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Introduction of polar groups on the naphthalene scaffold of molecular tongs inhibiting wild-type and mutated HIV-1 protease dimerization†

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A new series of naphthalene-based molecular tongs containing polar groups at the 3-position of the naphthalene scaffold was synthesized and its anti-dimerization activity was evaluated against HIV-1 protease. The polar groups were introduced mainly *via* metal-catalysed cross coupling reactions such as the Buchwald–Hartwig amination, the Sonogashira reaction and the Beller cyanation. Kinetic analyses showed that by introducing specific polar substituents on the naphthalene scaffold the inhibitory activity of molecular tongs against wild-type and mutated HIV-1 proteases is maintained, while increasing their water solubility.

Introduction

The human immunodeficiency virus type 1 (HIV-1) protease (PR) is an important target in the fight against AIDS, since its inhibition results in the production of an uninfectious virus.[1,2] The PR inhibitors (PIs) used in highly active antiretroviral therapy (HAART) act as transition-state analogues by targeting the PR active site, however the emergence of several mutations within or outside the active site[3,4] can lead to PI resistance. The mature PR is only active as a homodimer (99 residues per monomer), in which the energy of monomer–monomer interactions and consequently the energy of dimerization is not uniformly distributed along the monomer–monomer interface. The PR homodimer is mainly stabilised by a four-stranded antiparallel β-sheet that involves the N-(H-Pro(1)-Gln(2)-Ile(3)-Thr(4)) and C-termini (Cys(95)-Thr(96)-Leu(97)-Asn(98)-Phe(99)-OH) from both monomers. This four-stranded region contains the main hotspot interface residues, providing more than 50% of the hydrogen bonds along the dimerization interface[5] and contributing to close to 75% of the total Gibbs free energy of PR dimerization.[6] Interestingly, this PR termini β-sheet interface appears to be free of mutations.[7] Therefore, targeting this hotspot could prevent the essential dimerization process or disrupt the dimer, and as a consequence constitute an alternative to drugs that target the active site.[8,9] Several dimerization inhibitors targeting the β-sheet region have been described in the literature, namely, C- and N-terminal mimetic peptides,[10–12] lipopeptides,[13–15] bicyclic guanidinium and alkyl hydroxybenzoic acid derivatives[16] and cross-linked peptides with flexible[17] or semi-rigid spacers.[18] Our strategy for inhibiting PR dimerization consisted of designing organised molecular tongs displaying a rigid spacer to provide an entropic benefit.[19–23] Initially, we described the design of molecular tongs based on a naphthalene or a quinoline scaffold in which two identical or non-identical peptide strands were attached through a carboxypropyloxy linker to enable the formation of an antiparallel β-sheet with the N- and C-termini of one PR monomer.[19,20] Afterwards, one or two peptide fragments were replaced by peptidomimetic ones while keeping the same array of hydrogen bonds.[21,22] Inserting a 5-acetamido-2-methoxybenzohydrazide group into one or two strands (for example, molecular tongs **1** and **5**, Fig. 1 and 2 respectively) provided efficient inhibitors of wild-type (PR) *in vitro* (K_{id} of 0.22 and 0.28 μM for **1** and **5** respectively), with an increased metabolic stability.[21,22] However, one drawback of our previously reported molecular tongs was their lack of water solubility. More recently, our efforts have focused on the development of more water-soluble non-peptidic molecular tongs. For that purpose, two strategies have been established. In a first attempt hydrophilicity was

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† Electronic supplementary information (ESI) available: Experimental part with the synthesis procedures and characterisation of all the new compounds; characteristic ^{1}H, ^{13}C and 2D NMR spectra of a few molecular tongs; HPLC chromatograms of molecular tongs **3** and **8** and evolution with time of the concentration of molecular tongs **3** and **8** incubated at 37 °C in RPMI culture medium containing 20% fetal calf serum. See DOI: 10.1039/c4md00032c

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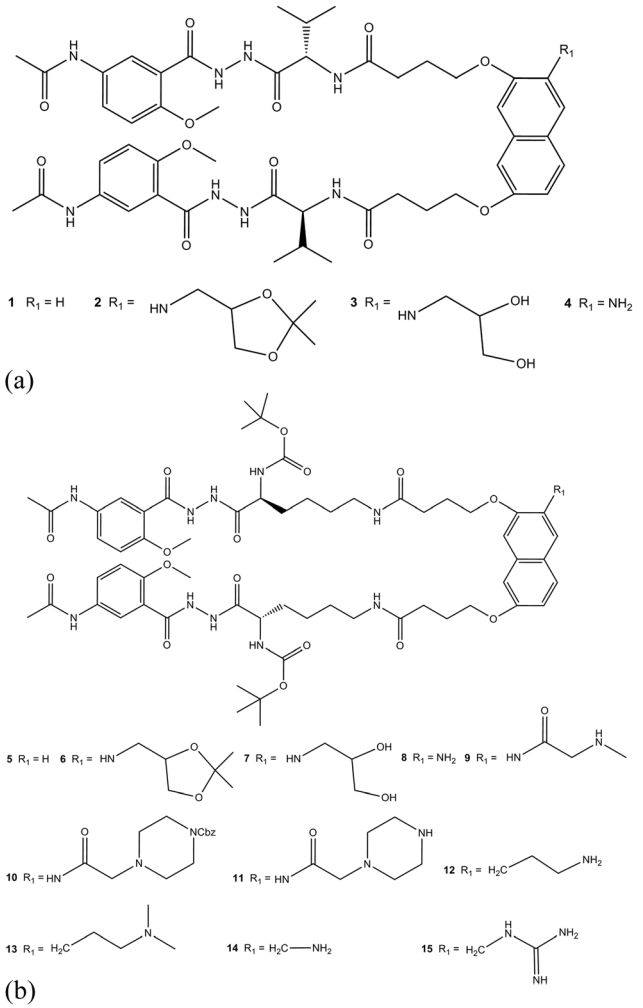

