7.35 and 7.52 (br s, 2 H, amide H), 7.52 (s, 2 H 2′,6′ Ar-H), 7.67, (d, 2 H, 3″,5″ Ar-H, J = 8.6 Hz), 7.79 (d, 2 H, 2″,6″ H, J = 8 Hz). Anal. (C, H, N, Cl). Further elution yielded **3a** (0.351 g, 16.6%), which was identical with that prepared via Scheme I. Table V summarizes the reaction conditions, stoichiometry, and results of the triazole alkylations.

Acknowledgment. Microanalyses were carried out at Merck & Co. by Jane Wu and her staff. We wish to thank Drs. H. Mrozik, T. Blizzard and D. Ostlind for their technical support and discussions, Mr. J. Smith and Dr. J. Liesch for recording the mass spectra of the analogues, and Dr. R. Reamer for selected NMR examinations.

Registry No. **2c**, 93272-19-6; **3a**, 99519-84-3; **3b**, 135365-03-6; **3c**, 135340-66-8; **3d**, 99519-83-2; **3e**, 99519-81-0; **3f**, 99880-60-1; **3g**, 135340-67-9; **3h**, 135340-68-0; **3i**, 135340-69-1; **3j**, 135340-70-4; **3k**, 135340-71-5; **3l**, 135340-72-6; **3m**, 135340-73-7; **3n**, 99519-91-2; **3o**, 135340-74-8; **3p**, 135340-75-9; **3q**, 135340-76-0; **3r**, 99519-82-1; **3s**, 99533-74-1; **3t**, 4342-08-9; **3u**, 132269-38-6; **3v**, 133992-52-6; **3w**, 99613-59-9; **4a**, 99508-23-3; **4b**, 135340-37-3; **4c**, 135340-38-4; **4d**, 99519-97-8; **4e**, 5395-79-9; **4f**, 99508-46-0; **4g**, 135340-39-5; **4h**, 135340-40-8; **4i**, 135340-41-9; **4j**, 135340-42-0; **4k**, 135340-43-1; **4l**, 135340-44-2; **4m**, 135340-45-3; **4n**, 99508-49-3; **4o**, 135340-46-4; **4p**, 135340-47-5; **4q**, 135340-48-6; **4r**, 35256-82-7; **4s**, 99520-00-0; **4t**, 108-88-3; **4u**, 106-43-4; **4v**, 25186-47-4; **5a**, 99508-24-4; **5b**, 135340-49-7; **5c**, 135340-50-0; **5d**, 99519-98-9; **5e**, 91457-11-3; **5f**, 99508-47-1; **5g**, 135340-51-1; **5h**, 135340-52-2; **5i**, 135340-53-3; **5j**, 135340-54-4; **5k**, 135340-55-5; **5l**, 135340-56-6; **5m**, 135340-57-7; **5n**, 99508-50-6; **5o**, 135340-58-8; **5p**, 135340-59-9; **5q**, 135340-60-2; **5r**, 35278-62-7; **5s**, 99520-01-1; **5t**, 100-39-0; **5u**, 622-95-7; **5v**, 7778-01-0; **5w**, 18880-04-1; **6a**, 99508-26-6; **6d**, 99519-99-0; **6e**, 99519-95-6; **6f**, 135340-61-3; **6g**, 135340-62-4; **6k**, 135340-63-5; **6m**, 135340-64-6; **6n**, 99508-51-7; **6p**, 135340-65-7; **6q**, 135340-83-9; **6r**, 99519-96-7; **6s**, 99520-02-2; **6t**, 622-79-7; **6u**, 27032-10-6; **6v**, 133992-55-9; **6w**, 99613-63-5; **7**, 4342-07-8; **8a**, 135340-77-1; **16a**, 99520-05-5; **16b**, 135340-36-2; **16c**, 50-43-1; **16d**, 7697-25-8; **16e**, 99-94-5; **16f**, 50-84-0; **16g**, 99-04-7; **16h**, 50-30-6; **16i**, 6342-60-5; **17a**, 99508-22-2; **17b**, 135365-02-5; **17c**, 4136-95-2; **17d**, 21900-53-8; **17e**, 874-60-2; **17f**, 89-75-8; **17g**, 1711-06-4; **17h**, 4659-45-4; **17i**, 21900-50-5; **18a**, 615-65-6; **18b**, 95-81-8; **19**, 135340-78-2; **20a**, 135340-79-3; **29b**, 21423-84-7; **21a**, 135340-80-6; **21b**, 64597-37-1; **22a**, 618-61-1; **22b**, 499-08-1; **23**, 52215-41-5; **24a**, 93857-90-0; **24b**, 25186-47-4; **24c**, 108-70-3; 3-diazonium-5-nitrotoluene hexafluorophosphate salt, 135340-82-8; chlorobenzene, 108-90-7; benzene, 71-43-2; fluorobenzene, 462-06-6; trichlorovinylbenzene, 700-60-7; *o*-dichlorobenzene, 95-50-1; toluene, 108-88-3; 3-chlorotoluene, 108-41-8; 2-cyanoacetamide, 107-91-5; 4-chlorobenzoyl chloride, 122-01-0; 4-bromobenzoyl chloride, 586-75-4; 4-iodobenzoyl chloride, 1711-02-0; 3,5-dichlorobenzoyl chloride, 2905-62-6.

