under the plasma concentration versus time curve (AUC), clearance (CL/F), terminal half-life ($t_{1/2}$), and apparent volume distribution (Vd_{ss}) were calculated as appropriate. Bioavailability (F) of an oral dose of compound was calculated for each dog using Microsoft Excel (Version 7.0, Microsoft Corp., Redmond, WA) according to the following formula:

$$F\ (\%) = 100(AUC_{po}/AUC_{iv})(DOSE_{iv}/DOSE_{po})$$

Statistical data are expressed as the mean ± the standard deviation.

Chemical Procedures. High field ^{1}H NMR spectra were recorded on a Bruker AC-360 spectrometer at 360 MHz with tetramethylsilane as an internal standard, and coupling constants are given in hertz (Hz). All mass spectra were obtained by electrospray technique (MH^+) on a Hewlett-Packard Series 1050 spectrometer. Melting points were determined on a MEL-TEMP II melting point apparatus and are uncorrected. Microanalyses were performed at Robertson Microlit Laboratories, Inc., Madison, NJ. Final compounds were purified by recrystallization/precipitation from common organic solvents and/or column chromatography using Merck silica gel 60. Purities were assessed on a combination Waters/Beckman HPLC System and a Phenomenex Ultra-carb column (100 × 4.6 mm) using an aqueous TFA/acetonitrile mobile phase (typically 10% MeCN with 90% water containing 0.1% TFA); all compounds exhibited >95% purity.

Materials. Arylboronic acids were purchased from Lancaster Synthesis, Inc. (*R*)-Nipecotic acid ethyl ester tartrate was purchased from Chemi S.P.A. and converted to *N*-Boc-(*R*)-nipecotic acid as published.[15] 2-Chlorotrityl chloride resin was purchased from Novabiochem. All other reagents were purchased from Aldrich Chemical.

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(3-pyridyl)]propionic Acid (2).** A mixture of **4** (50.3 g, 0.47 mol), EtOH (1 L), NH_4OAc (36.2 g, 1 mol equiv), and malonic acid (48.9 g, 1 mol equiv) was heated at reflux for 6 h, cooled, and filtered. The white solid was washed with EtOH and MeOH and dried. This solid was dissolved in 2:1 acetone/water (360 mL), treated with triethylamine (72.7 g, 0.72 mol) and phenylacetyl chloride (55.6 g, 0.36 mol), and stirred for 22 h. The mixture was evaporated and the residue dissolved in water (500 mL) and adjusted to pH 12 (1 N NaOH). The aqueous layer was adjusted to pH 2 (concentrated HCl), extracted with Et_2O, and evaporated to a white foam. The foam was purified by silica gel chromatography (10% MeOH/DCM) to give **5** (DCM = CH_2Cl_2). A solution of compound **5** (62.5 g, 0.22 mol) in water (600 mL) at room temperature was adjusted to pH 7.5 using KOH (3.0 N) and treated with penicillin amidase (91 520 units, Sigma).[20] This mixture was stirred for 47 h, acidified to pH 1 with HCl (concentrated), and the resultant precipitate filtered through Celite. The filtrate was extracted with Et_2O (3 × 300 mL), concentrated in vacuo, and treated with MeOH/concentrated NH_4OH (9:1). This product-containing solution was purified by silica gel chromatography (eluent DCM/MeOH/NH_4OH, 78:18:4) to give (*S*)-3-phenylacetamido-3-(3-pyridyl)propionic acid ammonium salt (19.5 g). This product was treated with HCl (6.0 N, 292 mL), heated at reflux for 5 h, cooled to 23 °C, and extracted with Et_2O (3 × 200 mL). The aqueous layer was adjusted to pH 12, concentrated in vacuo, and the resultant solid triturated with MeOH (2 × 300 mL). This solution was evaporated to give ca. 14 g of sodium salt. This material was treated with MeOH (500 mL), 2,2-dimethoxypropane (44 mL), and HCl (4 N in dioxane, 84 mL), and stirred for 90 h at 23 °C. This mixture was filtered and the filtrate concentrated in vacuo. The resultant off-white solid was triturated with Et_2O (2 × 150 mL) and dried to give **6** (16.7 g, 96% enantiomeric excess) as a white, amorphous solid. To a cooled (5 °C) solution of *N*-Boc-(*R*)-nipecotic acid (6.3 g, 0.028 mol) and **6** (7.0 g, 1 mol equiv) in MeCN (500 mL) was added HBTU (10.4 g, 1 mol equiv), HOBT (3.8 g, 1 mol equiv), and NMM (9.2 mL, 3 mol equiv; NMM = *N*-methylmorpholine). This mixture was stirred for 15 h, diluted with water (30 mL), and evaporated. The residue was diluted with EtOAc (300 mL), the layers separated, and the organic layer was dried (Na_2SO_4) and evaporated to give a white foam (10.6 g). The foam was treated with HCl (2 N in dioxane, 60 mL), stirred for 3 h, and evaporated to **7** as a foam. Compound **7** (9.8 g, 0.027 mol) was dissolved in MeCN (400 mL), cooled to 5 °C, and treated with *N*-Boc-4-piperidinepropanoic acid (6.9 g, 1 mol equiv), HBTU (10.0 g, 1 mol equiv), HOBT (3.6 g, 1 mol equiv), and NMM (8.8 mL, 3 mol equiv) with stirring for 6 h. The mixture was diluted with water (30 mL), evaporated, and diluted with EtOAc (300 mL), and the layers separated. The organic layer was dried, evaporated, and purified by silica gel chromatography (7% EtOH/DCM) to give a foam (7.3 g). To a solution of the foam (7.3 g, 0.014 mol) in THF (40 mL) cooled in an ice bath was added $LiOH{\cdot}H_2O$ (0.69 g dissolved in 50 mL of water) dropwise. This mixture was stirred for 1.5 h, acidified with AcOH (4.5 mL), and warmed to room temperature. This solution was diluted with $CHCl_3$ (150 mL) and the layers separated. The organic layer was dried (Na_2SO_4) and evaporated to give a white foam (7.1 g). The foam (7.1 g, 0.014 mol) was dissolved in dioxane (50 mL) and anisole (1.5 mL), cooled in an ice bath, treated with HCl (40 mL, 4.0 N in dioxane), and stirred for 3 h to give a precipitate. The precipitate was filtered and washed with Et_2O (300 mL) and MeCN (50 mL) to give **2** as a white powder (6.1 g): mp 81−89 °C; ^{1}H NMR (DMSO-d_6) δ 1.2−2.0 (m, 12 H), 2.3 (m, 3 H), 2.5 (m, 1 H), 2.8 (m, 5 H), 3.2 (d, J = 8 Hz, 2 H), 3.8 (m, 2 H), 4.2 (m, 2 H), 5.2 (m, 1 H), 7.8 (t, J = 4 Hz, 1 H), 8.3 (t, J = 4 Hz, 1 H), 8.5 (m, 1 H), 8.7 (m, 1 H), 8.9 (m, 2 H); MS *m/e* 417 (MH^+); $[\alpha]^{25}_D$ −32.5° (c 0.12, DMSO).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(3,4-methylenedioxyphenyl)]propionic Acid (3).** Compound **3** was prepared as previously reported[15] as a white powder (1.78 g): mp 190−200 °C; ^{1}H NMR (DMSO-d_6) δ 1.2−1.7 (m, 11 H), 1.81 (d, J = 10 Hz, 2 H), 2.31 (d, J = 7 Hz, 2 H), 2.6 (m, 3 H), 2.7 (m, 2 H), 3.0 (m, 1 H), 3.15 (d, J = 10 Hz, 2 H), 3.7 (m, 1 H), 4.1−4.3 (m, 1 H), 5.08 (dd, J = 5 Hz, 11, 1 H), 5.95 (s, 2 H), 6.7 (m, 1 H), 6.79 (d, J = 5 Hz, 1 H), 6.83 (d, J = 5 Hz, 1 H), 8.4 (m, 1 H), 8.6 (m, 1 H), 8.9 (m, 1 H), MS *m/e* 460 (MH^+); $[\alpha]^{25}_D$ −0.478° (*c* 1.00, MeOH).