Fig. 1 Structure of molecular tongs **1–15**.

increased within the strands of the molecular tongs by synthesizing new molecular tongs containing carbonylhydrazide (CONHNHCO) and oligohydrazide (azatide) peptidomimetic fragments displaying increased water solubility.[23] The inhibitory potency depended on the peptidomimetic strand structure suggesting that an appropriate balance between hydrophilicity and hydrophobicity had to be reached in order to obtain powerful inhibitors.[23] In this report, a second strategy is described based on the introduction of hydrophilic groups on the 3-position of the naphthalene scaffold, while keeping the structure of the hydrophobic strands, in order to change the physicochemical properties in the non-pharmacophore region of the molecular tongs. Keeping the two strands of molecular tongs **1** and **5** (those with the 5-acetamido-2-methoxybenzohydrazide group attached to the carboxypropyloxy linker through a valine or the ε-amino group of a lysine residue), the main challenge was to obtain similar or increased anti-dimerization PR efficiency, while introducing the naphthalene moiety groups whose steric and/or electronic effects do not destabilize the interaction with PR monomer termini. In molecular tongs **3** and **4**, (both derivatives of molecular tong **1**) an aminopropanediol and an amine were respectively introduced (Fig. 1). It has been well established that when the number of polar groups (hydroxyl, carboxylic acid or amine groups) increases in a molecule, water solubility is enhanced since new dipole–dipole interactions (such as those involved in hydrogen bonding) and thus molecule–solvent interactions are rendered possible.[24] For instance, the introduction of a *N*-1,2-dihydroxypropyl moiety has been previously used to improve water solubility and thus enhance drug-likeness properties.[25] The protected precursor **2** was also evaluated in order to study the influence of the presence or absence of a diol moiety on the PR dimerization inhibitory activity. Starting from molecular tong **5**, which is deprived of any peptidic character,[22] additional diversity was introduced on the polar groups (Fig. 1). An aminopropanediol and a primary amine were attached to the naphthalene scaffold (molecules **7** and **8** respectively). The dioxolane precursor of **7** was also evaluated (molecule **6**). In molecules **9** and **11**, a secondary or a tertiary amine (methylamino or piperazine) was introduced on the scaffold *via* an alkylamide function. The protected precursor **10** was also evaluated for its PR dimerization inhibitory potency. Primary or tertiary amines were also inserted using a propyl or methyl chain as a linker in molecules **12**, **13** and **14**. Finally, in molecule **15** a guanidine group, which has been previously shown to increase water solubility,[26] was introduced. The amine and guanidine groups become charged at biologically relevant pH values and thus should increase the water solubility of molecules **9–15**. Most of the polar groups present on the naphthalene scaffold were introduced by metal-catalysed cross coupling reactions.

Results and discussion

Chemistry

The synthetic procedure designed to obtain the modified naphthalene scaffolds relies on the initial introduction of a bromine atom at the 3-position of the naphthalene-2,7-diol **16**, to allow further functionalization through metal-catalysed reactions, such as Buchwald–Hartwig aminations and Sonogashira couplings.

Bromination of naphthalene-2,7-diol with bromine in acetic acid gave rise to a mixture of two dibromo derivatives, the 1,3- and 1,6-isomers,[27] which by an *in situ* monodebromination using Sn powder[27,28] afforded the desired 3-bromonaphthalene-2,7-diol **17**, in excellent yield (94%, Scheme 1). Compound **17** was then alkylated with benzyl-4-bromobutanoate or ethyl-4-bromobutanoate to afford the dibenzyl ester **18** (89% yield) and the diethyl ester **19** (82% yield) respectively (Scheme 1).

Then amination reactions on the bromonaphthalene derivatives **18** were performed (Scheme 2). Firstly, we investigated the best conditions for coupling bromonaphthalene derivative **18** with a primary amine bearing a 2,2-dimethyl-1,3-dioxolane methyl group, which would be the precursor of the polar aminopropanediol group found in the target molecular tongs **3** and **7** (Fig. 1 and 2). Nucleophilic aromatic substitution of **18** was initially investigated using a copper catalyst (CuI, 20 mol%) in the presence of a base (Ullmann conditions),[29] without (Table 1, entry 1) or in the presence of a ligand (Table 1, entries 2–6). No

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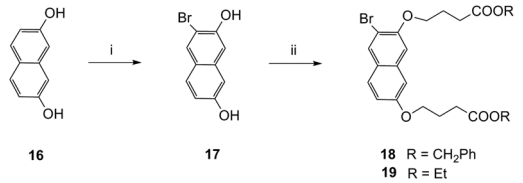
substitution was observed in the absence of a ligand (entry 1). The introduction of L-proline[30,31] as a ligand (entries 2 and 3) was not successful, resulting in complex crude mixtures. The use of the bidentate ligand DMEDA (entries 4–6)[32,33] afforded the desired substituted product **20**, albeit in low yields. In this case, the best conditions screened were: DMEDA (10 equiv.) in the presence of potassium carbonate (2 equiv.) in dioxane at 110 °C for 24 h, which allowed the synthesis of **20**, but still in unsatisfactory yield (25%) (Table 1, entry 5). As an alternative to the copper-catalyzed reaction, the Buchwald–Hartwig cross-coupling reaction was attempted,[34,35] in which C(sp^2)–N bond formation is palladium-catalysed, in the presence of the bidentate ligand, 9,9-dimethyl-4,5-bis(diphenylphosphino)-xanthene (xantphos)[36,37] and a base (Table 2). The best results (Table 2, entries 2 and 3) were obtained using palladium(II) acetate as a catalyst and potassium carbonate as a base, in dry dioxane. The reaction was complete and **20** was obtained in satisfactory yield (70%), only when a large excess of base was used (20 equiv., Table 2, entry 3). The same observation was already reported in the literature for the amination of chloro-pyridines and chloro-pyridazinones.[38,39] On the other hand, the use of stronger bases (*e.g.*, sodium-*tert*-butoxide, Table 2, entry 5) mainly led to degradation products and only traces of the desired substituted product **20** were recovered. The catalyst tris(dibenzylideneacetone)dipalladium was less efficient than palladium(II) acetate (Table 2, entry 6).

