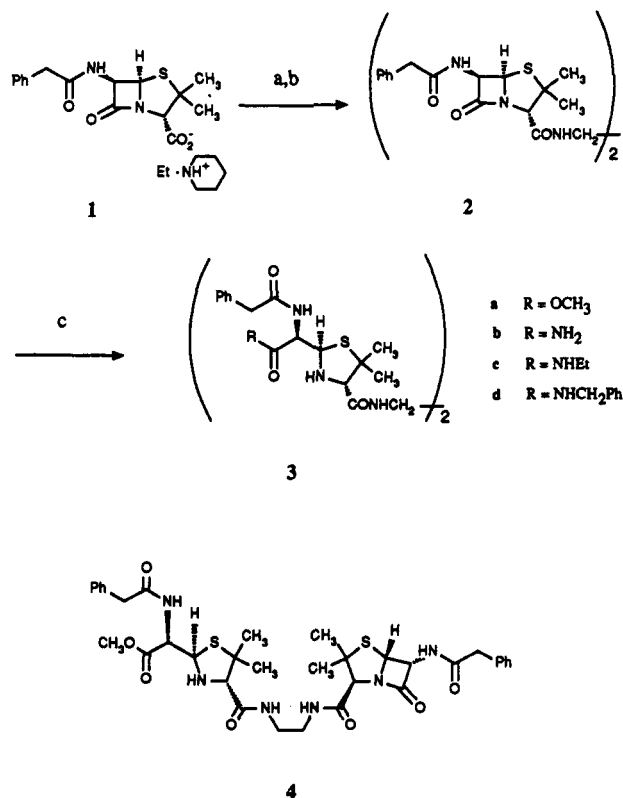


Penicillin Derived C_2 -Symmetric Dimers as Novel Inhibitors of HIV-1 Proteinase

The human immunodeficiency virus type 1 (HIV-1) encodes a proteinase required for post-translational modification of certain viral encoded polypeptides to produce the structural and core proteins and enzymes necessary for viral replication.¹ As inactivation of HIV-1 proteinase leads to the formation of immature and non-infectious virions,² this enzyme has been identified as a major chemotherapeutic target for the treatment of AIDS.³ The enzyme is a member of the aspartyl proteinase family and has been shown to function as a C_2 -symmetric homodimer.^{4,5} A number of groups have developed peptide-based inhibitors of HIV-1 proteinase using the natural substrate sequences as starting points in conjunction with the transition-state analogue concept previously employed in the renin area.⁶ Many of these inhibitors have been shown to block the replication of HIV-1 in vitro.

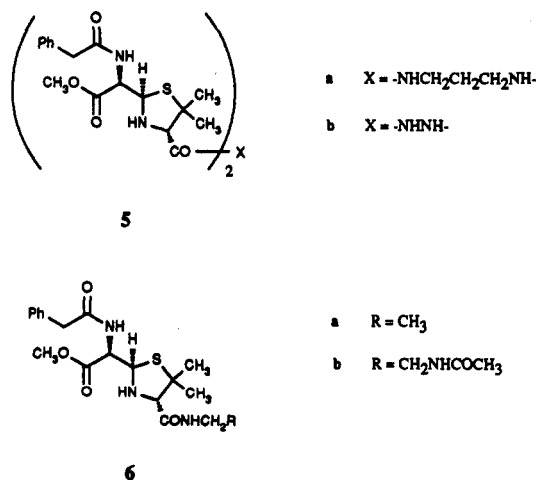
As an alternative to a peptide-based approach, we embarked on a screening program⁷ in order to identify novel inhibitors of HIV-1 proteinase. This led to the identification of a crude sample of the penicillin dimer 2 (Scheme I) as having inhibitory activity. Chromatography (silica, CHCl_3 - CH_3OH , 9:1) of the test sample provided pure 2, which was inactive (Table I) and the activity of the original material was attributed to impurities arising from opening of the β -lactam rings of 2. However the C_2 -symmetric diester 3a, which was an artefact of the purification process, transpired to be a potent inhibitor of the enzyme (IC_{50} 60 nM). We report here the syntheses and antiviral activities of a series of novel and readily prepared inhibitors of HIV-1 related to this lead compound.