Design and Synthesis of HIV Protease Inhibitors. Variations of the Carboxy Terminus of the HIV Protease Inhibitor L-682,679

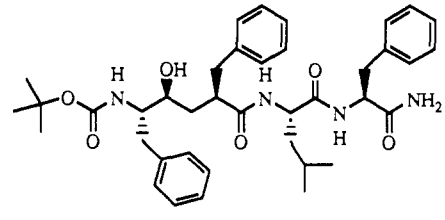
S. Jane deSolms,* Elizabeth A. Giuliani, James P. Guare, Joseph P. Vacca, William M. Sanders, Samuel L. Graham, J. Mark Wiggins, Paul L. Darke, Irving S. Sigal, Joan A. Zugay, Emilio A. Emini, William A. Schleif, Julio C. Quintero, Paul S. Anderson, and Joel R. Huff

Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486. *Received March 11, 1991*

A series of tetrapeptide analogues of **1** (L-682,679), in which the carboxy terminus has been shortened and modified, was prepared and their inhibitory activity measured against the HIV protease in a peptide cleavage assay. Selected examples were tested as inhibitors of virus spread in cell culture. Compound **12** was a 10-fold more potent enzyme inhibitor than **1** in vitro and 30-fold more potent in inhibiting the viral spread in cells.

The HIV protease has been characterized biochemically and structurally as a member of the aspartyl protease family of enzymes. Inactivation of this protease results in the production of noninfectious virons and consequent inhibition of the spread of viral infection in susceptible cells.[1] Inhibition of this enzyme should provide an attractive therapeutic goal in the treatment of AIDS. Our strategy, like that of others,[2-4] was based on the transition-state mimetic concept which proved successful in the design of renin inhibitors. We found early on[5] that incorporation of a hydroxyethylene isostere as a dipeptide mimic provided compounds which were potent and selective inhibitors of HIV protease. The prototype, L-682,679 (**1**), essentially a pentapeptide, is a potent and

P_1 P_1' P_2' P_3'



1

L- 682, 679

(1) 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.

(2) Dreyer, G. B.; Metcalf, B. W.; Tomaszek, T. A., Jr.; Carr, T. J.; Chandler, A. C. III.; Hyland, L.; Fakhoury, S. A.; Magaard, V. W.; Strickler, J. E.; Debouck, C.; Meek, T. D. Inhibition of Human Immunodeficiency Virus 1 Protease in vitro: Rational Design of Substrate Analog Inhibitors. *Proc. Natl. Acad. Sci. U.S.A* **1989**, *86*, 9752–9756.

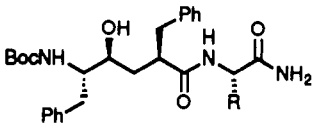
(3) Roberts, N. A.; Martin, J. A.; Kinchington, D.; Broadhurst, A. V.; Craig, J. C.; Duncan, I. B.; Galpin, S. A.; Handa, B. K.; Kay, J.; Krohn, A.; Lambert, R. W.; Merrett, J. H.; Mills, J. S.; Parkes, K. E. B.; Redshaw, S.; Ritchie, A. J.; Taylor, D. L.; Thomas, G. J.; Machin, P. J. Rational Design of Peptide-Based HIV Proteinase Inhibitors. *Science* **1990**, *248*, 358–361.