Finally, cleavage of the benzyl ester by catalytic hydrogenation using Pd/C as catalyst afforded the desired compound **21** (Scheme 2). The Buchwald–Hartwig amination reaction was then applied under the conditions previously described (palladium acetate 10 mol%, xantphos 20 mol%, K_2CO_3 20 equiv., in dry dioxane at 110 °C) for the introduction of the amino (NH_2) group on the naphthalene scaffold. For that purpose, benzophenone imine was engaged as a surrogate for ammonia in the Pd-catalyzed reaction. This reagent is commercially available and allows an easy deprotection of the amino function under mild conditions.[40] A Buchwald–Hartwig reaction was performed on compounds **18** and **19**, using benzophenone imine to afford intermediates that were not isolated but immediately hydrolysed, using methanol in the presence of sodium acetate and hydroxylamine hydrochloride, to afford amines **22** (62% over two steps) and **23** (51% over two steps) in satisfactory yields (Scheme 3).

Derivatives **22** and **23** were then converted into halides **24** (80% yield) and **25** (75% yield) by reaction with chloroacetyl chloride in dry dichloromethane (Scheme 3). Treatment of compound **24** with methylamine in the presence of triethylamine and a catalytic amount of potassium iodide in DMF afforded amine **26** (60% yield). Following the same procedure, intermediate **25** was coupled to the mono protected Cbz-piperazine to afford compound **28** in good yield (86%). In this case, we used the naphthalene scaffold bearing the carboxypropyl linker protected as an ethyl ester in order to obtain an orthogonal protection with respect to the Cbz protected piperazine ring (Scheme 3). Finally, cleavage of the benzyl ester of **26** by catalytic hydrogenation, using Pd/C as a catalyst, afforded **27** (84% yield), and basic hydrolysis of the ethyl ester of **28**, by aqueous NaOH 2 N in methanol, gave **29** (86% yield).

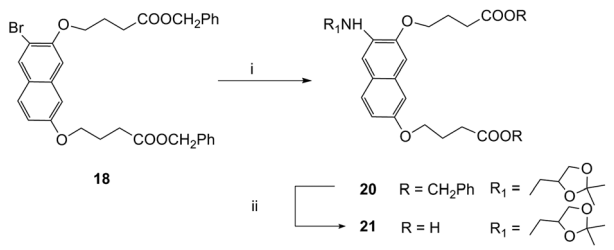
The second goal of this chemistry section was to introduce alkylamino chains into the naphthalene scaffold. The Sonogashira coupling, consisting of a palladium catalysed sp^2–sp coupling reaction between an aryl halide and a terminal alkyne, in the presence of an amine playing the role of a base, has become the most important method to prepare aryl alkynes,[41] used here as precursors of arylalkanes. Sonogashira also reported that addition of a catalytic amount of copper(I) iodide greatly accelerates the reaction.[42] The Sonogashira reaction is usually performed using a palladium-phosphane ligand complex (*e.g.* $Pd(PPh_3)_4$ or $PdCl_2(PPh_3)_2$) as a catalyst in the presence of a catalytic amount of copper(I) salt and an amine (as a solvent or in large excess) under homogeneous conditions.

Starting from the bromo derivative **18**, the coupling with the *N*,*N*-dimethylpropargylamine in the presence of $Pd(PPh_3)_4$ as a catalyst (5%) and copper iodide (10%) as a co-catalyst, triethylamine and using DMF as a solvent, at 80 °C afforded compound **30** in satisfactory yield (60%, Scheme 4). The use of freshly prepared $PdCl_2(PPh_3)_2$ (from $PdCl_2$ and PPh_3) or longer reaction times did not lead to higher yields. Compound **30** was then treated with H_2 and Pd/C in a (1 : 1) mixture of MeOH and EtOAc in order to reduce the triple bond, and concomitantly cleave the benzyl ester by hydrogenolysis, affording compound **31** in excellent yield (98%). The Sonogashira coupling of compound **19** with *N*,*N*-di(benzyloxycarbonyl)propargylamine, using the same conditions as for **18**, afforded compound **32** in moderate yield (41%, Scheme 4). Compound **32** was then treated with aqueous NaOH 2 N in methanol to afford the corresponding acid **33** after treatment with aqueous HCl 1 N (61%, Scheme 4). It is worth noting the loss of one Cbz moiety during the basic hydrolysis of the methyl ester.



16 **17** **18** R = CH_2Ph; **19** R = Et

Scheme 1 Reagents and conditions: (i) Br_2, CH_3COOH, 1.5 h, then Sn powder, H_2O, 80 °C, 24 h. (ii) $Br(CH_2)_3COOCH_2Ph$ or $Br(CH_2)_3COOEt$, K_2CO_3, dry DMF, 50 °C, overnight.



18

Scheme 2 Reagents and conditions: (i) (2,2-dimethyl-[1,3]-dioxolan-4-yl)-methylamine, amination reaction. (ii) H_2, 10% Pd/C, CH_3OH–EtOAc (1 : 1, v/v), r.t., 24 h.

