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FUNCTIONALIZED ALIPHATIC P2/P2' ANALOGS OF HIV-1 PROTEASE INHIBITOR DMP323

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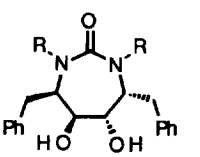
Abstract: A series of analogs of HIV protease inhibitor DMP323 containing functionalized aliphatic P2/P2' groups was prepared and evaluated for HIV protease inhibition and antiviral activity in a cell-based assay. Asymmetric compounds with a 5-hydroxypentyl substituent at P2 and a benzylic substituent at P2' showed increased potency over the corresponding symmetrically substituted analogs.

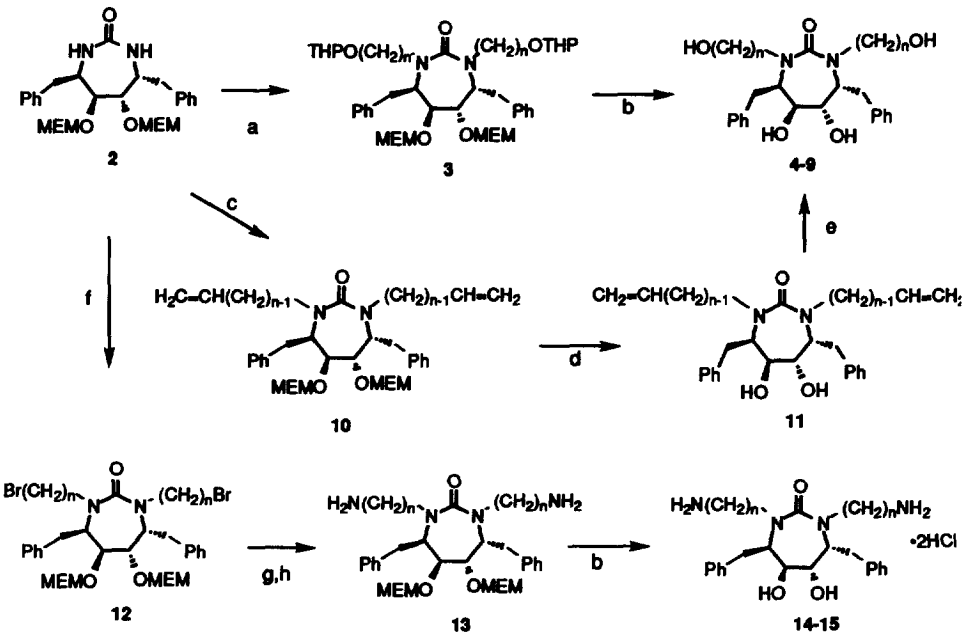
Despite recent introduction of new drugs for the treatment of Acquired Immune Deficiency Syndrome (AIDS), the search for effective long-term therapy remains a major focus of pharmaceutical research. HIV protease is the viral enzyme responsible for processing viral polyprotein precursors into the structural proteins and enzymes needed for viral maturation.[1,2] The solution of the crystal structure of the enzyme both alone[3] and bound to various inhibitors[4] has facilitated the rapid design of additional potent and selective nonpeptide inhibitors of HIV protease by numerous research groups.[5] Furthermore, clinical studies with several such compounds have established the efficacy of this class of drugs for the treatment of AIDS in humans.[6] The design and synthesis of seven-membered cyclic ureas as low molecular weight nonpeptide inhibitors of HIV-1 protease has been previously reported.[7] The cyclic scaffold in these molecules was designed to displace the structural water molecule found in linear inhibitor/protease complexes. In addition, the high degree of preorganization inherent in the geometry of the R,S,S,R stereoisomer allows optimum projection of the four substituents into the S1/S1' and S2/S2' binding pockets. The combination of these features has provided a large array of highly potent compounds. The first example of this structural class to reach clinical trials was DMP323, 1. DMP323 is a potent inhibitor of HIV protease, which showed promising bioavailability in animal models.[8] Unfortunately, the low solubility of this compound resulted in variable blood levels after oral dosing in man, which precluded advancement of this compound past Phase I clinical trials.[9,10]



1
DMP323
R = 4-hydroxymethylbenzyl

In our continuing effort to find HIV protease inhibitors with more favorable pharmacokinetic properties, we prepared a series of analogs with substituted alkyl groups of varying chain length at P2/P2'.

