Retro-Inverso Concept Applied to the Complete Inhibitors of Enkephalin-Degrading Enzymes

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Peptide retro-inverso modification was applied to the complete hydroxamate inhibitors of the three zinc metallopeptidases (neutral endopeptidase 24-11 (NEP, EC 3.4.24.11), aminopeptidase N (APN, EC 3.4.11.2), and a dipeptidylaminopeptidase (DAP) involved in the in vitro enkephalin degradation by brain tissues. Compounds corresponding to the general formula $RN(OH)CO(CH_2)_nCH(CH_2Ph)NHCOCH(R')COOH$ (n = 0, 1) were synthesized. In the first series of inhibitors (n = 0), the "retro-inverso" modification induced a large decrease in inhibitory potency for NEP as compared to that of the parent compounds. In contrast, the presence of a methylene group between the hydroxamate and CHα in the second series (n = 1) led to derivatives with inhibitory potencies in the nanomolar range, similar to their analogues with a natural amide bond. On the other hand, the retro-inverso modification led to a slight improvement in the inhibition of DAP and APN, in the first series of inhibitors, while the inverse result occurred in the second series. Thus, compounds containing an α-amino acid moiety in P'_1 position behave as weak inhibitors of the three enzymes, with IC_{50} values in the micromolar range, and compounds bearing a β-amino acid moiety in the same position are more specific than the parent compounds for NEP inhibition.

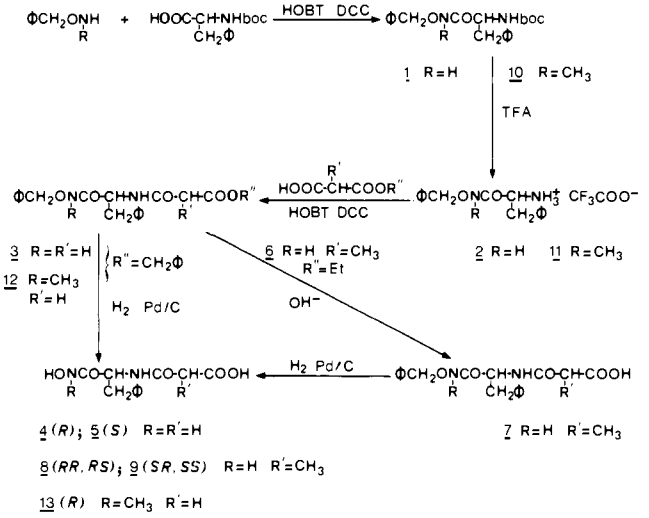
To study the physiological responses associated with opioid receptor stimulation by the endogenous morphine-like peptides Met-enkephalin (Tyr-Gly-Gly-Phe-Met) and Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu), the in vivo stability of these molecules should be increased. It has been established that the enkephalins are rapidly degraded in vivo by at least two brain enzymes, the neutral endopeptidase (EC 3.4.24.11, NEP) sometimes termed enkephalinase, which cleaves the Gly^3-Phe^4 bond of these peptides,[1,2] and aminopeptidase N (EC 3.4.11.2, APN), which releases the N-terminal tyrosine.[3,4] The physiological relevance of these peptidases has been clearly demonstrated by the analgesic responses induced by icv administration of thiorphan ($HSCH_2CH(CH_2Ph)CONHCH_2COOH$), an NEP inhibitor,[5] and bestatin, an aminopeptidase inhibitor.[6,7] Furthermore, a dipeptidylaminopeptidase activity (DAP) that cleaves the Gly^2–Gly^3 bond of enkephalins has been evidenced by in vitro experiments,[8] although its exact role in enkephalin catabolism has not been clearly established.[9,10] This heterogeneous degradation pathway led us to investigate the potential usefulness of complete inhibitors of the three peptidases. As expected, kelatorphan ($OHNHCOCH_2CH(CH_2Ph)CONHCH(CH_3)COOH$), a bidentate-containing derivative, which represents the first complete inhibitor of enkephalin degradation,[11,12] exhibits biochemical and pharmacological properties better than those produced by the association of bestatin and thiorphan.[13-17]

It has previously been shown that retro inversion of the thiorphan amide bond, in *retro*-thiorphan ($HSCH_2CH(CH_2Ph)NHCOCH_2COOH$),[18] leads to an NEP inhibitor as efficient as the parent compound. This compound represents the first application of the concept of retro inversion in enzyme inhibitor synthesis. The success of this approach in the thiorphan–*retro*-thiorphan series prompted us to develop a new series of inhibitors derived from kelatorphan by retro inversion of the amide bond. Based on the previously described bidentate inhibitors, two series of compounds, corresponding to the general formula $HONHCO(CH_2)_nCH(CH_2Ph)NHCOCH(R)COOH$ with n = 0, 1, were synthesized, and their biochemical properties were studied on the three enkephalin-degrading enzymes.

Results

(1) **Synthesis.** The synthesis of the first series of inhibitors, corresponding to *N*-(2-carboxy-1-oxoethyl)-phenylalanine N'-hydroxyamide (**4**, **5**) and their derivatives, **8**, **9**, and **13**, involved two main steps: creation of

Scheme I. Synthesis of (*R*)- and (*S*)-*N*-(2-Carboxy-1-oxoethyl)phenylalanine *N'*-Hydroxyamide (**4**, **5**) and Their Derivatives



phenylalanine N'-hydroxyamide (**4**, **5**) and their derivatives, **8**, **9**, and **13**, involved two main steps: creation of

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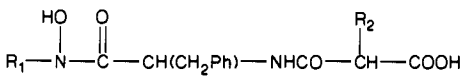
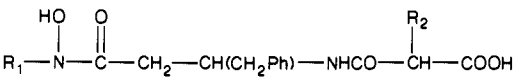
Table I. Inhibitory Potency of the Retro-Inverso Hydroxamates on the Three Enkephalin-Degrading Activities

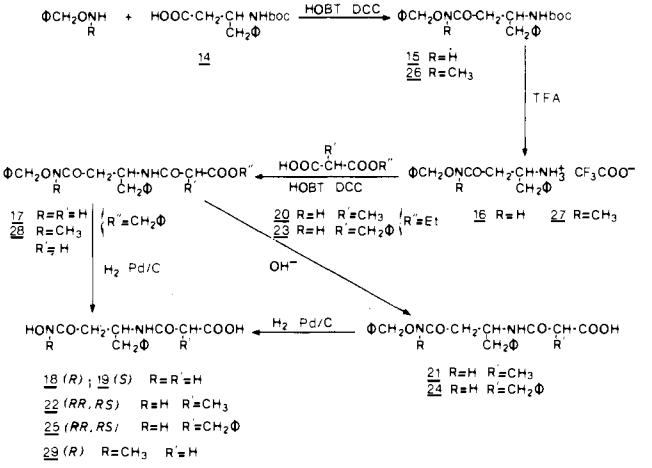
no.	R_1	R_2	stereochem	IC_{50},[a] nm NEP[b]	APN[c]	DAP[c]
			Series I			
						