***N*-3-(4-*N*-Methylpiperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(3,4-methylenedioxyphenyl)]propionic Acid (27).** Compound **27** was prepared as described for **2**, except *N*-Me-4-piperidinepropanoic acid was employed in the second coupling step (57% yield; see Scheme 1, step h) to give a tan powder (0.76 g): ^{1}H NMR (CD_3OD) δ 1.3−2.0 (m, 11 H), 2.4 (m, 3 H), 2.7 (m, 2 H), 2.84 (s, 3 H), 3.0 (m, 3 H), 3.2 (m, 1 H), 3.50 (d, J = 7 Hz, 2 H), 3.8 (m, 1 H), 4.3 (m, 1 H), 5.22 (t, J = 3 Hz, 1 H), 5.90 (s, 2 H), 6.8 (m, 3 H), 8.2−8.4 (m, 3 H); MS *m/e* 474 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(3,4-methylenedioxyphenyl)]propionic Acid Ethyl Ester (28).** Compound **28** was prepared as previously reported[15] except the lithium hydroxide ester saponification step was omitted, to give a white powder (1.34 g): MS *m/e* 488 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*R*)-3-amino-3-(2-phenylethynyl)]propionic Acid (29).** Compound **29** was prepared as described for **2**, except methyl [(*R*)-3-amino-3-(2-phenylethynyl)] propionate[6b] was employed in the first coupling step (77% yield; see Scheme 2) to give a white powder (1.25 g): ^{1}H NMR (DMSO-d_6) δ 1.1−1.9 (m, 10 H), 2.3 (m, 3 H), 2.7 (m, 5 H), 3.2 (m, 3 H), 3.4 (m, 2 H), 3.8 (m, 1 H), 4.3 (m, 1 H), 5.1 (m, 1 H), 7.36 (s, 5 H), 8.8 (m, 3 H); MS *m/e* 440 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(2-phenylethynyl)]propionic Acid (30).** Compound **30** was prepared as described for **2**, except methyl [(*S*)-3-amino-3-(2-phenylethynyl)]propionate[6b] was employed in the first coupling step (80% yield; see Scheme 2) to give a white powder (0.76 g): MS *m/e* 440 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl-[(*R*)-3-amino-3-(2-*tert*-butylethynyl)]propionic Acid (31).** Compound **31** was prepared as described for **2**, except methyl [(*R*)-3-amino-3-(2-*tert*-butylethynyl)]propionate[6b] was employed in the first coupling step (72% yield; see Scheme 2) to give a white powder (0.12 g): ^{1}H NMR (CD_3OD) δ 1.19 (s, 9 H), 1.3−2.0 (m, 11 H), 2.5 (m, 3 H), 2.6 (m, 2 H), 2.9 (m, 4 H), 3.4 (m, 3 H), 3.9 (m, 1 H), 4.2 (m, 1 H), 4.5 (m, 1 H), 4.8 (m, 2 H), 5.0 (t, J = 3 Hz, 1 H); MS *m/e* 420 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(2-*tert*-butylethynyl)]propionic Acid (32).** Compound **32** was prepared as described for **2**, except methyl [(*S*)-3-amino-3-(2-*tert*-butylethynyl)] propionate[6b] was employed in the first coupling step (81% yield; see Scheme 2) to give a white powder (0.33 g): ^{1}H NMR (CD_3OD) δ 1.21 (s, 9 H), 4.9 (m, 1 H); MS *m/e* 420 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(3-quinolinyl)]propionic Acid (33).** Compound **33** was prepared as described for **2**, except methyl (*S*)-3-amino-3-(3-quinolinyl)propionate (prepared by penicillin amidase resolution as for **2**) was employed in the first coupling step (65% yield; see Scheme 1) to give white flakes (1.11 g): mp 142−144 °C; MS *m/e* 467 (MH^+); $[\alpha]^{25}_D$ −173° (*c* 1.00, MeOH).