Scheme 1



(a) 5.0 equiv NaH, 7.5 equiv $Br(CH_2)_nOTHP$, TBAI, DMF; (b) HCl/1,4-dioxane/MeOH; (c) $Br(CH_2)_{n-2}CH{=}CH_2$ (d) HCl, H_2O, MeOH; (e) O_3, $NaBH_4$, CH_2Cl_2, -78 °C; (f) NaH, DMF, xs $Br(CH_2)_nBr$; (g) NaN_3; (h) H_2/Pd/C

The rationale for this approach was twofold. We expected compounds with less rigid aliphatic sidechains would be more soluble. In addition, the conformationally unconstrained alkyl chains would allow us to probe the P2/P2' pocket for additional hydrophilic interactions.

The synthesis of targets wherein P2/P2' are both hydroxyalkyl (compounds **4–9**) is achieved in a straightforward manner from unsubstituted cyclic urea **21**[10,11] as depicted in Scheme 1. Bisalkylation of **2** is carried out using an excess of sodium hydride and the appropriate tetrahydropyranyloxyalkylbromide in DMF to provide **3**. Subsequent acid hydrolysis to remove all four hydroxyl protecting groups provides the tetraols, **4–9**.[12] The requisite alkylating agents are prepared from the commercially available diols by monoprotection with dihydropyran followed by treatment with carbon tetrabromide and triphenylphosphine to provide the bromides. Alternately, compounds **4–9** can be obtained by alkylation of **2** with the C4-C9 alkenylbromides to provide diolefins **10**. The MEM-protecting groups are then removed by acid hydrolysis to give the diols. Ozonolysis followed by reductive work-up provides the tetraols.

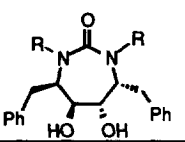
Diamine analogs (**14** and **15**) are also easily prepared by alkylation of **2** with an excess of the corresponding dibromoalkanes to provide the dibromides, **12**. Displacement of bromine with azide, catalytic hydrogenation of the azides to the amines **13** and hydrolysis of the diol protecting groups provide the diaminediols. The remaining compounds in Table 1 are prepared from the corresponding bis-MEM protected diols (the THP ethers are selectively deprotected by treatment with HOAc in aqueous THF at 50 °C for 2 h) or amines **13** by standard methods.

The compounds were tested for inhibitory activity against HIV protease.[13] Antiviral activity was assessed in a cell-based assay which measures the inhibition of viral RNA production in HIV-1-infected MT-2 cells.[14] The results are shown in Tables 1 and 2. A comparison of the straight-chain alcohols to the corresponding alkyls[10] shows that the introduction of the hydroxyl group is detrimental at chain lengths of 3 or 4, but significantly enhances the activity at longer chain lengths with a peak in activity at n = 5 or 6. This observation demonstrates that a minimum five carbon hydrophobic chain is necessary to satisfy the lipophilic interactions in the S2/S2' pocket before hydrogen bond donors on the P2/P2' side chain of the inhibitor can find sites for favorable H-bonding interactions.

Jones oxidation of compound **7** to the corresponding diacid results in compound **16**, which retains the enzyme inhibitory potency of **7**, but this compound shows poor translation in the whole cell assay, due presumably to the negatively-charged acidic groups. This loss of translation could be remedied by conversion to the dimethyl ester **17**; however, a 100-fold loss in potency compared to the parent acid was observed. A similar loss in activity is seen in going from alcohol **7** to methyl ether **18**, indicating the preference for a H-bond donor in this position.

The protease inhibition potency is adversely affected by the introduction of positively charged groups at P2/P2' as can be seen by the poor activity of the basic amines, **14** and **15**, suggesting a preference for a neutral H-bonding interaction in this region. Various attempts to produce metabolically stable inhibitors by providing neutral H-bond donors in the form of an amide, sulfonamide or urea derivative of compound **14** did not provide potent compounds.

