New Constrained "Molecular Tongs" Designed To Dissociate HIV-1 Protease Dimer

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New "molecular tongs" based on naphthalene and quinoline scaffolds linked to two peptidic strands were synthesized. They were designed to prevent dimerization of HIV-1 protease by targeting the antiparallel β-sheet involving N- and C-termini of each monomer. Compared to "molecular tongs" previously described (Bouras, A.; Boggetto, N.; Benatalah, Z.; de Rosny, E.; Sicsic, S.; Reboux-Ravaud, M. *J. Med. Chem.* **1999**, *42*, 957−962), two main different structural features were introduced: positively charged quinoline as a new scaffold and two peptidic strands displaying different sequences. Seventeen new "molecular tongs" with dipeptidic or tripeptidic strands were synthesized. These molecules were assayed on HIV-1 protease using the Zhang kinetic technique. Eleven molecules behaved as pure dimerization inhibitors, mostly at the submicromolar range. Compared to a naphthalene scaffold, the quinoline one was shown in several cases to favor dimerization inhibition. The simplified hydrophobic Val-Leu-Val-OMe strand was confirmed as particularly favorable. The C-terminal analogue strand Thr-Leu-Asn-OMe was shown to be the best one for inducing dimerization inhibition (K_{id} of 80 nM for compound **30**). The mechanism of inhibition was ascertained using ANS binding and gel filtration. Experimental results are in agreement with the dissociation of the HIV-1 protease dimeric form in the presence of the synthesized molecular tongs.

Introduction

HIV-1 protease (PR) has been widely studied due to its importance as a valuable therapeutic target for developing anti-AIDS drugs since its inhibition results in the production of noninfectious virus.[1,2] Today seven HIV-1 PR inhibitors including saquinavir, ritonavir, amprenavir, indinavir, nelfinavir, lopinavir, and atazanavir have been approved and marketed as anti-AIDS drugs.[3−6] Their efficacy was shown to be remarkable in tritherapy protocols, combined with two reverse transcriptase inhibitors, reducing the viral load and improving the number of CD4 lymphocytes in AIDS patients. However, a rapid emergence of mutations that confer resistance to all protease inhibitors is observed. Multidrug resistance is triggered by mutations located at different regions of the protease molecule (within the active site, or not) due to rapid amino acid mutations of the enzyme.[7,8] All the protease inhibitors used in therapy are active site competitive inhibitors which bind across the active site formed by both subunits of the active dimeric enzyme. Residues in the dimer interface are well conserved from the points of view of both genetic variation and drug resistance.[9,10] A network of interactions around the active site and termini of the mature protease are critical for dimerization.[11] More particularly, the antiparallel β-sheet formed between the four monomeric termini contributes over 75% to the stabilizing force of the dimer[12] and has been found to be highly conserved in HIV-1 and most HIV-2 isolates.[13] Blocking the formation or disrupting the homodimer was shown to be a means of inhibiting protease activity.[14] C- and N-terminal mimetics were the first reported dimerization inhibitors.[15−17] Variation of peptide sequence and addition of a lipophilic terminal group favored targeting the antiparallel β sheet and improved the efficiency.[18,19] The cross-linking of interface peptides with flexible[20] or semirigid[21] spacers was explored. Recently, guanidinium derivatives displaying short peptide mimics and a lipophilic group have been designed as dimerization inhibitors.[22] Our contribution to the antidimer strategy was the synthesis of the first "molecular tongs" based on a conformationally constrained scaffold attached to two peptidic strands through two carboxypropyl links.[23] The advantage of this strategy is that the two peptidic strands could be suitably oriented by the scaffold allowing the putative formation of an antiparallel β-sheet with the C-terminal end of one HIV-1 PR monomer, thus leading to an entropy benefit (Figure 1). The integrity of inner C-terminal strands is essential for dimer formation.[11] Two scaffolds based on naphthalenediol and pyridinediol were found to lead to active molecules as antidimers conversely to resorcinol ($K_{id} = 0.560$ μM for the best inhibitor).[23] The "β-sheet nucleator" 4-(2-aminoethyl)-6-dibenzopropionic acid introduced by Kiso to connect two peptide fragments was less efficient with $K_{id} = 5.4$ μM.[24] In order to improve the activity of this type of inhibitor, it was tempting to introduce a new scaffold and modifications of the peptidic strands. Our previous studies showed that the naphthalenediol spacer was sterically more

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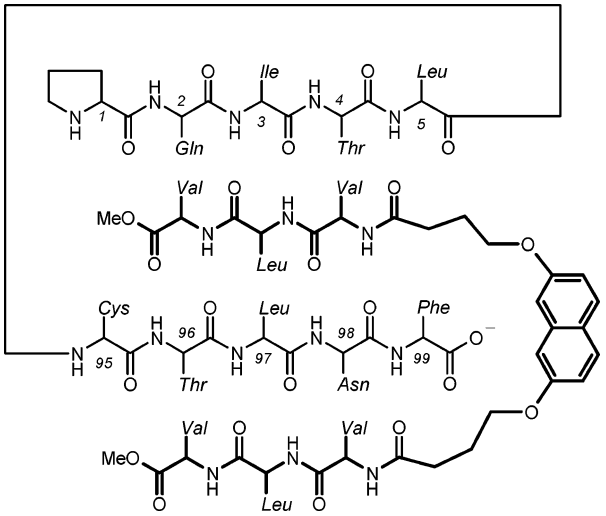


Figure 1. Model of interaction of a typical "molecular tong" with one monomer of HIV-1 protease.

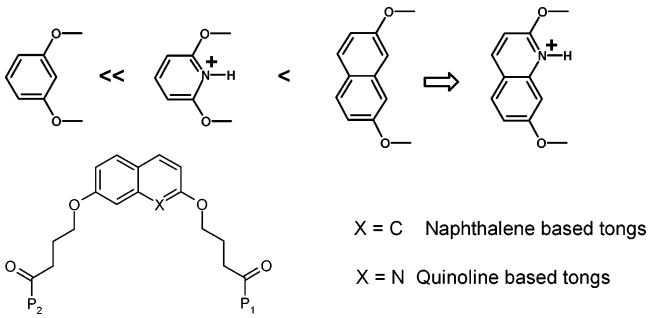


Figure 2.

favorable than the pyridinediol one, but the latter could form a favorable ionic interaction with the negatively charged Phe-O^- 99 at the C-terminus strand of the monomer.[23] To get advantage of the structure of these two previous scaffolds, we have introduced a quinolinediol spacer which has the steric structure of naphthalenediol and an electronic structure close to that of pyridinediol (Figure 2). We conserved the same carboxypropyl linker between the quinolinediol and peptide strands. In our previous study, identical peptidic strands were introduced ("symmetric tongs") leading to the identification of the best sequence Val-Leu-Val-OMe, a simplified mimetic of the C-terminal strand of the monomer. In the new molecular tongs, the peptide Val-Leu-Val-OMe constitutes one strand and the nature of the second one has varied: essentially elements of the N-terminal end of the monomer, in one case of the C-terminus, and of YEL found in the powerful lipopeptides.[19,25] Corresponding molecular tongs displaying a naphthalenediol spacer were synthesized for comparison.

Chemistry

Nonsymmetric naphthalene-based molecules were synthesized after differential protection of the two OH groups of 2,7-dihydroxynaphthalene. Product **4** was synthesized from commercially available 2,7-dihydroxynaphthalene **1** using classical reactions according to Figure 3. Using 0.9 equiv of ethyl-4-bromobutyrate, monoester **2** was obtained in a 36% yield, along with diester and unchanged product **1**. Subsequent benzylation of the other OH group gave compound **3**, giving rise to distinguishable OH functions. Compound **3** was then hydrolyzed under basic conditions to afford scaffold **4**.