***N*-3-(4-Piperidinepropionyl)-(*S*)-(−)-nipecotyl[(*S*)-3-amino-3-(3-quinolinyl)]propionic Acid (34).** Compound **34** was prepared as described for **2**, except methyl (*S*)-3-amino-3-(3-quinolinyl)propionate (prepared by penicillin amidase resolution as for **2**) was employed in the first coupling step with *N*-Boc-(*S*)-nipecotic acid (71% yield; see Scheme 1) to give a white solid (0.057 g): MS *m/e* 467 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(2-thienyl)]propionic Acid (35).** Compound **35** was prepared as described for **2**, except methyl (*S*)-3-amino-3-(2-thienyl) propionate (prepared by penicillin amidase resolution as for **2**) was employed in the first coupling step with *N*-Boc-(*R*)-nipecotic acid (38% yield; see Scheme 1) to give a glass (0.093 g): mp 99−109 °C; MS *m/e* 422 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(5-chloro-2-thienyl)]propionic Acid (36).** Compound **36** was formed as a byproduct during the final HCl/dioxane-mediated Boc removal step during the synthesis of **35**, isolated by reverse phase HPLC (gradient 5−50% MeCN/0.1%TFA/water on a C18 column), and converted to the HCl salt using 1 N HCl to give a glass (0.12 g): mp 94−105 °C; MS *m/e* 457 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl-(*R*)-3-aminobutyric Acid (37).** Compound **37** was prepared on a 2-chlorotrityl resin support as reported[15] using *tert*-butyl (*R*)-3-aminobutyrate (purchased from Oxford Asymmetry) and was isolated as a tan powder (0.050 g, TFA salt): ^{1}H NMR (CD_3OD) δ 1.17 (d, J = 9 Hz, 3 H), 1.3 (m, 2 H), 1.5 (m, 6 H), 1.7 (m, 2 H), 2.0 (m, 2 H), 2.4 (m, 2 H), 2.7 (m, 2 H), 2.9 (m, 3 H), 3.2 (m, 1 H), 3.3 (m, 3 H), 3.5 (m, 2 H), 3.9 (m, 1 H), 4.2 (m, 1 H), 4.5 (m, 1 H); MS *m/e* 354 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl-(*R*)-3-amino-4-phenylbutyric Acid (38).** Compound **38** was prepared on a 2-chlorotrityl resin support as reported[15] using *tert*-butyl (*R*)-3-amino-4-phenylbutyrate (purchased from Oxford Asymmetry) and was isolated as a tan glass (0.030 g, TFA salt): ^{1}H NMR (DMSO-d_6) δ 1.3 (m, 2 H), 1.5 (m, 6 H), 1.7 (m, 2 H), 2.1 (m, 2 H), 2.4 (m, 2 H), 2.7 (m, 2 H), 2.9 (m, 3 H), 3.0 (d, J = 5 Hz, 2 H), 3.2 (m, 1 H), 3.3 (m, 2 H), 3.5 (m, 2 H), 3.9 (m, 1 H), 4.2 (m, 1 H), 4.5 (m, 1 H) 7.1 (m, 5 H), 8.0 (m, 2 H); MS *m/e* 430 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(2*S*)-hydroxy-(3*R*)-amino-4-phenyl]butyric Acid (39).** Compound **39** was prepared as described for **2**, except *tert*-butyl [(2*S*)-hydroxy-(3*R*)-amino-4-phenyl]butyrate (purchased from Oxford Asymmetry), was employed in the first coupling step with *N*-Boc-(*R*)-nipecotic acid (99% yield; see Scheme 1) to give a white foam (2.07 g): mp 73−77 °C; MS *m/e* 446 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*R,S*)-2-hydroxy-3-amino]propionic Acid (40).** Compound **40** was prepared on a 2-chlorotrityl resin support as reported[15] using methyl (*R,S*)-2-hydroxy-3-aminopropionate, and was isolated as the TFA salt: pink glass (0.050 g): ^{1}H NMR (DMSO-d_6) δ 1.0−1.4 (m, 10 H), 1.8 (m, 4 H), 2.2 (m, 3 H), 2.6 (m, 1 H), 2.8 (m, 3 H), 3.2 (m, 3 H), 3.7 (m, 1 H), 4.2 (m, 2 H), 7.6 (m, 1 H), 8.2 (m, 1 H), 8.5 (m, 1 H); MS *m/e* 356 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(3-chloro-5-trifluoromethyl-2-pyridyl)]propionic Acid (41).** Compound **41** was prepared as described for **2**, except methyl(*S*)-3-amino-3-(3-chloro-5-trifluoromethyl-2-pyridyl)propionate (prepared by kinetic resolution of the (−)-ephedrine salt of *N*-Boc-3-amino-3-(3-chloro-5-trifluoromethyl-2-pyridyl)propionic acid as for **43**) was employed in the first coupling step (97% yield; see Scheme 1) to give a white foam (1.72 g): mp 78−80 °C; MS *m/e* 519 (MH^+); $[\alpha]^{25}_D$ −50.2° (*c* 0.22, MeOH).