4	H	H	*R*	3350 ± 500	~20,000	~32 000
5	H	H	*S*	2500 ± 250	~400,000	2000 ± 110
8	H	CH_3	*RR* + *RS*	945 ± 55	6100 ± 830	2700 ± 300
9	H	CH_3	*SR* + *SS*	320 ± 50	~100 000	71 ± 8
13	CH_3	H	*R*	6070 ± 225	ND[d]	ND
			Series II			
						
18	H	H	*R*	0.5 ± 0.05	2000 ± 500	140 ± 60
19	H	H	*S*	2.5 ± 0.5	150,000	450 ± 150
22	H	CH_3	*RR* + *RS*	1.6 ± 0.6	8600 ± 500	17.5 ± 2.5
25	H	CH_2Ph	*RR* + *RS*	3.7 ± 1.3	6200 ± 2000	32.5 ± 7.5
29	CH_3	H	*R*	1730 ± 225	>100 000	ND

[a] Values are the mean ± SEM from four independent experiments computed by log probit analysis of five inhibitor concentrations. [b] Concentration inhibiting 50% of NEP activity, with 20 nM of [^{3}H]D-Ala^2-Leu-enkephalin as substrate. [c] Concentration inhibiting 50% of APN or DAP activity, with 10 nM of [^{3}H]Leu-enkephalin as substrate. [d] ND = not determined.

the hydroxamate function by reaction of a O-protected hydroxylamine derivative on the carboxylate group of a L- or D-phenylalanine followed by formation of the *retro*-amide bond through a coupling step between the amino group of the D- or L-phenylalanine hydroxamate and various substituted malonic derivatives.

Compounds **4** and **5** were obtained by using Boc-D- and -L-phenylalanine, respectively, as starting material. After coupling with *O*-benzylhydroxylamine by the classical DCC/HOBT method, the α-amino group of L- and D-phenylalanine was deprotected by trifluoroacetic acid and condensed with the monobenzyl ester of malonic acid. The hydrogenolysis of the two benzyl protecting groups led finally to compounds **4** and **5** (Scheme I). Similarly, compounds **8** and **9** were obtained by using the monoethyl ester of 2-methylmalonic acid in the second coupling step in place of the monobenzyl ester of malonic acid. For these two derivatives, a sequential release of the protecting groups was carried out by alkaline hydrolysis for the ethyl ester and hydrogenolysis for the *O*-benzyl group (Scheme I). The last compound of this series, **13**, was obtained by the same method with *N*-methyl-*O*-benzylhydroxylamine in place of *O*-benzylhydroxylamine. This *N*-methyl derivative of the commercially available *O*-benzylhydroxylamine was synthesized as previously described.[19] The synthesis of the second series of inhibitors (compounds **18**, **19**, **22**, **25**, and **29**) followed the same sequential steps, from

Scheme II. Synthesis of (*R*)- and (*S*)-*N*-(2-Carboxy-1-oxoethyl)-β-phenylalanine *N'*-Hydroxyamide (**18**, **19**) and Their Derivatives



Boc-(*R*)- and -(*S*)-3-amino-4-phenylbutanoic acid corresponding to D- and L-β-phenylalanine (Scheme II). These two β-amino acids were obtained from the corresponding α-amino acids by the method of Arndt–Eistert.[20] The Boc-phenylalanine was first converted into the diazo ketone analogue, which then underwent the Wolff rearrangement with Ag_2O. The β-amino acids obtained by this method retain their full configuration. Consequently, the various molecules obtained in these syntheses contain either one asymmetric center (compounds **18**, **19**, and **29**) borne on the amino acid moiety and, in this case, the absolute configuration of this carbon is known or two asymmetric centers (compounds **22** and **25**) borne on the amino acid and the malonic moieties, respectively. In the latter case the molecules were synthesized as diastereoisomeric mixtures, which were not separated. It has been shown that 2-substituted malonic acids or amides are subject to fast racemization due to keto–enol exchange involving the asymmetric carbon,[21] and recent studies on

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partially retro-inverso peptides[22] or inhibitors[23] have shown that after separation by HPLC the pure isomers are epimerized at 25 °C in few hours.

(2) Inhibitory Potency of Retro Inhibitors on the Enkephalin Degrading Enzyme Activities. The inhibitory potency of the two series of hydroxamates was tested on rabbit kidney NEP with [^{3}H]D-Ala^2-Leu-enkephalin as substrate (K_m = 20 μM), on pig kidney APN and a membrane-bound DAP purified from rat brain, with [^{3}H]Leu-enkephalin as substrate (K_m = 50 and 25 μM on APN and DAP, respectively).[12]

(a) NEP Inhibition. As shown in Table I, the first series of molecules were relatively poor inhibitors of NEP with IC_{50} values in the micromolar range. A small influence of the absolute configuration of the phenylalanine moiety on inhibitory potency was noted with the *S* isomer (compound **5**) being slightly more efficient than the *R* isomer (compound **4**). This result was rather unexpected since, due to the inversion of the amide bond, the *R* isomer possesses a spatial orientation of the lateral chain identical with that of a natural amino acid. Owing to the racemic form of the malonyl moiety, compounds **8** and **9** correspond to mixtures of diastereoisomers, which are somewhat more active than **4** and **5**, and a comparison of their IC_{50}'s indicates the same influence of the phenylalanine stereochemistry. The methylation of the hydroxamate function (compound **13**) induces a 2-fold loss of affinity.

In contrast, for the second series of inhibitors, characterized by a methylene spacer between the hydroxamate group and the phenylalanine moiety, a large increase in the inhibitory potency was observed, with IC_{50} values in the nanomolar range for compounds **18**, **19**, **22**, and **25**. Contrasting with the previous series, a comparison of the potency of compounds **18** and **19** shows the expected stereochemical dependence of activity since the *R* isomer is slightly more efficient (by a factor of 5) than the *S* isomer. The introduction of the more hydrophobic benzyl moiety on the malonyl part did not significantly modify the inhibition of NEP. Finally, the methylation of the hydroxamate group, in compound **29**, produced a dramatic loss of activity, by a factor of 1000. However, it is interesting to note that the IC_{50} obtained for compound **29** (1730 nM) is better than that determined for compound **4** and by extension for compound **13**.