Compound 3a was prepared from penicillin-G *N*-ethylpiperidine salt 1 by the route shown in Scheme I.⁸ The salt 1 was reacted with ethyl chloroformate and the resulting mixed anhydride treated without isolation with ethylenediamine to give the penicillin dimer 2. Dimer 2 was treated with methanol to provide the diester 3a. A small amount of compound 4 in which only one β -lactam has been opened was isolated by extensive chromatography of the mother liquors of the reaction. Related analogues 3b, 3c, and 3d were prepared in a similar fashion by reacting 2 with the appropriate amine nucleophile. Com-

Scheme I^a

^a (a) ClCO_2Et , CH_2Cl_2 , -10°C for 2 h; (b) $\text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2$, 0°C to room temperature for 2 h; (c) MeOH for 3a, or RNH_2 , CH_2Cl_2 for 3b, 3c, 3d, room temperature for 16-48 h.

pounds 5a and 5b, with different linker portions, and 6a and 6b derived from a single penicillin unit, were prepared by the same general procedure by reacting the intermediate mixed anhydride with other diamines or amines in place of ethylenediamine. Compound 8 bearing an ester linker was prepared by way of penicillin-G 2-chloroethyl ester 7⁹ as depicted in Scheme II.



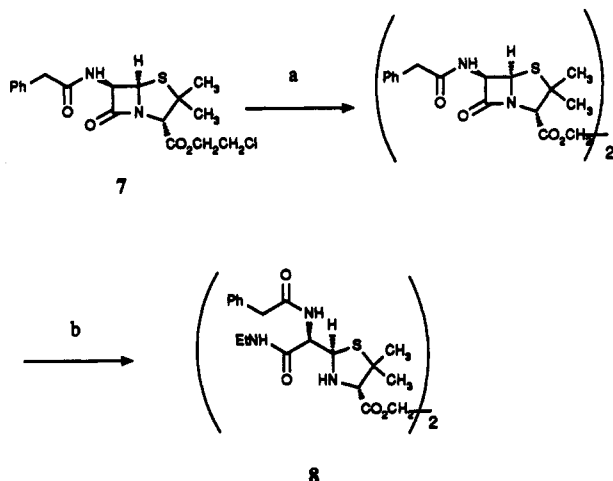
The asymmetric ester 4 in which only one of the β -lactam rings was opened showed weak activity (Table I). Compounds 5a and 5b with modified linkers derived from propylenediamine and hydrazine respectively and compound 8 with an ester linker were all essentially inactive. This indicates that not only is the length of the linker

- (1) Kay, J.; Dunn, B. M. Viral Proteinases: Weakness in Strength. *Biochim. Biophys. Acta* 1990, 1048, 1-18.
- (2) Kohl, N. E.; Emini, E. A.; Schleif, W. A.; Davis, L. J.; Heimbach, J. C.; Dixon, R. A. F.; Scolnick, E. M.; Sigal, I. S. Active Human Immunodeficiency Virus Protease is Required for Viral Infectivity. *Proc. Natl. Acad. Sci. U.S.A.* 1988, 85, 4686-4690.
- (3) Huff, J. R. HIV Protease: A Novel Chemotherapeutic Target for AIDS. *J. Med. Chem.* 1991, 34, 2305-2314.
- (4) Navia, M. A.; Fitzgerald, P. M. D.; McKee, B. M.; Leu, C.-T.; Heimbach, J. C.; Herber, W. K.; Sigal, I. S.; Darke, P. L.; Springer, J. P. Three-Dimensional Structure of Aspartyl Protease from Human Immunodeficiency Virus HIV-1. *Nature* 1989, 337, 615-620.
- (5) 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. Conserved Folding in Retroviral Proteinases: Crystal Structure of a Synthetic HIV-1 Protease. *Science* 1989, 245, 616-621.
- (6) See ref 3 for leading references.
- (7) A solid-phase assay utilizing an immobilized radiolabelled substrate peptide was developed: Orr, D. C., unpublished results. The assay is similar to that recently published by other workers: Wondrak, E. M.; Copeland, T. D.; Louis, J. M.; Orszan, S. A Solid Phase Assay for the Protease of Human Immunodeficiency Virus. *Anal. Biochem.* 1990, 188, 82-85.
- (8) All new compounds gave satisfactory combustion analysis and ^1H NMR data and were homogeneous by TLC and/or HPLC.

- (9) Ramaiah, M. A New Convenient Method for Esterification Using the $\text{Ph}_3\text{P}/\text{CCl}_4$ System. *J. Org. Chem.* 1985, 50, 4991-4993.

Table I. Inhibition of HIV-1 Proteinase by Penicillin-Derived Dimers and Related Compounds

no.	IC ₅₀ , ^a nM
2	>70000
3a	60
3b	3.0
3c	4.8
3d	0.9
4	500
5a	>12000
5b	6800
6a	>63000
6b	>55000
8	>12000

^a Assay procedure as in ref 7.**Scheme II^a**^a (a) 1 (K salt), DMF, 100 °C for 4 h; (b) EtNH₂, CH₂Cl₂ for 24 h.

important for inhibitory activity, but that the amide groups in the linker of 3a are possibly involved in important H-bonding interactions with the enzyme.

