Design and Synthesis of Potent, Selective Inhibitors of Endothelin-Converting Enzyme

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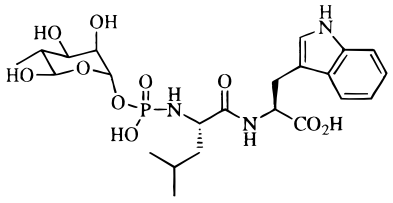
Endothelin-1 is the most potent peptidic vasoconstrictor discovered to date. The final step of posttranslational processing of this peptide is the conversion of its precursor by endothelin-converting enzyme-1 (ECE-1), a metalloprotease which displays high amino acid sequence identity with neutral endopeptidase 24.11 (NEP) especially at the catalytic center. A series of potent and selective arylacetylene-containing ECE-1 inhibitors have been prepared. (*S*,*S*)-3-Cyclohexyl-2-[[5-(2,4-difluorophenyl)-2-[(phosphonomethyl)amino]pent-4-ynoyl]amino]propionic acid (**47**), an arylacetylene amino phosphonate dipeptide, was found to inhibit ECE-1 and NEP with IC_{50} values of 14 nM and 2 μM, respectively. Similarly, (*S*)-[[1-[(2-biphenyl-4-ylethyl)-carbamoyl]-4-(2-fluorophenyl)but-3-ynyl]amino]methyl]phosphonic acid (**56**), an arylacetylene amino phosphonate amide, had IC_{50}'s of 33 nM and 6.5 μM for ECE-1 and NEP, respectively. Slight modification of the aryl moiety was found to have dramatic effects on ECE-1/NEP selectivity. The 2-fluoro dipeptide analogue, (*S*,*S*)-2-[[5-(2-fluorophenyl)-2-[(phosphonomethyl)-amino]pent-4-ynoyl]amino]-4-methylpentanoic acid (**40**), showed a 72-fold selectivity for ECE-1 over NEP, while the 3-fluoro dipeptide analogue, (*S*,*S*)-2-[[5-(3-fluorophenyl)-2-[(phosphonomethyl)amino]pent-4-ynoyl]amino]-4-methylpentanoic acid (**22**), was equipotent for ECE-1 and NEP. Several of these inhibitors were shown to be potent in blocking ET-1 production in vivo as demonstrated by the big ET-1-induced pressor response in rats. These potent inhibitors are the most selective for ECE-1 reported to date and are envisaged to have a variety of therapeutic applications.

Introduction

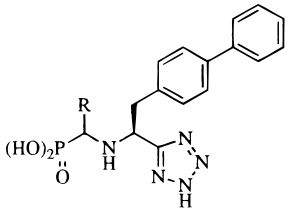
Endothelin-1 (ET-1), a 21-amino acid peptide, is the most potent vasoconstrictor known.[1] Elevated plasma levels of ET-1 have been found in several disease states including asthma, hypertension, cerebral vasospasm, congestive heart failure, and chronic and acute renal failure.[2] For these reasons, blockade of the ET-1 system could have therapeutic utility in these areas.

ET-1 is biosynthesized from its inactive 38-amino acid precursor, big ET-1, by specific proteolytic cleavage. The enzyme responsible for this last step of posttranslational processing of big ET-1 is a membrane-bound zinc metalloprotease, endothelin-converting enzyme-1 (ECE-1) (Scheme 1).[3] It is expected that inhibition of ECE-1 will suppress the production of ET-1 and thus its pathophysiological effects.[4]

Inhibitors of ECE-1 have been reported, including phosphoramidon **1** and amino phosphonates **2**.[5] However, all of the ECE-1 inhibitors reported to date have neutral endopeptidase 24.11 (NEP) inhibitory activity as well.[6] The fact that ECE-1 inhibitors are also inhibitors of NEP is not surprising since NEP is known to be a membrane-bound zinc metalloprotease and shares 37% amino acid sequence identity with ECE-1.[7] It is believed that ECE-1 and NEP share a high degree of homology in their active sites.[3,8]



1
Phosphoramidon
IC_{50}(ECE) 1.2 μM
IC_{50}(NEP) 30 nM
NEP/ECE = 0.025



2a
CGS 26303 R = H
IC_{50}(ECE) 410 nM
IC_{50}(NEP) 1 nM
NEP/ECE = 0.0024

2b
CGS 31447 R = CH_2CH_2Naph
IC_{50}(ECE) 17 nM
IC_{50}(NEP) 5 nM
NEP/ECE = 0.29

NEP cleaves a variety of substrates including atrial natriuretic factor (ANF) and bradykinin, a bronchoconstrictor.[9,10] ANF is a 28-amino acid peptide whose biological effects include sodium excretion and vasorelaxation.[11] Potentiation of ANF levels has been an active area of research for treatment of hypertension

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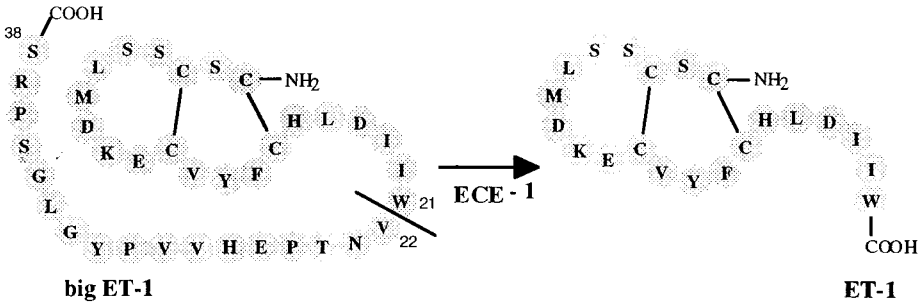
Scheme 1. Proteolytic Cleavage of Big ET-1 to ET-1 by ECE-1

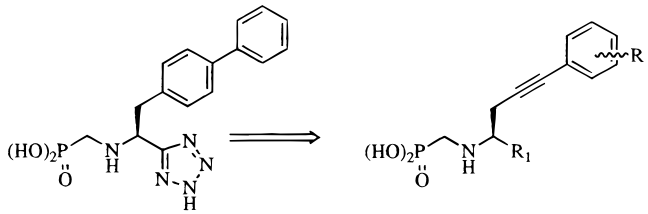


and congestive heart failure. Inhibition of NEP has been one of the major targets in raising ANF levels as such inhibitors have shown similar effects to ANF infusion in animal models.[12] In addition, ECE-1/NEP inhibition, which is believed to combine elevating ANF levels with lowering of ET-1 levels, has shown positive effects in several animal models of vascular pathophysiology.[4,5b,c,13]

While little progress has been made in the design and development of selective ECE-1 inhibitors, there remains a need for such agents. Selective ECE-1 inhibitors have potential application in respiratory diseases since treatment with a dual ECE-1/NEP inhibitor is likely to increase the levels of bradykinin. Also, ET-1 has been shown to be a substrate for NEP,[14] so that inhibitors of NEP may raise ET-1 levels. Finally, without selective ECE-1 inhibitors, the involvement of overproduction of ET-1 in various disease states could never be definitively validated. All in vivo studies on dual inhibitors would be complicated by the fact that the observed response could be due to ECE-1 or NEP inhibition, or both. For these reasons and the potential therapeutic benefit of blocking ET-1 biosynthesis, research efforts were focused on the design of potent and selective ECE-1 inhibitors.

Screens of NEP inhibitors for ECE-1 activity identified CGS 26303, a potent nonpeptidic NEP inhibitor with moderate ECE-1 inhibitory activity.[5a] Previous studies on optimization of the ECE-1 inhibitory activity of CGS 26303 have shown that alterations to the core of CGS 26303 result in significantly weaker inhibitors. These studies also showed that a dramatic increase in ECE-1 inhibitory activity, while maintaining potent NEP inhibition, can be achieved by introduction of arylethyl side chains at the P_1 binding subsite (see CGS 31447).[5d] However, these ECE-1 inhibitors with P_1 side chains lack the desired in vivo profile. Subsequent optimization of the P_1' binding subsite of CGS 31447 has implied that selectivity for ECE-1 over NEP could be obtained.[5f] Recent efforts have focused on decreasing NEP inhibition while maintaining potent ECE-1 inhibition. In the search for selective inhibitors, arylacetylenes were investigated as potential novel biphenyl surrogates (Scheme 2).