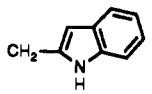
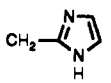
(4) McQuade, T. J.; Tomasselli, A. G.; Liu, L.; Karacostas, V.; Moss, B.; Sawyer, T. K.; Heinrikson, R. L.; Tarpley, W. G. A Synthetic HIV-1 Protease Inhibitor with Antiviral Activity Arrests HIV-Like Particle Maturation. *Science*, **1990**, *247*, 454–456.

(5) Huff, J. R.; Anderson, P. S.; Britcher, S. F.; Darke, P.; deSolms, S. J.; Dixon, R. A. F.; Emini, E.; Hoffman, J. M.; Lyle, T. A.; Randall, W. C.; Rooney, C. S.; Sigal, I. S.; Sweet, C. S.; Thompson, W. J.; Vacca, J. P.; Young, S. D. The Design and Synthesis of HIV Protease Inhibitors. Symposium on Molecular and Cellular Biology, Frisco, Colorado, 2/27–3/4/90. *J. Cell Biochem. Suppl.* **1990**, *14C*, 222.

0022-2623/91/1834-2852$02.50/0

Table I



no.	R	mp, °C	% yield[a]	formula[b]	IC_{50}, nM
3	$CH(CH_3)_2$	214–216	52	$C_{29}H_{41}N_3O_5$	1.1
13	H	149–151	58	$C_{26}H_{35}N_3O_5$	550
14	CH_3	174–176	62	$C_{27}H_{37}N_3O_5$	7
15	$CH_2CH(CH_3)_2$	185–187	32	$C_{30}H_{43}N_3O_5$	10
16	$CH(CH_3)CH_2CH_3$	226–228	20	$C_{30}H_{43}N_3O_5$	2.7
17	C_6H_5	215–216		$C_{32}H_{39}N_3O_5$	0.9
18	C_6H_{11}	232–234	30	$C_{32}H_{45}N_3O_5$	5.2
19	$CH_2C_6H_5$	227–230	62	$C_{33}H_{41}N_3O_5$	29
20	$CH_2C_6H_{11}$	203–205	10	$C_{33}H_{47}N_3O_5$	64
21	CH_2OH	180–182	25	$C_{27}H_{37}N_3O_6$	606
22		202–204	35	$C_{35}H_{42}N_4O_5$	60
23		130–133	20	$C_{30}H_{39}N_5O_5$	770
24	D-$CH_2CH(CH_3)_2$	216–218	29	$C_{30}H_{43}N_3O_5$	3100

[a] Yields are combined for coupling and desilylation. [b] Satisfactory analyses (C, H, and N; ±0.4% of theoretical values) were obtained for all compounds.

selective inhibitor of HIV protease with an IC_{50} of 0.6 nM in the peptide cleavage assay. It was also an effective inhibitor of the spread of the virus in cell culture with a minimum inhibitory concentration (MIC_{100}) of 6 μM. Structural manipulation of various portions of this molecule provided compounds with enhanced potency, selectivity, and solubility. In this paper we describe the systematic study of the carboxy terminus of this molecule with special emphasis on the variation of the P_2' amino acid and the elimination/replacement of the P_3' amino acid.