Diester 3a failed to block the cytopathic effect of HIV-1 in MT-4 cells.¹⁰ However, diamides 3c and 3d, both potent inhibitors of the proteinase enzyme, had EC₅₀ activities of 5.4 and 0.29 μM, respectively, in HIV-1 infected MT-4 cells. The more hydrophilic diamide 3b failed to show an antiviral effect possibly because of lack of penetration into the cells. Compounds 3c and 3d inhibit syncytia formation of HIV-1 infected C8166 cells at concentrations of 1.11 and 0.06 μM, respectively, and also the expression of p24 core antigen in H9 cells at concentrations of 0.42 and 0.025 μM, respectively.¹¹ Neither compound exhibited cytotoxicity in any of the cellular assays, nor did they show activity against other aspartyl proteinases (renin, pepsin, or cathepsin D) at concentrations up to 100 μg/mL.

Crystal structures of HIV-1 proteinase both in its native form and complexed with various inhibitors have been

described.⁶ In view of the activity of the symmetric dimers 3 and the contrasting inactivity of the amides 6, it was reasonable to speculate that they bind symmetrically at the active site. An X-ray structure¹² of the diamide 3c complexed to recombinant enzyme¹³ has shown that the interaction is indeed symmetrical.

Workers at Abbott Laboratories have designed an elegant series of C₂-symmetric inhibitors of HIV-1 proteinase which also inhibit HIV-1 replication.^{14,15} Our compounds also exhibit C₂ symmetry but have the advantage of being less peptidic in character. Studies are in progress to assess the potential of this novel series of compounds as chemotherapeutic agents for the treatment of AIDS.

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(12) Wonacott, A.; et al., unpublished results.

(13) Montgomery, D. S.; Singh, O. M. P.; Gray, N. M.; Dykes, C. W.; Weir, M. P.; Hobden, A. N. Expression of an Autoprocessing CAT-HIV-1 Proteinase Fusion Protein: Purification to Homogeneity of the Released 99 Residue Proteinase. *Biochem. Biophys. Res. Commun.* 1991, 175, 784-794.(14) 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. Design, Activity and 2.8 Å Crystal Structure of a C₂-Symmetric Inhibitor Complexed to HIV-1 Protease. *Science* 1990, 249, 527-533.(15) Kempf, D. J.; Norbeck, D. W.; Codacovi, L. M.; Wang, X. C.; Kohlbrenner, W. E.; Wideburg, N. E.; Paul, D. A.; Knigge, M. F.; Vasavanonda, S.; Craig-Kennard, A.; Saldivar, A.; Rosenbrook, W., Jr.; Clement, J. J.; Plattner, J. J.; Erickson, J. Structure-Based C₂-Symmetric Inhibitors of HIV Protease. *J. Med. Chem.* 1990, 33, 2687-2689.

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Disodium

(R,R)-5-[2-[[2-(3-Chlorophenyl)-2-hydroxyethyl]-amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL 316,243). A Potent β-Adrenergic Agonist Virtually Specific for β₃ Receptors. A Promising Antidiabetic and Antiobesity Agent

β-Adrenoceptors have been subclassified as β₁ and β₂ since 1967.¹ Increased heart rate is the primary consequence of β₁-receptor stimulation, while bronchodilation and smooth muscle relaxation typically result from β₂ stimulation. Rat adipocyte lipolysis was initially thought to be a β₁-mediated process.¹ However, more recent results indicate that the receptor-mediating lipolysis is neither β₁ nor β₂, but "atypical" in nature.² These "atypical" re-

(10) The assay procedure employed was essentially as described by Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; DeClercq, E. Rapid and Automated Tetrazolium-Based Colorimetric Assay for the Detection of Anti-HIV Compounds. *J. Virol. Methods* 1988, 20, 309-321.(11) Assays for inhibition of syncytium formation and expression of p24 core antigen were as previously detailed: Coates, J. A. V.; Inggall, H. J.; Pearson, B. A.; Penn, C. R.; Storer, R.; Williamson, C.; Cameron, J. M. Carbovir: The (-) Enantiomer is a Potent and Selective Antiviral Agent Against Human Immunodeficiency Virus *In Vitro*. *Antiviral Res.* 1991, 15, 161-168.(1) Lands, A. M.; Arnold, A.; McAuliff, J. P.; Luduena, F. P.; Brown, T. G., Jr. Differentiation of receptor systems activated by sympathomimetic amines. *Nature* 1967, 214, 597-598.