Scheme 2. Arylacetylene Amino Phosphonates



CGS 26303
IC_{50}(ECE) 410 nM
IC_{50}(NEP) 1 nM
NEP/ECE = 0.0024

I
R_1 = tetrazole, amino acids, 2° amides

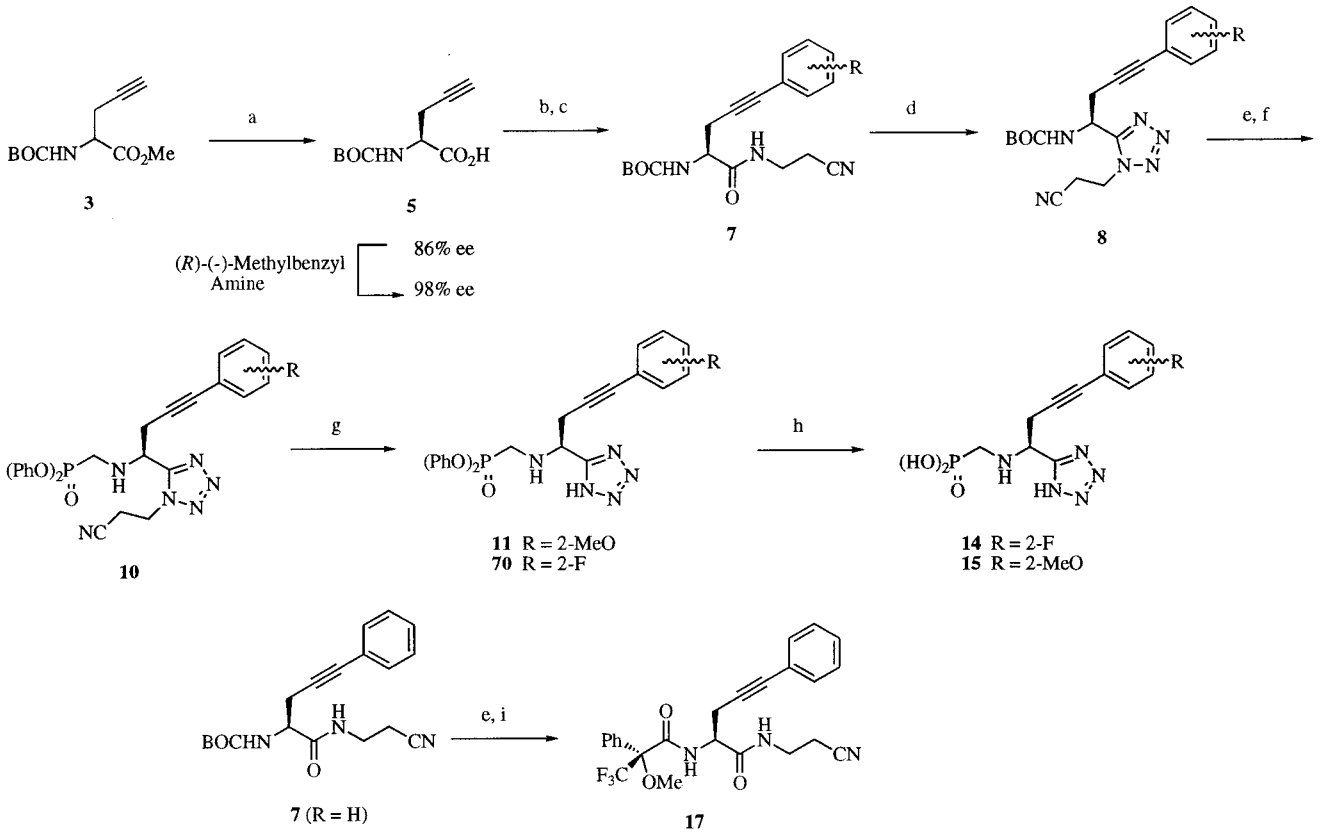
Chemistry

Target compounds **12**−**15** could be prepared from racemic propargylglycine which was prepared as its 2-*tert*-butoxycarbonylamino methyl ester **3** following literature procedures.[15] Alternatively, both propargylglycine and *N*-acetylpropargylglycine are commerically available in achiral form. The route for chiral, nonracemic derivatives **14** and **15** is outlined in Scheme 3. (*S*)-2-[(*tert*-Butoxycarbonyl)amino]pent-4-ynoic acid (**5**) was prepared by enzymatic resolution of **3** with alcalase. This gave 96% yield (based on 50% recovery) of (*S*)-*N*-Boc-propargylglycine (**5**) having 86% ee which could be crystallized with (*R*)-(−)-methylbenzylamine to optical purity. Amide formation was followed by Pd(0)-catalyzed cross-coupling with 2-iodoanisole to give the arylacetylene amino amide **7**.[16] Amide **7** was then converted to the tetrazole amino phosphonate **15** with slight modifications of previously reported procedures.[5a,12d] The Mosher amide[17] derivative **17** was prepared and compared to the Mosher amide of the racemate to show that the Pd(0)-catalyzed cross-coupling reaction proceeded without epimerization of the asymmetric center (Scheme 3).

The leucine dipeptides **22**−**25** were synthesized from (*S*)-*N*-Boc-propargylglycine (**5**) using standard methods as outlined in Scheme 4.

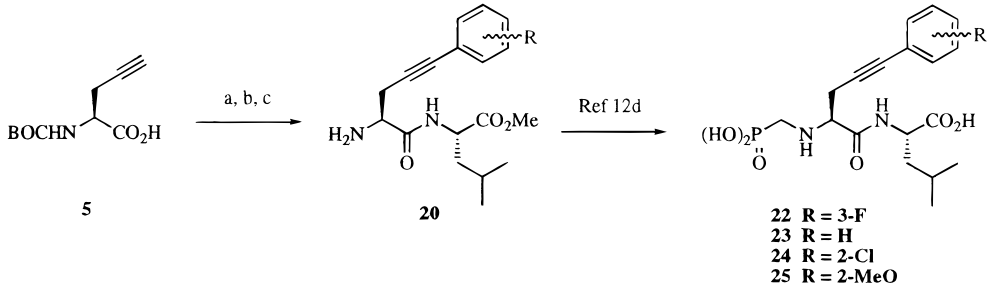
2-Fluoro- and 2,4-difluoroarylacetylene amino phosphonate amides, dipeptides, and tripeptides **38**−**69** were prepared by the procedure outlined in Scheme 5. Racemic 2-[(*tert*-butoxycarbonyl)amino]pent-4-ynoic acid methyl ester (**3**)[15] was subjected to Pd(0) cross-coupling with 2,4-difluoroiodobenzene to afford the arylacetylene amino ester in 85% yield.[16] Enzymatic resolution with alcalase gave the (*S*)-amino acid **27** in 96% yield (based on 50%) along with the (*R*)-amino ester **28**. The enantiomeric excess of **27** was determined to be 96% by examination and comparison of the derived Mosher amides **29** and **30** by ^{19}F NMR. Removal of the BOC group and alkylation of the primary amine as previously described[5a,12d] were followed by selective hydroylsis of the ester with NaOH to give the dimethyl phosphonate carboxylic acid **36**. Coupling of acid **36** with (L)-leucine methyl ester hydrochloride was accomplished with BOP reagent. Deprotection yielded the arylacetylene amino phosphonate **41**.

Scheme 3. Synthesis of Tetrazole Analogues[a]



[a] (a) Alcalase, 0.2% $NaHCO_3$, MeCN; (b) 3-aminopropionitrile, EDCI, HOBt, Et_3N, DMF; (c) $PdCl_2$(dppf), CuI, ArI, Et_3N, DMF; (d) $TMSN_3$, Ph_3P, DIAD, MeCN; (e) HCO_2H; (f) $(CH_2O)_n$, EtOAc; $(PhO)_2POH$, PhMe; (g) DBU, MeCN; (h) $NaHCO_3$(aq), MeCN; (i) (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, Et_3N, CH_2Cl_2.

Scheme 4. Synthesis of Leucine Dipeptide Analogues[a]



[a] (a) L-Leucine methyl ester hydrochloride, EDCI, HOBt, Et_3N, DMF; (b) $PdCl_2$(dppf), CuI, ArI, Et_3N, DMF; (c) HCO_2H.

Results

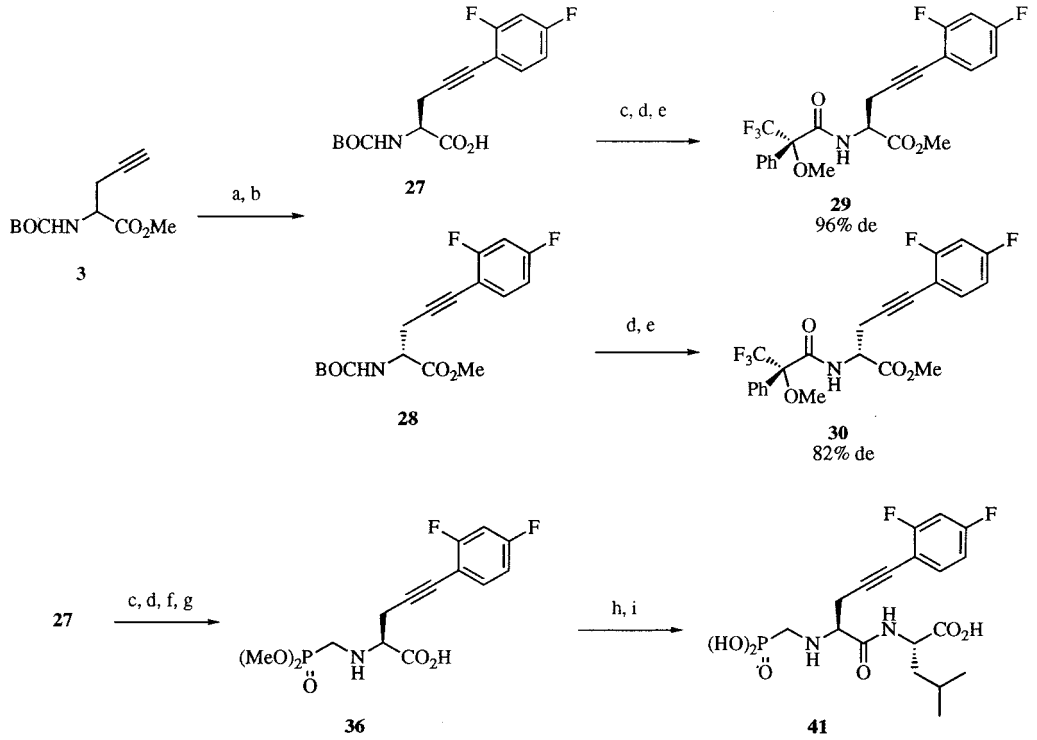
In Vitro Activity. In the search for more potent and selective ECE-1 inhibitors, arylacetylene analogues of CGS 26303 were investigated. Direct replacement of the P_1' biphenyl moiety of CGS 26303 with phenylacetylene **12** led to a nearly equipotent ECE-1 inhibitor and a weaker NEP inhibitor (see Table 1). This inhibitor was the first nonbiaryl amino phosphonate to show potent ECE-1 inhibition.[5c] In addition, while **12** was still more potent for NEP than ECE-1, it was 85-fold less potent than CGS 26303 for NEP. Encouraged by these results, we focused our efforts on optimization of arylacetylene amino phosphonates.

Examination of the aryl ring of **12** was undertaken first (Table 1). This study showed that placement of an *o*-fluoro substituent on the aryl ring resulted in an increase in ECE-1 inhibitory activity (see **14**). A similar increase in NEP inhibitory activity was also observed. Interestingly, the *o*-methoxy derivative **15** was a poor ECE-1 inhibitor. Finally, arylacetylene seemed to be of optimal length in the S_1' binding pocket of ECE-1, as the tolyl derivative **13** resulted in a dramatic loss of activity.

In search of greater ECE-1 selectivity, attention was turned to replacement of the tetrazole moiety. Preparation of the carboxylic acid **38** led to a selective ECE-1 inhibitor (Table 2). A slight reduction in ECE-1 inhibition was observed when compared to **14**. However, NEP activity was decreased dramatically from 14 nM for **14** to 11 μM for **38**. The 2,4-difluoroarylacetylene carboxylate **39** was also a selective ECE-1 inhibitor (Table 2).