Results and Discussion

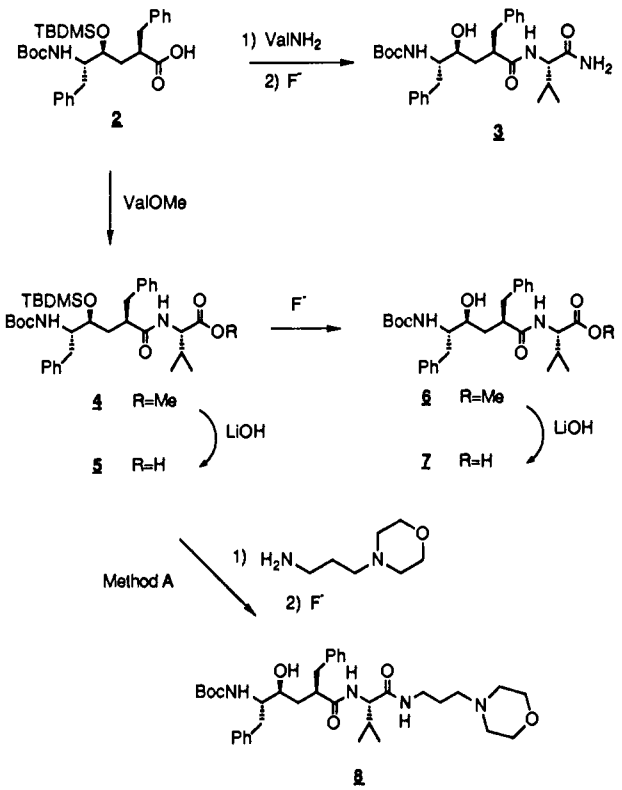
Synthesis. All of the compounds were prepared from a common intermediate, protected acid **2**, described previously.[6] This acid was readily coupled to an amino acid amide such as valinamide by using standard conditions (EDC, HOBT) to provide compound **3** after desilylation with tetrabutylammonium fluoride (Scheme I). Compounds **13–24**, shown in Table I were prepared in this manner.

To prepare analogues of **1** in which phenylalanine in P_3' is replaced by an amine, acid **2** was coupled to an amino acid ester such as valine methyl ester to give **4**, which was hydrolyzed with base to acid **5**. Subsequent coupling of **5** to an amine such as 4-(3-aminopropyl)morpholine followed by deprotection gave **8** (method A, Scheme I). A more efficient synthesis was achieved by first coupling an active ester of requisite P_2' amino acid **9** with the desired amine such as 2-(aminomethyl)benzimidazole to provide **10**. Deprotection with hydrochloric acid and subsequent coupling to acid **2** gave **12** (method B, Scheme II) after desilylation. Compounds **25–52** in Table II were prepared by one of these methods.

In Vitro Activity. Initial evaluation of these compounds as HIV protease inhibitors was carried out with the HIV-1 enzyme substrate cleavage assay, which has been described elsewhere.[7] Each compound was dissolved in DMSO and added to the enzyme mixture to provide the required concentrations of inhibitor. The IC_{50} values were calculated from the extent of substrate cleavage as determined by HPLC.[7]

The in vitro activities are shown in Tables I and II. The amino terminal *tert*-butyloxycarbonyl group, as well as the

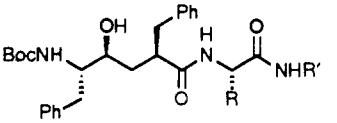
Scheme I

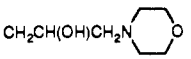
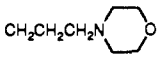
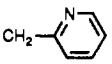
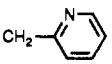
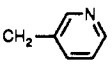
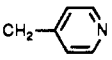
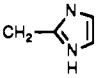
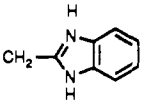
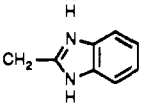
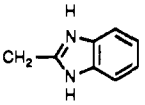


(6) Evans, B. E.; Rittle, K. E.; Homnick, C. F.; Springer, J. P.; Hirshfield, J.; Veber, D. F. A Stereocontrolled Synthesis of Hydroxyethylene Dipeptide Isosteres Using Novel, Chiral Aminoalkyl Epoxides and γ-(Aminoalkyl) γ-Lactones. *J. Org. Chem.* **1985**, *50*, 4615–4625.

(7) Heimbach, J. C.; Garsky, V. M.; Michelson, S. R.; Dixon, R. A. F.; Sigal, I. S.; Darke, P. L. Affinity Purification of the HIV-1 Protease. *Biochem. Biophys. Res. Commun.* **1989**, *164*, 955–960.