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Table 1 Ullmann-type conditions used in the amination reaction of **18** with CuI as the copper source

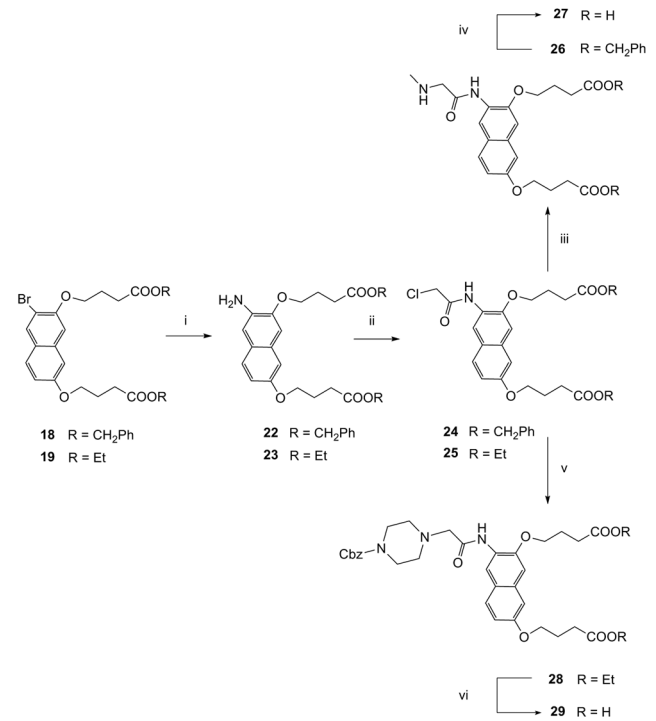
Entry	Ligand (mol%)	Base (equiv.)	Solvent	Temp	Duration	Result
1	—	K_2CO_3 (3)	DMSO	135 °C	48 h	SM
2	Proline (40)	K_2CO_3 (3)	DMSO	135 °C	48 h	DP
3	Proline (20)	K_2CO_3 (2)	DMSO	110 °C	24 h	DP
4	DMEDA (10)	K_2CO_3 (2)	Dioxane	75 °C	24 h	SM
5	DMEDA (10)	K_2CO_3 (2)	Dioxane	110 °C	24 h	25%[a] (+SM)
6	DMEDA (20)	K_2CO_3 (2)	Dioxane	125 °C	24 h	8%[a] (SM + DP)

[a] Yield of a pure isolated compound. Abbreviations used in the table: SM = starting material, DP = degradation products.

Table 2 Buchwald–Hartwig conditions used in the amination reaction of **18** (in dioxane, at 110 °C, for 15 h)

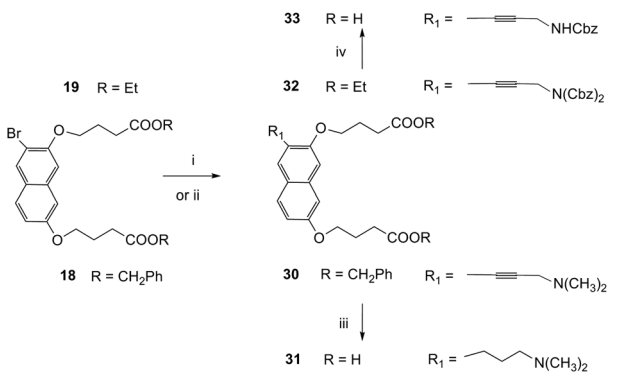
Entry	Pd source (10 mol%)	Ligand (mol%)	Base (equiv.)	Result[a]
1	$Pd(OAc)_2$	Xantphos (20)	K_2CO_3 (5)	27%
2	$Pd(OAc)_2$	Xantphos (20)	K_2CO_3 (10)	66%
3	$Pd(OAc)_2$	Xantphos (20)	K_2CO_3 (20)	70%
4	$Pd(OAc)_2$	Xantphos (20)	Cs_2CO_3 (20)	51%
5	$Pd(OAc)_2$	Xantphos (20)	NaO-*t*-Bu (2)	4%[b]
6	$Pd_2(dba)_3$	Xantphos (20)	K_2CO_3 (20)	50%

[a] Yield of a pure isolated compound. [b] The reaction mainly led to degradation products.



Scheme 3 Reagents and conditions: (i) benzophenone imine, $Pd(OAc)_2$ (10 mol%), xantphos (20 mol%), K_2CO_3 (20 equiv.), dry dioxane, 110 °C, 15 h, then $NH_2OH{\cdot}HCl$, $NaOAc{\cdot}3H_2O$, CH_3OH, r.t., 6 h. (ii) $ClCH_2COCl$, Et_3N, CH_2Cl_2, 0 °C, 10 min, then r.t., 2 h. (iii) **24**, $CH_3NH_2{\cdot}HCl$, Et_3N, DMF, r.t., 48 h. (iv) H_2, 10% Pd/C, CH_3OH, r.t., 18 h. (v) **25**, *N*-Cbz-piperazine, Et_3N, DMF, r.t., 24 h. (vi) $NaOH_{aq}$ 2 N, CH_3OH, r.t., 35 h, then HCl_{aq} 1 N.