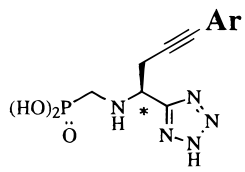
Biaryl dipeptide amino phosphonates were shown to be potent NEP inhibitors, but they have not been shown to inhibit ECE-1.[12d] Dipeptides of the arylacetylene amino phosphonates were examined to see if they behaved similarly to the biaryl series. Several dipep-

Scheme 5. Synthesis of Amide, Dipeptide, and Tripeptide Analogues[a]



[a] (a) $PdCl_2$(dppf), CuI, ArI, Et_3N, DMF; (b) alcalase, 0.2% $NaHCO_3$, MeCN; (c) $TMSCHN_2$; (d) HCO_2H; (e) (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, Et_3N; (f) $(MeO)_2OPCH_2OSO_2CF_3$, DIPEA; (g) 1 N NaOH; (h) L-leucine methyl ester hydrochloride, BOP, NMM, DMF; (i) 1 N NaOH; TMSBr, DIPEA, CH_2Cl_2.

Table 1. Tetrazole Arylacetylene Amino Phosphonates[a]



compd	Ar	*	IC_{50} (nM) rhECE-1	IC_{50} (nM) NEP	IC_{50} NEP/ IC_{50} ECE
12	Ph	*R,S*	590 ± 170	170 ± 7	0.3
13	4-Me-C_6H_4	*R,S*	45% at 1 μM	ND	ND
14	2-F-C_6H_4	*S*	150 ± 6	14 ± 1	0.1
15	2-MeO-C_6H_4	*S*	0% at 1 μM	ND	ND

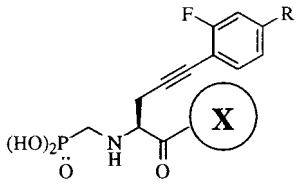
[a] The IC_{50} values are expressed in mean ± SEM ($n = 3$). rhECE-1, recombinant human ECE-1.

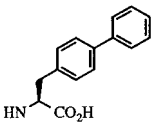
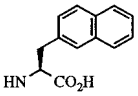
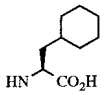
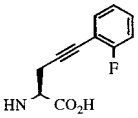
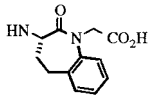
tides were prepared and found to be potent and selective ECE-1 inhibitors (Table 2). For example, leucine derivative **48** was found to be a highly potent and selective ECE-1 inhibitor (IC_{50} of 28 nM for ECE-1 and 6.3 μM for NEP), as were derivatives **41** and **47**. In addition to dipeptides, two tripeptides were prepared (Table 2). These were also selective ECE-1 inhibitors. For example, **49** exhibited excellent potency and selectivity with IC_{50} values of 8 and 5800 nM for ECE-1 and NEP, respectively.

A study of the leucine arylacetylene amino phosphonate dipeptides showed the sensitivity of ECE-1 and NEP to aryl substitution (Table 3). The 2-fluoro and 2,4-difluoro derivatives **40** and **41** were shown to be potent and selective inhibitors of ECE-1. Preparation of the 3-fluoro derivative **22** led to a 3-fold weaker ECE-1 inhibitor. Strikingly, the 3-fluoro derivative **22** was 21-fold more active against NEP than the 2-fluoro derivative **40**. This illustrated that a minute structural modification could have a dramatic effect on potency and selectivity. The unsubstituted analogue **23** fell between **40** and **22** with IC_{50} values of 510 and 948 nM for ECE-1 and NEP, respectively. Interestingly, the 2-chloro and 2-methoxy derivatives **24** and **25** were not tolerated by either enzyme.

Good potency and selectivity were also obtained with a series of amides of arylacetylene amino phosphonates (Table 4). The phenethyl and tryptamine derivatives **52**−**55** showed potent inhibitory activity and selectivity (IC_{50} values for ECE-1 inhibition ranged from 76 to 230 nM, while selectivity for ECE-1 over NEP ranged from 83- to 136-fold). The biphenethyl amides showed an increase in ECE-1 inhibitory activity as compared to the phenethyl derivatives (compare **56** and **57** to **54** and **55**). Both 1- and 2-naphthylethyl amides were well-tolerated (**62** and **63**), indicating a large lipophillic binding pocket. Interestingly, in the 1-naphthylethyl series, the 2,4-difluoro derivative **62** was found to be almost 4-fold more potent than the 2-fluoro analogue **61** in ECE-1 inhibition (34 and 130 nM, respectively). While the arylethyl amides seemed to be optimal for ECE-1 inhibitory activity, the arylpropyl derivative **58** was still significantly active, but the arylbutyl **59** was inactive (IC_{50} of 150 nM and 0% inhibition at 1 μM, respectively). The arylpropyl amide **58** was much more potent in NEP inhibition than the arylethyl amide derivative **55** (IC_{50} values of 640 and 15 000 nM, respectively). This differential inhibition of ECE-1 and NEP was significant and has yet to be fully explored. Finally, the complete loss of activity of tertiary amide **60** shows the importance of the hydrogen-bonding role of the secondary amide **55** in the putative ECE-1 active site.

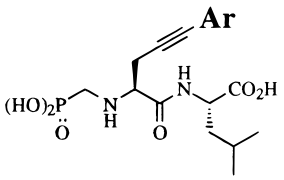
Table 2. Carboxylic and Peptidic Arylacetylene Amino Phosphonates[a]



compd	X	R	IC50 (nM) rhECE-1	IC50 (nM) NEP	IC50 NEP/IC50 ECE
38	OH	H	240 ± 42	11000 ± 280	46
39	OH	F	170 ± 48	26000 ± 160	153
40	Leu-OH	H	120 ± 3	8600 ± 150	72
41	Leu-OH	F	51 ± 1	6700 ± 72	131
42	Met-OH	H	230 ± 35	4060 ± 130	18
43	Val-OH	H	110 ± 15	6300 ± 210	57
44	Phe-OH	H	56 ± 5	1200 ± 50	21
45		H	13 ± 1	250 ± 7	19
46		H	25 ± 4	1300 ± 67	52
47		F	14 ± 2	2070 ± 13	148
48		H	28 ± 1	6300 ± 210	225
49	Leu Ala-OH	F	8 ± 2	5800 ± 190	725
50		F	82 ± 8	730 ± 15	9

[a] The IC_{50} values are expressed in mean ± SEM ($n = 3$).

Table 3. Leucine Arylacetylene Amino Phosphonate Dipeptides[a]



compd	Ar	IC50 (nM) rhECE-1	IC50 (nM) NEP	IC50 NEP/ IC50 ECE
40	2-F-C_6H_4	120 ± 3	8600 ± 150	72
41	2,4-diF-C_6H_4	51 ± 1	6700 ± 72	131
22	3-F-C_6H_4	400 ± 22	407 ± 11	1.0
23	Ph	510 ± 54	948 ± 31	1.9
24	2-Cl-C_6H_4	4300 ± 380	*b*	ND
25	2-MeO-C_6H_4	0% at 1 μM	*c*	ND

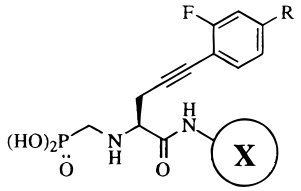
[a] The IC_{50} values are expressed in mean ± SEM ($n = 3$). [b] 38% at 10 μM. [c] 12% at 10 μM.

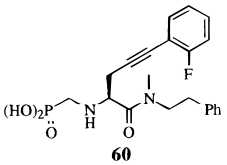
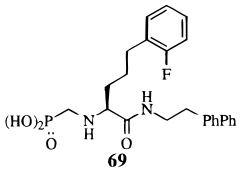
From these studies, it appears that the selectivity between ECE-1 and NEP is a function of both the P_1' and P_2' binding subsites. Interestingly, P_1' biaryl acids, amides, and peptides do not inhibit ECE-1,[5d,12d] whereas the arylacetylene analogues (Tables 2 and 4) were found to be potent and selective ECE-1 inhibitors suggesting that a combination of the P_1' and P_2' binding sites may play a role. One explanation for the observed selectivity of the arylacetylene derivatives is that the binding of these inhibitors in the P_1' subsite alters the overall binding mode of the inhibitor so that the enzyme will tolerate acids, amides, and peptides in the P_2' subsite. This effect of the P_1' subsite of ECE-1 is enhanced by the 2-fluorophenyl derivative as seen in Table 3.

No ECE-1/NEP selectivity was observed for the tetrazole analogues **12** and **14** (Table 1). Perhaps the P_2' tetrazole has a stronger influence on the binding mode, and this effect overshadows any effect the arylacetylenes may have in the P_1' binding subsite. A definative explanation and understanding awaits further structural information on the ECE-1 and NEP active sites.

In Vivo Activity. The ability of selected compounds to inhibit the increase in mean arterial pressure (MAP) produced by big ET-1 in vivo is shown in Figure 1. Intravenous bolus injection of 1 nmol/kg big ET-1 in anesthetized, ganglion-blocked rats treated with vehicle resulted in an 86 ± 4 mmHg rise in MAP. Pretreatment of the animals with compound **14**,[18] **48**, **41**, or **49**, at 10 mg/kg iv, was associated with an 85 ± 4%, 56 ± 4%, 51 ± 11%, and 64 ± 3% inhibition of the big ET-1 pressor response, respectively. By comparison, the administration of phosphoramidon at 15 and 30 mg/kg iv caused a 59 ± 9% and 79 ± 2% inhibition of the response to big ET-1 (data not shown).

Table 4. Amide Arylacetylene Amino Phosphonates[a]



compd	X	R	IC50 (nM) rhECE-1	IC50 (nM) NEP	IC50 NEP/ IC50 ECE
51	CH_2Ph	H	250 ± 85	55000 ± 3800	220
52	tryptamine	H	76 ± 6	10000 ± 120	132
53	tryptamine	F	111 ± 17	9700 ± 120	87
54	$(CH_2)_2Ph$	H	230 ± 58	19000 ± 720	83
55	$(CH_2)_2Ph$	F	110 ± 3	15000 ± 100	136
56	$(CH_2)_2PhPh$	H	33 ± 8	6500 ± 110	197
57	$(CH_2)_2PhPh$	F	28 ± 4	2500 ± 32	89
58	$(CH_2)_3Ph$	F	150 ± 23	640 ± 4	4
59	$(CH_2)_4Ph$	H	0% at 1 μM	ND	ND
60	 **60**		3% at 1 μM	ND	ND
61	$(CH_2)_2$-1-Nap	H	130 ± 3	10700 ± 58	82
62	$(CH_2)_2$-1-Nap	F	34 ± 8	6200 ± 310	182
63	$(CH_2)_2$-2-Nap	F	53 ± 9	7000 ± 95	132
64	$(CH_2)_2CHMe_2$	F	140 ± 34	23000 ± 88	164
65	$(CH_2)_2p$Br-C_6H_4	H	110 ± 12	40000 ± 1200	364
66	$(CH_2)_2p$Br-C_6H_4	F	43 ± 8	860 ± 7	20
67	$(CH_2)_2$(3,4-diMeOC_6H_3)	F	160 ± 43	*b*	ND
68	$(CH_2)_2$-2-pyridinyl	F	310 ± 41	10000	32
69	 **69**		8% at 1 μM	ND	ND

[a] The IC_{50} values are expressed in mean ± SEM ($n = 3$). [b] 21% at 10 μM.