Table 1



Compound	R	K_i(nM)[a]	IC_{90}(nM)[a]
1	-$CH_2C_6H_4$(4-CH_2OH)	0.34	136 ± 55[d]
4	-$(CH_2)_3OH$	51	NA[e]
5	-$(CH_2)_4OH$	42	22,671
6	-$(CH_2)_5OH$	1.4 ± 0.6[b]	953 ± 251[c]
7	-$(CH_2)_6OH$	0.47 ± 0.15[c]	1508 ± 588[b]
8	-$(CH_2)_7OH$	34 ± 10[c]	9013
9	-$(CH_2)_8OH$	500	17,158
14	-$(CH_2)_4NH_2$	1250	NA
15	-$(CH_2)_5NH_2$	1250	NA
16	-$(CH_2)_5COOH$	0.24 ± 0.08[c]	NA
17	-$(CH_2)_5COOMe$	29	30,889
18	-$(CH_2)_5OMe$	96	NA
19	-$(CH_2)_5NHCOCH_3$	350	NA
20	-$(CH_2)_5NHSO_2CH_3$	270	NA
21	-$(CH_2)_5NHCONHCH_3$	270	NA
22	-$(CH_2)_5NHCOPh$	59	NA
23	-$(CH_2)_5NHCOPh$(3-F)	14	8773
24	-$(CH_2)_5NHCOPh$(4-F)	18	NA
25	-$(CH_2)_5NHCOCH_2$(3-pyr)	8.9	3129
26	-$(CH_2)_5NHCO$(4-pyr)	16	9903

[a]Values without standard deviations represent an n of 1. [b]n of 3 [c]n of 2 [d]n of 181 [e]Highest concentration tested was 50 μg/ml.

The three-dimensional structure of HIV-1 PR complexed with one of the aliphatic alcohols, **7**, was determined with 1.7 Å diffraction data.[15] An examination of the bound conformation confirms that the alkyl chain of **7** is located in the hydrophobic S2/S2' pockets formed by the residues Asp29/29', Asp30/30', Ile47/47', Gly48/48', Gly49/49', and Ile50/50'. The terminal hydroxyl groups of P2/P2' are situated at hydrogen bonding distances to residues Asp30/30' (Fig. 1).

The symmetric inhibitor was bound at the active site in a symmetric mode while retaining the pseudo twofold symmetry of the 99 residues homodimeric protease. The root mean square

