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RESTRICTING THE FLEXIBILITY OF CROSSLINKED, INTERFACIAL PEPTIDE INHIBITORS OF HIV-1 PROTEASE

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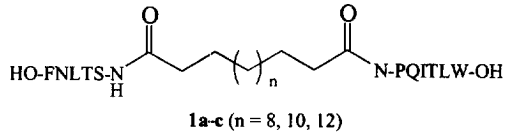
Abstract: Interfacial peptides of HIV-1 protease were crosslinked with varying length alkyl-chains containing either a single *cis* or *trans* double bond, or a triple bond to remove degrees of freedom within the tethers. The synthesis of these compounds and their effects on the activity of HIV-1 protease are described.

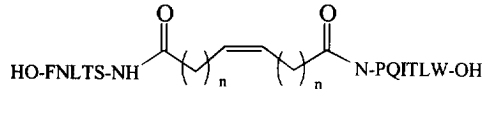
Introduction

HIV-1 protease (PR) is a dimeric, aspartyl-protease that processes the gag and gag/pol HIV polypeptide precursors.[1,2] As such, PR is essential for viral replication, and viruses expressing nonfunctional, mutant forms of PR are noninfectious.[3] Because of its essential role in HIV replication, PR has been the focus of a wide range of active site inhibitor studies,[4] culminating recently in the use of PR inhibitors in the clinical treatment of AIDS. These agents have proven very effective in lowering viral loads in HIV-infected individuals,[5] but drug resistence problems have demonstrated the need to develop PR inhibitors with novel mechanisms of action.[6]

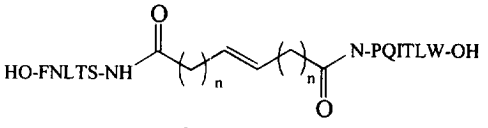
The importance of dimerization in PR activity has lead to the design of inhibitors that target the dimeric interface of PR. Indeed, dominant-negative forms of PR have been used as inhibitors, and have demonstrated that decreasing PR activity by 50-fold is sufficient to prevent the formation of infectious particles.[7] The interfacial region of PR is composed of an N- and C-terminal, four-stranded β-sheet, and this region has been the target for inhibitor design.[8,9] One class of inhibitors contained interfacial peptides of PR that were crosslinked by flexible alkyl-tethers (**1**).[9a] In an effort to decrease the flexibility of these agents, a single sp^2 or sp center was designed into the midpoint of the tether, thereby removing one torsional degree of freedom within the tethering moiety. The nature of the unsaturation may play an essential role in the relative orientation of the interfacial peptides, therefore, agents containing a triple bond (**2a-c**), or a *cis*- (**3a-c**) or *trans*- (**4a-c**) double bond within tethers composed of an additional 10, 12, or 14 methylene units were prepared and evaluated for PR inhibition.



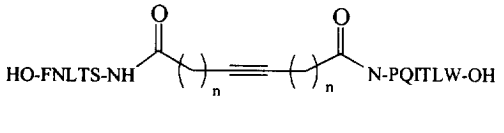
1a-c (n = 8, 10, 12)



3a-c (n = 5, 6, 7)



2a-c (n = 5, 6, 7)



4a-c (n = 5, 6, 7)