Nonsymmetric quinoline-based molecules were synthesized from compound **5** obtained as described in the literature[26] (Figure 4). The 7-OH group of **5** was selectively protected as the benzyl ether **6**, allowing the O-alkylation of the 2 position with 71% yield. Reaction of **6** with ethyl 4-bromobutyrate gave ester **7** obtained in 40% yield along with the N-alkylated derivative **8** obtained in 42% yield. The two isomers were easily separated and well identified by their NMR and IR spectra. These results are not surprising as it is well-known that alkylation of 2(1*H*)-quinoline derivatives involves an ambident anion and leads to a mixture of N- and O-alkylated products.[27,28] The yields were comparable with those reported in the literature. Compound **7** was subsequently hydrolyzed to afford the desired acid **9**.

After scaffolds **4** and **9** were obtained, nonsymmetric naphthalene- and quinoline-based tongs were obtained according to Figure 5. In a first step, the carboxylic acid scaffold **4** or **9** was coupled via a classical peptide condensation to H-Val-Leu-Val-OMe to afford compound **10** or **11** respectively in good yields (90% and 93%, respectively). After a debenzylation step, compounds **12** and **13** were O-alkylated with benzyl 4-bromobutyrate,[29] giving compounds **14** and **15**, respectively. These benzyl-protected compounds were used in order to selectively generate compounds **16** and **17** respectively in the subsequent step of deprotection without modifying the terminal ester of the tripeptide strand. In a last step, condensation of **16** or **17** with a dipeptidic or tripeptidic strand was done to afford final naphthalene-based tongs **18**−**20** and **22**−**24** (X = C), and quinoline-based tongs **25**−**27** and **29**−**32** (X = N) in moderate yields of 63% to 80% (Supporting Information). Compounds **20** and **27** which are ortho-protected at the side chain level of glutamate with a *tert*-butyl group, were hydrolyzed under acidic conditions to afford respectively compounds **21** and **28** (Supporting Information).

In order to compare activities of these new compounds with activities of former ones,[23] the two symmetric quinoline-based tongs **36** and **37** having Val-Leu-Val-OMe or Thr-Ile-Val-OMe respectively as peptidic strands were also synthesized in three steps from molecule **5** (Figure 6). In a first step dialkylation of **5** with ethyl bromobutyrate afforded the N,O-dialkylated compound **33** along with O,O′-dialkylated compound **34**, which gave compound **35** after separation and hydrolysis under basic conditions. This compound was then submitted to a condensation step using the HBTU coupling method with H-Val-Leu-Val-OMe giving rise to compound **36**, or with H-Ile-Thr-Val-OMe giving rise to compound **37** in moderate yields (66% and 70%) (Supporting Information).

The peptidic strands Z-Ile-Thr-NH_2, Z-(O-*t*Bu)Glu-Leu-NH_2, Z-Gln-Ile-Thr-NH_2, Z-Tyr-(O-*t*Bu)Glu-Leu-NH_2, and Z-Ile-Thr-Leu-OMe were synthesized according to a linear peptide coupling method which consists of condensing the acid group of one N-protected amino acid with the amino group of another O-protected amino

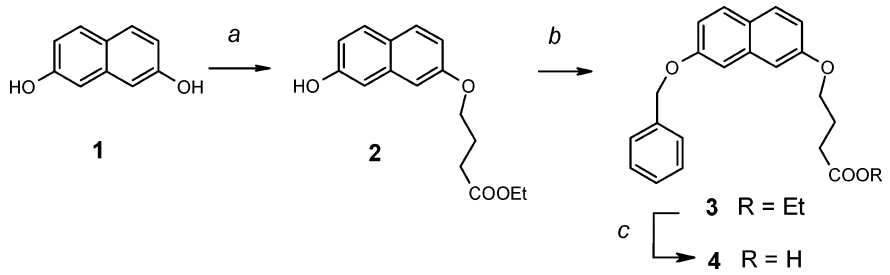


Figure 3. (a) K_2CO_3, $Br(CH_2)_3COOEt$, DMF, 24 h, rt. (b) K_2CO_3, BrBn, CH_3CN, 4 h, reflux. (c) Methanolic KOH 10%, 4 h, reflux.

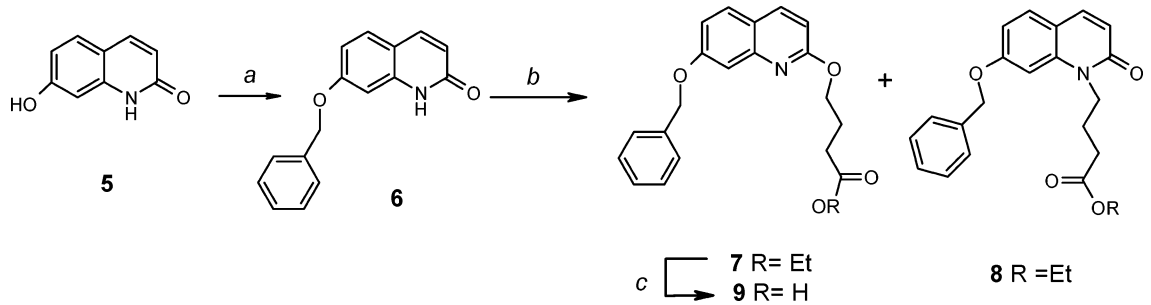


Figure 4. (a) DBU, BrBn, *i*PrOH, 4 h, reflux. (b) K_2CO_3, $Br(CH_2)_3COOEt$, DMF, 24 h, rt. (c) Methanolic KOH 10%, 3 h, reflux.

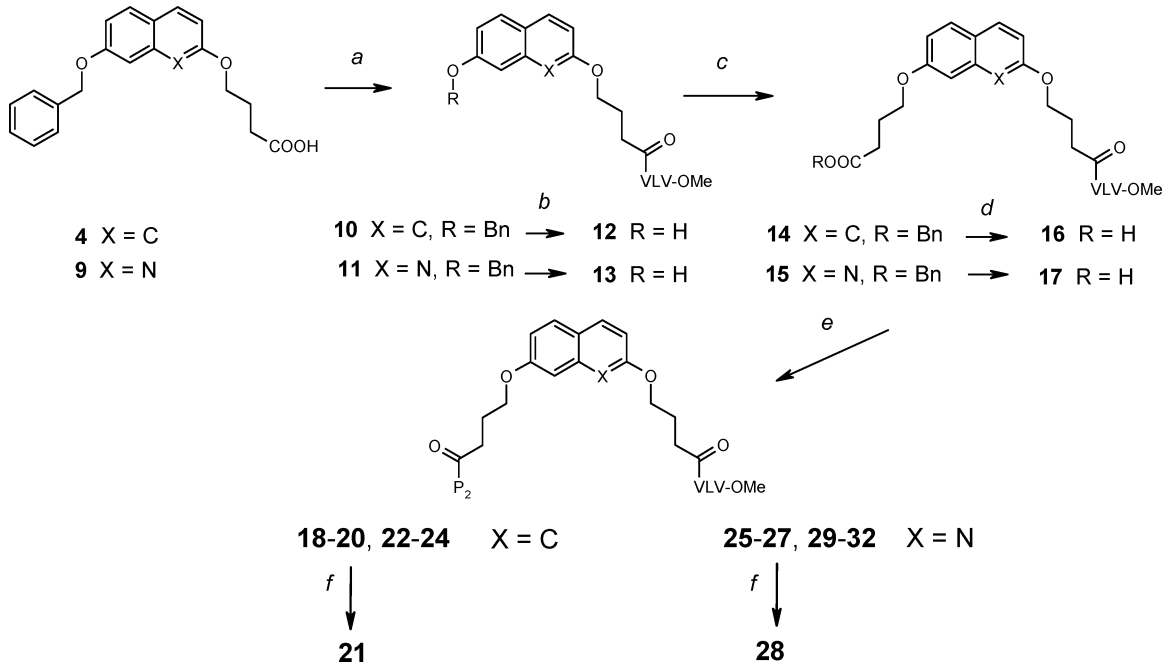


Figure 5. (a) HBTU, triethylamine, H-VLV-OMe, DMF, 24 h, rt. (b) H_2 Pd/C, MeOH, 24 h. (c) K_2CO_3, $Br(CH_2)_3COOBn$, DMF, 24 h, rt. (d) H_2 Pd/C, DMF, 24 h. (e) HBTU, triethylamine, H-P_2, DMF, 24 h, rt. (f) Trifluoroacetic acid, 6 h, rt.