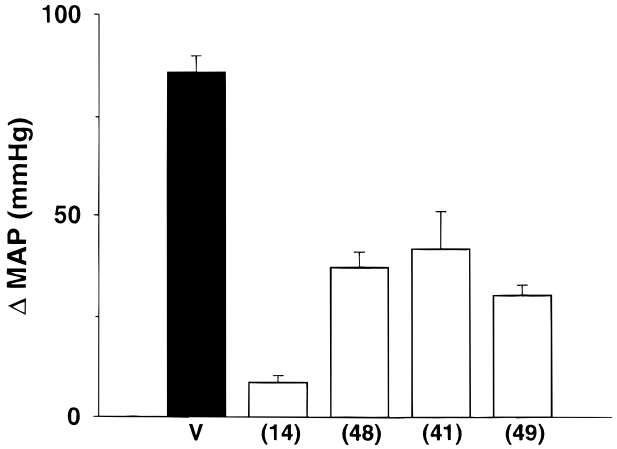


Figure 1. Maximum increases in MAP produced by big ET-1 in anesthetized, ganglion-blocked rats. Rats were treated with vehicle (V, 0.05 N NaOH at 1 mL/kg iv) or compound **14**, **48**, **41**, or **49** at 10 mg/kg iv and challenged with 1 nmol/kg iv big ET-1 15 min later. Values are the mean ± SEM of 15, 4, 4, 6, and 2 animals for the vehicle, **14**, **48**, **41**, and **49** groups, respectively.

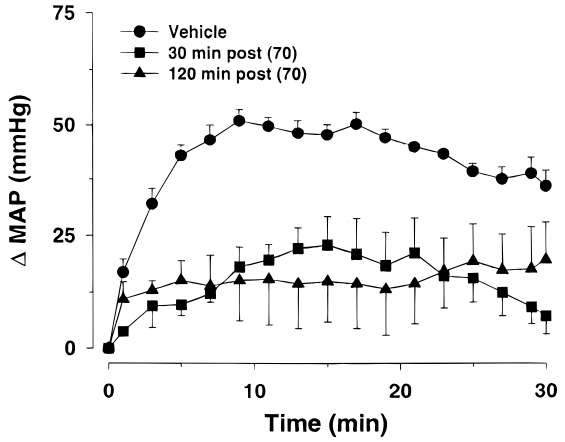


Figure 2. Changes in MAP produced by big ET-1 (1 nmol/kg iv) at 30 and 120 min after oral administration of **70** at 30 mgequiv/kg or vehicle (PEG 400, 1 mL/kg) to conscious rats. Values are the mean ± SEM for 4−8 animals.

The effect of compound **70**, the diphenyl phosphonate prodrug of **14**, on the big ET-1 pressor response in conscious rats is shown in Figure 2. Treatment with **70** at 30 mgequiv/kg po was associated with a 63% and 65% reduction of the pressor effect of big ET-1 at 30 and 120 min after dosing.

Conclusions

Arylacetylenes were found to be excellent surrogates for biphenyl as ECE-1 inhibitors. Within the tetrazole

series, phenylacetylene amino phosphonate **12** (Table 1) was found to be as active against ECE-1 as the biphenyl analogue CGS 26303. Amino phosphonate arylacetylene di- and tripeptides and amides exhibited high potency and selectivity as ECE-1 inhibitors (Tables 2 and 4). These derivatives were shown to be the most selective ECE-1 inhibitors reported to date. For example, dipeptide **47** had an IC_{50} of 14 nM for ECE-1 and an IC_{50} of 2.0 μM for NEP. Amide **56** had an IC_{50} of 33 nM for ECE-1 and an IC_{50} of 6.5 μM for NEP. In contrast, phosphoramidon, the most widely used ECE-1 inhibitor, had IC_{50} values of 1.2 μM and 30 nM for ECE-1 and NEP inhibition, respectively. In addition, 2-fluoroarylacetylenes showed an increase in ECE-1 inhibitory activity compared to phenylacetylene (see Table 1, derivatives **12** and **14**). Interestingly, the 2-fluoro substituent appears to play a critical role in NEP selectivity as seen in dipeptide derivatives **40** and **22** (Table 3). The role of the 2-fluoro substituent in increased potency and selectivity for ECE-1 inhibition is not clear at this time nor is the origin of the selectivity of these inhibitors. One explanation is that the binding of these inhibitors in the P_1' subsite alters the overall binding mode of the inhibitor (vide supra). Further study, including three-dimensional structural information on the ECE-1 and NEP active sites, is needed to elucidate these effects. Finally, these inhibitors have shown the ability to block ET-1 production in vivo as demostrated by the big ET-1-induced pressor response (Figures 1 and 2). These selective ECE-1 inhibitors could find broad application in vascular biology.

Experimental Section

Melting points (mp) were determined on either a Thomas-Hoover or Mel-TempII melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Nicolet 5SXB FTIR spectrometer. ^{1}H NMR spectra were recorded on either a Bruker AC-250 or Bruker AC-300 spectrometer using $CDCl_3$, D_2O, or DMSO-d_6 as internal standard. When needed, trifluoroacetic acid (a few drops) was used to solubilize amino phosphonic acids in DMSO-d_6. ^{31}P NMR spectra were recorded on a Bruker AC-300 spectrometer. ^{31}P chemical shifts are reported relative to 85% aqueous phosphoric acid as external standard. ^{19}F NMR spectra were recorded on a Bruker ARX-500 instrument. Optical rotations were measured with a Jasco DIP-370 instrument. Mass spectra were obtained on a MicroMass Platform II spectrometer. Microanalyses were performed at Robertson Laboratory, Inc., Madison, NJ. All organic solvents used were of anhydrous grade. Chromatographic separations were performed on either silica gel 60, Diol Gel, or reverse-phase MCI gel under N_2 pressure. Some chromatographic separations were performed on a Biotage Flash40 apparatus with silica gel. Alcalase (food grade) was aquired from Novo Nordisk (Franklinton, NC).

Enzyme Assays. 1. ECE-1 Assay: To assess the effect of an inhibitor on ECE-1 activity, 10 μg of protein of membrane preparation of CHO cells expressing human ECE-1 was preincubated with the compound at a desired concentration for 20 min at room temperature in 50 mM Tes, pH 7.0, and 0.005% Triton X-100 in a volume of 10 μL. Human big ET-1 (5 μL) was then added to a final concentration of 0.2 μM, and the reaction mixture was further incubated for 2 h at 37 °C. The reaction was stopped by adding 500 μL of radioimmunoassay (RIA) buffer containing 0.1% Triton X-100, 0.2% bovine serum albumin, and 0.02% NaN_3 in phosphate-buffered saline.

2. Quantitation of ET-1: Diluted samples (200 μL) obtained from the above enzyme assay were incubated at 4 °C overnight with 25 μL each of [^{125}I]ET-1 (10 000 cpm/tube; New England Nuclear, Boston, MA) and 1:20000-fold diluted rabbit antibodies that recognize specifically the carboxyl terminal tryptophan of ET-1. Goat anit-rabbit antibodies coupled to magnetic beads (70 μg) were then added to each tube, and the reaction mixture was further incubated for 30 min at room temperature. The beads were pelleted using a magnetic rack. The supernatant was decanted, and the radioactivity in the pellet was counted in a gamma counter. Total and nonspecific binding were measured in the absence of nonradioactive ET-1 and anit-ET antibodies, respectively. Under these conditions, ET-1 and big ET-1 displaced [^{125}I]ET-1 binding to the antibodies with IC_{50} values of 21 ± 2 and 260 000 ± 66 000 fmol (mean = SEM, n = 3−5), respectively.

3. Data Analysis: Duplicate samples were carried out in each experiment. To determine IC_{50} value of an inhibitor, the full concentration−response curve of each inhibitor was performed at least three times. A nonlinear least-squares curve-fitting program was used to fit data to a one-site model.

NEP assay was performed as previously described.[12d]

Big ET-1 Pressor Test. Male Sprague−Dawley rats were anesthetized with Inactin (100 mg/kg ip) and instrumented with catheters in the femoral artery and vein to record mean arterial pressure (MAP) and adminster compounds, respectively. A tracheostomy was performed and a cannula inserted into the trachea to ensure airway patency. The body temperature of the animals was maintained at 37 ± 1 °C by means of a heating blanket. Following surgery, MAP was allowed to stabilize for 30 min before interrupting autonomic neurotransmission with chlorisondamine (3 mg/kg iv) and challenging with big ET-1 (1 nmol/kg iv) 15 min later. The data are reported as the maximum increase in MAP produced by the big ET-1 injection.

Compound **70**, a prodrug of **14**, was also evaluated following oral administration in conscious rats. To perform this study, male Spraque−Dawley rats were anesthetized with methohexital sodium (75 mg/kg ip) and instrumented with catheters in the femoral artery and vein to measure MAP and administer big ET-1, respectively. The catheters were then threaded through a swivel system which enabled the animals to move freely after regaining consciousness. On the following day, MAP was recorded via the femoral artery catheter and compound at 30 mg/kg or vehicle (PEG 400, 1 mL/kg) was administered po. The animals were challenged with big ET-1 (1 nmol/kg iv) at 30 and 120 min after dosing. The changes in MAP produced by big ET-1 were reported at 2-min intervals.

Chemistry. Derivatives **12**−**15** could be prepared from either D,L- or L-*N*-(*tert*-butoxycarbonyl)propargylglycine**.** Derivative **15** is representative.

