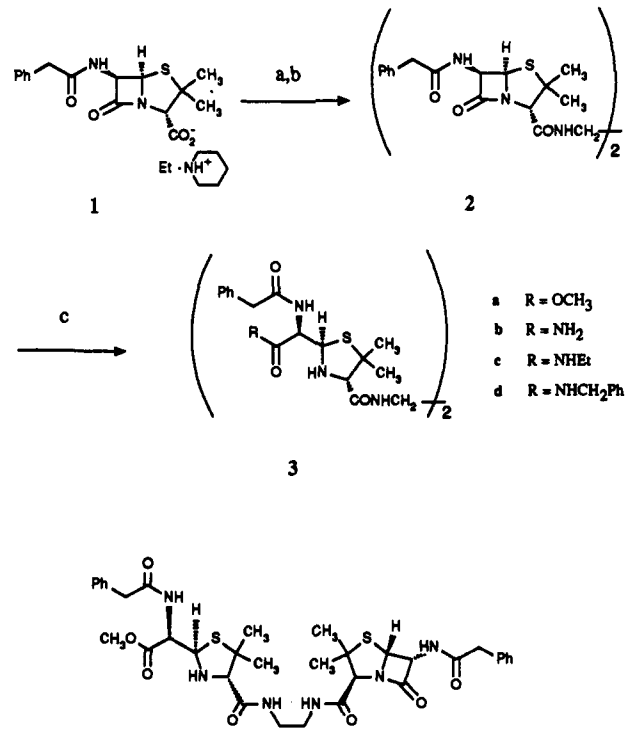
Penicillin Derived C_2-Symmetric Dimers as Novel Inhibitors of HIV-1 Proteinase

The human immunodeficiency virus type 1 (HIV-1) encodes a proteinase required for post-translational modification of certain viral encoded polyproteins 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 program[7] 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.

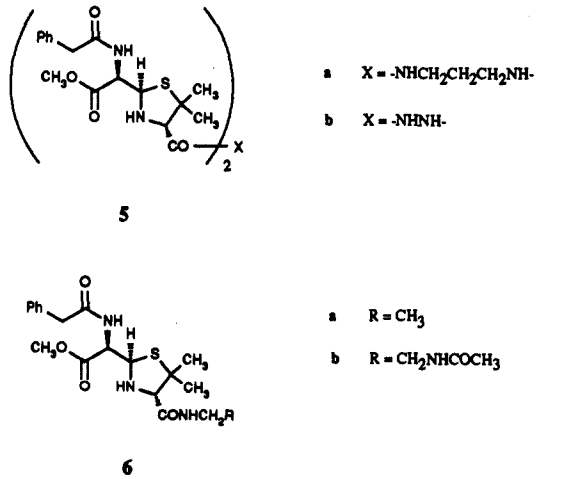
Compound **3a** was prepared from penicillin-G *N*-ethylpiperidine salt **1** by the route shown in Scheme I.[8] The salt **1** was reacted with ethyl chloroformate and the resulting mixed anhydride treated without isolation with ethylenediamine to give the penicillin dimer **2**. Dimer **2** was treated 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. Compounds **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 **7**[9] as depicted in Scheme II.

Scheme I[a]



1 2 3 4

[a] (a) $ClCO_2Et$, CH_2Cl_2, −10 °C for 2 h; (b) $H_2NCH_2CH_2NH_2$, 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.



5 6

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

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(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.; Oroszlan, 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.
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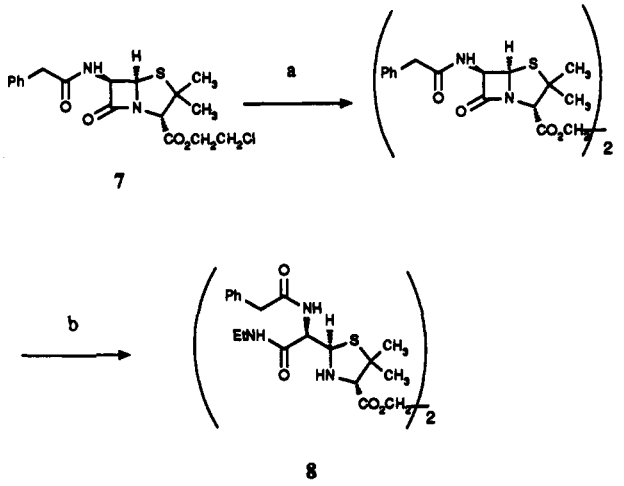
0022-2623/92/1835-3080$03.00/0

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) $EtNH_2$, CH_2Cl_2 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 EC_{50} 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 structure[12] of the diamide **3c** complexed to recombinant enzyme[13] has shown that the interaction is indeed symmetrical.

Workers at Abbott Laboratories have designed an elegant series of C_2-symmetric inhibitors of HIV-1 proteinase which also inhibit HIV-1 replication.[14,15] Our compounds also exhibit C_2 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.

Acknowledgment. We thank Dr. Stuart B. Laing and Dr. Peter S. Marshall for help with HPLC purifications and Miss Rachel Potter for assistance with the enzyme assays. We are grateful to the Medical Research Council AIDS Reagent Project for supplying the viruses and cells necessary for this study.

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Received April 24, 1992

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 β_3 Receptors. A Promising Antidiabetic and Antiobesity Agent

β-Adrenoceptors have been subclassified as β_1 and β_2 since 1967.[1] Increased heart rate is the primary consequence of β_1-receptor stimulation, while bronchodilation and smooth muscle relaxation typically result from β_2 stimulation. Rat adipocyte lipolysis was initially thought to be a β_1-mediated process.[1] However, more recent results indicate that the receptor-mediating lipolysis is neither β_1 nor β_2, but "atypical" in nature.[2] These "atypical" re-

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