(b) APN Inhibition. As shown in Table I, the inhibitory potencies of all the compounds synthesized were relatively low, especially for the first series of molecules. A large influence of the absolute configuration of the α carbon of the phenylalanine moiety was observed by comparison of the IC_{50} values of compounds **4** and **5**, **8** and **9**, and finally **18** and **19**. In the two series, the *R* isomer was more efficient, as expected, if it is accepted that the benzyl moiety is located in the S'_1 subsite of the peptidase. The introduction of a methylmalonyl group as the P'_2 residue in compound **8** led to a large increase in inhibitory potency, as compared to compound **4**, but curiously, in the second series the presence of a methyl (compound **22**) or a benzyl (compound **25**) group on the P'_2 moiety produced a decrease in affinity as compared to the unsubstituted compound **18**. The best inhibitor of aminopeptidase activity was compound **18** with a value of IC_{50} of 2 μM.

(c) DAP Inhibition. As reported in Table I, the IC_{50} values of all compounds on DAP activity reflect the important role played by both the stereochemistry of the P'_1 residue and the nature of the P'_2 moiety. Compounds **5** and **9**, which contain a L-Phe component, *S* isomers, were much more efficiently recognized by DAP than the *R* isomers (compounds **4** and **8**). The opposite effect occurred in the second series, with the R isomer (compound **18**) slightly more efficient (factor of 3) than the *S* isomer (compound **19**). Furthermore, the introduction of hydrophobic malonyl moieties in compounds **9**, **22**, and **25** induced a significant increase in the DAP inhibition. Finally, among these new inhibitors, it is very interesting to notice a change in the selectivity for compound **9**, which becomes more potent against DAP than against the two other peptidases.

Discussion

(1) Stereochemical Aspects of the Recognition of the Three Enkephalin-Degrading Enzymes by "*retro*-Hydroxamate" Inhibitors. The similarity in IC_{50} values on NEP for the enantiomers **4** and **5**, on one hand, and **18** and **19**, on the other hand, shows that the influence of the absolute configuration of the benzyl moiety is very weak. The same phenomenon, already observed with thiol[23] and hydroxamate-containing inhibitors,[12] contrasts with the results obtained in *retro*-thiorphan series in which a large difference in the inhibitory potency of *R* and *S* isomers was found.[23]

For compounds **4** and **5**, which are relatively weak NEP inhibitors, it may be assumed that the relatively low stabilizing interactions are not greatly influenced by the spatial orientation of the amino acid moiety. However the IC_{50} ratio of 5 between *R* (compound **18**) and *S* (compound **19**) isomers, as compared to the IC_{50} ratio of 100 obtained between the *R* and *S* isomers of *retro*-thiorphan is striking. Since the thiol and the hydroxamate inhibitors differ only by the nature of the zinc-chelating group, the geometrical parameters of their complex with the metal atom could explain the differences between both series. In complexes between thermolysin and thiol inhibitors, the zinc cation is tetracoordinated in a pseudotetrahedric structure[24] whereas it is pentacoordinated in a distorted tetragonal pyramide in the case of hydroxamate-containing inhibitors.[25] Furthermore, the conformational space accessible to the thiol group is smaller than that accessible to the hydroxamate function. Indeed, starting from the α carbon, there is only one degree of freedom (a rotation around the $C\alpha$–CH_2 bond) in *retro*-thiorphan and two degrees of freedom (rotations around the $C\alpha$–CH_2 and CH_2–CO bonds) in the hydroxamates. The present data suggest that the association of these two structural parameters allows the hydroxamate group of an (S)-β-amino acid to interact as strongly as that of an (*R*)-β-amino acid.

The comparison of the inhibitory potencies of compounds **4**, **5**, **18**, and **19** on DAP seems to indicate the same type of stereochemical requirements as observed for NEP: in the first series (compounds **4** and **5**) the *S* isomer has a higher affinity than the *R* isomer, and the reverse effect is obtained in the second series (compounds **18** and **19**). The influence of the absolute configuration of the amino acid moiety is more important in the first series (factor of 12) than in the second series (factor of 3). It is known that this enzyme contains highly hydrophobic subsites in the S'_1 and S'_2 position,[10] and the increased affinity of compounds **8** and **9** versus **4** and **5** or of compounds **22** and **25** versus **18** and **19** emphasizes the importance of this parameter in DAP recognition.

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Table II. Inhibitory Potency of Retro-Inverso Hydroxamates and Their Parent Compounds on Neutral Endopeptidase, Aminopeptidase N, and Dipeptidylaminopeptidase Activities

compound	no.	IC_{50},[a] nM NEP[b]	APN[c]	DAP[c]
HONH-CO-CH(CH_2Ph)-NHCO-CH_2-COOH (*R*)	**4**	3350 ± 500	~20 000	~32 000
HONH-CO-CH(CH_2Ph)-NHCO-CH_2-COOH (*S*)	**5**	2500 ± 300	400 000	2000 ± 200
HONH-CO-CH(CH_2Ph)-CONH-CH_2-COOH (*R,S*)	A	12 ± 1	100 000 ± 5000	35 000 ± 5000
HONH-CO-CH_2-CH(CH_2Ph)-NHCO-CH_2-COOH (*R*)	**18**	0.5 ± 0.05	2000 ± 500	140 ± 60
HONH-CO-CH_2-CH(CH_2Ph)-NHCO-CH_2-COOH (*S*)	**19**	2.5 ± 0.5	150 000 ± 5000	450 ± 150
HONH-CO-CH_2-CH(CH_2Ph)-CONH-CH_2-COOH (*R,S*)	B	1.4 ± 0.4	2000 ± 200	21 ± 5
HONH-CO-CH_2-CH(CH_2Ph)-NHCO-CH(CH_3)-COOH (*RR* + *RS*)	**22**	1.6 ± 0.6	8600 ± 500	17.5 ± 2.5
HONH-CO-CH_2-CH(CH_2Ph)-CONH-CH(CH_3)-COOH (*RS*)	C	1.7 ± 0.6	380 ± 50	0.9 ± 0.1
HONH-CO-CH_2-CH(CH_2Ph)-NHCO-CH(CH_2Ph)-COOH (*RR* + *RS*)	**25**	3.7 ± 1.3	6200 ± 2000	32.5 ± 7.5
HONH-CO-CH_2-CH(CH_2Ph)-CONH-CH(CH_2Ph)-COOH (*RS*)	D	2.5 ± 0.7	130 ± 10	2.5 ± 0.6

[a] Values are the mean ± SEM from four independent experiments computed by log probit analysis of five inhibitor concentrations. [b] Concentrations inhibiting 50% of NEP activity, with 20 nM of [^{3}H]D-Ala^2-Leu-enkephalin as substrate. [c] Concentration inhibiting 50% of APN or DAP activity, with 10 nM of [^{3}H]Leu-enkephalin as substrate.