(*S*)-2-[(*tert*-Butoxycarbonyl)amino]pent-4-ynoic Acid (5). To a stirred solution of 2-[(*tert*-butoxycarbonyl)amino]-pent-4-ynoic acid methyl ester (**3**)[15] (11.1 g, 48.9 mmol) in CH_3CN (165 mL) and 0.2 M $NaHCO_3$ (325 mL) was added alcalase (2.2 mL). The mixture was placed in a preheated oil bath at 40 °C and stirred for 2 h. The reaction mixture was cooled to room temperature, Et_2O was added, and the mixture stirred for several minutes. The layers were separated, and the aqueous layer was extracted several times with Et_2O. The combined ether extracts were dried over $MgSO_4$, filtered, and concentrated to give the (*R*)-amino ester **4** as an orange oil (5.54 g): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.38−5.35 (1H, bm), 4.48−4.42 (1H, m), 3.75 (3H, s), 2.72−2.70 (2H, m), 2.07−2.02 (1H, m), 1.43 (9H, s). The aqueous layer was acidified to pH 2−3 with 1 N HCl; then some of the water was evaporated in vacuo. The residue was extracted several times with EtOAc. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated to give 5.01 g (96%) of the (*S*)-amino acid **5**: IR (KBr) 1743, 1679, 1523, 1209 cm^{-1}; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 5.35−5.30 (1H, bd), 4.55−4.40 (1H, m), 2.8−2.7 (2H, m), 2.05−2.00 (1H, m), 1.40 (9H, s); $[\alpha]^{25}_D$ +20.2 (*c*, 12.4 mg/mL MeOH) (lit.[15] $[\alpha]^{25}_D$ +23.5 (*c*, 9.1 mg/mL MeOH) (86% ee)); MS (ES$^-$) *m*/*z* 212 (M − H). Optically pure **5** was obtained by crystallization with (*R*)-(+)-α-methylbenzylamine. (*R*)-(+)-α-Methylbenzylamine (4.36 g, 20.47 mmol) was added to a stirred solution of acid **5** (86% ee) in Et_2O (100 mL) which resulted in

a white precipitate. The reaction mixture was heated to 40 °C in a water bath, and absolute EtOH (~150 mL) was added until a clear solution resulted. The reaction mixture was cooled to room temperature and allowed to stand for 16 h. The resultant crystalline needles were collected by filtration and washed with Et_2O. After drying in vacuo, the crystals were suspended in Et_2O and washed with 1 N HCl and brine. The clear organic layer was dried ($MgSO_4$) and concentrated to give 3.42 g (78%) of (*S*)-amino acid **5** as an oil: $[\alpha]^{25}_D$ +22.2 (*c*, 12.4 mg/mL MeOH) (lit.[15] $[\alpha]^{25}_D$ +23.5 (*c*, 9.1 mg/mL MeOH) (95% ee)).

(*S*)-[1-[(2-Cyanoethyl)carbamoyl]but-3-ynyl]carbamic Acid *tert*-Butyl Ester (6). To a stirred solution of acid **5** (3.21 g, 15.07 mmol) in DMF (50 mL) at room temperature under N_2 atmosphere was added HOBt (2.24 g, 16.58 mmol) followed by triethylamine (2.73 mL, 19.59 mmol). The solution was stirred for 10 min before 3-aminopropionitrile (1.11 mL, 15.07 mmol) was added. After the mixture stirred for 5 min, EDCI (3.76 g, 19.59 mmol) was added. The reaction mixture was stirred for 16 h and then partitioned between EtOAc and H_2O. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with H_2O, 1 N HCl, saturated $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to give 3.42 g (86%) of amide **6** as a white solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.00 (1H, bt, J = 7.5 Hz), 5.30 (1H, bd, J = 9.0 Hz), 4.32 (1H, bs), 3.57 (2H, dt, J = 13.5, 6.8 Hz), 2.82−2.55 (4H, m), 2.14 (1H, t, J = 3.8 Hz), 1.46 (9H, s).

(*S*)-[1-[(2-Cyanoethyl)carbamoyl]-4-(2-methoxyphenyl)but-3-ynyl]carbamic Acid *tert*-Butyl Ester (7). The method of Robertson and Crisp was employed.[16] To a solution of amide **6** (1.57 g, 5.92 mmol) in DMF (30 mL) was added $PdCl_2$(dppf) (0.24 g, 0.30 mmol), followed by 2-iodoanisole (0.85 mL, 6.52 mmol), triethylamine (1.65 mL, 11.84 mmol), and CuI (0.23 g, 1.18 mmol). The reaction mixture was degassed with house vacuum, placed under N_2 atmosphere, and stirred for 0.5 h. The reaction mixture was partitioned between EtOAc and H_2O. The layers were separated, and the aqueous layer was extracted several times with EtOAc. The combined organic extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by flash column chromatography (SiO_2, 1:1 hexanes−EtOAc) gave 1.38 g (63%) of arylacetylene **7** as a white foam: IR (thin film) 3300, 2250, 1715, 1669, 1498, 1269, 1142 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40−7.31 (2H, m), 7.04 (1H, bs), 6.95−6.89 (2H, m), 5.71 (1H, bs), 4.41 (1H, bs), 3.93 (3H, s), 3.60−3.51 (2H, m), 3.12 (1H, dd, J = 16.5, 6.0 Hz), 2.79 (1H, dd, J = 16.5, 7.5 Hz), 2.62−2.56 (2H, m), 1.48 (9H, s); MS (ES+) m/z 372 (M + H), 272 (M + H − Boc).

(*S*)-[1-[1-(2-Cyanoethyl)-1*H*-tetrazol-5-yl]-4-(2-methoxyphenyl)but-3-ynyl]carbamic Acid *tert*-Butyl Ester (8). The method of Duncia et al. was employed with minor modification.[19] To a solution of amide **7** in MeCN (15 mL) was added triphenylphosphine (2.26 g, 8.63 mmol). The reaction mixture was gently heated with a heat gun until a clear solution resulted. Upon cooling to 0 °C, a precipitate formed. Diisopropyl azodicarboxylate (1.70 mL, 8.63 mmol) and $TMSN_3$ (1.15 mL, 8.63 mmol) were added dropwise by syringe, alternating 10 drops at a time, starting with diisopropyl azodicarboxylate. During addition reaction became a clear-yellow solution. Within minutes of completion of addition, an off-white precipitate formed. Reaction mixture was warmed to room temperature and stirred for 1 h at 40 °C and then at room temperature for 16 h. The reaction mixture was diluted with EtOAc and water, the layers were separated, and the organic layer was washed with saturated $NaHCO_3$ solution and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated. Purification by flash column chromatography (eluent: 60:40 hexanes−EtOAc) gave 1.18 g (86%) of tetrazole **8** as a white solid: IR (thin film) 2254, 1701, 1600, 1494, 1264, 1166 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.32−7.23 (2H, m), 6.88 (2H, t, J = 7.5 Hz), 5.57 (1H, d, J = 10.5 Hz), 5.32−5.23 (1H, m), 4.81 (2H, t, J = 7.5 Hz), 3.88 (3H, s), 3.31−3.00 (4H, m), 0.91 (9H, s); MS (ES+) m/z 397 (M + H).

(*S*)-3-[5-[1-Amino-4-(2-methoxyphenyl)but-3-ynyl]tetrazol-1-yl]propionitrile (9). A solution of tetrazole **8** (1.11 g, 2.80 mmol) in formic acid (7 mL) was heated at 40 °C for 5 h. The reaction was cooled to room temperature and concentrated to near dryness. The residue was taken up in EtOAc and slowly made slightly basic (pH 8) with saturated $NaHCO_3$. The layers were separated, and the aqueous layer was extracted twice with EtOAc. The combined organic extracts were washed once with brine, dried over $MgSO_4$, filtered, and concentrated to give 0.80 g (97%) of the amine **9** as a near-colorless oil which was carried forward without purification: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.43 (2H, t, J = 6.8 Hz), 6.95−6.87 (2H, m), 4.95 (2H, ddt, J = 36.0, 13.5, 7.5 Hz), 4.57 (1H, t, J = 7.5 Hz), 3.87 (3H, s), 3.22 (2H, dd, J = 9.8, 3.0 Hz), 3.12 (2H, t, J = 9.8 Hz), 2.00 (2H, bs).

(*S*)-[[[1-[1-(2-Cyanoethyl)-1*H*-tetrazol-5-yl]-4-(2-methoxyphenyl)but-3-ynyl]amino]methyl]phosphonic Acid Diphenyl Ester (10). Phosphonate **10** was prepared as described previously.[5b,12d] To a solution of amine **9** (0.80 g, 2.70 mmol) in EtOAc (15 mL) at 0 °C was added a 37% solution of formaldehyde in H_2O (0.26 mL, 9.49 mmol). After 1 h, the reaction mixture was warmed to room temperature and stirred for an additional 16 h. The reaction mixture was diluted with EtOAc and washed with cold H_2O and cold brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated to give a white foam. To a solution of this foam in 3:1 PhMe−THF (20 mL) was added diphenyl phosphite (0.93 mL, 4.86 mmol; contains 10−15% phenol). The reaction mixture was heated to 65 °C with stirring for 1.5 h. Cooling, concentration, and purification by flash column chromatography gave 1.14 g (78%) of amino phosphonate **10** as a white solid: IR (thin film) 2256, 1601, 1260, 1186, 935 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38−7.23 (8H, m), 7.23−7.12 (4H, m), 7.10 (2H, d, J = 9.0 Hz), 6.92−6.81 (2H, m), 4.89−4.68 (3H, m), 3.82 (3H, s), 3.61−3.47 (1H, m), 3.40−2.92 (5H, m), 2.62 (1H, m); ^{31}P NMR ($CDCl_3$, 125 MHz) δ 17.78; MS (ES+) m/z 543 (M + H).

(*S*)-[[[4-(2-Methoxyphenyl)-1-(1*H*-tetrazol-5-yl)but-3-ynyl]amino]methyl]phosphonic Acid Diphenyl Ester (11). Tetrazole **11** was prepared as described previously.[5a,b] To a solution of tetrazole **10** (0.71 g, 1.31 mmol) in CH_2Cl_2 (10 mL) was added DBU (0.59 mL, 3.93 mmol). After 2 h, the reaction mixture was diluted with EtOAc and washed with 1 N HCl and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated. Purification by flash column chromatography on DIOL gel (eluent: 1:1 hexanes−EtOAc) gave 0.47 g (73%) of tetrazole **11** as a foamy white solid: IR (thin film) 1589, 1490, 1263, 1189, 943 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.43−7.10 (12H, m), 7.02−6.92 (2H, m), 4.61 (1H, t, J = 3.8 Hz), 4.05 (3H, s), 3.51−3.29 (2H, m), 3.09 (2H, dq, J = 16.5, 6.8 Hz); ^{31}P NMR ($CDCl_3$, 125 MHz) δ 16.68; MS (ES+) m/z 490 (M + H).