Table II



no.	R	R'	method	mp, °C	% yield[a]	formula[b]	IC_{50}, nM
1	$CH_2CH(CH_3)_2$	$PheNH_2$	A + B	218–219	60	$C_{39}H_{52}N_4O_6$	0.6
25	$CH(CH_3)CH_2CH_3$	$PheNH_2$	B	118–119	86	$C_{39}H_{52}N_4O_6$	0.25
26	$CH_2CH(CH_3)_2$	CH_2Ph	B	171–173	32	$C_{37}H_{49}N_3O_5$	1.4
27	$CH(CH_3)CH_2CH_3$	CH_2Ph	B	204–206	31	$C_{37}H_{49}N_3O_5$	0.71
28	$CH(CH_3)_2$	CH_2Ph	B	204–206	71	$C_{36}H_{47}N_3O_5$	0.24
29	C_6H_5	CH_2Ph	B	194–196	25	$C_{39}H_{45}N_3O_5$	5.8
30	$CH_2CH(CH_3)_2$	CH_2CH_2Ph	A	164–165	37	$C_{38}H_{51}N_3O_5$	15
31	$CH_2CH(CH_3)_2$	$CH(CH_2OH)CH_2Ph$	A	189–191	44	$C_{39}H_{53}N_3O_6$	1.5
32	$CH_2CH(CH_3)_2$	CH_2CH_2OH	B	173–175	33	$C_{32}H_{47}N_3O_6$	2.1
33	$CH(CH_3)CH_2CH_3$	CH_2CH_2OH	A	202–204	46	$C_{32}H_{47}N_3O_6$	0.46
34	$CH(CH_3)_2$	CH_2CH_2OH	B	188–189	59	$C_{31}H_{45}N_3O_6$	0.44
35	$CH(CH_3)CH_2CH_3$	$CH_2CH(OH)CH_2OH$	B	171–172	36	$C_{33}H_{49}N_3O_7$	0.15
36	$CH(CH_3)_2$	$CH_2CH(OH)CH_2OH$	B	170–171	31	$C_{32}H_{47}N_3O_7$	0.05
37	$CH_2CH(CH_3)_2$	$CH_2CH_2CH_2N(CH_3)_2$	B	155–156	36	$C_{35}H_{54}N_4O_5$	23
38	$CH(CH_3)CH_2CH_3$	$CH_2CH_2CH_2N(CH_3)_2$	A	195–196	40	$C_{35}H_{54}N_4O_5$	7.9
39	$CH(CH_3)_2$	$CH_2CH_2CH_2N(CH_3)_2$	B	175–177	57	$C_{34}H_{52}N_4O_5$	3.4
40	C_6H_5	$CH_2CH_2CH_2N(CH_3)_2$	B	162–164	14	$C_{37}H_{51}N_4O_5$	26
41	$CH(CH_3)CH_2CH_3$	$CH_2CH_2N(CH_3)_2$	A	195–197	39	$C_{34}H_{52}N_4O_5$	7.1
42	$CH(CH_3)_2$	$CH_2CH_2NHCH_3$	A	168–170	3	$C_{36}H_{52}N_4O_4$	1.73
43	$CH(CH_3)_2$	$CH_2CH_2NH_2$	B	192–194	76	$C_{31}H_{46}N_4O_5$	0.46
44	$CH(CH_3)_2$	$CH_2CH(OH)CH_2N(C_2H_5)_2$	A	152–156	42	$C_{36}H_{56}N_4O_6$	6.3
45	$CH(CH_3)_2$		A	141–143	44	$C_{36}H_{54}N_4O_7$	0.88
8	$CH(CH_3)_2$		A	176–178	77	$C_{36}H_{54}N_4O_6$	2.9
46	$CH(CH_3)CH_2CH_3$		A	187–189	33	$C_{36}H_{46}N_4O_5$	1.3
47	$CH(CH_3)_2$		A	159–161	8.5	$C_{35}H_{46}N_4O_5$	0.75
48	$CH(CH_3)_2$		A	154–155	22	$C_{35}H_{46}N_4O_5$	0.69
49	$CH(CH_3)_2$		A	168–170	17	$C_{35}H_{46}N_4O_5$	0.62
50	$CH(CH_3)CH_2CH_3$		B	231–233	38	$C_{34}H_{47}N_5O_5$	0.88
51	$CH(CH_3)_2$		B	204–206	27	$C_{37}H_{47}N_5O_5$	0.2
52	C_6H_5		B	171–173	30	$C_{40}H_{45}N_5O_5$	0.07
12	$CH(CH_3)CH_2CH_3$		B	233–235	31	$C_{38}H_{49}N_5O_5$	0.06

[a] Yields are combined for coupling and desilylation. [b] Satisfactory analyses (C, H, and N; ±0.4% of theoretical values) were obtained for all compounds.