On the other hand, the lack of potency of N-methylated hydroxamates **13** and **29**, as compared to their analogues **4** and **18**, is in accordance with previously reported results on thermolysin-[26] and enkephalin-degrading enzymes,[11] which have shown an important decrease in the activity of N-substituted bidentate inhibitors. This has been interpreted, from crystallographic data on thermolysin,[25] as the consequence of a steric hindrance between the methyl group of the bidentate inhibitor and the residue Ala-113 of the protein. The steric contact reduces the stabilizing interactions between these two entities. This phenomenon seems to be true for other metallopeptidases, and the results obtained in this study are in accordance with the mechanism proposed. It would appear that the higher the affinity of the inhibitor for the enzyme, the greater the loss of potency caused by methylation.

The data for aminopeptidase N inhibition provide some interesting informations on the structural requirements of the S'_1 position of this enzyme's active site. A very large difference appears in the IC_{50} values of the two enantiomers **4** and **5** or **18** and **19** with the *R* isomer being more potent in both cases. It should be recalled that, in these retro inhibitors, the spatial orientation of the functional groups in the *R* isomer is similar to that occurring in a natural amino acid. These results are in accordance with the data reported by Nishizawa et al.[27] on the inhibition of leucine aminopeptidase by various stereoisomers of bestatin. Indeed, for this molecule a change in the absolute configuration of the leucine residue, which interacts with the S'_1 subsite, induces a loss of potency by factors ranging from 40 to 350, depending on the stereochemistry of the 3-amino-2-hydroxy-4-phenylbutanoic acid (AHPA) moiety.

(2) Structural Aspects of the Differential Recognition of "Hydroxamate" and "*retro*-Hydroxamate" Inhibitors by the Three Enzymes. In order to determine the influence of the retro inversion on the inhibition of the three enkephalin-degrading enzymes, the molecules were compared with their parent compounds, which contain a standard amide bond[12] (Table II).

However, from a stereochemical point of view, direct comparison of the inhibition constants is difficult. Compounds **4** and **5**, on one hand, and **18** and **19**, on the other hand, are pure stereoisomers while the parent compounds A and B, respectively, are racemic mixtures. Furthermore, compounds **22** and **25**, which correspond to diastereoisomeric mixtures due to the racemization of the methyl and benzyl malonyl moieties (P'_2 chain), have to be compared with C and D, which possess a well-defined absolute configuration. However, taking these limitations into account a number of observations can be made.

(a) NEP Recognition. The comparative study of the inhibitory potency of compounds **4** and **5** with their analogue A on NEP activity shows that retroinversion is a very unfavorable influence. Conversely, compounds **18** and **19** have the same range of inhibitory activity as their analogue B. Similar potencies were also observed for compounds **22** and C and compounds **25** and D, respectively. The results obtained with this second series of inhibitors confirm the ability of NEP to recognize a *retro*-amide bond as well as a standard amide bond.

(b) Aminopeptidase N Recognition. There are three different aspects to the comparison of the inhibitory potency of these various compounds: (i) the IC_{50}'s of compounds **4**, **5**, and A are very high and more precise information cannot be extracted from these data; (ii) the IC_{50}'s of compounds **18** and B are identical; this suggests that APN is able to accept the retro inversion of the amide bond; however, (iii) the presence of substituants on the malonyl part of **22** and **25** plays an inverse role to that induced by these groups on the amino acid moiety in C and D. This latter result is interesting, since it is well known that the S'_1 and S'_2 subsites of aminopeptidase N are hydrophobic.[28] A hypothesis may be proposed to explain this apparent discrepancy, Holmes and Matthews[25] have shown by a crystallographic analysis of the complex thermolysin–hydroxamate, that the malonyl moiety presents a planar structure due to the tautomeric form of this group in the active site of the enzyme. If the same structure exists in the *retro*-hydroxamate for the C-terminal malonyl moiety of compounds **22** and **25**, it is possible that the methyl and the benzyl chains induce steric hindrance in the active site of the enzyme. However to verify this hypothesis crystallographic data on the APN inhibitor complex would be required.

(c) Dipeptidylaminopeptidase Recognition. As with APN inhibition, the comparison between the high IC_{50} values of compounds **4**, **5**, and A on DAP activity does not provide very significant data on the ability of this peptidase to recognize a retroamide bond. On the other hand the systematic increase in the IC_{50}'s of the retro inhibitors **18**, **19**, **22**, and **25**, as related to their parent analogues B, C, and D, reflects a small but significant unfavorable influence of the amide bond retro inversion on DAP recognition.

Conclusion

The main result of this study is the demonstration that NEP can be strongly inhibited by analogues of retrodi-

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peptides containing an hydroxamate group as zinc-chelating agent. However, for these compounds the presence of a methylene spacer between the hydroxamate and the benzyl moiety, assumed to interact with the hydrophobic S'_1 subsite, is essential for an optimal recognition of the enzyme's active site. With IC_{50}'s in the nanomolar range, compound 18 (*N*-(2-carboxy-1-oxopropyl)-3-amino-4-phenyl-*N'*-hydroxybutanamide) and its derivatives **22** and **25** are at least as efficient as thiorphan or kelatorphan in inhibiting NEP. APN and DAP may be also inhibited by "*retro*-hydroxamate" containing a β-phenylalanine moiety in the P'_1 position. However for DAP, the affinity constants of the various retro inhibitors are greater than those obtained with the parent compounds, whatever the substituant introduced on the P'_2 malonyl moiety. For APN inhibition, an unexpected inverse effect of the substitution on the P'_2 residue is observed for the "*retro*-hydroxamates" as compared to the standard inhibitors. Consequently, the retro inversion of the amide bond in the kelatorphan series led to highly efficient and more selective NEP inhibitors.

Experimental Section

Biological Tests. [^{3}H]Leu-enkephalin (35 Ci/mmol) was obtained from New England Nuclear and [^{3}H]Tyr^1-D-Ala^2-Leu-enkephalin (32 Ci/mmol) was obtained from the Centre d'Etude Atomique (CEA, France).

Purification of Enkephalin Degrading Enzymes. Enkephalinase (NEP, EC 3.4.24.11) was purified to homogeneity from rabbit kidney.[29] A single band at 94 kD was observed by polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$. The membrane-bound dipeptidylaminopeptidase was partially purified from rat brain as previously reported.[11]

Aminopeptidase N purified from pig kidney was obtained from Boehringer and used without further purification.