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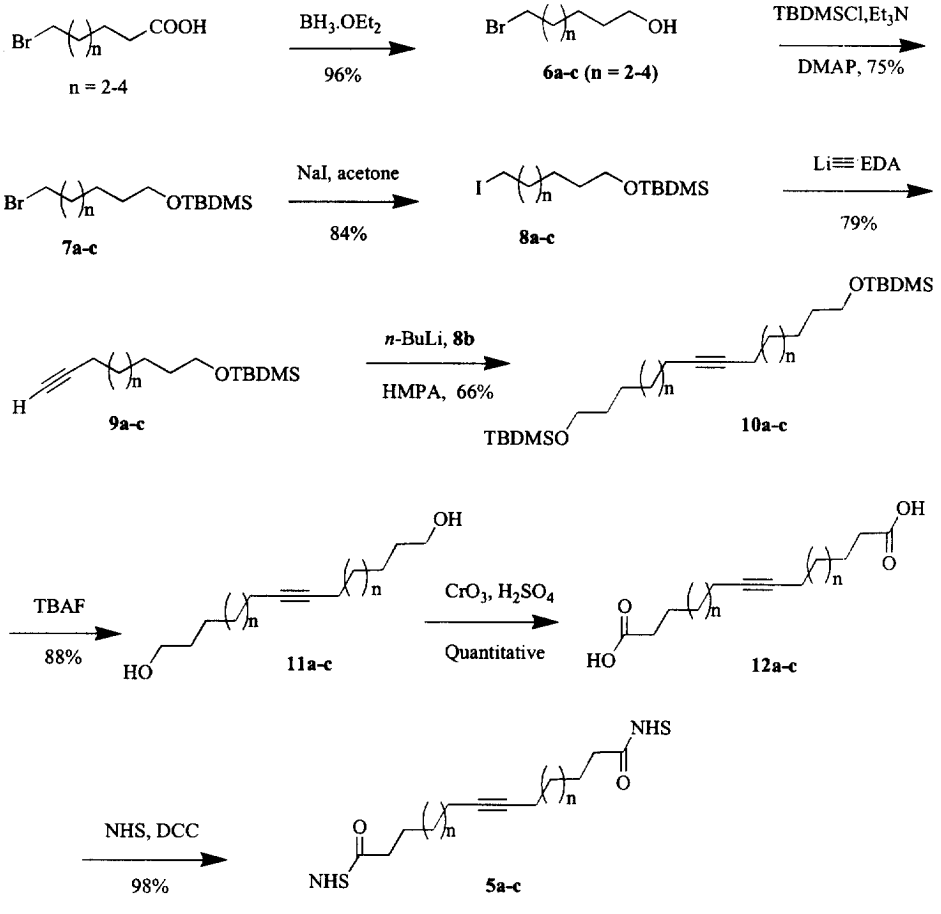
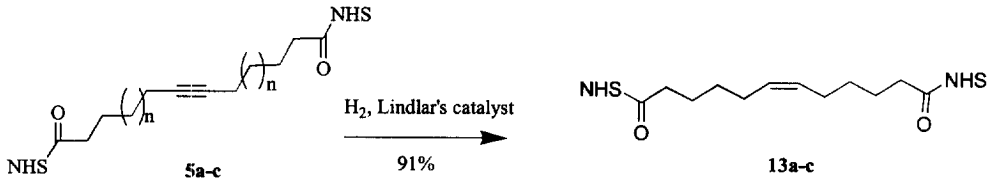
Results and Discussion

Synthesis of acetylenic-tethers (5a-c). A typical starting material for this class of compounds was the commercially available 6-bromohexanoic acid, 7-bromoheptanoic acid, or 8-bromooctanenitrile. When beginning the synthesis of **5c**, the 8-bromooctanenitrile was hydrolyzed with HCl/HOAc to generate 8-bromooctanoic acid. For the synthesis of **5b**, for example, 7-bromoheptanoic acid was reduced with diborane in THF to produce the corresponding alcohol **6b** (Scheme 1). Treatment of the alcohol, **6**, with TBDMS-Cl provided a 75% yield of the desired protected alcohol, **7b**, and this compound was treated with NaI in refluxing acetone to produce the corresponding iodide **8b**. Displacement of the iodide within **8b** was achieved with Li-acetylide ethylenediamine complex in DMSO to afford the alkynyl-precursor **9b**. Treatment of **9b** with *n*-BuLi at -78 °C, followed by the addition of an equimolar amount of the TBDMS-protected iodoalcohol, **8b**, produced the symmetrical alkyne, **10b**, in 66% yield. Deprotection of the TBDMS-protecting group with TBAF produced the diol **11b** in 89% yield, followed by Jones oxidation to produce the desired diacid, **12b**. Treatment of **12b** with DCC, N-hydroxysuccinimide and a catalytic amount of DMAP generated the di-NHS ester, **5b**, in a 95% yield.[10]



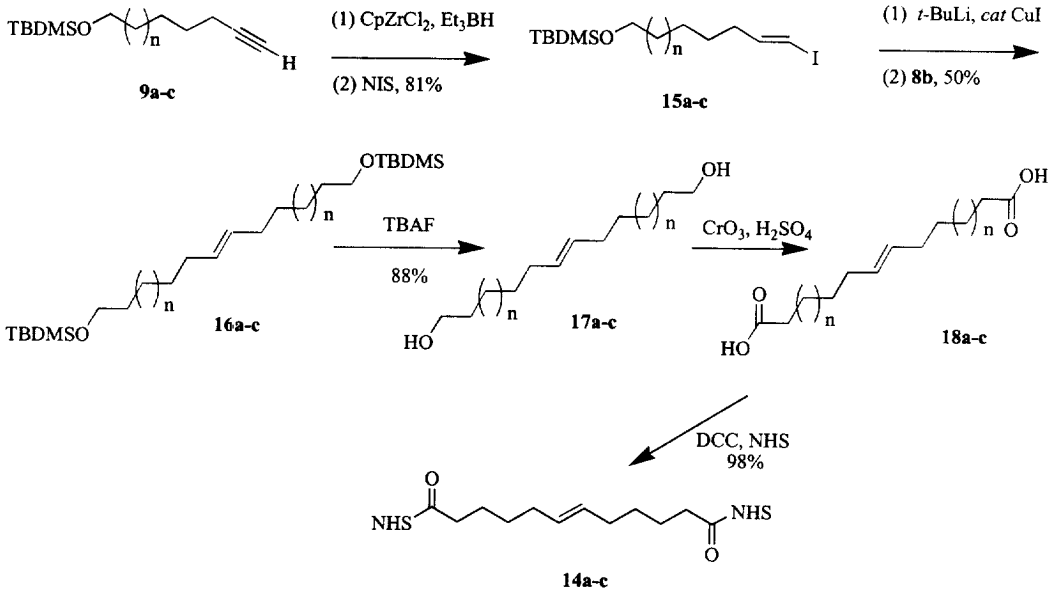
Scheme 1.