In order to enlarge the scope of the substitution pattern of the main bromonaphthalene scaffold, a cyano group was introduced, and subsequently reduced to an aminomethyl group. Aromatic nitriles have been prepared in numerous ways. These methods include the Rosemund-von Braun reaction of aryl halides[43] and the diazotization of anilines with subsequent Sandmeyer reaction.[44] More recently, the transition metal-catalysed cyanation of aryl halides has been widely employed.[45–47] In a first attempt, a Stille coupling, which is a versatile C–C bond forming reaction between a stannane and an aryl halide, was performed. Therefore, compound **19** was treated with Bu_3SnCN (2 equiv.) in the presence of tetrakis palladium (20 mol%) as a catalyst in 1,2-dichloroethane at 70 °C, but no reaction occurred. The general problem with metal-catalysed cyanation is the high affinity of cyanide species for the metal center, which results in the fast deactivation of the catalytic system by formation of stable cyanide complexes. Beller *et al.* described copper-catalysed cyanation with potassium ferrocyanate ($K_4[Fe(CN)_6]$), which has the advantage of being essentially the least toxic cyanide source conceivable.[48] This method foresees the use of imidazole ligands to control the stability and selectivity of the copper catalyst. Using this methodology, compound **19** was treated with $K_4[Fe(CN)_6]$ in the presence of CuI (10 mol%) as a catalyst and 1-butyl-1*H*-imidazole as a ligand in dry toluene at reflux affording compound **34** in satisfactory



Scheme 4 Reagents and conditions: (i) **18**, *N*,*N*-dimethylpropargylamine, CuI (10 mol%), $Pd(PPh_3)_4$ (5 mol%), Et_3N, DMF, 80 °C, 6.5 h. (ii) **19**, *N*,*N*-di(benzyloxycarbonyl)propargylamine, CuI (10 mol%), $Pd(PPh_3)_4$ (5 mol%), Et_3N, DMF, 80 °C, 15 h. (iii) H_2, 10% Pd/C CH_3OH–EtOAc 1 : 1 (v/v), r.t., 14 h. (iv) $NaOH_{aq}$ 2 N, CH_3OH–THF 1 : 1 (v/v), r.t., 5.5 h, then HCl_{aq} 1 N.

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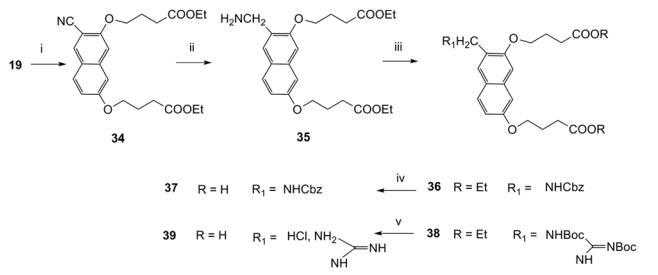
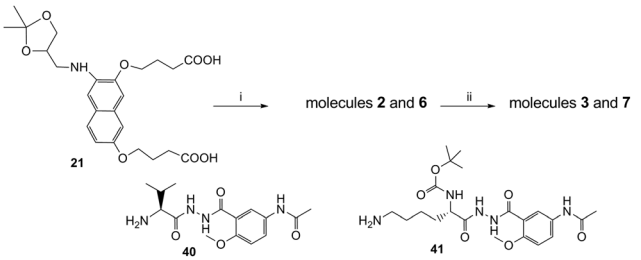
yield (70%, Scheme 5). The cyano group was then reduced into the aminomethyl derivative by treatment of compound **34** with Ni-RANEY® in a hydrogen atmosphere (60 psi) in ethanol, and in the presence of ammonia, affording derivative **35** in 98% yield (Scheme 5). Compound **35** was further treated with CbzCl in the presence of triethylamine in dry dichloromethane affording the corresponding N-protected amine **36** (83% yield, Scheme 5). Compound **36** was then converted into the free carboxylic acid derivative **37** by treatment with aqueous NaOH 2 N in methanol (96% yield, Scheme 5). Amine **35** was reacted with 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea in the presence of $HgCl_2$ and triethylamine in dry dichloromethane to give compound **38** in good yield (62%, Scheme 5). Finally, compound **38** was treated with aqueous NaOH 2 N in MeOH–THF and, subsequently, with HCl 4 N in dioxane, to hydrolyse the esters and cleave the Boc moieties respectively, affording compound **39** in quantitative yield (Scheme 5).

Molecular tongs **2** and **6** were synthesised from scaffold **21** and peptidomimetics **40** (ref. 21) and **41** (ref. 22) respectively, in satisfactory yields (50% and 59%) using a standard coupling protocol (using HBTU and HOBt, in the presence of DIPEA, in dry DMF) (Scheme 6). The 2,2-dimethyl-1,3-dioxolane group of molecule **2** was then cleaved by HCl in EtOH–THF, affording molecular tong **3**, in satisfactory yield (60%). Since molecule **6** contained acid labile protecting groups (Nα-Boc) in its strands, the diol deprotection step was performed with Amberlyst 15 resin in methanol,[49] affording molecular tong **7** in low yield (30%). An alternative to the selective dimethyl-1,3-dioxolane cleavage was tested in the presence of *p*-toluenesulfonic acid in methanol,[50] affording molecular tong **7** with a similar yield.

The synthesis of molecular tongs **4** and **8–11** is described in Scheme 7. Scaffold **22** was deprotected by catalytic hydrogenolysis with hydrogen and Pd/C in MeOH–EtOAc affording the corresponding carboxylic acid **42** in quantitative yield. Compound **42** was then coupled to peptidomimetic arms **40** and **41** (Scheme 6) using the same standard coupling protocol described above (HBTU–HOBt, DIPEA, in dry DMF), to afford molecular tongs **4** and **8** in satisfactory yields (68% and 72%, respectively). In the same manner, scaffolds **27** and **29** were coupled to peptidomimetic arm **41** to afford molecular tongs **9**



Scheme 5 Reagents and conditions: (i) $K_4[Fe(CN)_6]$, CuI, 1-butyl-1*H*-imidazole, toluene, reflux, 46 h. (ii) H_2, RANEY® Ni, NH_3 1 N in EtOH, DMF, 4 bar, r.t., 2 days. (iii) CbzCl, Et_3N, CH_2Cl_2, 0 °C, 30 min, then r.t. overnight or 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea, $HgCl_2$, NEt_3, CH_2Cl_2, r.t., 17 h. (iv) $NaOH_{aq}$ 2 N, CH_3OH, r.t. 2 h, then HCl_{aq} 1 N. (v) $NaOH_{aq}$ 2 N, CH_3OH–THF 1 : 1 (v/v), r.t., 4 h, HCl_{aq} 1 N then HCl 4 N in dioxane, r.t., overnight.