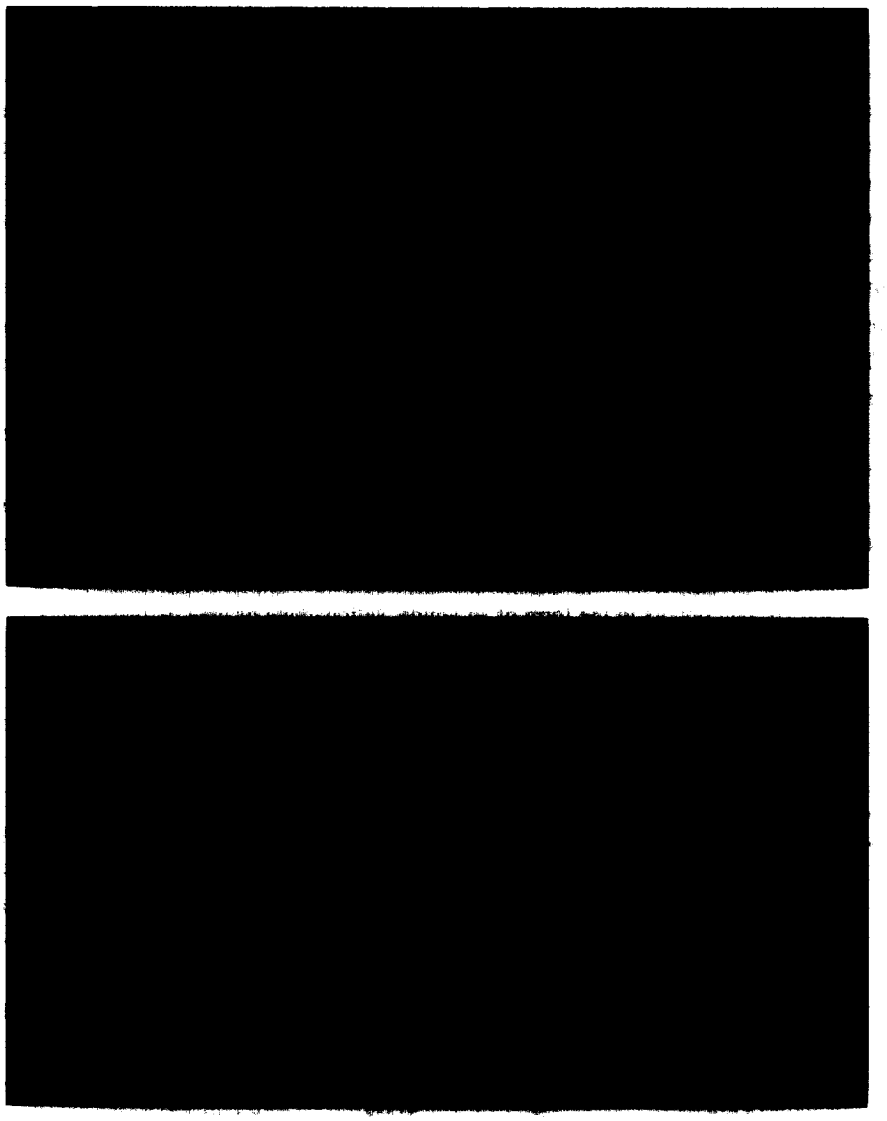


Figure 1: (Top) X-ray crystal structure of compd 7 bound to HIV-1 PR showing hydrogen bonds between the inhibitor and the protein. Residues 24–30 at the active site and 46–53 at the flap are shown. Light-blue circles depict amide nitrogen atoms forming hydrogen bonds with the inhibitor. Water molecules are shown as dark blue balls. (Bottom) Bound conformation of compd 7 shown from above.

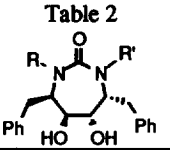
(rms) deviation of the 99 Cα between the two monomers after the superposition using a twofold axis is 0.25 Å. With the superposition of symmetrical portions of the inhibitor, the rms is 0.64 Å. In the absence of P2/P2', the rms is 0.11 Å, which is comparable to that of the cyclic urea inhibitors with aromatic rings at P2/P2'. The largest deviation, 1.59Å, occurs at the third carbon from the ring nitrogen due to the opposite torsional rotation at C1-C2 to C1'-C2'. Still, the terminal oxygen atoms of P2 and P2' occupy symmetrically equivalent positions in the S2/S2' pockets and each forms two hydrogen bonds with the amide nitrogen and the carboxyl oxygen atoms of Asp30/30'. Furthermore, there are two water molecules located near each terminal oxygen atom. These water molecules form hydrogen bonds to the carboxyl oxygen atoms of Asp29/29' as well as to the terminal oxygen atoms of **7**. In this binding mode, a minimum of five (5) atoms are required in the alkyl chain for the terminal oxygen atom to form a hydrogen bond with the amide nitrogen of Asp30/30' which is consistent with the observed poor inhibition potency of compounds **4** and **5**.

Since both compounds **7** and **8** were potent protease inhibitors as compared with DMP323, we decided to make hybrid molecules with one aliphatic and one benzylic P2/P2' side chain. These asymmetrical analogs were prepared by a modification of Scheme 1 using stepwise alkylation of unsubstituted cyclic urea **2**. Careful alkylation of **2** in the presence of 2.5 equiv sodium hydride with a tetrahydropyranyloxyalkylbromide provides a monoalkylated product in 50–70% yield after chromatographic separation from the bisalkylated by-product. A second alkylation with an appropriately protected arylmethyl bromide followed by deprotection in the usual manner provided the asymmetric compounds listed in Table 2.