Synthesis of cis-alkenyl-tethers (13a-c). The desired *cis*-alkenyl tethers **13a-c** were obtained in one step by treating compounds **5a-c** individually with Lindar's catalyst under an atmosphere of hydrogen to produce the desired materials in 90% yield (Scheme 2).[10]



Scheme 2.

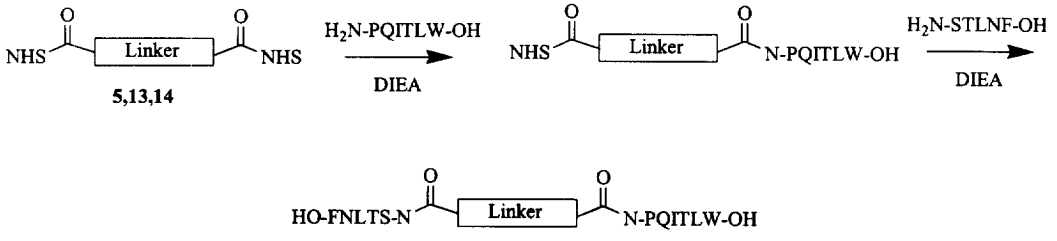
Synthesis of trans-alkenyl-tethers (14a-c). In a typical reaction, the alkynyl-derivative **9b** was treated with Schwartz's reagent,[11] zirconocene hydride generated in situ from zirconocene dichloride and lithium triethylborohydride, to produce the required *trans*-vinyl zirconocene, which was treated immediately with N-iodosuccinimide to sterospecifically produce the *trans*-vinyl iodide, **15b**, in an 81% yield (Scheme 3). Treatment of **15b** with 2 equiv of *t*-BuLi and a catalytic amount CuI at -78 °C, followed by the addition of the TBDMS-protected iodoalcohol, **8**, in HMPA produced the symmetrical *trans*-alkene, **16b**, in 50% yield. The TBDMS-protecting group was removed with TBAF, and the resulting diol, **17b**, was oxidized with Jones reagent to the corresponding diacid, **18b**, in an 80% overall yield for the 2 steps. Treatment of **18b** with DCC, N-hydroxysuccinimide and a catalytic amount of DMAP generated the di-NHS ester, **14b**, in a 98% yield.[10]



Scheme 3.

Synthesis of crosslinked interfacial peptides (2-4). With the appropriate tethers in hand, their coupling to previously optimized peptide sequences (NH-PQITLW-OH, and NH_2-STLNF-OH) was undertaken (Scheme 4). In a typical reaction a DMSO solution of NH-PQITLW-OH (1 mM) was treated with 1 equiv of the appropriate

di-NHS ester (**5a-c**, **13a-c**, and **14a-c**), and 1 equiv of DIEA at 60 °C, and the progress of the reaction was monitored by reverse-phase HPLC.[9a] After approximately 24 h, 1 equiv of NH_2-STLNF-OH in DMSO was added with an additional equivalent of DIEA. After approximately 24 h at 60 °C, the reaction mixture was separated by reverse-phase HPLC to afford the desired agents **2-4** in a 10-15% overall yield.[12]



Scheme 4.

Evaluation of inhibition. HIV-1 protease (Bachem) inhibition was evaluated using a fluorogenic substrate assay developed by Toth and Marshall.[13] The effect of agents **2-4** (10% DMSO in final mixture) upon the hydrolysis of the fluorogenic substrate by PR was evaluated by monitoring the increase in fluorescence at 430 nm with respect to time.[9a] Ths increase in fluorescence was compared to controls of PR preincubated with DMSO, and IC_{50} values were obtained for each inhibitor (Table 1). In all cases, the agents with the shortest tethers (C12) were the least effective inhibitors, and further increasing the tether length led to more pote nt agents. The *trans*-alkenyl inhibitors (**4a-c**), for instance, displayed approximately a five-fold increase in activity upon going from a chain length of C12 (**4a**) to C14 (**4b**), although further chain extension to C16 (**4c**) resulted in about a 1.5-fold decrease in potency. Both the alkynyl (**2a-c**) and *cis*-alkenyl (**3a-c**) inhibitors showed steady increases in potency upon increasing chain length; increasing the chain length from C12 (**2a**, **3a**) to C16 (**2c**, **3c**) produced agents that were both approximately 8 times more potent. Interestingly, members of the series of agents, **2-4**, were almost equipotent with their more flexible counterparts (**1a-b**), but a longer chain length was needed to obtain these potencies than with the fully saturated tethers.