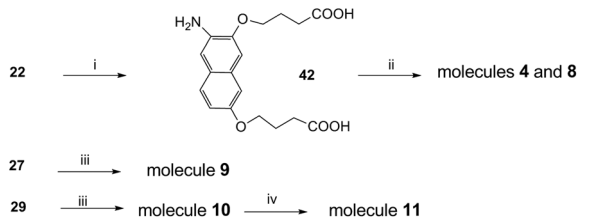


Scheme 6 Synthesis of molecular tongs **2–3** and **6–7**. Reagents and conditions: (i) peptidomimetic **40** or **41**, HBTU, HOBt, DIPEA, DMF, r.t., 48 h. (ii) **2**, HCl_{aq} 1 N, EtOH–THF, 55 °C, 1 h, or **6**, Amberlyst™ 15, MeOH, r.t., 4 h.

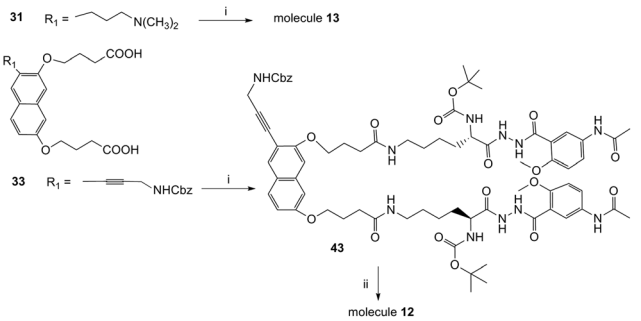
and **10** in good yields (93% and 61% respectively). The Cbz protecting group of the piperazinyl ring of molecule **10** was removed by hydrogenolysis using Pd/C in methanol, to give molecular tong **11** in quantitative yield.

The synthesis of molecular tongs **12** and **13** is described in Scheme 8. Scaffolds **31** and **33** were coupled to peptidomimetic arm **41** (using HBTU–HOBt, DIPEA, in dry DMF) to afford molecular tongs **13** and **43** in very good yields (86% for both compounds). Treatment of molecule **43** in a hydrogen atmosphere using 10% Pd/C in methanol afforded molecular tong **12** in excellent yield (92%).

The synthesis of molecular tongs **14–15** is described in Scheme 9. Scaffolds **37** and **39** were coupled to peptidomimetic



Scheme 7 Synthesis of molecular tongs **4** and **8–11**. Reagents and conditions: (i) H_2, 10% Pd/C, MeOH–EtOAc, r.t., 24 h. (ii) Peptidomimetics **40** or **41**, HBTU, HOBt, DIPEA, DMF, r.t., 48 h. (iii) Peptidomimetic **41**, HBTU, HOBt, DIPEA, DMF, r.t., 48 h. (iv) H_2, 10% Pd/C, MeOH, r.t., 19 h.



Scheme 8 Synthesis of molecular tongs **12** and **13**. Reagents and conditions: (i) peptidomimetic **41**, HBTU, HOBt, DIPEA, DMF, r.t., 48 h. (ii) H_2, 10% Pd/C, MeOH, r.t., 48 h.

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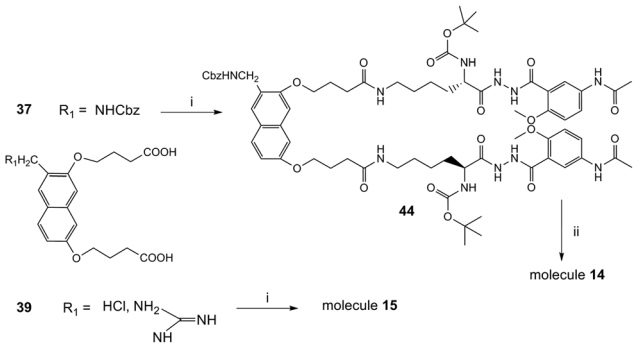

Scheme 9 Synthesis of molecular tongs **14–15**. Reagents and conditions: (i) peptidomimetic **41**, HBTU, HOBt, DIPEA, DMF, r.t., 48 h. (ii) H_2 (10 bar), 10% Pd/C, MeOH, 50 °C, 3 min.

arm **41** (using HBTU–HOBt, DIPEA, in dry DMF) to give molecular tongs **44** and **15** in satisfactory yields (65% for both compounds). Surprisingly, the classical deprotection conditions used previously (H_2, 10% Pd/C, MeOH) were inefficient for the cleavage of the Cbz moiety present on molecule **44**. Therefore, more drastic conditions were used, and treatment of molecule **44** in a hydrogen atmosphere under pressure (10 bar) using 10% Pd/C in hot methanol (50 °C) afforded molecular tong **14** in modest yield (32%).