acid or peptide. The coupling agents used were HBTU or chloroformates. The N-protective group Z was cleaved by hydrogenolysis with H_2 Pd/C 10% in MeOH or DMF since the N-protective group Boc was hydrolyzed with a solution of 3 N HCl/MeOH (Table 1 of Supporting Information). During the deprotection step of the amino group of dipeptides Z-Ile-Thr-OMe and Z-(O-*t*Bu)Glu-Leu-OMe, we observed that cyclization of the dipeptide occurred to afford lactam, when a methyl carboxylic acid protective group was used. Consequently we opted for the use of an amide carboxylic acid protective group to avoid the cyclization since an amide group is less sensitive to nucleophilic attack than an ester. The other peptide strands were synthesized as previously reported.[23]

Results and Discussion

In our previous work, we have shown that symmetric molecular tongs, displaying two identical peptidic strands and based on naphthalenediol and pyridinediol as rigid scaffolds, could lead to efficient inhibitors of HIV-1 PR dimerization ($K_{id} = 0.560\ \mu M$ for compound **38** in Table 1, spacer 2,7-naphthalenediol and peptide Val-Leu-Val-OMe).[23] To improve the potency of this family of HIV-1 PR dimerization inhibitors, we have modified the structure of the scaffold and also introduced nonsymmetric tongs, which consist of two different peptidic strands attached to each scaffold. For the first point, we decided to study a quinoline-based scaffold, as quinoline contains structural requirements of both naphthalene and pyridine (Figure 2). For the second point, we decided to

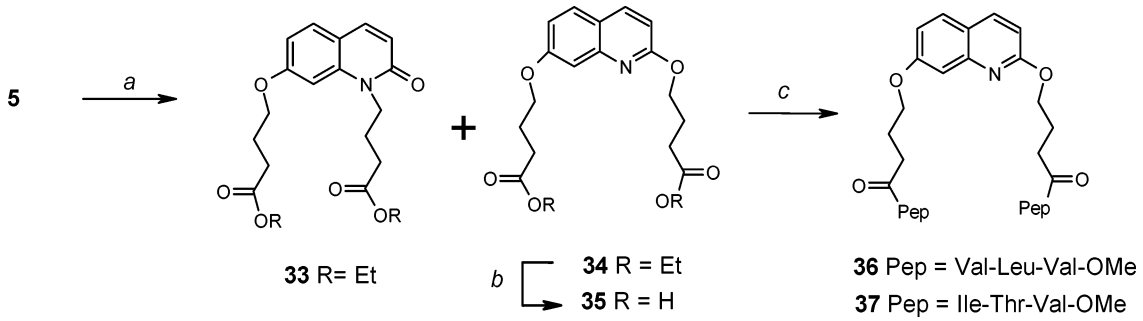
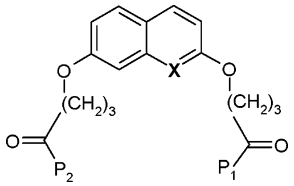


Figure 6. (a) K_2CO_3, $Br(CH_2)_3COOEt$, DMF, 24 h, rt. (b) KOH, MeOH 10%, 3 h, reflux. (c) HBTU, triethylamine, HN-pep, DMF, 24 h, rt.

Table 1. Biological Results of Molecular Tongs[a]



compd	X	P_1	P_2	IC_{50} (μM)	K_{ic} (μM)	K_{id} (μM)
18	C	VLVOMe	TLOMe	4	10.3	1.40
25	N	VLVOMe	TLOMe	1.4		1.50
19	**C**	VLVOMe	$ITNH_2$	4.3	6.2	1.50
26	N	VLVOMe	$ITNH_2$	1.7	4.3	0.25
20	C	VLVOMe	E^*LNH_2[b]	1.1		0.42
27	N	VLVOMe	E^*LNH_2	1.8		0.53
21	C	VLVOMe	$ELNH_2$	2.8		0.90
28	N	VLVOMe	$ELNH_2$	20% at 5.7 μM		
38[c]	C	VLVOMe	VLVOMe	3.5		0.56
36	N	VLVOMe	VLVOMe	1.4		0.13
37	N	TIVOMe	TIVOMe	45% at 5.7 μM		1.90
22	C	VLVOMe	ITLOMe	ND		
29	N	VLVOMe	ITLOMe	5.3		0.40
30	N	VLVOMe	TLNOMe	5.1		0.08
23	C	VLVOMe	$QITNH_2$	2.8		2.30
31	N	VLVOMe	$QITNH_2$	30% at 5.7 μM		
24	C	VLVOMe	YE^*LNH_2	1.1		0.23
32	N	VLVOMe	YE^*LNH_2	1.0		0.41

[a] Binding analysis used the Zhang procedure described previously.[23] [b] E* for *tert*-butyl ester of the glutamate. [c] From ref 23.

mimic the four-stranded β-sheet structure of HIV-1 PR. In order to easily establish a structure−activity relationship, it was decided to keep unchanged one of the two peptidic strands of these nonsymmetric tongs. According to our previous results, the peptidic strand P_1 was fixed to be Val-Leu-Val-OMe for all compouds except **37**. The other peptidic strand P_2 varied in size (di- or tripeptide) and sequence. In one case P_2 was a part of the C-terminal Thr-Leu-Asn-Phe strand of monomers of HIV-1 PR (**30**); in other cases it was a part of the N-terminal Pro-Gln-Ile-Thr-Leu strand (**19**, **22**, **23**, **26**, **29**, and **31**) of monomers. For compounds **18** and **25**, P_2 was fixed to Thr-Leu-OMe which is a part of both C-terminal Thr-Leu-Asn-Phe and N-terminal Gln-Ile-Thr-Leu strands of each monomer. The peptides Glu-Leu and Tyr-Glu-Leu and their O-protected glutamate analogues introduced in compounds **20**, **21**, **24**, **27**, **28**, and **32** are parts of the powerful dimerization inhibitor lipopeptide Pam-Tyr-Glu-Leu designed by Schramm.[19] Symmetric compounds **36** ($P_1 = P_2 =$ Val-Leu-Val-OMe) and **37** ($P_1 = P_2 =$ Thr-Ile-Val-OMe) based on the quinoline scaffold were also synthesized for comparison with symmetric tongs **38** based on the naphthalene scaffold, and with nonsymmetric quinoline-based tongs. Table 1 summarizes measured inhibitions by molecular tongs expressed as IC_{50}, calculated inhibition constants K_{ic} attributed to competitive inhibition, and calculated inhibition constants K_{id} attributed to dimerization inhibition. Inhibitions were obtained with all compounds except for compound **22**, which precipitated at 2 μM. Weak inhibitory effects were observed with compounds **28** and **31** (20−30% at 5.7 μM), and no inhibitory effect was detected for compound **22**, which precipitates at 2 μM. Furthermore, except for compounds **18**, **19,** and **26**, which led to mixed inhibition in which the inhibitors could bind to the interfacial region but also to the active site, the other tested compounds gave a series of parallel lines in a Zhang plot, which is diagnostic of pure dissociative inhibition.[23] The values of the inhibition constants K_{ic} (competitive inhibition) and K_{id} (dimerization inhibition) were determined as explained in the Experimental Section. The observed results may be discussed as follow: (i) The new symmetric quinoline-based tongs **36** and **37** behaved as dimerization inhibitors; more particularly, compound **36**, which is the quinoline analogue of the naphthalene-based compound **38**, showed a good $K_{id} = 0.13$ μM. This result is a first indication that the quinoline spacer is slightly more favorable to interface inhibition than the naphthalene spacer. The detrimental effect of sequence Thr-Ile-Val-OMe (compound **37**, $K_{id} = 1.90$ μM) confirms the superiority of the sequence Val-Leu-Val-OMe for dimerization inhibition. (ii) Compounds which contained P_2 as a part of the dimerization inhibitor Pam-Tyr-Glu-Leu (**20**, **27, 21**, **24**, and **32**) behaved as pure dimerization inhibitors. These results confirm the beneficial effect induced by Tyr-Glu-Leu for the interfacial inhibition, a good inhibition being also induced by only the dipeptide Glu-Leu. For both quinoline and naphthalene spacers, deprotection of the glutamate residue decreased the inhibitory potency (**21** vs **20**, **28** vs **27**), confirming a favorable influence of the hydrophobic character of the tong arms on efficiency.[22,23] In the case of compound **28**, self-association of the inhibitor by intramolecular or intermolecular ion pairing between glutamic acid carboxylate and quinolinium ion may be evoked to explain the noticeable decrease in the inhibitory efficiency compared to **21**. (iii) For other molecular tongs, it could be emphasized that reproducing in P_2 a part of C-terminal HIV-1 PR monomers Thr-Leu-Asn-Phe sequence is more favorable to interface inhibition than reproducing a part of the N-terminal monomer sequence. Thus, in quinoline-based series, compound **25** ($P_2 =$ Thr-Leu-OMe) is a better interface inhibitor than