Table 1. Inhibition of PR with Crosslinked Interfacial Peptides[a]

chain length	**C12**	**C14**	**C16**
agents			
2a-c (alkynyl)	51.5 μM	17.5 μM	6.3 μM
3a-c (cis-alkenyl)	32.7 μM	12.9 μM	4.4 μM
4a-c (trans-alkenyl)	21.0 μM	4.5 μM	7.2 μM
1a-c (alkyl)	13.0 μM	2.0 μM	4.0 μM

[a]IC_{50} values are ± 10%

Conclusions

A series of PR inhibitors was successfully synthesized that contain interfacial peptides from the dimerization interface of PR crosslinked with linkers containing a single *cis*- or *trans*-double bond or a triple bond moiety integrated in the center of a flexible alkyl chain. Interestingly within this series the tethers containing the *trans*-double bond were more potent with shorter tether lengths than the corresponding *cis*-analogs, perhaps due to the introduction of a kink within the *cis*-analog that would necessitate a longer tether length to span a similar distance as the *trans*-analog. In all cases the introduction of a single sp^2 or sp center within the center of the tethering moiety was not sufficient to increase the potency of the inhibitors over that obtained for the fully saturated analogs (**1a-c**), and removing degrees of freedom resulted in the need to increase the chain length to obtain similar potencies. This work points to the need to investigate other types of rigidifying analogs in an effort to increase the potency of PR inhibitors of this class.

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10. Characterization of tethers: **(5b)** ^{1}H NMR ($CDCl_3$, 300 MHz), δ 2.82 (s, 8 H), 2.61 (t, J = 7.52 Hz, 4 H), 2.15(t, J = 6.54 Hz, 4 H), 1.3-1.5 (m, 16 H). ^{13}C NMR ($CDCl_3$, 75 MHz), δ 18.72, 24.59, 25.05, 25.69, 28.31, 28.38, 28.83, 30.98, 80.23, 168.74, 169.33. MS (CI, 70 eV) $(M + H)^+$ 477 (100), 362 (71.01), 247 (49.83). HRMS (CI) calculated for $C_{24}H_{32}N_2O_8$: 477.2237. Found: 477.2251. **(13b)** ^{1}H NMR ($CDCl_3$, 300MHz) δ 1.39 (bs, 12 H), 1.75 (t, J = 7.12 Hz, 4 H), 2.01 (bs, 4 H), 2.58 (t, J = 2.44 Hz, 4 H), 2.81 (s, 8 H), 5.32

(t, J = 7.12 Hz, 2 H). ^{13}C NMR ($CDCl_3$, 75 MHz), δ 24.64, 25.69, 27.16, 28.78, 29.50, 31.02, 129.89, 168.77, 169.34. MS (CI, 70 eV) $(M + H)^+$ 451 (52.86), 336 (100), 221 (15.15), 116 (49.14), 100 (29.34). HRMS (CI) calcd for $C_{24}H_{34}N_2O_8$: 479.2393. Found: 479.2378. **(14b)** ^1H NMR ($CDCl_3$, 300 MHz) δ 5.37 (t, J = 3.61 Hz, 2 H), 2.83 (s, 8 H), 3.60 (t, J = 6.61 Hz, 4 H), 1.9 (s, 2 H), 1.96 (bt, 4 H), 1.54 (p, 4 H), 1.34 (s, 16 H). ^{13}C NMR ($CDCl_3$, 75 MHz), δ 25.84, 29.14, 29.39, 29.61, 32.63, 32.83, 63.07, 130.45. MS (CI, 70 eV) $(M + H)^+$ 479 (50.0), 364 (100), 338 (45.0). HRMS (CI) calcd for $C_{24}H_{34}N_2O_8$: 479.2393. Found: 479.2378.

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12. MS data for inhibitors: **2a** (PDMS): $(M + H)^+$ 1556.0, **2b** (PDMS): $(M + H)^+$ 1585.4, **2c** (PDMS): $(M + H)^+$ 1613.4, **3a** (PDMS): $(M + H)^+$ 1557.5, **3b** (PDMS): $(M + H)^+$ 1585.9, **3c** (PDMS): $(M + Na)^+$ 1623.0, **4a** (PDMS): $(M + H)^+$ 1557.0, **4b** (PDMS): $(M + H)^+$ 1587.3, **4c** (PDMS): $(M + Na)^+$ 1651.0

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