(*S*)-[[[4-(2-Methoxyphenyl)-1-(1*H*-tetrazol-5-yl)but-3-ynyl]amino]methyl]phosphonic Acid (15). Phosphonic acid **15** was prepared from phosphonate **11** as previously described.[5b] To a solution of phosphonate **11** (0.367 g, 0.750 mmol) in MeCN (7 mL) was added a 1.0 M solution of $NaHCO_3$ (7 mL) followed by H_2O (3 mL). The reaction mixture was heated to 50 °C, stirred for 16 h, diluted with H_2O, and extracted with EtOAc. The aqueous layer was acidified with 1 N HCl and cooled to 0 °C. After 1 h, a white precipitate had formed. The white solid was collected by filtration, washed with H_2O and Et_2O, and dried in vacuo. Recovered 0.146 g (58%) of phosphonic acid **15** as a white solid: mp 235 °C dec; IR (KBr) 2800 (b), 1608, 1261, 1071, 746 cm^{-1}; ^{1}H NMR DMSO-d_6−TFA, 300 MHz) δ 7.28 (1H, dt, J = 8.3, 3.0 Hz), 7.17 (1H, dd, J = 6.8, 3.0 Hz), 6.94 (1H, d, J = 8.3 Hz), 6.85 (1H, t, J = 6.8 Hz), 5.19 (1H, dd, J = 9.0, 6.0 Hz), 3.70 (3H, s), 3.52−3.17 (4H, m); ^{31}P NMR (DMSO-d_6−TFA, 125 MHz) δ 12.33; MS (ES−) m/z 336 (M − H). Anal. ($C_{13}H_{16}N_5O_4P$) C, H, N.

(*S,S*)-5-Phenyl-2-[(3,3,3-trifluoro-2-methoxy-2-phenylpropionyl)amino]pent-4-ynoic Acid (2-Cyanoethyl)amide (17). Stereochemical integrity of Pd(0) cross-coupling

was shown by preparing the Mosher amides of racemic and optically active (*S*)-[1-[(2-cyanoethyl)carbamoyl]-4-phenylbut-3-ynyl]carbamic acid *tert*-butyl ester (**7**, where R = H). The procedures for the racemate and single enantiomer were identical except (*R*)-Mosher acid chloride was used for the racemate and (*S*)-Mosher acid chloride was used for the single enantiomer. A solution of amide **7** (R = H) (0.150 g, 0.439 mmol) in formic acid (5 mL) was heated to 40 °C for 2 h. The reaction mixture was concentrated in vacuo to near dryness. The residue was taken up in EtOAc and slowly made slightly basic (pH 8) with saturated $NaHCO_3$ solution. The layers were separated, and the aqueous layer was extracted twice with EtOAc. The combined organic extracts were washed once with brine, dried over $MgSO_4$, filtered, and concentrated to give 0.110 g (quantitative yield) of the amine as a yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.99 (1H, bs), 7.42−7.27 (5H, m), 3.62−3.53 (3H, m), 2.97 (1H, dd, J = 16.0, 4.0 Hz), 2.84 (1H, dd, J = 16.0, 7.5 Hz), 2.64 (2H, m). To a solution of the above amine (0.060 g, 0.249 mmol) in CH_2Cl_2 (1 mL) was added triethylamine (0.038 mL, 0.273 mmol) followed by (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.051 mL, 0.273 mmol). After stirring for 1 h, the reaction mixture was diluted with EtOAc and washed with 1 N HCl, water, saturated $NaHCO_3$, water, and brine. The organic layer was dried over $MgSO_4$, filtered, concentrated, and passed through a plug of silica gel (eluent: 30% EtOAc in hexanes) to give 0.100 g (91%) of the Mosher amide **16**, (*R*,*R*)/(*R*,*S*)-5-phenyl-2-[(3,3,3-trifluoro-2-methoxy-2-phenylpropionyl)amino]pent-4-ynoic acid (2-cyanoethyl)amide: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.81 (1H, d, J = 9.0 Hz), 7.76 (1H, d, J = 9.0 Hz), 7.58−7.00 (20H, m), 4.86−4.72 (2H, m), 3.52−3.28 (10H, m), 2.98−2.80 (4H, m), 2.51 (2H, t, J = 7.5 Hz), 2.40 (2H, t, J = 7.5 Hz). Mosher amide **17** was prepared similarly, (*S*,*S*)-5-phenyl-2-[(3,3,3-trifluoro-2-methoxy-2-phenylpropionyl)amino]pent-4-ynoic acid (2-cyanoethyl)amide: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.81 (1H, d, J = 8.0 Hz), 7.51−7.25 (10H, m), 4.79 (1H, q, J = 7.1 Hz), 3.42 (1H, d, J = 1.2 Hz), 3.39 (3H, s), 3.29 (2H, q, J = 6.6 Hz), 2.86 (2H, d, J = 6.6 Hz), 2.36 (2H, t, J = 6.5 Hz).

Compounds **22**−**25** were prepared using standard peptide coupling, the Pd(0) cross-coupling described above,[16] and previously reported procedures.[5a,b,12d] Derivative **24** is representative.

(*S*,*S*)-2-[[2-[(*tert*-Butoxycarbonyl)amino]pent-4-ynoyl]-amino]-4-methylpentanoic Acid Methyl Ester (18). Dipeptide **18** (0.13 g, 83%) was prepared as described for amide **6** using acid **5** (0.10 g, 0.47 mmol) and L-leucine methyl ester hydrochloride (0.85 g, 0.47 mmol): IR (KBr) 2121, 1745, 1733, 1681, 1654 cm^{-1}; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 6.72 (1H, d, J = 7.4 Hz), 5.27 (1H, s), 4.64−4.56 (1H, m), 4.30−4.20 (1H, m), 3.70 (3H, s), 2.78 (1H, ddd, J = 18.2, 5.5, 2.7 Hz), 2.57 (1H, ddd, J = 18.2, 5.5, 2.7 Hz), 2.05 (1H, t, J = 2.6 Hz), 1.75−1.50 (3H, m), 1.43 (9H, s), 0.90 (6H, dd, J = 5.8, 2.2 Hz); MS (ES+) m/z 341 (M + H).

(*S*,*S*)-2-[[2-[(*tert*-Butoxycarbonyl)amino]-5-(2-chlorophenyl)pent-4-ynoyl]amino]-4-methylpentanoic Acid Methyl Ester (19). Arylacetylene **19** (0.386 g, 69%) was prepared as described for arylacetylene **7** using dipeptide **18** (0.420 g, 1.235 mmol) and 1-chloro-2-iodobenzene (0.17 mL, 1.420 mmol):[16] IR (thin film) 2256, 1739, 1720, 1679 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.48−7.40 (2H, m), 7.24−7.17 (2H, m), 6.78 (1H, bs), 5.51 (1H, bs), 4.68−4.62 (1H, m), 4.42 (1H, bs), 3.69 (3H, s), 3.15 (1H, dd, J = 17.3, 7.5 Hz), 2.86 (1H, dd, J = 18.8, 7.5 Hz), 1.60−1.54 (3H, m), 1.52 (9H, s), 0.85 (6H, dd, J = 16.5, 7.5 Hz); MS (ES+) m/z 451 (M + H).

(*S*,*S*)-2-[[2-Amino-5-(2-chlorophenyl)pent-4-ynoylamino]-4-methylpentanoic Acid Methyl Ester (20). Amine **20** (0.271 g, 90%) was prepared as previously described for amine **9** using arylacetylene **19** (0.386 g, 0.857 mmol) and carried forward without purification: IR (thin film) 2227, 1706, 1689 cm^{-1}; MS (ES+) m/z 351 (M + H).

(*S*,*S*)-2-[[5-(2-Chlorophenyl)-2-[[(dimethoxyphosphoryl)-methyl]amino]pent-4-ynoyl]amino]-4-methylpentanoic Acid Methyl Ester (21). Phosphonate **21** was prepared following literature procedures.[5a,12d] A solution of dimethyl-[[[(trifluoromethyl)sulfonyl]oxy]methyl]phosphonate[20] (0.263 g, 0.966 mmol) in CH_2Cl_2 (2 mL) was added to a solution of amine **20** (0.271 g, 0.773 mmol) and diisopropylethylamine (0.20 mL, 1.16 mmol) in CH_2Cl_2 at room temperature. After stirring for 16 h, the reaction mixture was diluted with EtOAc and washed with brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Purification using Flash40 chromatography system on silica gel (eluent: EtOAc) gave 0.226 g (62%) of phosphonate **21** as a film: IR (thin film) 2246, 1741, 1675, 1243 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.70 (1H, d, J = 7.5 Hz), 7.48−7.38 (2H, m), 7.24−7.18 (2H, m), 4.67−4.55 (1H, m), 3.88 (3H, d, J = 4.5 Hz), 3.82 (3H, d, J = 4.5 Hz), 3.72 (3H, s), 3.52 (1H, bs), 3.28−2.89 (4H, m), 1.70−1.56 (3H, m), 0.95−0.80 (6H, m); MS (ES+) m/z 473 (M + H).

(*S*,*S*)-2-[[5-(2-Chlorophenyl)-2-[(phosphonomethyl)amino]pent-4-ynoyl]amino]-4-methylpentanoic Acid (24). Phosphonic acid dipeptide **24** was prepared with slight modification of literature procedures.[12d] To a solution of phosphonate **21** (0.226 g, 0.478 mmol) in MeOH (2 mL) was added 1 N NaOH (1.5 mL). After 1.5 h, the reaction mixture was concentrated under reduced pressure to give the monomethyl phosphonate (92% pure, trace of dimethyl phosphonate): ^{1}H NMR (D_2O, 300 MHz) δ 7.45−7.38 (2H, m), 7.28−7.18 (2H, m), 4.18 (1H, dd, J = 9.8, 1.5 Hz), 3.50 (3H, d, J = 9.8 Hz), 3.50−3.43 (1H, m), 2.91 (2H, d, J = 3.0 Hz), 2.89−2.69 (2H, m), 1.57−1.38 (3H, m), 0.59 (6H, d, J = 4.5 Hz); ^{31}P NMR (D_2O, 121 MHz) δ 23.05. To a stirred slurry of the monomethyl phosphonate in CH_2Cl_2 (7 mL) was added diisoproylethylamine (0.42 mL, 2.39 mmol) followed by TMSBr (1.26 mL, 9.56 mmol). After 0.5 h, the reaction mixture was concentrated under reduced pressure without heat. To the recovered residue was added 50 mL of H_2O, and the resulting slurry was stirred for 0.5 h. The light-brown solid which formed was collected by filtration and washed with Et_2O. Recovered 0.092 g (45%) of phosphonic acid dipeptide **24** as a light-brown solid: mp 195 °C dec; IR (KBr) 1658, 1198, 1159 cm^{-1}; ^{1}H NMR (DMSO-d_6−TFA, 300 MHz) δ 7.56 (1H, dd, J = 7.4, 2.2 Hz), 7.48 (1H, d, J = 7.7 Hz), 7.38−7.28 (2H, m), 4.30−4.26 (2H, m), 3.32−3.12 (4H, m), 1.64−1.54 (3H, m), 0.86−0.83 (6H, m); ^{31}P NMR DMSO-d_6−TFA, 121 MHz) δ 12.29; MS (ES−) m/z 429 (M − H). Anal. ($C_{18}H_{24}ClN_2O_6P$) C, H, N.