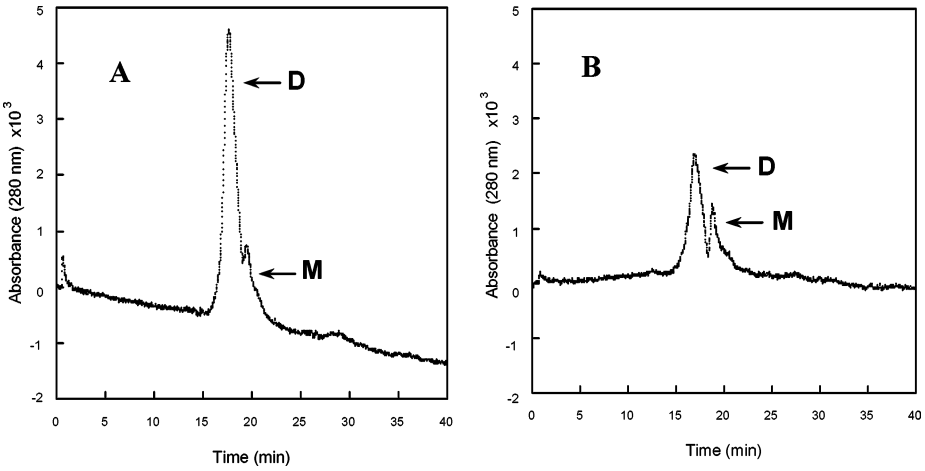


Figure 7. Analytical gel filtration chromatogram of PR in the absence (A) or presence of compound **25** (B). Arrows indicate peaks corresponding to the dimeric (D) and monomeric (M) forms of PR (Superdex 75; flow rate 0.1 mL/min; 100 mM sodium acetate; 5% (v/v) DMSO, pH = 4.7 and 25 °C).

compound **26** (P_2 = Ile-Thr-OMe), and compound **30** (P_2 = Thr-Leu-Asn-OMe) is better than compounds **29** (P_2 = Ile-Thr-Leu-OMe) and **31** (P_2 = Gln-Ile-Thr-OMe). Also in naphthalene-based series, compound **38** (P_2 = Val-Leu-Val-OMe) is a better interface inhibitor than compound **23** (P_2 = Gln-Ile-Thr-OMe). (iv) Finally, we observed that the quinoline spacer which is protonated in the assay medium improves the interface inhibition of molecular tongs in several cases (**25** vs **18**, **26** vs **19**, and **36** vs **38**) where the P_2 strand has a hydrophobic character. This phenomenon was not observed when the P_2 strand possesses a more polar character (**27** vs **20**, and **32** vs **24**). Due to the fact that the quinoline ring is weakly basic and that biological assays were done at pH 4.7, the importance of this interpretation should be modulated for physiological mediums which are at pH 7.0. Further evaluation under neutral conditions could address this aspect. Taken together all these results show that dimerization inhibition may be promoted, when the two peptidic strands P_1 and P_2 reproduce a part of the C-terminal Thr-Leu-Asn-Phe sequence of HIV-1 PR monomers or when one strand fulfills this condition and the other one reproduces a part of the Pam-Tyr-Glu-Leu sequence. Furthermore, as observed by Bouras,[23] tripeptidic sequences are more convenient than dipeptidic sequences to promote a good dimerization inhibition. This is exemplified by tongs **24**, **30**, **32**, and **36** which are sub-micromolar dimerization inhibitors. Finally, the good potency of the quinoline-based tong **30** (K_{id} = 80 nM) should be pointed out, since this result represents a new advance in the research of potent dimerization inhibitors of HIV-1 PR.

To further probe the mechanism of inhibition we performed two types of experiments. Analytical gel filtration chromatography was used to test the ability of compound **25** to disassemble the dimeric protease.[30] As seen in Figure 7, gel filtration of HIV-1 protease in the presence of **25** led to apparition of two peaks, one at 17.2 min corresponding to dimeric protease and the other at 19.5 min attributed to dissociated monomers. Clearly the ratio monomer over dimer increased in the presence of compound **25**, in agreement with a dissociative effect for this compound.

The ANS binding technique was also performed. It is well-known that ANS binding to protein promoted an

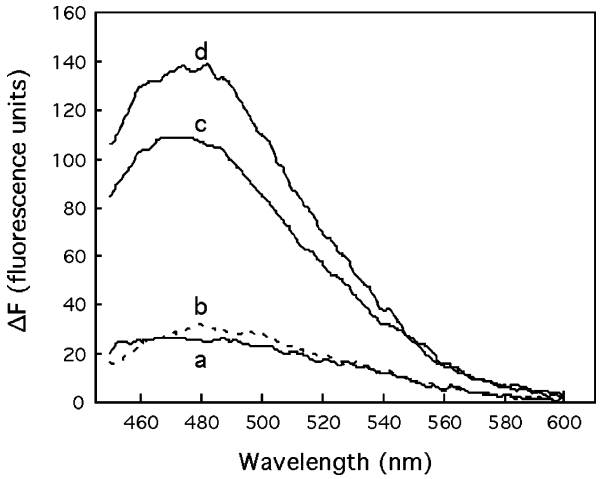


Figure 8. ANS emission spectra at pH 4.7 and 25 °C (λ_{ex} = 376 nm): PR (0.35 μM) was incubated in the absence of inhibitor (a), or in the presence of 0.1 μM acetylpepstatin (b), 3.75 μM **23** (c), or 1.66 μM **32** (d). The fluorescence intensity was corrected from the fluorescence due to the same concentration of ANS (30 μM) without enzyme (a) and without enzyme and inhibitor (b−d).