Determination of Inhibitory Potencies. Inhibitory potencies were measured via a procedure previously described.[12] NEP (at a final concentration of 1 pmol/100 μL) was preincubated for 15 min at 25 °C with or without increasing concentrations of inhibitor in a total volume of 100 μL in 50 mM Tris·HCl buffer (pH 7.0). [^{3}H]D-Ala^2-Leu-enkephalin (K_m = 30 μM) was added to a final concentration of 20 nM, and the reaction was stopped after 30 min by adding 10 μL of 0.5 M HCl. The dipeptidylaminopeptidase and the aminopeptidase activity was measured by the same procedure by using [^{3}H]Leu-enkephalin, at final concentration of 10 nM, as substrate (K_m = 25 μM for DAP; K_m = 50 μM for APN). The tritiated metabolites formed were separated on polystyrene beads.[30]

Chemistry. The protected amino acids were from Bachem (Switzerland). Dibenzyl malonate, diethyl methylmalonate, diethyl benzylmalonate, and *O*-benzylhydroxylamine were from Janssen-Chimica (Belgium). All the other reagents and solvents (Normapur label) were from Prolabo (France). The monosaponification of the various malonyl diester was performed by using the procedure of Curtius and Sieber.[31] L- and D-Phe-diazo ketones were prepared according to Sharpe and Szelke.[32] The purity of all the synthesized compounds was checked by thin-layer chromatography on silica gel plates (E. Merck) with the following solvent systems (v/v): A, $CHCl_3$/MeOH (9:1); B, $CHCl_3$/MeOH/AcOH (7:3:0.5); C, $CHCl_3$/MeOH/AcOH (9:1:0,1); D, $CHCl_3$/MeOH (7:3); E, $CHCl_3$/MeOH (8:2); F, butanol/AcOH/H_2O (4:1:1); G, CH_2Cl_2/MeOH/AcOH (8:2:0.2). 1-Hydroxybenzotriazole was used in its hydrated form. The solution of this reagent in THF was therefore dried by shaking over 4-Å molecular sieves for 10 min before use. The purity of the final compounds was checked also by HPLC on a reversed-phase μ Bondapak C_{18} column (Waters). The eluted peaks were monitored at 257 nm. The structure of all the compounds was confirmed by ^{1}H NMR spectroscopy (Brüker WH 270 MHz) in Me_2SO-d_6 (5×10^{-3} M). Chemical shifts (in parts per million ± 0.02) relative to HMDS as internal reference are reported only for the final compounds. Melting points of the crystallized compounds were determined on a Kofler apparatus (±2 °C) and are reported uncorrected. The following abreviations are used: MeOH, methanol; EtOH, ethanol; AcOH, acetic acid; Et_2O, diethyl ether; THF, tetrahydrofuran; EtOAc, ethyl acetate; TFA, trifluoroacetic acid; Me_2SO-d_6, hexadeuteriodimethyl sulfoxide; HMDS, hexamethyldisiloxane; *t*-Boc, *tert*-butyloxycarbonyl.

***N*-*t*-Boc-D-phenylalanine *N'*-(Benzyloxy)amide (1). Procedure A.** To a solution of 3 g (11.3 mmol) of *N*-Boc-D-phenylalanine in $CHCl_3$ (15 mL), cooled to 0 °C, were added successively 1.8 g (11.3 mmol) of *O*-benzylhydroxylamine hydrochloride and 1.6 mL of triethylamine in $CHCl_3$ (15 mL), 1.73 g (11.3 mmol) of 1-hydroxybenzotriazole in THF (20 mL), and 2.56 g (12.43 mmol) of dicyclohexylcarbodiimide in $CHCl_3$ (15 mL). After 1 h at 0 °C, the mixture was stirred at room temperature overnight. After filtration of dicyclohexylurea (DCU) and evaporation of the solvents, the residue was dissolved in EtOAc and washed successively with water (30 mL), 10% citric acid (2 × 30 mL), water (30 mL), 10% $NaHCO_3$ (2 × 30 mL), water (30 mL), and, finally, saturated NaCl (30 mL). The organic layer was dried over Na_2SO_4 and evaporated in vacuo. A white powder was obtained (3.3 g, 79%): mp 123 °C; R_f (A) 0.75.

(*R*)-Phenylalanine *N'*-(Benzyloxy)amide Trifluoroacetate (2). Procedure B. To a solution of 2.8 g (8.46 mmol) of (*R*)-*N*-*t*-Boc-phenylalanine *N'*-(benzyloxy)amide in dry CH_2Cl_2 (8.5 mL), cooled to 0 °C, was added 8.5 mL of TFA. After 1 h at 0 °C, solvents were evaporated in vacuo and the residue was washed with Et_2O. A white solid was obtained (1.8 g, 55.4%): mp 134 °C, R_f (A) 0.36.

***N*-[2-(Benzyloxycarbonyl)-1-oxoethyl]-D-phenylalanine *N'*-(Benzyloxy)amide (3).** This compound was obtained via procedure A. From 500 mg (1.3 mmol) of (*R*)-phenylalanine *N'*-(benzyloxy)amide trifluoroacetate and 252 mg (1,3 mmol) of malonic acid monobenzyl ester, a white solid was obtained (460 mg, 80%): mp 120 °C; R_f (A) 0.64. Anal. ($C_{26}H_{26}N_2O_5$) C, H, N.

(*R*)-*N*-(2-Carboxy-1-oxoethyl)phenylalanine *N'*-Hydroxyamide (4). To a 10% Pd on charcoal (124 mg) suspension in MeOH (5 mL), saturated by hydrogen, was added 460 mg (1.03 mmol) of the preceding compound in MeOH (5 mL). The mixture was stirred for 4 h at room temperature. After filtration, the solution was evaporated in vacuo. A white solid was obtained after recrystallization in MeOH/H_2O (260 mg, 94%): mp 83 °C; R_f (B) 0.35; ^{1}H NMR δ 2.8 (CH_2 β-Phe), 3.05 (CH_2 malonate), 4.32 (CH α-Phe), 7.15 (Ph), 8.37 (NH Phe), 8.83 (OH), 10.63 (ONH), 12.33 ppm (COOH); HPLC $AcONH_4$/MeCN (85:15), t_R 4 min 12 s. Anal. ($C_{12}H_{14}N_2O_5$) C, H, N.

(*S*)-*N*-(2-Carboxy-1-oxoethyl)phenylalanine *N'*-Hydroxyamide (5). This compound was synthesized via the procedure described for compound 4 with *N*-*t*-Boc-L-phenylalanine as starting material. A white solid was obtained. Physical characteristics were identical with those of compound 4. Anal. ($C_{12}H_{14}N_2O_5$) C, H, N.

(*R*)-*N*-[(*R*,*S*)-2-Carbethoxy-1-oxopropyl]phenylalanine *N'*-(Benzyloxy)amide (6). This compound was obtained via procedure A. From 500 mg (1.3 mmol) of (*R*)-phenylalanine *N'*-(benzyloxy)amide trifluoroacetate and 190 mg (1.3 mmol) of methylmalonic acid monoethyl ester was obtained a white solid (467 mg, 90%): mp 130 °C; R_f (A) 0.65. Anal. ($C_{22}H_{26}N_2O_5$) C, H, N.