Biology

The ability of compounds **2–4** and **6–15** to inhibit PR (recombinant wild-type protease) was evaluated in a fluorimetric assay at the optimum pH (4.7) and 30 °C.[19–22] Zhang–Poorman kinetic analyses were performed to identify the mechanism of inhibition. Plots of $[E]_0/\sqrt{vi}$ *versus* $\sqrt{vi}$ were constructed, where vi is the initial rate. The results are summarised in Table 3. Compounds **2–4**, **6–8** and **14** efficiently inhibited PR. Parallel lines were obtained for these compounds, demonstrating that they act as pure dimerization inhibitors (Fig. 2a). Compound **15** was able to inhibit PR, however it acted as a competitive inhibitor since altered slopes and the unaltered *y*-axis intercept were obtained (Fig. 2b). Compounds **10** and **13** were not inhibitors at the highest tested concentration (28 μM), whereas compounds **9**, **11** and **12** inhibited the enzyme poorly (18, 18 and 35% inhibition at 28 μM, respectively). The most efficient inhibitors **3**, **4**, **8** and **14** were also assayed against the multidrug-resistant mutated protease ANAM-11 (which bears 11 mutations: Leu10Ile/Met36Ile/Ser37Asp/Met46Ile/Arg57-Lys/Leu63Pro/Ala71Val/Gly73Ser/Ile84Val/Leu90Met/Ile93Leu, Fig. 2c).[51] This protease is analogous to that found in multi-resistant viruses.

Biological evaluation of the new molecular tongs showed that the introduction of a small amino group directly attached to the naphthalene scaffold in molecules **4** and **8** almost preserved the dimerization inhibitory efficiency (0.40 μM for **4** *versus* 0.22 μM for **1** (ref. 22) and 0.55 μM for **8** *versus* 0.28 μM for **5** (ref. 22)). The aminopropanediol group decreased the dimerization inhibitory efficiency only slightly in the case of molecule **3** (0.60 μM for **3** relative to 0.22 μM for **1** (ref. 22)) and noticeably in the case of **7** (2.75 μM for **7** relative to 0.28 μM for **5** (ref. 22)). It is noteworthy that both dioxolane precursors (compounds **2** and **6**) exhibited dramatically decreased inhibition (2.70 μM for **2** *versus* 0.60 μM for the aminopropanediol **3** and 5.90 μM for **6** *versus* 2.75 μM for the aminopropanediol **7**). This decreased inhibitory activity of molecular tongs **2** and **6** suggests a possible alteration of the hypothesised binding mode due to the presence of the 2,2-dimethyl-1,3-dioxolane methyl group. Even if introduced in the non-pharmacophore region, such a group might weaken the interaction between the peptidomimetic strands and the PR-termini. The methylamino group preserved the dimerization inhibitory potency of molecular tong **14** (0.30 μM for **4** *versus* to 0.22 μM for **1** (ref. 22)). Remarkably, the four new molecular tongs **3**, **4**, **8** and **14** behaved as antidimers against the multi-mutated protease ANAM-11 (Table 3). However, when the amino group was attached through a longer alkyl or alkylamide chain the inhibitory activity is dramatically decreased (molecules **9**, **11** and **12**) or even suppressed (molecules **10** and **13**). These results might be attributed to the length and flexibility of the alkyl or

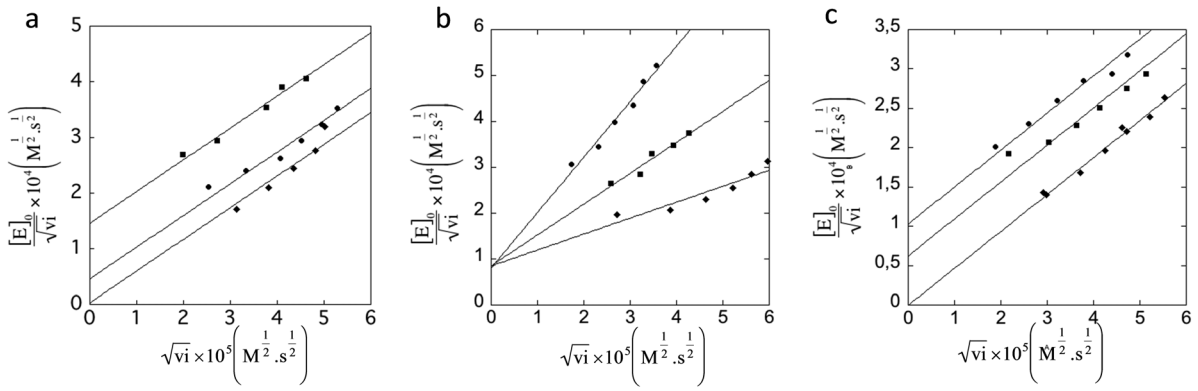

Fig. 2 Zhang kinetic analyses of PR by compounds **3** (a) and **15** (b), and ANAM-11 by compound **8** (c), at pH 4.7 and 30 °C. PR inhibition: [PR] = 5.38 − 18 nM; $[S]_0 = 5.2$ μM; [**3**] = 17 μM (■) and 11 μM (▲); [**15**] = 22 μM **2** (■) and 11 μM **2** (▲). ANAM-11 inhibition: [PR] = 5.33 − 18 nM; $[S]_0 = 5.2$ μM; [**8**] =11 μM (■) and 5.6 μM **2**. Enzyme activity was also always measured without inhibitor (●). Each experiment was at least performed in triplicate.

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Table 3 Inhibition of PR and mutated HIV-1 protease ANAM-11 by compounds **1–15** (30 °C and pH 4.7)

Comp	K_{id}[a] (μM) PR	ANAM-11	*C* log *P*	Hydrophobic surface area (Å^2)	Hydrophilic surface area (Å^2)	Retention time[c] (min)
1	0.22 (ref. 22)	nd	6.5	1305	359	13.8
2	2.70	nd	7.3	1455	368	14.2
3	0.60	0.94	4.8	1316	456	11.3
4	0.40	0.94	5.6	1261	402	10.6
5	0.28 (ref. 22)	2.00	8.7	1681	489	14.8
6	5.90	nd	9.5	1844	519	15.2
7	2.75	nd	6.9	1713	600	12.7
8	0.55	0.83	7.7	1635	536	12.2
14	0.30	2.45	7.4	1664	558	11.1