increase of fluorescence of ANS. As ANS is a hydrophobic molecule, it is generally considered that binding of this molecule occurred at hydrophobic surfaces of proteins.[31] As seen in Figure 8 the fluorescence due to ANS binding at the protease surface is considerably enhanced in the presence of compounds **23** and **32** if compared to the negligible increase observed in the presence of the active site inhibitor acetylpepstatin. This is consistent with the exposure of hydrophobic areas located at the interface between the two monomers upon binding of the putative dimerization inhibitor which dissociates or destabilizes the dimer.[25] Conversely to active site inhibitor binding, fixation of dimerization inhibitors unmasks hydrophobic protein surfaces. This agrees with dissociation or destabilization of the dimer enzyme favoring the exposure of hydrophobic areas located at the interface between monomers.[25]

Conclusions

Our previous finding of "molecular tongs" as interfacial inhibitors of HIV-1 protease[23] prompted us to

continue this work in order to get new insights in the structure−activity relationship of this kind of inhibitor. Thus, we have introduced quinoline as a new rigid scaffold, along with modifications of the structure of peptidic strands attached to naphthalene- and quinoline-based scaffolds. The main results are (i) quinoline is a valuable scaffold, as most of quinoline-based tongs were found as pure interfacial inhibitors of HIV-1 PR leading to efficient molecules (K_{id} = 80 nM for **30**); (ii) the attached tripeptidic strands induce generally better inhibitions than the dipeptidic ones; and (iii) hydrophobicity of the attached peptidic strands is a favorable property to induce inhibition. However two points remain to be clearly established: the superiority of inhibitors with two different tripeptides over inhibitors with two identical ones, and the superiority of the quinoline scaffold over the naphthalene one. These two points are under current evaluation by preparation of a quinoline scaffold endowed with two Thr-Leu-Asn-OMe chains and of the naphthalene counterpart of compound **30**. Further studies on the HIV dimer inhibitors reported here will include (i) optimization of the electrostatic interaction with the C-terminus, including evaluation at pH values closer to neutrality; (ii) establishment of the antiviral efficacy in cell culture assays; (iii) evaluation of binding to the proteases from other strains of the HIV virus to assess the potential, if any, of differential binding; (iv) creating proteolysis-resistant inhibitors by replacement of the peptide strands by pseudopeptides; and (v) assessment of binding specificity with other β-sheet proteins as a early indicator of toxicity.

Experimental Section

Materials. Usual solvents were purchased from commercial sources. Dimethylformamide (DMF) was distilled on BaO. Tetrahydrofuran (THF) was distilled on sodium/benzophenone; acetonitrile was distilled on $CaCl_2$. Liquid chromatography was performed on Merck silica gel 60 (70/30 mesh); TLC was done on silica gel, 60F-250 (0.26 mm thickness) plates. Benzyl bromide, ethyl 4-bromobutyrate, 2,7-dihydroxynaphthalene **1**, protected amino acids, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were purchased from commercial sources. Benzyl 4-bromobutyrate,[29] H-Val-Leu-Val-OMe, H-Thr-Leu-Asn-OMe, H-Thr-Ile-Val-OMe,[23] and 7-hydroxy-1*H*-quinolin-2-one **5**[26] were prepared according to methods reported in the literature. Melting points were determined on a Kofler melting point apparatus. NMR spectra were performed on a Bruker AMX 200 (^{1}H, 200 MHz; ^{13}C, 50 MHz) or a Bruker AVANCE 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz). Unless otherwise stated $CDCl_3$ was used as solvent. Chemical shifts δ are in ppm, and the following abbreviations are used: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), multiplet (m), broad triplet (bt), and broad singlet (bs). IR spectra were performed on a Bruker Vector 22 apparatus. Mass spectra were obtained using a Bruker Esquire electrospray ionization apparatus. Elemental analyses were performed at the Microanalysis Service of the Faculty of Pharmacy at Châtenay-Malabry (France).

HIV-1 PR, from the HIV (BRU) DNA clone (subclone of λJ19),[32] was expressed in *Escherichia coli* BL21(DE3)pLysS, isolated and purified as described by Billich et al.[33] 1-Anilino-8-naphthalene sulfonate (ANS) was purchased from Sigma-Aldrich Chimie. Fluorescence experiments were performed on a Perkin-Elmer LS 50 B luminescence spectrofluorometer equipped with a thermostated cell holder. A Suprasil quartz cell with a 1-cm path length from Hellma (Germany) was used for all experiments.

Enzyme and Inhibition Assays. The proteolytic activity of PR was assessed by using the fluorogenic substrate (DABCYL-γ-Abu-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-EDANS) at pH 4.7 and 30 °C in 100 mM sodium acetate containing 1 mM EDTA and 1 M NaCl. Excitation and emission wavelengths were 340 and 490 nm, respectively. Substrate alone (blank) or substrate and inhibitor previously dissolved in DMSO (final concentration: 3% v/v) were preincubated for 3 min at 30 °C. Enzyme was added to start the reaction. The final enzyme concentration was 7.5 nM. The increase of fluorescence (λ_{ex} = 340 nm; λ_{em} = 490 nm) was followed during 3 min. The IC_{50} values were determined using 0.52 μL of a 3 mM substrate solution and 8.5 μL of 8−10 different concentrations of inhibitor in a total volume of 300 μL. The enzymatic reaction was begun by adding of 1.6 μL of enzyme (prediluted in buffer with BSA). IC_{50} values were calculated by fitting experimental data to the equation % inhibition = $100[I]_0/(IC_{50} + [I]_0)$. The Zhang's kinetic analyses were carried out at constant initial substrate and using at least four concentrations of enzyme (4.7−13.6 nM). Inhibition of PR was measured at different concentrations of inhibitor: **18** (2.5 and 4 μM); **19** (4.33 μM); **20** (0.8 μM); **21** (1.5 μM); **23** (2 and 4.33 μM); **24** (0.66 μM); **25** (1 and 2.33 μM); **26** (2 and 5.66 μM); **27** (1.33 and 2.83 μM); **29** (4 μM); **30** (1 and 3 μM); **32** (0.66 and 1.66 μM); **36** (1.5 and 4 μM) and **37** (4 μM). The K_{ic} and K_{id} values were determined as previously described.[16,22,23]

Fluorescence Experiments. The experiments were performed by diluting the PR from a protein stock solution (stored in ice) into the appropriate buffer: 100 mM sodium acetate, 1 mM EDTA, 1 M NaCl, 3% DMSO (v/v), pH 4.7 and 25 °C at a final concentration of 350 nM. Then, PR was preincubated in the absence or in the presence of the inhibitor (compound **23** at 3.75 μM or compound **32** at 1.66 μM) prior to the addition of ANS (20 or 30 μM). The excitation wavelength was 376 nm. Excitation and emission bandwidths were 5 and 20 nm, respectively. Acetylpepstatin (0.1 μM) was studied under the same experimental conditions. Each spectrum was collected three times and averaged. The spectrum for buffer containing ANS + inhibitor or ANS + DMSO was subtracted from the corresponding curve obtained in the presence of enzyme. It has been verified that the intrinsic fluorescence of PR and of compounds **23** and **32** was negligible under the experimental conditions used.

Analytical Gel Filtration Chromatography. Inhibitor **25** (5 mM) was incubated for 30 min at 25 °C in 100 mM sodium acetate (pH 4.7) containing PR (95 nM). The blank (no added inhibitor) contained the same final DMSO concentration (5% v/v). The mixture was loaded onto a column of Superdex 75 (30 cm × 3.2 mm^2, Amersham Biosciences). The preequilibrated column was eluted with the same sodium acetate buffer (100 μM, pH 4.7), flow rate 0.1 mL·min^{-1}. Absorbance at 280 nm was followed using the Empower program (Waters). The column was previously calibrated with cytochrome *c*, horse skeletal muscle myoglobin, bovine serum albumin, and human thrombin. Bichromate potassium and dextran blue were used for determination of the column total and void volumes.