(*R*)-*N*-[(*R*,*S*)-2-Carboxy-1-oxopropyl]phenylalanine *N'*-(Benzyloxy)amide (7). Procedure C. To a solution of 460 mg (1.16 mmol) of the preceding compound in EtOH (5 mL), cooled to 0 °C, was slowly added 1.3 mL of 1 N aqueous NaOH (1.1 equiv). After 15 min at 0 °C and 2 h at room temperature, EtOH was evaporated, and the residue was dissolved in water (10 mL), washed with AcOEt (5 mL), and then acidified to pH 2 with 3 M HCl and extracted with AcOEt (3 × 5 mL). The organic layer was washed with H_2O (8 mL) and saturated NaCl (8 mL), dried over Na_2SO_4, and evaporated in vacuo. A white solid was obtained (360 mg, 84%): mp 104 °C; R_f (C) 0.39. Anal. ($C_{20}H_{22}N_2O_5$) C, H, N.

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(*R*)-*N*-[(*R*,*S*)-2-Carboxy-1-oxopropyl]phenylalanine *N*'-Hydroxyamide (8). The preceding compound (250 mg, 0.68 mmol) was hydrogenated via the procedure described for compound 4. Recrystallization ($MeOH/H_2O$) gave a white solid (178 mg, 94.7%): mp 140 °C; R_f (D) 0.28; ^1H NMR δ 0.98 (CH_3), 2.77 (CH_2 β-Phe), 3.25 (CH malonate), 4.3 (CH α-Phe), 7.17 (Ph), 8.26 (NH Phe), 8.80 (OH), 10.62 (ONH), 12.27 ppm (COOH); HPLC $AcONH_4$/MeCN (85:15) 4 min 48 s and 6 min (two diastereoisomers). anal. ($C_{13}H_{16}N_2O_5$) C, H, N.

(*S*)-*N*-[(*R*,*S*)-2-Carboxy-1-oxopropyl]phenylalanine *N*'-Hydroxyamide (9). This compound was obtained via the procedure described for compound 8 with *N*-*t*-Boc-L-phenylalanine as starting material. A white solid was obtained. Physical characteristics were identical with those of compound 8. Anal. ($C_{13}H_{16}N_2O_5$) C, H, N.

(*R*)-*N*-*t*-Boc-phenylalanine *N*'-(Benzyloxy)-*N*'-methylamide (10). This compound was obtained from 244 mg (0.92 mmol) of *N*-*t*-Boc-D-phenylalanine and 126 mg (0.92 mmol) of *N*-methyl-*O*-benzylhydroxylamine[19] via the procedure A. An oily product was obtained (269 mg, 76.2%): R_f (A) 0.82.

(*R*)-Phenylalanine *N*'-(Benzyloxy)-*N*'-methylamide Trifluoroacetate (11). This compound was synthesized via procedure B from 259 mg (0.68 mmol) of the preceding compound. An oily product was obtained (246 mg, 86%): R_f (A) 0.39.

(*R*)-*N*-[2-(Benzyloxycarbonyl)-1-oxoethyl]phenylalanine *N*'-(Benzyloxy)-*N*'-methylamide (12). This compound was prepared from 267 mg (0.68 mmol) of the preceding compound and 131 mg (0.68 mmol) of malonic acid monobenzyl ester, via procedure A. An oily product was obtained (64 mg, 20.5%): R_f (A) 0.76. Anal. ($C_{27}H_{28}N_2O_5$) C, H, N.

(*R*)-*N*-(2-Carboxy-1-oxoethyl)phenylalanine *N*'-Hydroxy-*N*'-methylamide (13). This inhibitor was prepared via the procedure described for compound 4. From 60 mg (0.13 mmol) of the preceding compound, 30 mg (82.4%) of the title compound was obtained as a white solid after recrystallization ($MeOH/H_2O$): mp 70–75 °C; R_f (B) 0.45; ^1H NMR δ 2.8 (CH_2 β-Phe), 3.07 (CH_2 malonate), 3.25 (CH_3), 5.02 (CHα Phe), 7.15 (Ph), 8.18 (NH), 10.1 (OH), 12.3 ppm (COOH); HPLC $AcONH_4$/MeCN (85:15) 7 min 48 s. Anal. ($C_{13}H_{16}N_2O_5$) C, H, N.

(*R*)-*N*-*t*-Boc-3-Amino-4-phenylbutanoic Acid (14). To a suspension of 217 mg (0.93 mmol) of fresh Ag_2O, 528 mg (4.98 mmol) of Na_2CO_3, and 296 mg (1.19 mmol) of $Na_2S_2O_3 \cdot 5H_2O$ in water (20 mL), heated to 50 °C, was slowly added a solution of (*R*)-*N*-*t*-Boc-phenylalanine diazo ketone (1.5 g, 5.19 mmol) in dioxane (10 mL).[33] The mixture was then refluxed for 1 h, cooled, diluted with water, filtered, washed with AcOEt (3 × 25 mL), acidified to pH 1–2 with diluted HNO_3, and extracted by AcOEt (3 × 25 mL). The organic layer was washed with saturated NaCl (30 mL), dried over Na_2SO_4, and evaporated in vacuo. A white solid was obtained after recrystallization (Et_2O) (1.3 g, 90%): mp 88–90 °C; R_f (A) 0.46.

(*R*)-*N*-*t*-Boc-3-amino-4-phenyl-*N*'-(benzyloxy)butanamide (15). This compound was obtained via procedure A. From 264 mg (0.95 mmol) of the preceding compound and 151 mg (0.95 mmol) of benzylhydroxylamine chlorhydrate, a white solid was obtained (190 mg, 52.4%): mp 96 °C; R_f (A) 0.65.

(*R*)-3-Amino-4-phenyl-*N*'-(benzyloxy)butanamide Trifluoroacetate (16). The preceding compound (186 mg, 0.48 mmol) was treated via procedure B. An orange oil was obtained (150 mg, 75%): R_f (E) 0.45.

(*R*)-*N*-[2-(Benzyloxycarbonyl)-1-oxoethyl]-3-amino-4-phenyl-*N*'-(benzyloxy)butanamide (17). This compound was obtained via procedure A from 140 mg (0.35 mmol) of the preceding compound and 68 mg (0.35 mmol) of malonic acid monobenzyl ester. A white solid was obtained after recrystallization (ether) (110 mg, 68%): mp 159 °C; R_f (A) 0.60. Anal. ($C_{27}H_{28}N_2O_5$) C, H, N.