Representative Solid-Phase Synthesis for 42b−n: *N*-3-(4-Piperidinepropionyl)nipecotyl[3-amino-3-(5-phenyl-3-pyridyl)]propionic Acid (42l). A 25 mL sintered-glass vessel under nitrogen was charged with 2-chlorotrityl chloride resin (0.24 g, 0.36 mmol, Novabiochem) and DMF (5 mL). The resin was agitated and swelled with nitrogen for 5 min and the DMF removed. The resin was treated with DMF (5 mL), DIPEA (0.31 mL, 5 mol equiv), and allyl 3-(4-piperidine)-propionate·HCl (0.20 g, 2.4 mol equiv), sequentially, and agitated for 8 h. The resultant dark green solution was removed, and the resin washed with DMF (3 × 5 mL), aqueous DMF (25%, 3 × 5 mL), THF (3 × 5 mL), DCM (3 × 5 mL), and Et_2O (5 mL). The resin was swelled with DCE (5 mL; DCE = $ClCH_2CH_2Cl$) and treated with a mixture of tetrabutylammonium fluoride hydrate (0.28 g, 3 mol equiv), azidotrimethylsilane (0.38 mL, 10 mol equiv), tetrakis(triphenylphosphine)-palladium (0.084 g, 20 mol %), and DCE (5 mL). The resin was agitated for 15 h and the orange solution removed. The resin was washed with DCM (3 × 5 mL), DMF (3 × 5 mL), THF (3 × 5 mL), and Et_2O (5 mL). The resin was swelled with DMF (5 mL) and treated with DIPEA (0.18 mL, 3 mol equiv), racemic allyl nipecotate·HCl (0.17 g, 3 mol equiv), DIC (0.17 mL, 3 mol equiv; DIC = diisopropylcarbodiimide), and HOBT (1 mg). The resin was agitated for 15 h and then the reaction solution removed. The resin was washed with DMF (3 × 5 mL), aqueous DMF (25%, 3 × 5 mL), THF (3 × 5 mL), DCM (3 × 5 mL), and Et_2O (5 mL). The resin was swelled with DCE (5 mL) and treated with a mixture of tetrabutylammonium fluoride hydrate (0.28 g, 3 mol equiv), azidotrimethylsilane (0.38 mL, 10 mol equiv), tetrakis(triphenylphosphine)palladium (0.084 g, 0.02 mol equiv), and DCE (5 mL). The resin was agitated for 15 h and the orange solution removed. The resin was washed with DCM (3 × 5 mL), DMF (3 × 5 mL), THF (3 × 5 mL), and Et_2O (5 mL). The resin was swelled with DMF (5 mL) and treated with DIEA (0.18 mL, 3 mol equiv), methyl d,L-3-amino-3-(5-bromopyridyl)propionate·HCl (0.36 g, 3 mol equiv), DIC (0.17 mL, 3 mol equiv), and HOBT (1 mg). The resin was agitated for 17 h and then the reaction solution removed. The resin was washed with DMF (3 × 5 mL), aqueous DMF (25%, 3 × 5 mL), THF (3 × 5 mL), DCM (3 × 5 mL), and Et_2O (5 mL). The resin was transferred to a round-bottomed flask, swelled with DME (5 mL; DME = $MeOCH_2CH_2OMe$), treated with phenylboronic acid (0.09 g, 2 mol equiv) and aqueous sodium carbonate (0.45 mL, 2.0 M), and heated at 65 °C for 18 h. This mixture was cooled, the reaction solution removed, and the resin washed with DMF (3 × 5 mL), aqueous DMF (25%, 3 × 5 mL), THF (3 × 5 mL), DCM (3 × 5 mL), and Et_2O (5 mL). The resin was swelled with THF (5 mL) and treated with a solution of KOTMS (0.23 g, 10 mol equiv) and THF (2 mL). The resin was agitated for 18 h and then the reaction solution removed. The resin was washed with DMF (3 × 5 mL), HOAc/THF (1:1, twice), aqueous DMF (25%, 3 × 5 mL), THF (3 × 5 mL), DCM (3 × 5 mL), and Et_2O (5 mL). The resin was treated with TFA/DCM (1:1, 10 mL), agitated for 15 min, and the resultant red solution collected. This solution was evaporated and the resultant oil triturated with Et_2O (3 × 5 mL) and dried to afford compound **42l** as a clear glass (0.045 g, 17%): ^{1}H NMR (CD_3OD) δ 1.1−1.7 (m, 10 H), 1.8 (m, 4 H), 2.4 (m, 3 H), 2.6 (m, 1 H), 2.9 (m, 4 H), 3.2 (d, J

= 5 Hz, 2 H), 3.8 (d, J = 5 Hz, 1 H), 4.2 (m, 1 H), 5.4 (m, 1 H), 7.5 (m, 5 H), 8.1 (d, J = 8 Hz, 1 H), 8.5 (d, J = 3 Hz, 1 H), 8.7 (m, 3 H), 9.0 (m, 1 H).

Using the same general solid-phase synthesis technique as described for **42l**, compounds **42a**−**o** were prepared as shown in Scheme 4. All compounds gave satisfactory mass spectral analysis. Representative compounds gave satisfactory ^{1}H NMR spectra (five compounds per matrix). Products exhibited purities of 90−99%.[15]

***N*-3-(4-Piperidinepropionyl)nipecotyl[3-amino-3-(5-bromo-3-pyridyl)]propionic Acid (42a).** ^{1}H NMR (DMSO-d_6) δ 1.0−1.7 (m, 10 H), 1.8 (m, 4 H), 2.3 (m, 3 H), 2.5 (m, 1 H), 2.7 (m, 4 H), 3.2 (d, J = 5 Hz, 2 H), 3.8 (d, J = 5 Hz, 1 H), 4.2 (m, 1 H), 5.2 (m, 1 H), 7.9 (d, J = 8 Hz, 1 H), 8.5 (d, J = 3 Hz, 1 H), 8.7 (m, 3 H), 9.0 (m, 1 H).

***N*-3-(4-Piperidinepropionyl)nipecotyl[3-amino-3-(5-(2-benzofuranyl)-3-pyridyl)]propionic Acid (42c)**. ^{1}H NMR (CD_3OD) δ 1.1−1.7 (m, 10 H), 1.8 (m, 4 H), 2.4 (m, 3 H), 2.6 (m, 1 H), 2.9 (m, 4 H), 3.2 (d, J = 5 Hz, 2 H), 3.8 (d, J = 5 Hz, 1 H), 4.2 (m, 1 H), 5.4 (m, 1 H), 7.2−7.5 (m, 4 H), 7.6 (d, J = 6 Hz, 1 H), 8.1 (d, J = 8 Hz, 1 H), 8.3 (d, J = 3 Hz, 1 H), 8.7 (m, 3 H), 9.1 (m, 1 H).