4-(7-Hydroxy-naphthalen-2-yloxy)butyric Acid Ethyl Ester (2). To a solution of **1** (5 g, 31 mmol) and K_2CO_3 (6.5 g, 47 mmol) in anhydrous DMF (50 mL) was added dropwise ethyl 4-bromobutyrate (4 mL, 28 mmol). The mixture was stirred for 24 h, and then solvent was evaporated in vacuo. The residue was dissolved in CH_2Cl_2, and the solution was washed with water (5 × 30 mL), dried (Na_2SO_4), and concentrated in vacuo. The resulting crude product was chromatographed on silica gel (petroleum ether/ethyl acetate 4:1) to give 2.8 g (36%) of **2** as white needles, mp = 91−93 °C. ^{1}H NMR: δ 7.6 (d, 2H), 7.0−6.9 (m, 4H), 5.5 (bs, 1H), 4.2−4.0 (m, 4H), 2.5 (t, 2H), 2.1 (m, 2H), 1.2 (t, 3H). ^{13}C NMR: δ 174.4, 157.2, 136.3, 129.3, 123.4, 116.2, 115.5, 108.4, 105.8, 66.7, 60.2, 30.5, 24.6, 14.4. Anal. ($C_{16}H_{18}O_4$) C, H.

4-(7-Benzyloxy-naphthalen-2-yloxy)butyric Acid Ethyl Ester (3). To a solution of **2** (6.5 g, 24.4 mmol) and K_2CO_3 (4.1 g, 29.3 mmol) in anhydrous CH_3CN (70 mL) was added benzyl bromide (3.48 mL, 29.3 mmol). The mixture was

refluxed for 3 h and stirred at room temperature for 24 h. After filtration, the filtrate was concentrated in vacuo, the residue was dissolved in CH_2Cl_2 (70 mL), and the solution was washed with water (3 × 10 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated. The crude product was chromatographed on silica gel (petroleum ether/ethyl acetate 9:1) to give 4.98 g (60%) of **3** as white crystals, mp = 84−85 °C (petroleum ether/ethyl acetate 95:5). ^{1}H NMR: δ 7.7−6.9 (m, 11H), 5.1 (s, 2H), 4.2−4.0 (m, 4H), 2.5 (t, 2H), 2.1 (m, 2H), 1.3 (t, 3H). ^{13}C NMR: δ 174.4, 157.2, 136.3, 129.3, 128.0, 123.4, 116.2, 115.5, 108.4, 105.8, 69.5, 66.7, 60.2, 30.5, 24.6, 14.4. Anal. ($C_{23}H_{24}O_4$) C, H.

4-(7-Benzyloxy-naphthalen-2-yloxy)butyric Acid (4). A suspension of **3** (0.9 g, 2.5 mmol) in a 10% KOH methanolic solution (25 mL) was stirred at reflux for 4 h. The solid obtained after evaporation of the solvent was suspended in water (10 mL) and the mixture acidified with 1 N HCl. Product was then extracted twice (methylene chloride), and the organic layers were dried ($MgSO_4$) and concentrated in vacuo. The crystalline crude product was recrystallized (ethyl acetate/petroleum ether 85:15) to give 0.76 g (92%) of the desired acid **4** as white crystals, mp = 132−134 °C. ^{1}H NMR: δ 7.9−7.6 (m, 11H), 5.1 (s, 2H), 4.1 (t, 2H), 2.5 (t, 2H), 2.1 (m, 2H). ^{13}C NMR: δ 174.4, 157.3, 157.1, 137.3, 136.0, 129.3, 128.0, 124.2, 116.4, 107.1, 106.5, 69.5, 66.9, 30.5, 24.6. Anal. ($C_{21}H_{20}O_4$) C, H.

7-Benzyloxy-1H-quinolin-2-one (6). To a solution of **5**[26] (1 g, 6.2 mmol) in 2-propanol (15 mL) were added benzyl bromide (0.9 mL, 7.52 mmol) and DBU (1.4 mL, 9.28 mmol). The mixture was refluxed for 4 h. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2 and the organic layer was washed with aqueous 10% Na_2CO_3 (20 mL), 1 N HCl (20 mL), and water (20 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo, and the solid residue was recrystallized (methanol) to afford 1.1 g (71%) of **6** as white crystals, mp = 219−221 °C. ^{1}H NMR (DMSO-d_6): δ 11.6 (s, 1H), 7.8 (d, 1H), 7.6 (d, 1H), 7.3−7.5 (m, 5H), 6.8−6.9 (dd, 2H), 6.3 (d, 1H), 5.1 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 162.2, 160.0, 140.5, 139.9, 136.4, 129.2, 128.4, 127.6, 118.7, 113.5, 110.9, 99.2, 69.4. IR: ν = 1658 cm^{-1}. Anal. ($C_{16}H_{13}O_2N$) C, H, N.

4-(7-Benzyloxy-quinoline-2-yloxy)butyric Acid Ethyl Ester (7) and 4-(7-Benzyloxy-quinoline-2-oxo-2H-quinoline-1-yl)butyric Acid Ethyl Ester (8). A solution of **6** (0.5 g, 2.0 mmol) and K_2CO_3 (0.41 g, 3 mmol) in anhydrous DMF (10 mL) was stirred for 1 h. Then ethyl 4-bromobutyrate (0,5 mL, 3.95 mmol) was added, the mixture was stirred for 24 h at room temperature. After evaporation of the solvent in vacuo, the residue was dissolved in CH_2Cl_2 and the solution washed with water. The organic layer was dried ($MgSO_4$) and evaporated in vacuo, and the crude product was chromatographed on silica gel, giving rise to the two isomers **7** and **8** (petroleum ether/AcOEt, 8:2). Ester **7**: 0.126 g (40%), mp = 105−107 °C (petroleum ether/ethyl acetate, 8:2); IR ν 1734 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.9 (d, 1H), 7.5 (d, 1H), 7.2−7.5 (m, 5H), 7.2 (dd, 1H), 7.0 (d, 1 H), 6.7 (d, 1H), 5.1 (s, 2H), 4.4 (t, 2H), 4.1 (m, 2H), 2.5 (t, 2H), 2.1 (q, 2H), 1.2 (t, 3H); ^{13}C NMR (DMSO-d_6) δ 173.1,162.4,160.0,148.2, 138.2, 136.6, 128.5, 127.4, 119.9, 116.3, 110.3, 107.7, 70.0, 64.6, 60.2, 31.0, 24.4, 14.3. Anal. ($C_{22}H_{23}O_4N$) C, H, N. Ester **8**: 0.132 g (42%), mp = 79−80 °C; IR ν 1727 and 1646 cm^{-1}; ^{1}H NMR δ 7.4 (d, 1H), 7.3−7.1 (m, 5H), 7.2 (d, 1H), 7.1 (bs, 1H), 6.7 (d, 1H), 6.3 (d, 1H) 5.1 (s, 2H), 4.4 (m, 2H), 4.1 (t, 2H), 2.4 (t, 2H), 2.1 (q, 2H), 1.2 (t, 3H); ^{13}C NMR (DMSO-d_6) δ 173.1, 162.4, 160.0, 148.2, 138.2, 136.6, 128.5, 127.4, 119.9, 116.3, 110.3, 107.7, 70.0, 64.6, 41.5, 31.0, 21.9, 14.1. Anal. ($C_{22}H_{23}O_4N$) C; H calcd 6.3, found 6.8; N.

4-(7-Benzyloxy-quinoline-2-yloxy)butyric Acid (9). The same procedure was used as for the preparation of **4**. Compound **9** was obtained, from the ester **7**, as white crystals (84%), mp 159−161 °C (methanol, ethyl acetate). ^{1}H NMR (DMSO-d_6): δ 7.9 (d, 1H), 7.7 (d, 1H), 7.5−7.3 (m, 5H), 7.3 (d, 1H), 7.1 (dd, 1H), 6.8 (dd, 1H), 5.2 (s, 2H), 4.5 (t, 2H), 2.6 (t, 2H), 2.2 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 178.8, 163.0, 160.6, 148.4, 139.0, 137.0, 129.0, 128.4, 120.4, 117.0, 110.8, 107.8, 70.5, 65.0, 31.3, 24.8. Anal. ($C_{20}H_{19}O_4N$) C, H, N.