These compounds were all highly potent protease inhibitors with good antiviral activity. Compounds **28** and **30–32** showed improved potency over the symmetrically substituted compounds from which they were derived.[13] These compounds were evaluated for oral bioavailability in rats. The asymmetric compounds had similar plasma C_{max} values to the parent symmetrically substituted benzylic compounds and slightly extended plasma half-lives.

We have shown that replacement of the highly preorganized P2/P2' groups of DMP323 with straight chain C5-C6 hydroxyalkyl groups results in minimal loss of potency. Furthermore, the incorporation of one aliphatic and one benzylic alcohol as P2/P2' substituents led to an improved biological profile. This leads the

Table 2



Compd	R	R'	K_i(nM)[a]	IC_{90}(nM)[a]
28	-$(CH_2)_5OH$	2-naphthylmethyl	0.24 ± 0.05	199 ± 90[c]
29	-$(CH_2)_6OH$	2-naphthylmethyl	1.7	264 ± 144
30	-$(CH_2)_5OH$	(4-hydroxymethylphenyl)methyl	0.10 ± 0.03	108 ± 22[b]
31	-$(CH_2)_5OH$	(3-hydroxymethylphenyl)methyl	0.058 ± 0.004	76 ± 30[d]
32	-$(CH_2)_5OH$	3-hydroxyphenylmethyl	0.081 ± 0.028	36 ± 10[c]
33	-$(CH_2)_5OH$	3-carboethoxyphenylmethyl	0.72 ± 0.30[b]	365
34	-$(CH_2)_5OH$	cyclopropylmethyl	0.99 ± 0.44	814
35	-$(CH_2)_5OH$	benzyl	0.68 ± 0.11	398

[a]Values with no standard deviation represent an n of 1. All others are n of 2 unless otherwise noted. [b]n of 3 [c]n of 5 [d]n of 6

way for further optimization by reintroduction of alternate steric constraints into the aliphatic chain to mimic the conformation observed in the bound inhibitor/enzyme complex.