Comp	K_{ic}[b] (μM)	*C* log *P*	Hydrophobic surface area (Å^2)	Hydrophilic surface area (Å^2)	Retention time[c] (min)
15	15	7.2	1647	632	11.2

Comp	% Inhibition at 28 μM (PR)	*C* log *P*	Hydrophobic surface area (Å^2)	Hydrophilic surface area (Å^2)	Retention time[c] (min)
9	18	7.1	1755	561	11.1
10	0	9.6	2032	599	13.8
11	18	6.2	1724	522	11.2
12	35	8.0	1719	569	11.4
13	0	9.2	1833	510	11.4

[a] Dimerization inhibition constant (dissociation constant of monomer–inhibitor complexes). [b] Competitive inhibition constant (dissociation constant of a dimer–inhibitor complex). Standard errors of initial rates are less than 5%. nd: not determined. *C* log *P* and solvent accessible surface areas are indicated. [c] Determined on a column Sunfire 2.1 × 100 mm 3.5 μm, flow rate 1 mL min^{-1}; detection at 254 nm; solvent (A) H_2O + 0.2% formic acid and (B) CH_3CN, mixture A/B: from 95/5 to 0/100 in 20 min.

alkylamide chain allowing the amino groups to contract hydrogen bonds with the peptidomimetic arms, thus precluding interactions with the PR termini. The introduction of a guanidinium group at the 3-position of the naphthalene scaffold (molecule **15**) did not lead to PR dimerization inhibition. Conversely to that reasonably expected, the positively charged guanidinium of **15** led to a shift from dimerization inhibition towards active-site competitive inhibition. The decrease or suppression of the dimerization inhibitory activity of molecules **9–13** and **15** might be assigned to a destabilization of the hypothesised binding mode with PR termini due to steric and/or electronic effects. In particular, the positive charge resulting from protonation of the amine (molecules **9–13** and **14**) and guanidine (molecule **15**) groups might form a salt bridge with the C-terminal carboxylate of Phe-99. It was previously reported that bicyclic guanidinium spacers introduce additional electrostatic hydrogen bonding interactions with the C-terminal Phe-99 carboxylate leading to dimerization inhibitors.[16]

Three physical properties (estimated log *P*, referred to as *C* log *P*, hydrophobic surface area and hydrophilic surface area) of molecular tongs **1–15** were calculated by molecular modeling (see ESI†[52–56]) in order to correlate the nature of the 3-substituent on the naphthalene scaffold with the hydropathic properties of the molecules (Table 3). The more favorable groups capable of decreasing *C* log *P* are the piperazine ring (factor of 1.40 between molecular tongs **5** and **11**) and the aminopropanediol (factor of 1.35 between molecular tongs **1** and **3** and factor of 1.26 between molecular tongs **5** and **7**). The amine in molecules **4** and **8**, the methylamine in molecules **14** and **9**, and the methylguanidine in molecule **15** have similar effects on the *C* log *P* (factors around 1.12–1.20). The aminopropyl group in **12** and the dimethylaminopropyl substituent in **13** have a negligible effect on the *C* log *P* (factor of 1.08 and 1.05 respectively). The hydrophilic surface provided by the polar groups is increased in all cases, while the hydrophobic surface provided by the strands is almost always preserved. The estimated *C* log *P* values were compared with the experimentally determined chromatographic retention times, because the reverse phase liquid chromatography has been previously reported as a model to estimate aqueous solubility.[57] In the first series of molecules **1–4**, retention time differences were in accordance with their *C* log *P*. Molecules **3** and **4** were less retained on the column than molecules **1** and **2** (Table 3). In the second series of molecules **5–15**, retention time differences were in accordance with their *C* log *P* for the neutral molecules **5–8** while ionisable compounds **9** and **11–15** have similar retention times (Table 3).[57c] Experimental solubility in water was determined for the more active and the more polar compounds **3** and **4** (≈0.3 mg mL^{-1}). These data show that submicromolar inhibitory activity can be conserved (K_{id} = 0.60 and 0.40 μM for molecules **3** and **4** respectively compared to 0.22 μM for molecular tong **1**) while solubility is increased.

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Although highlighting the difficulty involved, these data show the possibility of introducing polar groups on the naphthalene scaffold and finding a compromise between hydrophilicity provided by the polar groups and inhibitory activity essentially supported by the peptidomimetic strands.

Finally, we determined the stability of molecular tongs **3** and **8** in RPMI culture medium containing 20% fetal calf serum. We previously reported that the introduction of the 5-acetamido-2-methoxybenzohydrazide group in a single strand increased the metabolic stability of the molecular tongs.[21] Remarkably, compounds **3** and **8** were not significantly degraded after incubation during 48 h (see ESI†).

Conclusions

A new series of naphthalene-based molecular tongs containing polar groups at the 3-position of the naphthalene scaffold was designed and synthesised. The polar groups were introduced mainly *via* metal-catalysed cross coupling reactions on bromonaphthalene derivatives, in very satisfying yields. The ability of the new molecules to disrupt the PR termini β-sheet interface in order to inhibit PR was analysed. The best of our molecular tongs inhibited PR dimerization with an inhibition constant K_{id} of 0.3 μM. Furthermore, most of the polar groups decreased the *C* log *P* parameter of the molecules and thus their water solubility. We have demonstrated that the inhibitory efficiency is strictly related to the molecule structure, indicating that it is essential to have an appropriate balance between the length and/or bulkiness of the substituent and its hydrophilicity, in order to maintain effective anti-dimerization activity. As shown by inhibitors **3** and **4**, introducing an aminopropanediol moiety or an amino group on the naphthalene scaffold increases water solubility without compromising inhibitory efficiency. The ability of these proteolysis-resistant molecules to inhibit multi-mutated ANAM-11 suggests that they have the potential to successfully overcome the resistance presently encountered with classical PI protease inhibitors.

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