***N*-3-(4-Piperidinepropionyl)nipecotyl[3-amino-3-(5-(3-methoxyphenyl)-3-pyridyl)]propionic Acid (42f).** ^{1}H NMR (CD_3OD) δ 1.1−1.7 (m, 10 H), 1.8 (m, 4 H), 2.4 (m, 3 H), 2.6 (m, 1 H), 2.9 (m, 4 H), 3.2 (d, J = 5 Hz, 2 H), 3.8 (d, J = 5 Hz, 1 H), 3.91 (s, 3 H), 4.2 (m, 1 H), 5.4 (m, 1 H), 7.1 (m, 2 H), 7.3 (m, 2 H), 8.0 (d, J = 8 Hz, 1 H), 8.5 (d, J = 3 Hz, 1 H), 8.7 (m, 3 H), 9.2 (m, 1 H).

***N*-3-(4-Piperidinepropionyl)nipecotyl[3-amino-3-(5-(2-methoxyphenyl)-3-pyridyl)]propionic Acid (42j).** ^{1}H NMR (CD_3OD) δ 1.1−1.7 (m, 10 H), 1.8 (m, 4 H), 2.4 (m, 3 H), 2.6 (m, 1 H), 2.9 (m, 4 H), 3.2 (d, J = 5 Hz, 2 H), 3.8 (d, J = 5 Hz, 1 H), 3.88 (s, 3 H), 4.2 (m, 1 H), 5.4 (m, 1 H), 7.0 (m, 2 H), 7.4 (m, 2 H), 8.0 (d, J = 8 Hz, 1 H), 8.6 (d, J = 3 Hz, 1 H), 8.7 (m, 3 H), 9.0 (m, 1 H).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(6-methyl-3-pyridyl)]propionic Acid (43).** Compound **43** was prepared as shown in Schemes 1 and 3. The β-amino acid was resolved by kinetic resolution of racemic *N*-Boc-3-amino-3-(6-methyl-3-pyridyl)]propionic acid.[6b] For the resolution, racemic 3-amino-3-(6-methyl-3-pyridyl)]propionic acid (5.2 g, 0.029 mol), prepared as described for compound **2** in 61% yield, was dissolved in sodium hydroxide (1 N, 60 mL) and 1,4-dioxane (35 mL) treated with di-*tert*-butyl dicarbonate (6.5 g, 1 mol equiv), and stirred at 23 °C for 3 h. The dioxane was removed in vacuo, the aqueous layer was washed with hexane (50 mL), and the layers separated. The aqueous layer was acidified with citric acid (7 g) and extracted with chloroform (6 × 50 mL). The combined organic solutions were dried (Na_2SO_4) and evaporated to afford *N*-Boc-3-amino-3-(6-methyl-3-pyridyl)]propionic acid (3.9 g, 48%) as a white powder. To a slurry of the powder in EtOAc (170 mL) was added a solution of (1*R*,2*S*)-(−)-ephedrine (2.3 g, 1 mol equiv) in EtOAc (170 mL). This mixture was heated to reflux to afford a clear solution; the solution was allowed to cool to room temperature and was filtered. The filtered white solid was washed with EtOAc (50 mL) and Et_2O (50 mL) and dried to afford (*S*)-*N*-Boc-3-amino-3-(6-methyl-3-pyridyl)]propionic acid·(−)-ephedrine as a white powder (1.9 g, 31%, mp 152.5−153.0 °C). This powder was dissolved in 1 N sodium hydroxide (5.2 mL), it was washed with DCM (2 × 30 mL), and the layers were separated. The aqueous layer was acidified with citric acid (4 g) and extracted with chloroform (6 × 40 mL). The combined organic solutions were dried (Na_2SO_4) and evaporated to give white flakes. The flakes were dissolved in 4 N HCl in dioxane (10 mL), MeOH (25 mL), and 2,2-dimethoxypropane (1.0 mL) and stirred for 22 h at 23 °C. The solution was evaporated, triturated with Et_2O (100 mL), and dried to afford methyl (*S*)-3-amino-3-(6-methyl-3-pyridyl)]propionate·2HCl (**14**) as a tan foam (2.0 g). The foam was employed as described for compound **2** to prepare target **43** as a white foam (1.20 g): mp 99−105 °C; MS *m*/*e* 431 (MH^+); UV (MeOH) λ_{max} 269 nm.

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(5-bromo-3-pyridyl)]propionic Acid (44).** Compound **44** was prepared as shown in Scheme 1. The β-amino acid was resolved by kinetic resolution of racemic *N*-Boc-3-amino-3-(5-bromo-3-pyridyl)]propionic acid as described for **43** (35% yield). Compound **44** was isolated as a white foam (1.24 g): mp 98−101 °C; MS *m*/*e* 495 and 497 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(6-chloro-3-pyridyl)]propionic Acid (45).** Compound **45** was prepared as shown in Scheme 1. The β-amino acid was resolved by kinetic resolution of racemic *N*-Boc-3-amino-3-(6-chloro-3-pyridyl)]propionic acid as described for **43** (38% yield). Compound **45** was isolated as white flakes (1.54 g): mp 101−105 °C; MS *m*/*e* 451 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(6-(3-thienyl)-3-pyridyl)]propionic Acid (46).** Compound **46** was prepared as shown in Scheme 1. The β-amino ester was prepared as follows. Methyl (*S*)-*N*-Boc-3-amino-3-(6-chloro-3-pyridyl)]propionate (1.4 g, 0.0043 mol, see **45**) in THF (65 mL) was treated with 3-thiopheneboronic acid (0.60 g, 1.1 mol equiv), tetrakis(triphenylphosphine)palladium (0.25 g, 0.05 mol equiv), and K_2CO_3 (0.59 g, 1 mol equiv) and the resultant solution was heated at reflux for 18 h. The reaction was cooled, filtered, and diluted with DCM (130 mL) and saturated NH_4Cl (20 mL), and the layers separated. The organic layer was dried (Na_2SO_4) and evaporated to afford a dark green oil (1.51 g, 97%). The Boc group was removed with HCl (see **2**) to give methyl (*S*)-3-amino-3-(6-(3-thienyl)-3-pyridyl)]propionate (1.34 g), and the synthesis was completed as shown in Scheme 1. Compound **46** was isolated as a white foam (1.05 g): mp 110−114 °C; MS *m*/*e* 499 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(5,6-dichloro-3-pyridyl)]propionic Acid (47).** Compound **47** was prepared as shown in Scheme 1. The β-amino acid was resolved by kinetic resolution of racemic *N*-Boc-3-amino-3-(5,6-dichloro-3-pyridyl)]propionic acid as described for **43** (41% yield). Compound **47** was isolated as a white powder (1.28 g): mp 128−130 °C; MS *m*/*e* 486 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(5-chloro-6-methoxy-3-pyridyl)]propionic Acid (48).** Compound **48** was prepared as shown in Scheme 1. The β-amino acid was resolved by kinetic resolution of racemic *N*-Boc-3-amino-3-(5-chloro-6-methoxy-3-pyridyl)]propionic acid as described for **43** (41% yield). Compound **48** was isolated as a white foam (0.50 g): mp 145−151 °C; MS *m*/*e* 481 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(5-phenyl-3-pyridyl)]propionic Acid (49).** Compound **49** was prepared as shown in Scheme 1. The methyl (*S*)-3-amino-3-(5-phenyl-3-pyridyl)]propionate intermediate was prepared as described for **46** (81% yield, Suzuki coupling with phenylboronic acid). Compound **49** was isolated as a white foam (0.88 g): ^{1}H NMR (CD_3OD) δ 1.2−1.8 (m, 11 H), 2.0 (m, 3 H), 2.4 (m, 2 H), 2.6 (m, 4 H), 2.9 (m, 2 H), 3.3 (m, 3 H), 3.8 (m, 1 H), 4.3 (m, 1 H), 5.4 (m, 1 H), 7.4 (m, 1 H), 7.5 (t, J = 7 Hz, 2 H), 7.7 (d, J = 7 Hz, 2 H), 8.1 (m, 1 H), 8.50 (s, 1 H), 8.64 (s, 1 H); MS *m*/*e* 493 (MH^+); UV (MeOH) λ_{max} 249 nm.