4-(7-Benzyloxy-naphthalen-2-yloxy)butyryl-Val-Leu-Val-OMe (10). To a stirred solution of **4** (1.24 g, 3.68 mmol), HBTU (1.53 g, 4.04 mmol), and TEA (0.56 mL, 4.04 mmol) in anhydrous DMF (25 mL) were added H-VLVOMe (1.26 g, 3.68 mmol) and 0.5 mL of TEA. The mixture was stirred for 24 h, DMF was evaporated in vacuo, the residue was dissolved in CH_2Cl_2, and the solution was washed with 1 N HCl (30 mL), 1 N NaOH (30 mL), and water. The organic layer was dried ($MgSO_4$) and evaporated in vacuo to give 2.7 g (90%) of a crude product, which was recrystallized (ethyl acetate/methanol) to afford **10** as a white powder, mp 215−217 °C. ^{1}H NMR: δ 7.6 (t, 2H), 7.4−7.3 (m, 5H), 7.1 (bs, 1H), 7.05 (dd, 1H), 7.0 (bs, 1H), 6.9 (d, 1H), 5.1 (s, 2H), 4.6 (m, 1H), 4.5 (m, 1H), 4.4 (m, 1H), 4.1 (t, 2H), 3.7 (s, 3H), 2.6−2.4 (m, 2H), 2.2−2.1 (m, 3H), 1.9 (m, 1H), 1.8−1.5 (m, 2H), 0.9−0.8 (m, 18H). ^{13}C NMR: δ 173.0, 172.5, 172.0, 157.3, 157.1, 137.0, 128.9, 128.0, 124.3, 116.3, 116.2, 106.4, 106.1, 70.0, 66.5, 58.4, 57.2, 52.1, 51.9, 41.0, 31.2, 30.1, 29.6, 24.6, 24.2, 22.5, 22.2, 19.0, 17.7. Anal. ($C_{38}H_{51}N_3O_7$) C, H, N.

4-(7-Benzyloxy-quinoline-2-yloxy)butyryl-Val-Leu-Val-OMe (11). The same procedure as described for preparation of **10** was used: 93%, mp 214−216 °C (methylene chloride/methanol).^{1}H NMR (DMSO-d_6): δ 8.2 (d, 1H), 8.1−7.9 (m, 3H), 7.7 (d, 1H), 7.4−7.3 (m, 5H), 7.1 (bs, 1H), 6.9 (dd, 1H), 6.7 (d, 1H), 5.1 (s, 2H), 4.6 (m, 1H), 4.5 (m, 1H), 4.4 (m, 1H), 4.1 (t, 2H), 3.7 (s, 3H), 2.6−2.4 (m, 2H), 2.2−2.1 (m, 3H), 1.9 (m, 1H) 1.8−1.5 (m, 3H), 0.9−0.8 (m, 18H). ^{13}C NMR (DMSO-d_6): δ 172.0, 162.0, 160.0, 139.0, 129.0, 128.7, 128.3, 116.2, 106.4, 106.1, 70.0, 65.0, 51.9, 41.0, 32.0, 31.2, 30.1, 25.0, 24.0, 23.0, 17.7. Anal. ($C_{37}H_{50}N_4O_7$) C, H, N.

4-(7-Hydroxy-naphthalen-2-yloxy)butyryl-Val-Leu-Val-OMe (12). A solution of **10** (2.2 g, 3.32 mmol), and 10% Pd/C (0.3 g) in MeOH (50 mL) was stirred under hydrogen. After 24 h, the suspension was filtered through Celite, and the Celite bed was washed thoroughly with ethyl acetate (100 mL). The filtrate was evaporated to dryness to yield 1.88 g of a crude powder, which was purified by liquid chromatography (ethyl acetate/petroleum ether, 20/80) (1.72 g, 90%). ^{1}H NMR: δ 7.7−7.5 (m, 3H), 6.9−6.8 (m, 4H), 4.5−4.2 (m, 3H), 3.9 (t, 2H), 3.6 (s, 3H), 2.6−2.4 (m, 2H), 2.1−1.3 (m, 7H), 0.8−0.7 (m, 18H). ^{13}C NMR (DMSO-d_6): δ 173.0, 172.0, 156.2, 136.0, 128.8, 124.6, 116.0, 115.9, 106.0, 66.5, 58.0, 52.5, 52.0, 41.0, 31.2, 30.1, 29.0, 24.6, 24.2, 22.5, 22.2, 19.0, 18.8, 18.4, 17.7. Anal. ($C_{31}H_{45}N_3O_7$) C, H, N.

4-(7-Hydroxy-quinoline-2-yloxy)butyryl-Val-Leu-Val-OMe (13) was synthesized from **11** by the same procedure as described above: 99%, foam. ^{1}H NMR (DMSO-d_6): δ 8,1 (d, 1H), 8.0−7.9 (m, 3H), 7.6 (d, 1H), 7.3 (bs, 1H), 6.9 (dd, 1H), 6.7 (d, 1H), 4.4 (m, 3H), 4.5 (m, 2H), 3.6 (s, 3H), 2.4−2.3 (m, 2H), 2.1−1.9 (m, 4H), 1.6 (m, 1H), 1.4 (m, 2H), 0.9−0.8 (18H, m). ^{13}C NMR (DMSO-d_6): 172.6, 172.4, 172.3, 162.0, 160.0, 149.0, 139.0, 129.0, 119.5, 116.0, 109.5, 65.0, 57.5, 52.0, 50.5, 41.5, 31.5, 31.0, 25.0, 24.5, 22.5, 17.7. Anal. ($C_{30}H_{44}N_4O_7 \cdot {}^3/_2H_2O$) C, H, N.

4-[7-(3-Benzyloxycarbonyl-propoxy)naphthalen-2-yloxy]-butyryl-Val-Leu-Val-OMe (14). A solution of **12** (1.9 g, 3.32 mmol), K_2CO_3 (0.7 g, 5 mmol) and benzyl 4-bromobutyrate (1 g, 4 mmol) in anhydrous DMF (20 mL) was stirred for 24 h at 50 °C. The solvent was evaporated in vacuo, the residue was dissolved in CH_2Cl_2 (30 mL), and the solution was washed with Na_2CO_3 10% (30 mL), NH_4Cl 10% (30 mL), and water (30 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. The crude product was chromatographed on silica gel (methylene chloride/methanol: 95/5) to yield 2 g (80%) of the benzyl ester **14**. ^{1}H NMR: δ 7.8 (d, 1H), 7.6 (d, 1H), 7.5 (d, 1H), 7.3−7.2 (m, 5H), 7.2 (d, 1H), 7.0−6.9 (m, 4H), 5.1 (s, 2H), 4.7 (m, 1H), 4.6 (m, 1H), 4.5 (m, 1H), 4.1 (t, 4H), 3.7 (s, 3H), 2.6 (t, 2H), 2.5−2.4 (m, 2H), 2.2−2.1 (m, 4H), 2.0−1.9 (m, 2H), 1.7 (m, 1H), 1.6 (m, 1H), 1.5 (m, 1H), 0.9−0.8 (m, 18H). ^{13}C NMR (DMSO-d_6): δ 172.0−171.0, 170.0, 157.0, 135.0, 129.0, 128.0, 124.0, 116.0, 106.0, 67.0, 65.1, 57.0, 52.0, 51.0, 41.0, 32.5, 31.0, 24.0, 22.0, 18.0. Anal. ($C_{42}H_{57}N_3O_9$) C, H, N.