(*R*)-*N*-(2-Carboxy-1-oxoethyl)-3-amino-4-phenyl-*N*'-hydroxybutanamide (18). The preceding compound (70 mg, 0.15 mmol) was hydrogenated via the procedure described for compound 4. A white solid was obtained after recrystallization ($MeOH/H_2O$) (41 mg, 96%): mp 80–84 °C; R_f (F) 0.53; ^1H NMR δ 2.05 (CH_2CO), 2.67 ($CH_2\beta$) 2.98 (CH_2 malonate), 4.15 (CHα), 7.17 (Ph), 7.95 (NH), 8.72 (OH), 10.33 (ONH), 12.33 ppm (COOH); HPLC $AcONH_4$/MeCN (85:15) 4 min 40 s. Anal. ($C_{13}H_{16}N_2O_5$) C, H, N.

(*S*)-*N*-(2-Carboxy-1-oxoethyl)-3-amino-4-phenyl-*N*'-hydroxybutanamide (19). This compound was obtained via the procedure described for compound 18 with L-*N*-*t*-Boc-phenylalanine diazo ketone[32] as starting material. Physical characteristics are identical with that of compound 18. Anal. ($C_{13}H_{16}N_2O_5$) C, H, N.

(*R*)-*N*-[(*R*,*S*)-2-Carbethoxy-1-oxopropyl]-3-amino-4-phenyl-*N*'-(benzyloxy)butanamide (20). This compound was synthesized via procedure A from 434 mg (1.09 mmol) of (*R*)-3-amino-4-phenyl-*N*'-(benzyloxy)butanamide trifluoroacetate and 159 mg (1.09 mmol) of methylmalonate monoethyl ester. After recrystallization, a white solid was obtained (212 mg, 48%): mp 121 °C; R_f (A) 0.59. Anal. ($C_{23}H_{28}N_2O_5$) C, H, N.

(*R*)-*N*-[(*R*,*S*)-2-Carboxy-1-oxopropyl]-3-amino-4-phenyl-*N*'-(benzyloxy)butanamide (21). The preceding compound (100 mg, 0.24 mmol) was treated via procedure C to give a white solid (71 mg, 76%): mp 156 °C; R_f (C) 0.29. Anal. ($C_{21}H_{24}N_2O_5$) C, H, N.

(*R*)-*N*-[(*R*,*S*)-2-Carboxy-1-oxopropyl]-3-amino-4-phenyl-*N*'-hydroxybutanamide (22). A 67-mg (0.17-mmol) sample of the preceding compound was hydrogenated via the procedure described for compound 4. A white solid was obtained after recrystallization ($MeOH/H_2O$) (50 mg, 94%): mp 115 °C; R_f (G) 0.21; ^1H NMR δ 0.93–1.07 (CH_3) 2.08 ($COCH_2$), 2.65 ($CH_2\beta$), 3.1 (CH malonate), 4.12 (CHα), 7.13 (Ph), 7.85 (NH), 8.68 (OH), 10.27–10.37 (ONH), 12.23 ppm (COOH); HPLC AcOH 2%/MeCN (85/15) 8 min and 11 min 24 s (two diastereomers). Anal. ($C_{14}H_{18}N_2O_5$) C, H, N.

(*R*)-*N*-[(*R*,*S*)-2-Carbethoxy-1-oxo-3-phenylpropyl]-3-amino-4-phenyl-*N*'-(benzyloxy)butanamide (23). This compound was obtained via procedure A from 440 mg (1.1 mmol) of (*R*)-3-amino-4-phenyl-*N*'-(benzyloxy)butanamide trifluoroacetate and 245 mg (1.1 mmol) of benzylmalonate monoethyl ester. After chromatography on silica gel column (CH_2Cl_2/Et_2O (8/2) as eluant), an oil was obtained (200 mg, 40%): R_f (A) 0.65. Anal. ($C_{29}H_{32}N_2O_5$) C, H, N.

(*R*)-*N*-[(*R*,*S*)-2-Carboxy-1-oxo-3-phenylpropyl]-3-amino-4-phenyl-*N*'-(benzyloxy)butanamide (24). This compound was obtained via procedure C from 193 mg (0.39 mmol) of the preceding compound. A colorless oil was obtained (133 mg, 73%): R_f (E) 0.3. Anal. ($C_{27}H_{28}N_2O_5$) C, H, N.

(*R*)-*N*-[(*R*,*S*)-2-Carboxy-1-oxo-3-phenylpropyl]-3-amino-4-phenyl-*N*'-hydroxybutanamide (25). A 126-mg (0.27-mmol) sample of the preceding compound was hydrogenated via the procedure described for compound 4. A white solid was obtained after recrystallization ($MeOH/H_2O$) (90 mg, 89%): mp 86–88 °C; R_f (G) 0.27; ^1H NMR δ 1.92–2.03 ($COCH_2$), 2.58–2.73 ($CH_2\beta$), 2.73–2.87 (CH_2 benzyl), 3.33 (CH malonate), 4.12 (CHα), 6.97–7.15 (Ph), 7.93 (NH), 8.68 (OH), 10.33 (ONH), 12.33 ppm (COOH); HPLC AcOH 2%/MeCN (75:25) 13 min 24 s and 22 min 12 s (two diastereoisomers). Anal. ($C_{20}H_{22}N_2O_5$).

(*R*)-*N*-*t*-Boc-3-amino-4-phenyl-*N*'-(benzyloxy)-*N*'-methylbutanamide (26). This compound was prepared via procedure A. From 118 mg (0.86 mmol) of *N*-methyl-*O*-benzylhydroxylamine and 240 mg (0.86 mmol) of (*R*)-*N*-*t*-Boc-3-amino-4-phenylbutanoic acid, 236 mg (69%) of the title compound was obtained: R_f (A) 0.74. Anal. ($C_{23}H_{30}N_2O_4$) C, H, N.

(*R*)-3-Amino-4-phenyl-*N*'-(benzyloxy)-*N*'-methylbutanamide Trifluoroacetate (27). This compound was obtained via procedure B from 230 mg (0.58 mmol) of the preceding compound. An oil was obtained (245 mg, 89%): R_f (A) 0.3.

(*R*)-*N*-[2-(Benzyloxycarbonyl)-1-oxoethyl]-3-amino-4-phenyl-*N*'-(benzyloxy)-*N*'-methylbutanamide (28). This compound was obtained via procedure A. From 234 mg (0.57 mmol) of the preceding compound and 110 mg (0.57 mmol) of malonic acid monobenzyl ester, an oil was obtained and purified by silica gel chromatography (CH_2Cl_2/Et_2O, 85/15) to give a white solid (105 mg, 39%): mp 140 °C; R_f (A) 0.74. Anal. ($C_{28}H_{30}N_2O_5$) C, H, N.