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl-[(*S*)-3-amino-3-(5-phenethynyl-3-pyridyl)]propionic Acid (50).** Compound **50** was prepared as shown in Scheme 1. Intermediate **18a** was prepared as described for **18b** (see **52**); the methyl (*S*)-3-amino-3-(5-phenethynyl-3-pyridyl)]propionate intermediate was prepared/resolved as described for **43**. Compound **50** was isolated as a white foam (0.44 g): ^{1}H NMR (CD_3OD) δ 1.3−1.9 (m, 11 H), 2.0 (m, 3 H), 2.4 (m, 2 H), 2.6 (m, 4 H), 2.9 (m, 2 H), 3.3 (m, 3 H), 3.8 (m, 1 H), 4.3 (m, 1 H), 5.4 (m, 1 H), 7.4 (m, 3 H), 7.7 (d, J = 7 Hz, 2 H), 8.6 (m, 2 H), 8.8 (m, 2 H); MS *m*/*e* 517 (MH^+); UV (MeOH) λ_{max} 281 nm.

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(5-phenethyl-3-pyridyl)]propionic Acid (51).** Compound **51** was prepared as shown in Scheme 1. The methyl (*S*)-3-amino-3-(5-phenethyl-3-pyridyl)]propionate intermediate (**19b**) was prepared by hydrogenation of **19a** over 5% palladium-on-carbon in MeOH at 50 psig and 23 °C (96% yield). Compound **51** was isolated as white flakes (0.25 g): ^{1}H NMR

(CD_3OD) δ 1.3−1.9 (m, 11 H), 2.0 (m, 7 H), 2.4 (m, 2 H), 2.6 (m, 4 H), 2.9 (m, 2 H), 3.3 (m, 3 H), 3.8 (m, 1 H), 4.3 (m, 1 H), 5.3 (m, 1 H), 7.4 (m, 3 H), 7.7 (d, J = 7 Hz, 2 H), 8.3 (m, 1 H), 8.42 (s, 1 H), 8.72 (s, 1 H), 8.8 (m, 1 H); MS *m/e* 521 (MH^+); UV (MeOH) λ_{max} 269 nm.

***N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(5-ethynyl-3-pyridyl)]propionic Acid (52).** Compound **52** was prepared as shown in Scheme 1. The synthesis of **18b** was carried out as follows. A solution of ethyl 5-bromonicotinate (38 g, 0.16 mol), triethylamine (69 g, 4.3 mol equiv), and EtOAc (150 mL) was degassed with nitrogen for 15 min, treated with trimethylsilylacetylene (75 g, 4.8 mol equiv), bis(triphenylphosphine)palladium chloride (0.56 g, 0.05 mol equiv), and copper iodide (0.30 g, 0.01 mol equiv), and stirred at 50 °C for 9 h. This dark mixture was cooled, filtered through Celite, and evaporated to give a dark brown oil. The oil was dissolved in EtOH (500 mL), treated with K_2CO_3 (1.6 g), and stirred for 20 h at 23 °C. The solvent was evaporated, and the dark residue was treated with water (150 mL) and extracted with EtOAc (3 × 300 mL). The combined organics were dried (Na_2SO_4) and evaporated to give dark brown crystals. The crystals were dissolved in EtOAc/hexane (9:1, 500 mL) and filtered through silica gel (300 mL), and the solvent evaporated to give yellow crystals (26 g, 93% for two steps). The crystals were dissolved in THF (600 mL), and this solution cooled to −78 °C. The solution was treated with lithium aluminum hydride (6.2 g, 1.1 mol equiv), stirred for 4 h, and quenched with dropwise addition of water (50 mL). This mixture was warmed to 23 °C, filtered through Celite, and evaporated. The resultant red oil was dissolved in THF/DCM (1:1, 300 mL), treated with charcoal, filtered, and evaporated to give an orange solid (15.4 g). A solution of the solid in DCM (400 mL) at room temperature was treated with pyridinium dichromate (40 g, 0.11 mol), stirred for 22 h, and filtered through silical gel (300 g). The filtrate was evaporated to give **18b** as a white solid (7.0 g, 46% yield). The methyl (*S*)-3-amino-3-(5-ethynyl-3-pyridyl)]propionate intermediate was prepared/resolved as described for **43**. Compound **52** was isolated as a tan foam (0.15 g): ^{1}H NMR (CD_3OD) δ 1.3−1.8 (m, 10 H), 2.0 (m, 7 H), 2.4 (m, 2 H), 2.5 (m, 3 H), 2.9 (m, 2 H), 3.3 (m, 3 H), 3.8 (m, 1 H), 4.1 (m, 1 H), 4.31 (s, 1 H), 5.4 (m, 1 H), 8.64 (s, 1 H), 8.87 (s, 1 H), 8.92 (s, 1 H); MS *m/e* 441 (MH^+).