benzyl substituents at the P_1 and P_1' sites have been kept constant. Systematic variation at these sites will be the subject of subsequent papers. Removal of the P_3' phenylalanine of 1 and replacement with an NH_2 provides 15, which is 17-fold less active than 1. Replacement of the primary amide with methyl ester 6 (IC_{50} = 25 nM) or free carboxylic acid 7 (IC_{50} = 161 nM), as illustrated in the P_2' valine series, decreases intrinsic potency 100-fold. As the R group of the P_2' amino acid increases in size from H to methyl to isopropyl, potency approaches that of 1. When R is a branched substituent like isobutyl (Ile-16), isopropyl (Val-3), or phenyl (Phg-17), maximal potencies are achieved. As the size of the R group increases to benzyl or cyclohexylmethyl or as polar groups such as hydroxymethyl are added, potency drops. The substitution of a D-amino acid amide in place of the natural L-amino acid amide, e.g. D-leucine (24), leads to a 300-fold drop in activity.

Variation of the P_3' terminus using either leucine, isoleucine, valine, or phenylglycine as the P_2' spacer provides compounds with activities covering a wide range. Replacement of phenylalaninecarboxamide in 1 with benzylamine gives compounds with potencies equivalent to that of 1. Homologation by one carbon (30) leads to a 25-fold loss in potency. Phenylalaninecarboxamide can be replaced with phenylalaninol (31) with slight loss of activity. Removal of the benzyl group to give the aminoethanol amide (32–34) does not affect potency significantly. The addition of a second hydroxyl group to provide aminoethanediols 35 and 36 increases potency.

Although water solubility improves when an aliphatic amino group is incorporated into the terminal amide, the

Scheme II

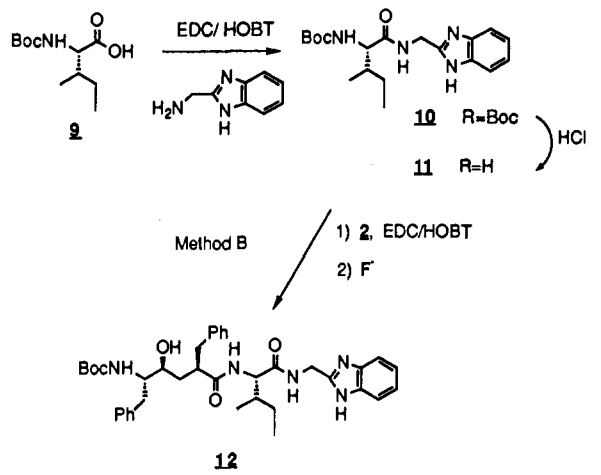


Table III. Viral Spread

no.	IC_{50}, nM	MIC_{100}, nM	no.	IC_{50}, nM	MIC_{100}, nM
1	0.6	6000	**32**	2.1	3000
4	1.1	780	**33**	0.46	400
14	7.0	6000	**36**	0.05	1500
16	2.7	3000	**8**	2.9	780
17	0.9	1500	**46**	1.3	780
26	1.4	3000	**49**	0.88	400
27	0.71	1500	**12**	0.06	200

compounds are less potent. Substitution with 3-(dimethylamino)propylamine gives compounds (**37–40**) with IC_{50} values in the 5–25 nM range. Shortening the aliphatic chain (**41**) has no effect on potency, but removing one (**42**) or both (**43**) of the methyls on nitrogen increases potency. Substitution of a less basic amine at the amide terminus, as in 3-(aminopropyl)morpholine (**8**), improves potency. When the hydroxyl moiety as well as a terminal amine is incorporated into the carboxy terminus, the activity is directed by the potency of the amine, with diethylamino compound **44** being less potent than morpholino derivative **45**. Replacement of the amide terminus with (aminomethyl)pyridines, with the pyridine nitrogen in any position, or (aminomethyl)imidazole gives compounds (**46–50**) with activities in the subnanomolar range. Substitution by (aminomethyl)benzimidazole provides the most potent compounds (**51**, **52**, **12**) in this series with IC_{50} values less than 100 pM. The imidazole portion of **12** appears to be mimicking a carboxamide and the phenyl portion may be contributing additional hydrophobic binding in essentially a P_4' site.