4-[7-(3-Benzyloxycarbonyl-propoxy)quinolin-2-yloxy]-butyryl-Val-Leu-Val-OMe (15) was synthesized from **13** by the same procedure as described above: 65%, mp 139−140 °C. ^{1}H NMR: δ 8.1−7.8 (m, 5H), 7.5 (m, 5H), 7.0−6.9 (dd, 2H), 6.6 (d, 1H), 5.2 (s, 2H), 4.2−4.1 (m, 3H), 4.0−4.1 (m, 4H), 3.6 (s, 3H), 2.2 (m, 2H), 2.1−1.9 (m, 5H), 1.4−1.3 (m, 4H), 0.9 (m, 18H). ^{13}C NMR: δ 138.0, 130.0, 128.0, 117.0, 109.0, 107.0, 72.0, 66.0, 60.0, 58.0, 51.0, 41.0, 33.3, 32.2, 31.0, 25.0, 23.0, 21.5. Anal. ($C_{41}H_{56}N_4O_9$) C, H, N.

4-[7-(3-Carboxy-propoxy)naphthalen-2-yloxy]butyryl-Val-Leu-Val-OMe (16). A solution of **14** (0.32 g, 0.42 mmol) and 10% Pd/C (0.03 g) in DMF (10 mL) was stirred under hydrogen. After 24 h, the suspension was filtered through Celite, and the Celite bed was washed thoroughly with ethyl acetate (100 mL). The filtrate was evaporated to one-fourth volume, the final product precipitated as a white powder after addition of water (2 mL), and the precipitate was dried to give 0.2 g (80%) of the desired acid. ^{1}H NMR: δ 7.6 (d, 2H), 7.3 (d, 1H), 7.1 (d, 1H), 7.0 (m, 2H), 6.9 (m, 2H), 6.8 (d, 1H), 4.5−4.4 (m, 2H), 4.3 (m, 1H), 4.1 (t, 2H), 4.0 (t, 2H), 3.7 (s, 3H), 2.6 (t, 2H), 2.5−2.4 (m, 2H), 2.2−2.1 (m, 5H), 2.1−1.9 (m, 1H), 1.7−1.4 (m, 3H), 0.9−0.8 (m, 18H). ^{13}C NMR: δ 173.1, 171.8, 157.8, 128.9, 124.3, 116.3, 116.2, 107.0, 70.0, 67.0, 58.6, 58.1, 51.7, 50.9, 41.0, 32.4, 31.2, 30.1, 29.6, 24.6, 24.8, 24.2, 22.5, 22.2, 19.0, 17.7. Anal. ($C_{35}H_{51}N_3O_9$) C, H, N.

4-[7-(3-Carboxy-propoxy)quinolin-2-yloxy]butyryl-Val-Leu-Val-OMe (17) was synthesized from **15** by the same procedure as described above: 71%. ^{1}H NMR (DMSO-d_6): δ 8.2 (d, 1H), 8.0 (m, 3H), 7.8 (d, 1H), 7.2 (d, 1H), 7.1 (dd, 1H), 6.9 (d, 1H), 4.4−4.3 (m, 3H), 4.2 (m, 4H), 3.7 (s, 3H), 2.4 (m, 4H), 2.2−2.0 (m, 6H), 1.7 (m, 2H), 1.6 (m, 1H), 0.8 (m, 18H). ^{13}C NMR (DMSO-d_6): δ 174.0, 172.7, 172.0, 162.0, 160.1, 148.0, 139.0, 129.0, 120.0, 116.0, 110.5, 107.5, 67.7, 66.1, 58.3, 52.0, 51.4, 42.0, 32.0, 30.0, 25.6, 24.5, 24.0, 18.0. Anal. ($C_{34}H_{50}N_4O_9$) C, H; N calcd 8.4, found 8.9.

General Synthesis of Nonsymmetric Tongs (18−20, 22−27, 29−32): Example of 19. To a stirred solution of **16** (0.143 g, 0.21 mmol), HBTU (0.09 g, 0.24 mmol), and TEA (33.24 μL, 0.24 mmol) in DMF (5 mL) was added a solution of Ile-Thr-NH_2 (0.05 g, 0.21 mmol) in DMF (2 mL). The mixture was stirred for 24 h. The solvent was removed, and the residue was dissolved in CH_2Cl_2 (10 mL). A product precipitated, which was filtered, washed with 1 N NaOH (10 mL), 1 N HCl (10 mL), water (10 mL), methylene chloride (10 mL), and methanol, and dried at 60 °C in vacuo.

4-[7-(3-Ethoxycarbonyl-propoxy)-2-oxo-2*H*-quinoline-1-yl]butyric Acid Ethyl Ester (33) and 4-[7-(3-Ethoxycarbonyl-propoxy)quinoline-7-yloxy]butyric Acid Ethyl Ester (34). To a solution of **5** (0.5 g, 3 mmol) and K_2CO_3 (0.63 g, 9 mmol) in DMF (5 mL) was added ethyl 4-bromobutyrate (0.43 mL, 6 mmol). The mixture was stirred for 24 h. The solvent was evaporated, and the crude product was chromatographed on silica gel (petroleum ether/ethyl acetate, 60:40) to give the N-alkylated product **33** and its O-alkylated isomer **34**. Product **33**: 49%; IR ν = 1646 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.7 (d, 1H), 7.5 (d, 1H), 7.0 (d, 1H), 6.9 (d, 1H), 6.7 (d, 1H), 4.5 (t, 2H), 4.4 (m, 6H), 2.6 (m, 4H), 2.3 (m, 4H), 1.2 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 173.2, 173.1, 162.5, 160.0, 148.0, 138.0, 128.0, 119.0, 116.0, 110.0, 107.0, 67.0, 64.7, 41.0, 31.1, 30.9, 24.0, 14.0. Anal. ($C_{21}H_{27}NO_6$) C, H, N. Product **34**: 43%; ^{1}H NMR (DMSO-d_6) δ 7.9 (d, 1H), 7.6 (d, 1H), 7.2 (d, 1H), 7.0 (d, 1H), 6.7 (d, 1H), 4.5 (t, 2H), 4.2 (m, 6H), 2.6 (m, 4H), 2.2 (m, 4H), 1.3 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 173.2, 173.1, 162.5, 160.1, 148.3, 138.2, 128.3, 119.8, 116.2, 110.3, 107.2, 66.8, 64.7, 60.3, 31.1, 30.8, 24.5, 14.1. Anal. ($C_{21}H_{27}NO_6$) C, H, N.

4-[7-(3-Carboxy-propoxy)quinolin-7-yloxy]butyric Acid (35). The carboxylic acid **35** was obtained from **34** by the same procedure as described for **4** and **8**: 90%. ^{1}H NMR (DMSO-d_6): δ 12.1 (s, 2H), 8.1 (d, 1H), 7.8 (d, 1H), 7.2 (d, 1H), 7.0 (d, 1H), 6.9 (d, 1H), 4.4 (t, 2H), 4.1 (m, 2H), 2.4 (m, 4H), 2.0 (m, 4H).^{13}C NMR (DMSO-d_6): δ 173.2, 173.1, 162.5, 160.1, 148.3, 140.0, 129.3, 119.8, 116.2, 110.3, 107.2, 67.0, 65.0, 30.0, 24.5. Anal. ($C_{17}H_{19}NO_6$) C, H, N.

Synthesis of Symmetric Tongs 36 and 37. Tongs **36** and **37** were synthesized using HBTU coupling methods from **35**, as described in the general synthesis of nonsymmetric tongs.

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Supporting Information Available: NMR data of synthesized peptides not described and analytical data of compounds **18**, **20**, **22**−**27**, **29**−**32**, **36**, and **37**. Table of elemental analysis of compounds **18**−**32**, **36**, and **37**. This material is available free of charge via the Internet at http://pubs.acs.org.

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