Compounds **38**−**69** were prepared using standard peptide coupling, the alcalase enzymatic resolution, and Pd(0) cross-coupling described above[16] and previously reported procedures.[5a,12d] Derivative **41** is representative.

2-[(*tert*-Butoxycarbonyl)amino]-5-(2,4-difluorophenyl)pent-4-ynoic Acid Methyl Ester (26). The procedure described for arylacetylene **7** was employed.[16] Using propargylglycine methyl ester **3** (10.87 g, 47.8 mmol) and 2,4-difluoro-1-iodobenzene (6.3 mL, 52.7 mmol) gave 13.83 g (85%) of arylacetylene **26**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.33 (1H, q, J = 7.5 Hz), 6.79 (2H, t, J = 7.5 Hz), 5.38 (1H, d, J = 7.5 Hz), 4.56 (1H, dt, J = 10.7, 5.0 Hz), 3.79 (3H, s), 2.95 (2H, t, J = 5.00 Hz), 1.45 (9H, s).

(*S*)-2-[(*tert*-Butoxycarbonyl)amino]-5-(2,4-difluorophenyl)pent-4-ynoic Acid (27). The enzymatic resolution procedure described for acid **5** was employed. Using 19 g (56.0 mmol) of methyl ester **26**, alcalase resolution gave 8.72 g (96%, based on 50% recovery) of acid **27** and ester **28**. The resolved acid was found to be of 96% enantiomeric excess: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.64 (1H, bs), 7.33 (1H, q, J = 6.3 Hz), 6.78 (2H, t, J = 7.5 Hz), 5.40 (1H, d, J = 7.5 Hz), 4.65−4.53 (1H, m), 3.05−2.92 (2H, m), 1.46 (9H, s).

The enantiomeric excess of acid **27** and the 2-fluoro derivative were determined by examination of the ^{19}F NMR spectra of the derived Mosher amides. For comparison, the Mosher amides of the enantiomers, isolated from the resolution, were prepared. These were prepared as described above for amides **16** and **17** using (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride.

(*R*,*S*)-5-(2,4-Difluorophenyl)-2-[(3,3,3-trifluoro-2-methoxy-2-phenylpropionyl)amino]pent-4-ynoic acid methyl ester (29): ^{19}F NMR ($CDCl_3$, 470 MHz) δ −69.92 (98%), −70.08 (2%); diastereomeric excess found to be 96%.

(*R*,*R*)-5-(2,4-Difluorophenyl)-2-[(3,3,3-trifluoro-2-methoxy-2-phenylpropionyl)amino]pent-4-ynoic acid methyl ester (30): ^{19}F NMR ($CDCl_3$, 470 MHz) δ −69.91 (9%); −70.07 (91%); diastereomeric excess estimated to be 82%.

(*R*,*S*)-5-(2,4-Fluorophenyl)-2-[(3,3,3-trifluoro-2-methoxy-2-phenylpropionyl)amino]pent-4-ynoic acid methyl ester (31): ^{19}F NMR ($CDCl_3$, 470 MHz) δ −69.92; diastereomeric excess found to be 98.8%.

(*R*,*R*)-5-(2,4-Fluorophenyl)-2-[(3,3,3-trifluoro-2-methoxy-2-phenylpropionyl)amino]pent-4-ynoic acid methyl ester (32): ^{19}F NMR ($CDCl_3$, 470 MHz) δ −69.92 (8%), −70.05 (92%); diastereomeric excess estimated to be 84%.

(*S*)-2-[(*tert*-Butoxycarbonyl)amino]-5-(2,4-difluorophenyl)pent-4-ynoic Acid Methyl Ester (33). To a stirred solution of acid **27** (3.36 g, 10.3 mmol) in 4:1 PhMe−MeOH (50 mL) was added a 2.0 M solution of $TMSCHN_2$ in hexanes (13.0 mL, 26.0 mmol). After 1.5 h, the reaction mixture was quenched by the addition of acetic acid and then concentrated under reduced pressure. Ester **33** was recovered in quantatitive yield: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.33 (1H, q, J = 7.5 Hz), 6.79 (2H, t, J = 7.5 Hz), 5.38 (1H, d, J = 7.5 Hz), 4.56 (1H, dt, J = 10.7, 5.0 Hz), 3.79 (3H, s), 2.95 (2H, t, J = 5.00 Hz), 1.45 (9H, s).

(*S*)-2-Amino-5-(2,4-difluorophenyl)pent-4-ynoic Acid Methyl Ester (34). The method described for amine **9** was employed. Using 5.45 g (16.06 mmol) of **33** 3.68 g (96%) of amine **34** was recovered as a light-yellowish oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38 (1H, q, J = 7.5 Hz), 6.81 (2H, t, J = 7.5 Hz), 3.69 (3H, s), 3.72−3.62 (1H, m), 2.90 (2H, d, J = 7.5 Hz), 1.93 (2H, bs).

(*S*)-5-(2,4-Difluorophenyl)-2-[[(dimethoxyphosphoryl)-methyl]amino]pent-4-ynoic Acid Methyl Ester (35). The method described for phosphonate **21** was employed. Using 4.20 g (17.56 mmol) of amine **34** and 4.78 g (17.56 mmol) of dimethyl[[[(trifluoromethyl)sulfonyl]oxy]methyl]phosphonate gave 4.08 g (64%) of phosphonate **35** as an oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38 (1H, q, J = 7.5 Hz), 6.88−6.78 (2H, m), 3.83 (3H, s), 3.80 (6H, s), 3.69 (1H, t, J = 5.3 Hz), 3.15 (2H, dt, J = 54.8, 15.0 Hz), 2.92 (2H, d, J = 7.5 Hz).

(*S*)-5-(2,4-Difluorophenyl)-2-[[(dimethoxyphosphoryl)-methyl]amino]pent-4-ynoic Acid (36). To a stirred solution of phosphonate **35** (4.08 g, 11.29 mmol) in MeOH (60 mL) was added a 1.0 N solution of NaOH (11.3 mL). After 3.5 h, the reaction mixture was extracted with Et_2O. The remaining aqueous layer was acidified with 1 N HCl and extracted with EtOAc several times. The combined EtOAc extracts were washed with H_2O and brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give 3.29 g (84%) of acid **36** as an oil: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.52 (1H, q, J = 7.5 Hz), 7.46 (1H, dt, J = 7.5, 1.5 Hz), 7.11 (1H, dt, J = 7.5, 1.5 Hz), 3.68 (6H, d, J = 9.8 Hz), 3.54 (1H, t, J = 5.3 Hz), 3.07 (2H, app p, J = 15.0 Hz), 2.81 (2H, t, J = 3.8 Hz); ^{31}P NMR (DMSO-d_6, 121 MHz) δ 28.85; MS (ES+) m/z 348 (M + H).

(*S*,*S*)-2-[[5-(2,4-Difluorophenyl)-2-[[(dimethoxyphosphoryl)methyl]amino]pent-4-ynoyl]amino]-4-methylpentanoic Acid Methyl Ester (37). To a stirred solution of acid **36** (0.310 g, 0.890 mmol) and L-leucine methyl ester hydrochloride (0.162 g, 0.890 mmol) in DMF (15 mL) was added *N*-methylmorpholine (0.29 mL, 2.64 mmol) followed by benzotriazolyl-*N*-oxytris(dimethylamino)phosphonium hexafluorophosphate (0.592 g, 1.340 mmol). After 16 h, the reaction mixture was partitioned between EtOAc and 1 N HCl and the layers were separated. The organic phase was washed with H_2O, saturated $NaHCO_3$ solution, and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Purification using Flash40 chromatography system on silica gel (eluent: 95:5 EtOAc−EtOH) gave 0.337 g (79%) of dipeptide phosphonate **37** as an oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.57 (1H, q, J = 9.0 Hz), 7.43−7.32 (1H, m), 6.88−6.79 (2H, m), 4.67−4.58 (1H, m), 3.85 (3H, d, J = 6.8 Hz), 3.81 (3H, d, J = 6.8 Hz), 3.74 (3H, s), 3.46 (1H, dd, J = 8.3, 6.0 Hz), 3.25−2.79 (4H, m), 1.70−1.52 (3H, m), 0.89 (6H, t, J = 7.5 Hz); ^{31}P NMR ($CDCl_3$, 121 MHz) δ 27.98; MS (ES+) m/z 475 (M + H).

(*S*,*S*)-2-[[5-(2,4-Difluorophenyl)-2-[(phosphonomethyl)-amino]pent-4-ynoyl]amino]-4-methylpentanoic Acid (41). The method described for phosphonic acid dipeptide **24** was employed. Using 0.337 g (0.711 mmol) of phosphonate **37** gave 0.168 g (55%) of phosphonic acid **41**: mp 200 °C dec; IR (KBr) 1659, 1615, 1591, 1507, 1268, 1144, 1101, 850 cm^{-1}; ^{1}H NMR DMSO-d_6−TFA, 300 MHz) δ 7.56 (1H, dd, J = 14.7, 8.5 Hz), 7.25 (1H, dt, J = 9.6, 2.6 Hz), 7.09−7.04 (1H, m), 4.29−4.23 (2H, m), 3.27−3.11 (4H, m), 1.64−1.53 (3H, m), 0.86−0.82 (6H, m); ^{31}P NMR DMSO-d_6−TFA, 121 MHz) δ 12.26; MS (ES-) m/z 431 (M − H). Anal. ($C_{18}H_{23}F_2N_2O_6P$) H, N; C: calcd, 50.00; found, 49.35.

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Supporting Information Available: Experimental data for compounds **12**−**14**, **22**, **23**, **25**, **38**−**40**, and **42**−**70** (12 pages). Ordering information is given on any current masthead page.

References

(1) (a) Rubanyi, G. M.; Polokoff, M. A. Endothelins: Molecular Biology, Biochemistry, Pharmacology, Physiology, and Pathophysiology. *Pharmacol. Rev.* **1994**, *46*, 325−415. (b) Patel, T. R. Therapeutic Potential of Endothelin Receptor Antagonists in Cerebrovascular Disease. *CNS Drugs* **1996**, *5*, 293−310. (c) Benigni, A.; Remuzzi, G. The Renoprotective Potential of Endothelin Receptor Antagonists. *Exp. Opin. Ther. Patents* **1997**, *7*, 139−149.