Viral Spread Activity. Representative compounds in this series were also tested for their ability to inhibit HIV-1 infection in cell culture by using the protocol described previously.[8] Compounds were tested at concentrations up to 12 μM in H9 human T-lymphoid cells without observing any morphological changes or a change in metabolism. In a cell-based assay described in ref 8 these compounds blocked the processing of the viral *gag* precursor protein, p55, to the mature p24 and p17 core and matrix proteins, implying that the inhibition of viral infectivity is due to their ability to inhibit the viral protease. Table III lists the minimum inhibitory concentration (MIC_{100}) necessary to stop the spread of HIV virus in cell culture (i.e. 100% inhibition) as well as the IC_{50} values in the peptide cleavage assay for these compounds. Pentapeptide **1** and diol **36** are considerably less potent in cell culture than would have been predicted by their IC_{50} values. This may reflect their inability to penetrate the hydrophobic cell membrane. Although their intrinsic potency is poorer, primary amides **4** and **16** are more potent inhibitors in cell culture than **1**. As the terminal amide increases in size (**26** and **27**) or polarity (**32** and **33**), the intrinsic potency improves, but no improvement is observed in MIC. Amides which contain a weakly basic site (**46** and **49**), although poorly water soluble, exhibit potent in vitro and in vivo activity. Benzimidazole **12** is the most potent inhibitor in this series.

Conclusions

Potent HIV protease inhibitors both in vitro and in cell-based assays have been prepared with the hydroxyethylene moiety to mimic the transition state. Optimization of the carboxy terminus by substituting an α-branched amino acid in the P_2'-position and capping it with numerous amides has provided inhibitors with subnanomolar activity. When the carboxy terminus is (aminomethyl)benzimidazole, as in **12**, the spread of virus in cells is stopped at an inhibitor concentration of 200 nM.

Experimental Section

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed on site and were within ±0.4% of the theoretical values. All compounds were routinely examined by proton NMR (Varian XL300) and TLC (silica gel). The primary amides listed in Table I were prepared from commerically available or known literature amines and acid **2** as exemplified by the procedure described for valine amide **3**. Two general methods, A (Scheme I) or B (Scheme II), were used to prepare the compounds listed in Table II, examples of which are given below.

***N*-[5(*S*)-[(*tert*-Butoxycarbonyl)amino]-4(*S*)-hydroxy-6-phenyl-2(*R*)-(phenylmethyl)hexanoyl]valine Amide (3).** 5(*S*)-[(*tert*-butoxycarbonyl)amino]-4(*S*)-(*tert*-butyldimethylsiloxy)-6-phenyl-2(*R*)-(phenylmethyl)hexanoic acid[5] (0.20 g, 0.38 mmol) was dissolved in DMF (2 mL) at ambient temperature and treated with 1-hydroxybenzotriazole hydrate (HOBT; 0.054 g, 0.40 mmol), 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC; 0.077 g, 0.40 mmol), and valinamide hydrochloride (0.062 g, 0.46 mmol) with stirring. The pH of the solution was adjusted to 8 with triethylamine (Et_3N; 0.12 mL, 0.86 mmol), and the mixture was stirred for 18 h. The DMF was removed under reduced pressure, and the residue partitioned between EtOAc and H_2O. The organic layer was separated, washed with H_2O, 10% citric acid solution, aqueous saturated $NaHCO_3$ solution, and brine, and dried (Na_2SO_4). Filtration and concentration to dryness gave 0.24 g of [5(*S*)-[(*tert*-butoxycarbonyl)amino]-4-(*S*)-(*tert*-butyldimethylsiloxy)-6-phenyl-2(*R*)-(phenylmethyl)-hexanoyl]valine amide, which was used without further purification: ^1H NMR ($CDCl_3$) δ 7.35–7.00 (m, 10 H, ArH), 6.16 (d, J = 9 Hz, 1 H, NH), 5.45 (br s, 2 H, NH_2), 4.81 (d, J = 12 Hz, 1 H, NH), 4.11 (t, J = 7 Hz, 1 H, CH), 3.80–3.70 (m, 1 H, CH), 2.85–2.47 (m, 5 H, CH_2), 2.15–2.10 (m, 1 H, CH), 1.88–1.65 (m, 2 H, CH_2), 1.35 (s, 9 H, CH_3), 0.97 (s, 9 H, CH_3), 0.93–0.75 (m, 6 H, CH_3), 0.12 (s, 6 H, CH_3). This product was dissolved in THF (3 mL) and treated with tetrabutylammonium fluoride (TBAF; 1.0 M in THF) (2 mL) with stirring at ambient temperature. After 20 h solvent was removed on a rotary evaporator, and the mixture was treated with 10% citric acid (20 mL)–H_2O (20 mL). The white solid precipitate was collected by suction filtration, washed with H_2O, dried, and chromatographed on a silica gel (2 mm) Chromatotron plate eluting with $CHCl_3$–MeOH (95:5) to give 0.10 g (52%) of **3**: mp 214–216 °C; MS (FAB) m/e 512 (M + 1); ^1H NMR (DMSO-d_6) δ 7.57 (d, J = 7 Hz, 1 H, NH), 7.26–7.10 (m, 10 H, ArH), 7.05 (s, 1 H, NH_2), 6.93 (s, 1 H, NH_2), 6.40 (d, J = 11 Hz, 1 H, NH), 4.57 (d, J = 4 Hz, 1 H, CH), 4.10–4.05 (m, 1 H, CH), 3.60–3.50 (m, 1 H, CH), 3.45–3.35 (m, 1 H, CH), 2.90–2.50