(*R*)-*N*-(2-Carboxy-1-oxoethyl)-3-amino-4-phenyl-*N*'-hydroxy-*N*'-methylbutanamide (29). This compound was

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obtained via the procedure described for compound 4 from 60 mg (0.13 mmol) of the preceding compound. A white solid was obtained after recrystallization ($MeOH/H_2O$) (30 mg, 76%): mp 95 °C; R_f (F) 0.65; ^1H NMR δ 2.45 ($COCH_2$), 2.68 ($CH_2\beta$), 2.88 (CH_2 malonate), 3.02 (NCH_3), 4.17 ($CH\alpha$), 7.13 (Ph), 7.97 (NH), 9.72 ppm (OH); HPLC $AcONH_4$/MeCN (85/15) 8 min 12 s. Anal. ($C_{14}H_{18}N_2O_5$) C, H, N.

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Registry No. (*R*)-1, 115364-50-6; (*S*)-1, 115364-82-4; (*R*)-**2**, 115364-52-8; (*S*)-**2**, 58207-46-8; (*R*)-**3**, 115364-53-9; (*S*)-**3**, 115364-85-7; **4**, 115364-54-0; **5**, 115364-55-1; (*R,R*)-**6**, 115364-56-2; (*R,S*)-**6**, 115364-89-1; (*S,R*)-**6**, 115364-90-4; (*S,S*)-**6**, 115364-91-5; (*R,R*)-**7**, 115364-57-3; (*R,S*)-**7**, 115364-86-8; (*S,R*)-**7**, 115364-87-9; (*S,S*)-**7**, 115364-88-0; (*R,R*)-**8**, 115364-58-4; (*R,S*)-**8**, 115364-83-5; (*S,R*)-**9**, 115364-59-5; (*S,S*)-**9**, 115364-84-6; **10**, 115364-60-8; **11**, 115364-62-0; **12**, 115364-63-1; **13**, 115364-64-2; (*R*)-14, 101555-61-7; (*S*)-14, 51871-62-6; (*R*)-**15**, 115364-65-3; (*S*)-**15**, 115364-92-6; (*R*)-**16**, 115364-67-5; (*S*)-**16**, 115364-94-8; (*R*)-**17**, 115364-68-6; (*S*)-**17**, 115364-95-9; **18**, 115364-69-7; **19**, 115364-70-0; (*R,R*)-**20**, 115364-71-1; (*R,S*)-**20**, 115364-97-1; (*R,R*)-**21**, 115364-72-2; (*R,S*)-**21**, 115383-46-5; (*R,R*)-**22**, 115364-73-3; (*R,S*)-**22**, 115364-99-3; (*R,R*)-**23**, 115364-74-4; (*R,S*)-**23**, 115364-98-2; (*R,R*)-**24**, 115364-75-5; (*R,S*)-**24**, 115364-96-0; (*R,R*)-**25**, 115364-76-6; (*R,S*)-**25**, 115365-00-9; **26**, 115364-77-7; **27**, 115364-79-9; **28**, 115364-80-2; **29**, 115364-81-3; DAP, 9032-67-1; BOC-D-Phe-OH, 18942-49-9; BOC-Phe-OH, 13734-34-4; $H_2NOCH_2Ph\cdot HCl$, 2687-43-6; $MeNHOCH_2Ph$, 22513-22-0; $HOOCCH_2COOCH_2Ph$, 40204-26-0; (±)-HOOCCH(CH_3)COOEt, 81110-31-8; (±)-HOOCCH(CH_2Ph)COOEt, 67682-05-7; (*R*)-(BOC)NHCH(CH_2Ph)$COCHN_2$, 115313-19-4; (*S*)-(BOC)NHCH(CH_2Ph)$COCHN_2$, 60398-41-6; EC 3.4.24.11, 82707-54-8; EC 3.4.11.2, 9054-63-1.

Phosphonoformate and Phosphonoacetate Derivatives of 5-Substituted 2′-Deoxyuridines: Synthesis and Antiviral Activity

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The synthesis of potential "combined prodrugs" wherein phosphonoformate or phosphonoacetate was attached to the 5′-position of 2′-deoxyuridine, 2′-deoxythymidine, 5-iodo-2′-deoxyuridine (IDU), 5-(2-chloroethyl)-2′-deoxyuridine (CEDU), or 5-(2-bromovinyl)-2′-deoxyuridine (BVDU) or to the 3′-position of CEDU is described. The antiviral activities of these derivatives and of reference compounds were compared in Vero, HEp-2, and primary rabbit kidney cells against herpes simplex virus types 1 and 2 (HSV-1 and -2). The CEDU and BVDU analogues were also evaluated against systemic and intracutaneous HSV-1 infection in mice. The nature of the 5-substituent proved critical for antiviral activity, since only the 5-iodo-, 5-(2-bromovinyl)-, and 5-(2-chloroethyl)-substituted derivatives were inhibitory to the herpesviruses. Furthermore, the type specificity is determined by the nature of the 5-substituent: the IDU analogues were similarly inhibitory to HSV-1 and -2 whereas the CEDU and BVDU analogues inhibited HSV-2 replication only at considerably higher concentrations than HSV-1. In vivo, several derivatives were shown to possess significant antiviral activity; however, none surpassed its respective parent compound, CEDU or BVDU, in potency. It seems improbable, therefore, that a synergistic effect between PFA or PAA and the nucleoside analogue occurred. The extent of in vitro and in vivo activity of the CEDU and BVDU 5′-phosphonoformates and 5′-phosphonoacetates is most plausibly explained by the ease by which the "combined prodrugs" are hydrolyzed and the parent compound, CEDU an BVDU, respectively, is released.

The class of 5-substituted pyrimidine nucleoside analogues comprises many compounds that possess significant and therapeutically useful antiherpesvirus activity.[1] Two of the most potent and selective antiviral representatives of this class are (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU)[2] and the recently described 5-(2-chloroethyl)-2′-deoxyuridine (CEDU).[3] Both compounds effectively inhibit herpes simplex virus type 1 (HSV-1) in vitro and in vivo.[2-10] Their selectivity is attested by their high antiviral indexes, which are 2000 and 5000 for CEDU and BVDU, respectively, as determined by the ratio of the minimum toxic dose for the normal host cell to the minimum inhibitory dose for HSV-1. In vivo CEDU is effective against systemic HSV-1 infection at a dose that is about 10-fold lower than those required for BVDU and the reference compound acyclovir (ACV), whereas in vitro BVDU is active at about 1/10 the concentration of CEDU.[3,9,10]

Phosphonoformic acid (PFA) and phosphonoacetic acid (PAA) have been reported to be inhibitory to herpesvirus replication in tissue culture and to be effective in the treatment of several herpesvirus infections of animals.[11]

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