References and Notes

1. Kohl, N. E.; Emini, E. A.; Schleif, W. A.; Davis, L. J.; Heimbach, J. C.; Dixon, R. A. F.; Scolnick, E. E.; Sigal, I.S. *Proc. Natl. Acd. Sci. U.S.A.* **1988**, *85*, 4686.
2. Gottlinger, H.; Sodroski, J.; Haseltine, W. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5781.
3. Navia, N.A.; Fitzgerald, P. M. D.; McKeever, B. M.; Leu, C-T.; Heimbach, J. C.; Herber, W. K.; Sigal, I. S.; Darke, P. L.; Springer, J. P. *Nature (London)* **1989**, *337*, 615. Wlodawer, A.; Miller, M.; Jaskolski, M.; Sathyanarayana, B. K.; Baldwin, E.; Weber, I. T.; Selk, L. M.; Clawson, L.; Schneider, J.; Kent, S. B. H. *Science* **1989**, *245*, 616. Lapatto, R.; Blundell, T.; Hemmings, A.; Overington, J.; Wilderspin, A.; Wood, S.; Merson, J. R.; Whittle, P. J.; Danley, D. E.; Geoghegan, K. F.; Hawrylik, S. J., Lee, S. E.; Scheld, K. G.; Hobart, P. M. *Nature (London)* **1989**, *343*, 299.
4. Miller, M.; Schneider, J.; Sathyanarayana, B. K.; Toth, M. V.; Marshall, G. R.; Clawson, L.; Selk, L.; Kent, S. B. H.; Wlodawer, A. *Science* **1989**, *246*, 1149. Erickson, J.; Neidhart, D. J.; VanDrie, J.; Kempf, D. J.; Wang, X. C.; Norbeck, D. W.; Plattner, J. J.; Rittenhouse, J. W.; Turon, M.; Wideburg, N.; Kohlbrenner, W. E.; Simmer, R.; Helfrich, R.; Paul, D. A.; Knigge, M. *Science*, **1990**, *249*, 527. Swain, A. L.; Miller, M. M.; Green, J.; Rich, D. H.; Schneider, J.; Kent, S. B. H.; Wlodawer, A. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 8805.
5. Darke, P.; Huff, J. *Adv. Pharmacol.* **1994**, *25*, 399.
6. Chong, K. T. *Exp. Opin. Invest. Drugs* **1996**, *5*, 115.
7. Lam. P. Y. S.; Jadhav, P. K.; Eyermann, D. J.; Hodge, C. N.; Ru, Y.; Bacheler, L. T.; Meek, J. L.; Otto, M. J.; Rayner, M. M.; Wong, N.Y.; Chang, C-Y.; Weber, P. C.; Jackson, D. A.; Sharpe, T. R.; Erickson-Viitanen, S. *Science* **1994**, *263*, 380.
8. Grubb, M. F.; Wong, Y. N.; Burcham, D. L.; Saxton, P. L.; Quon, C. Y.; Huang, S-M. *Drug Metab. Dispos.* **1994**, *22*, 709.
9. Shum, L. I.; Winslow, D. L.; Kornhauser, D. M. Unpublished data.
10. Pierce, M. E.; Harris, G. D.; Islam, Q.; Radesca, L. A.; Storace, L. Waltermire, R. E.; Wat, E.; Jadhav, P. K.; Emmett, G. C. *J. Org. Chem.* **1996**, *61*, 444.
11. All new compounds gave satisfactory ^{1}H NMR and MS data. Compounds were analyzed for purity by either elemental analysis or analytical HPLC (C18 column, 80% aqueous MeOH.)
12. For assay protocol see: Erickson-Viitanen, S.; Klabe, R. M.; Cawood, P. L.; O'Neal, P. L., Meek, J. L. *Antimicro. Agents Chemo.* **1994**, *38*, 1628.
13. For assay protocol see: Bacheler, L. T.; Paul, M.; Jadhav, P. K.; Otto, M.; Stone, B.; Miller, *J. Antiviral Chem. Chemo.* **1994**, *5*, 111.
14. Lam, P. Y. S.; Ru, Y.; Jadhav, P. K.; Aldrich, P. E.; DeLucca, G. V.; Eyermann, C. J.; Chang, C-H.; Emmett, G.; Holler, E. R.; Daneker, W. F.; Li, L.; Confalone, P. N.; McHugh, R. J.; Han, Q.; Li, R.; Markwalder, J. A.; Seitz, S. P.; Sharpe, T. R.; Jackson, D. A.; Erickson-Viitanen, S.; Hodge, C. N. *J. Med. Chem.* **1996**, *39*, 3514.
15. The complex of 7 and HIV protease was crystallized as described previously,[16] with unit cell dimensions of the complex being a = b = 63.2 Å, and c = 83.9 Å. Diffraction data were collected with an R-AXIS II imaging plate mounted on an RU200 Rigaku rotating anode generator operating at 50kV and 100 mA. The crystal diffracts up to 1.7 Å with a total of 74256 reflections, of which 19411 were unique reflections; the completeness of the data was 92%, and the Rsym was 8.4%. Difference maps calculated with the protein coordinate of XK263[7] revealed the corresponding inhibitor position. The structure was refined using the simulated annealing method, XPLOR.[17] The final R-factor was 0.209 with 110 water molecules. Standard geometry of the inhibitor was based on the single crystal structure of cyclic urea.
16. Erickson, J.; Neidhart, D. J.; VanDrie, J.; Kempf, D. J.; Wang, X. C.; Norbeck, D. W.; Plattner, J. J.; Rittenhouse, J. W.; Turon, M.; Wideburg, N.; Kohlbrenner, W. E.; Simmer, R.; Helfrich, R.; Paul, D. A.; Knigge, M. *Science* **1990**, *249*, 527.
17. Brünger, A. T.; Kuriyan, J.; Karplus, M. *Science* **1987**, *235*, 458.

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