(2) Battistini, B.; D'Orleans-Juste, P.; Sirois, P. Biology of Disease: Endothelins: Circulating Plasma Levels and Presence in Other Biologic Fluids. *Lab. Invest.* **1993**, *68*, 600−628.

(3) Turner, A. J.; Murphy, L. J. Molecular Pharmacology of Endothelin Converting Enzymes. *Biochem. Pharmacol.* **1996**, *51*, 91−102.

(4) Phosphoramidon (**1**), an NEP inhibitor with moderate ECE-1 inhibitory activity, has shown positive effects in several cardiovascular disease models: (a) Schultz, S. C.; Grady, B.; Cole, F.; Hamilton, I.; Burhop, K.; Malcolm, D. S. A Role for Endothelin and Nitric Oxide in the Pressor Response to Diaspirin Cross-Linked Hemoglobin. *J. Lab. Clin. Med.* **1993**, *122*, 301−308. (b) McMahon, E. G.; Palomo, M. A.; Moore, W. M. Phosphoramidon Blocks the Pressor Activity of Big Endothelin (1−39) and Lowers Blood Pressure in Spontaneously Hypertensive Rats. *J. Cardiovasc. Pharmacol.* **1991**, *17* (Suppl. 7), S29−S33. (c) Vemulapalli, S.; Watkins, R. W.; Brown, A.; Cook, J.; Bernardino, V.; Chiu, P. J. S. Disparate Effects of Phosphoramidon on Blood Pressure in SHR and DOCA-salt Hypertensive Rats. *Life Sci.* **1993**, *53*, 783−793. (d) Grover, G. J.; Sleph, P. G.; Fox, M.; Trippodo, N. C. Role of Endothelin-1 and Big Endothelin-1 in Modulating Coronary Vascular Tone, Contractile Function and Severity of Ischemia in Rat Hearts. *J. Pharmacol. Exp. Ther.* **1992**, *263*, 1074−1082. (e) Vemulapalli, S.; Chiu, P. J. S.; Chintala, M.; Bernardio, V. Attenuation of Ischemic Acute Renal Failure by Phosphoramidon in Rats. *Pharmacology* **1993**, *47*, 188−193. (f) Matsumura, Y.; Ikegawa, R.; Suzuki, Y.; Takaoka, M.; Uchida, T.; Kido, H.; Shinyama, H.; Hayashi, K.; Watanabe, M.; Morimoto, S. Phosphoramidon Prevents Cerebral Vasospasm Following Subarachnoid Hemorrhage in Dogs: The Relationship to Endothelin-1 Levels in the Cerebrospinal Fluid. *Life Sci.* **1991**, *49*, 841−848.

(5) (a) De Lombaert, S.; Blanchard, L.; Tan, J.; Sakane, Y.; Berry, C.; Ghai, R. D. Non-Peptidic Inhibitors of Neutral Endopeptidase 24.11: 1. Discovery and Optimization of Potency. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 145−150. (b) De Lombaert, S.; Blanchard, L.; Berry, C.; Ghai, R. D.; Trapani, A. J. Non-Peptidic Inhibitors of Neutral Endopeptidase 24.11: 2. Design and Pharmacology of Orally Active Phosphonate Prodrugs. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 151−154. (c) De Lombaert, S.; Ghai, R. D.; Jeng, A. Y.; Trapani, A. J.; Webb, R. L. Pharmacological Profile of a Non-

Peptidic Dual Inhibitor of Neutral Endopeptidase 24.11 and Endothelin-Converting Enzyme. *Biochem. Biophys. Res. Commun.* **1994**, *204*, 407−412. (d) De Lombaert, S.; Stamford, L. B.; Blanchard, L.; Tan, J.; Hoyer, D.; Diefenbacher, C. G.; Wei, D.; Wallace, E. M.; Moskal, M. A.; Savage, P.; Jeng, A. Y. Potent Non-Peptidic Dual Inhibitors of Endothelin-Converting Enzyme and Neutral Endopeptidase 24.11. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1059−1064. (e) Jeng, A. Y.; De Lombaert, S. Endothelin Converting Enzyme Inhibitors. *Curr. Pharm. Des.* **1997**, *3*, 541−558 and references therein. (f) De Lombaert, S.; Wallace, E. M.; Jeng, A. Y. Unpublished results.

(6) Chackalamannil and co-workers at Scherling-Plough have developed selective ECE-1 inhibitors. When our ECE-1 assays are normalized to phosphoramidon, their inhibitors show a greater than 2-fold selectivity for ECE-1 over NEP. Chankalamannil, S.; Chung, S.; Stamford, A. W.; McKittrick, B. A.; Wang, Y.; Tsai, H.; Cleven, R.; Fawzi, A.; Czarniecki, M. Highly Potent and Selective Inhibitors of Endothelin Converting Enzyme. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1257−1260.

(7) Schmidt, M.; Kröger, B.; Jacob, E.; Seulberger, H.; Subkowski, T.; Otter, R.; Meyer, T.; Schmalzing, G.; Hillen, H. Molecular Characterization of Human and Bovine Endothelin Converting Enzyme (ECE-1). *FEBS Lett.* **1994**, *356*, 238−243.

(8) Turner, A. J.; Tanzawa, K. Mammalian Membrane Metallopeptidases: NEP, ECE, KELL, and PEX. *FASEB J.* **1997**, *11*, 355−364.

(9) Stephenson, S. L.; Kenny, A. J. The Hydrolysis of Human Atrial Natriuretic Peptide by Pig Kidney Microvillar Membranes is Initiated by Neutral Endopeptidase-24.11. *Biochem. J.* **1987**, *243*, 183−187.

(10) Dusser, D. J.; Nadel, J. A.; Sekizawa, K.; Graf, P. D.; Borson, D. B. Neutral Endopeptidase and Angiotensin-Converting Enzyme Inhibitors Potentiate Kinin-Induced Contraction of Ferret Trachea. *J. Pharmacol. Exp. Ther.* **1988**, *244*, 531−536.

(11) Winquist, R. J.; Hintze, T. H. Mechanisms of Atrial Natriuretic Factor-Induced Vasodilation. *Pharmacol. Ther.* **1990**, *48*, 417−426.

(12) (a) Danilewicz, J. C.; Barclay, P. L.; Barnish, I. T.; Brown, S. F.; Campbell, S. F.; James, K.; Samuels, G. M. R.; Terrett, N. K.; Wythes, M. J. UK-69,578, a Novel Inhibitor of EC 3.4.24.11 which Increases Endogenous ANF Levels and is Natriuretic and Diuretic. *Biochem. Biophys. Res. Commun.* **1989**, *164*, 58−65. (b) Erdos, E. G.; Skidgel, R. A. Neutral Endopeptidase 24.11 (Enkephalinase) and Regulators of Peptide Hormones. *FASEB J.* **1989**, *3*, 145−151. (c) Gros, C.; Sougue, A.; Schwartz, J.-C.; Duchier, J.; Cournot, A.; Baumer, P.; Lacomte, J.-M. Protection of Atrial Natriuretic Factor Against Degradation, Diuretic and Natriuretic Responses after Inhibition of Enkephalinase (EC 3.4.24.11). *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7580−7585. (d) De Lombaert, S.; Erion, M. D.; Tan, J.; Blanchard, L.; El-Chehabi, L.; Ghai, R. D.; Sakane, Y.; Berry, C.; Trapani, A. J. *N*-Phosphonomethyl Dipeptides and Their Phosphonate Prodrugs, a New Generation of Neutral Endopeptidase (NEP, EC 3.4.24.11) Inhibitors. *J. Med. Chem.* **1994**, *37*, 498−511.

(13) (a) Trapani, A. J.; De Lombaert, S. D.; Kuzmich, S.; Jeng, A. Y. Inhibition of Big ET-1-Induced Pressor Response by an Orally Active Dual Inhibitor of Endothelin-Converting Enzyme and Neutral Endopeptidase 24.11. *J. Cardiovasc. Pharmacol.* **1995**, *26*, S69−S71. (b) Caner, H. H.; Kwan, A. L.; Arthur, A.; Jeng, A. Y.; Lappe, R. W.; Kassell, N. F.; Lee, K. S. Systemic Administration of an Inhibitor of Endothelin-Converting Enzyme for Attenuation of Cerebral Vasospasm Following Experimental Subarachnoid Hemorrhage. *J. Neurosurg.* **1996**, *85*, 917−922.

(14) (a) Abassi, Z.; Golomb, E.; Keiser, H. R. Neutral Endopeptidase Inhibition Increases the Urinary Excretion and Plasma Levels of Endothelin. *Metab. Clin. Exp.* **1992**, *41*, 683−685. (b) Sokolovsky, M.; Galron, R.; Kloog, Y.; Bdolah, A.; Indig, F. E.; Blumberg, S.; Fleminger, G. Endothelins are More Sensitive than Sarafotoxins to Neutral Endopeptidase: Possible Physiological Significance. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 4702−4706.

(15) Abood, N. A.; Nosal, R. Zinc Mediated Addition of Active Halides to a Glycine Cation Equivalent: Synthesis of *N*-BOC-*L*-propargylglycine. *Tetrahedron Lett.* **1994**, *35*, 3669−3672.

(16) Crisp, G. T.; Robertson, T. A. Palladium-Catalyzed Coupling of Propargylglycine Derivative. *Tetrahedron Lett.* **1992**, *48*, 3239−3250.

(17) Dale, J. A.; Dull, D. L.; Mosher, H. S. α-Methoxy-α-trifluoromethylphenylacetic Acid, a Versatile Reagent for the Determination of Enantiomeric Composition of Alcohols and Amines. *J. Org. Chem.* **1969**, *34*, 2543−2549.

(18) While **14** was shown to be a dual inhibitor of ECE-1 and NEP (Table 1), it has been previously shown that a pure NEP inhibitor has no effect in the big ET-1-induced pressor response.[5c]

(19) Duncia, J. V.; Pierce, M. E.; Santella, J. B., III. Three Synthetic Routes to a Sterically Hindered Tetrazole. A New One-Step Mild Conversion of an Amide into a Tetrazole. *J. Org. Chem.* **1991**, *56*, 2395−2400.

(20) Phillion, D. P.; Andrew, S. S. Synthesis and Reactivity of Diethyl Phosphonomethyl-triflate. *Tetrahedron Lett.* **1986**, *27*, 1477−1480.

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