Methyl *N*-3-(4-Piperidinepropionyl)-(*R*)-(−)-nipecotyl-[(*S*)-3-amino-3-(3-pyridyl)]propionate (53). Compound **53** was prepared as shown in Scheme 1 for **2**, with omission of saponification step I. Compound **53** was isolated as white flakes (0.56 g): mp 75−80 °C; ^{1}H NMR (DMSO-d_6) δ 1.2−1.9 (m, 11 H), 2.3 (m, 3 H), 2.5 (m, 1 H), 2.8 (m, 5 H), 3.2 (d, J = 8 Hz, 2 H), 3.60 (s, 3 H), 3.8 (m, 2 H), 4.2 (m, 2 H), 5.3 (m, 1 H), 7.9 (t, J = 4 Hz, 1 H), 8.4 (t, J = 4 Hz, 1 H), 8.6 (m, 1 H), 8.7 (m, 1 H), 9.0 (m, 2 H); MS *m/e* 431 (MH^+); UV (MeOH) λ_{max} 261 nm.

***N*-Formamidino-3-(4-piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(3-pyridyl)]propionic Acid (54).** A solution of **53** (1.0 g, 2.3 mmol) in EtOH (20 mL) and MeOH (1 mL) at room temperature was treated with ethyl formimidate·HCl (0.40 g, 1.6 mol equiv), stirred for 7 h, treated with Et_2O (15 mL), and refrigerated for 4 days to afford crystals. The crystals were filtered and dried, and then dissolved in 4 N HCl (15 mL). This solution was stirred for 28 h and evaporated. The resultant foam was dissolved in MeCN/water (9:1), cooled in an ice bath, and filtered to afford **54** as a white foam (0.56 g): mp 49−55 °C; ^{1}H NMR (DMSO-d_6) δ 1.2−1.9 (m, 11 H), 2.3 (m, 3 H), 2.5 (m, 1 H), 2.8 (m, 5 H), 3.2 (d, J = 8 Hz, 2 H), 3.8 (m, 2 H), 4.2 (m, 2 H), 5.3 (m, 1 H), 7.9 (t, J = 4 Hz, 1 H), 8.6 (m, 1 H), 8.7 (m, 1 H), 9.0 (m, 2 H), 9.32 (s, 1 H); MS *m/e* 444 (MH^+); UV (MeOH) λ_{max} 261 nm.

2-Cyclohexylethyl *N*-3-(4-piperidinepropionyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(3-pyridyl)]propionate (55). Compound **53** was prepared as shown in Scheme 1. A mixture of *N*-Boc-3-(4-piperidinepropionyl)-(*R*)-(−)-nipecotyl-[(*S*)-3-amino-3-(3-pyridyl)]propionic acid (0.60 g, 1.2 mmol, see **2**) in DCM (25 mL), 2-cyclohexylethyl alcohol (0.48 mL, 3 mol equiv), and NMM (0.25 mL, 3 mol equiv) at 23 °C was treated with EDC·HCl (0.44 g, 2 mol equiv), stirred for 8 h, and diluted with satd NH_4Cl (10 mL). The layers were separated, and the organic layer was dried (Na_2SO_4), evaporated, and purified by flash column chromatography (1% NH_4OH/8% EtOH/DCM) to afford of 2-cyclohexylethyl *N*-Boc-3-(4-piperidine-propionyl)-(*R*)-(−)-nipecotyl-[(*S*)-3-amino-3-(3-pyridyl)]propionate (0.45 g, 62%). This ester was deprotected with HCl (see **2**), and **55** was isolated as a white foam (0.26 g): mp 88−95 °C; ^{1}H NMR (DMSO-d_6) δ 0.9 (m, 2 H), 1.1−1.3 (m, 10 H), 1.4−1.9 (m, 10 H), 2.3 (m, 3 H), 2.5 (m, 1 H), 2.8 (m, 4 H), 3.2 (d, J = 8 Hz, 2 H), 3.60 (s, 3 H), 3.8 (m, 2 H), 4.0 (t, J = 4 Hz, 2 H), 4.2 (m, 2 H), 5.3 (m, 1 H), 7.9 (t, J = 4 Hz, 1 H), 8.4 (t, J = 4 Hz, 1 H), 8.6 (m, 1 H), 8.7 (m, 1 H), 8.9 (m, 2 H); MS *m/e* 527 (MH^+); UV (MeOH) λ_{max} 261 nm.

***N*-3-(4-Piperidineprop-2-enoyl)-(*R*)-(−)-nipecotyl[(*S*)-3-amino-3-(3-pyridyl)]propionic Acid (56).** Compound **56** was prepared as shown in Scheme 1. *N*-Boc-3-(4-piperidine)-prop-2-enoic acid was prepared as follows. To a solution of oxalyl chloride (24.8 mL, 50 mmol) in DCM (200 mL) at −78 °C was added DMSO (7.0 mL) dropwise. The mixture was stirred for 30 min, treated with *N*-Boc-4-piperidinemethanol (8.2 g, 0.038 mol), and stirred for 2 h. Triethylamine (31.7 mL, 6 mol equiv) was added dropwise, the mixture was warmed to 23 °C, and the mixture was diluted with water (30 mL). The layers were separated; the organic layer was washed with saturated NH_4Cl (30 mL) and saturated NaCl (30 mL), dried (Na_2SO_4), evaporated, and purified by silica gel chromatography (20% EtOAc/hexane) to give a white solid (7.3 g, 34 mmol). A solution of ethyl 2-(triphenylphosphoranylidene)acetate (13.1 g, 0.038 mol) and DCM (40 mL) at 5 °C was treated with this white solid (7.3 g), warmed to 23 °C, stirred for 2.5 h, and evaporated to dryness. This solid was treated with pentane (50 mL), and triphenylphosphine oxide was removed by filtration. The pentane solution was concentrated and the solid purified by silica gel chromatography (10% EtOAc/hexane) to afford a glass (8.4 g). The glass was dissolved in EtOH (60 mL), and this solution was treated with water (60 mL) and 1 N sodium hydroxide (59 mL) at 23 °C. The mixture was stirred for 4 h, acidified with citric acid (8 g), and extracted with DCM (3 × 100 mL). The combined organics were dried (Na_2SO_4) and evaporated to give *N*-Boc-3-(4-piperidine)prop-2-enoic as a white solid acid (7.5 g, 0.029 mol, 76% overall yield). This solid was reacted with **7** as shown in Scheme 1. Compound **56** was isolated as a foam (0.86 g): ^{1}H NMR (DMSO-d_6) δ 1.2−2.0 (m, 10 H), 2.3 (m, 1 H), 2.5 (m, 1 H), 2.8 (m, 5 H), 3.2 (d, J = 8 Hz, 2 H), 3.8 (m, 2 H), 4.2 (m, 2 H), 5.2 (m, 1 H), 6.5 (m, 2 H), 8.0 (t, J = 4 Hz, 1 H), 8.5 (d, J = 5 Hz, 1 H), 8.8 (d, J = 4 Hz, 1 H), 8.9 (m, 3 H); MS *m/e* 414 (MH^+).

***N*-3-(4-Piperidinepropionyl)-(4*R*)-(+)-piperazine-2-carbonyl[(*S*)-3-amino-3-(3-pyridyl)]propionic Acid (57).** Methyl 1-*N*-Boc-(*R*)-(+)-piperazine-2-carboxylate was prepared by the method of B. Aebischer,[25] coupled with *N*-Boc-3-(4-piperidine)propionic acid, saponified, and coupled with the appropriate β-amino ester as described for **2**. Compound **57** was isolated as a white powder (0.079 g): ^{1}H NMR (DMSO-d_6) δ 1.2−2.0 (m, 10 H), 2.3 (m, 3 H), 2.6 (m, 1 H), 2.8 (m, 5 H), 3.2 (d, J = 8 Hz, 2 H), 3.8 (m, 2 H), 4.2 (m, 2 H), 5.5 (m, 1 H), 8.0 (m, 1 H), 8.4 (m, 1 H), 8.6 (m, 1 H), 8.7 (m, 2 H), 9.0 (m, 2 H); MS *m/e* 418 (MH^+).

***N*-3-(4-Piperidineprop-2-enoyl)pyrrolidine-3-carbonyl[(*S*)-3-amino-3-(3-pyridyl)]propionic Acid (58).** Compound **58** was prepared as shown in Schemes 1 and 8. Racemic methyl pyrrolidine-3-carboxylate was prepared by the method of J. Saunders[26] and then carried forward as described for **56**. Compound **58** was isolated as a white powder (0.43 g): mp 118−122 °C; ^{1}H NMR (DMSO-d_6) δ 1.4−1.9 (m, 8 H), 2.3 (m, 1 H), 2.5 (m, 1 H), 2.8 (m, 5 H), 3.2 (d, J = 8 Hz, 2 H), 3.8 (m, 2 H), 4.2 (m, 2 H), 5.3 (m, 1 H), 6.2 (d, 1 H) 6.6 (m, 1 H), 8.0 (t, J = 4 Hz, 1 H), 8.6 (d, J = 5 Hz, 1 H), 8.8 (d, J = 4 Hz, 1 H), 9.1 (m, 3 H); MS *m/e* 401 (MH^+).

***N*-[(4,4′-Bipiperidin-1-yl)carbonyl]-(*R*)-(−)-nipecotyl-[(*S*)-3-amino-3-(3-pyridyl)]propionic Acid (59).** Intermediate **7** (2.0 g, 5.5 mmol) was dissolved in DCM (140 mL), cooled (5 °C), treated with 4-nitrophenylchloroformate (1.1 g, 1 mol

equiv) and NMM (2.0 mL, 3 mol equiv), and stirred for 2 h. The mixture was diluted with water (15 mL), the layers were separated, and the organic layer was dried (Na_2SO_4) and evaporated to an oil. The oil was dissolved in MeCN (70 mL), treated with *N*-Boc-4,4′-bipiperidine (1.3 g, 1.5 mol equiv) and DMAP (0.40 g, 1 mol equiv), and heated at reflux for 24 h. The mixture was cooled, evaporated to a solid, and partitioned between EtOAc (150 mL) and 1 N NaOH (20 mL). The layers were separated, and the organic layer was dried (Na_2SO_4), evaporated to a solid, and purified by silica gel chromatography (8% EtOH/DCM) to give methyl *N*-Boc-[(4,4′-bipiperidin-1-yl-)carbonyl]-(*R*)-(−)-nipecotyl-[(*S*)-3-amino-3-(3-pyridyl)]propionate as a green glass (0.90 g, 47%). The glass was saponified and deprotected as described previously (see **2**) to give **59** as a pale yellow powder (0.73 g): mp 121−125 °C; ^{1}H NMR (DMSO-d_6) δ 1.0 (m, 2 H), 1.1−1.4 (m, 6 H), 1.6 (m, 3 H), 1.8 (m, 4 H), 2.2 (m, 2 H), 2.7 (m, 6 H), 3.2 (d, J = 6 Hz, 4 H), 3.5 (m, 1 H), 3.7 (m, 2 H), 4.1 (m, 1 H), 5.2 (m, 1 H), 7.9 (t, J = 5 Hz, 1 H), 8.3 (d, J = 5 Hz, 1 H), 8.8 (m, 5 H); MS *m/e* 472 (MH^+); UV (MeOH) λ_{max} 261 nm.

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