(8) Vacca, J. P.; Guare, J. P.; deSolms, S. J.; Sanders, W. M.; Giuliani, E. A.; Young, S. D.; Darke, P. L.; Zugay, J. A.; Sigal, I. S.; Schleif, W. A.; Quintero, J. C.; Emini, E. A.; Anderson, P. S.; Huff, J. R. L-687,908, a Potent Hydroxyethylene-Containing HIV Protease Inhibitor. *J. Med. Chem.* **1991**, *34*, 1225–1228.

(m, 5 H, CH_2), 1.90–1.80 (m, 1 H, CH), 1.65–1.55 (m, 1 H, CH), 1.30 (s, 9 H, CH_3), 0.79–0.70 (m, 6 H, CH_3). Anal. ($C_{29}H_{41}N_3O_5$) C, H, N.

Compounds 13–24 were prepared in this manner.

***N*-[5(*S*)-[(*tert*-Butoxycarbonyl)amino]-4(*S*)-(*tert*-butyldimethylsiloxy)-6-phenyl-2(*R*)-(phenylmethyl)hexanoyl]valine Methyl Ester (4).** 5(*S*)-[(*tert*-Butoxycarbonyl)amino]-4(*S*)-(*tert*-butyldimethylsiloxy)-6-phenyl-2(*R*)-(phenylmethyl)hexanoic acid (0.50 g, 0.95 mmol) was dissolved in DMF (8 mL) and treated with HOBT (0.135 g, 1.0 mmol), EDC (0.192 g, 1.0 mmol), and valine methyl ester hydrochloride (0.191 g, 1.0 mmol) followed by Et_3N (0.30 mL, 1.14 mmol) to bring the pH of the solution to 8.5. After stirring for 18 h at ambient temperature, the reaction mixture was partitioned between EtOAc and H_2O. The organic layer was separated, washed with H_2O (3 × 20 mL), 10% citric acid (1 × 20 mL), aqueous saturated $NaHCO_3$ solution (1 × 20 mL), brine (1 × 20 mL), and dried (Na_2SO_4). Filtration and concentration to dryness gave 0.58 g (100%) of **4** as a white foam, which was used without further purification: ^1H NMR ($CDCl_3$) δ 7.33–7.02 (m, 10 H, ArH), 5.85 (d, J = 8.5 Hz, 1 H, NH), 4.68 (d, J = 9.5 Hz, 1 H, NH), 4.34 (dd, J = 5.7, 8.5 Hz, 1 H, CH), 3.95–3.92 (m, 1 H, CH), 3.73–3.62 (m, 1 H, CH), 3.59 (s, 3 H, CH_3), 2.84 (dd, J = 10.4, 14.8, 1 H, CH), 2.69–2.49 (m, 4 H, CH_2), 2.04–1.69 (m, 3 H, CH, CH_2), 1.32 (s, 9 H, CH_3), 0.94 (s, 9 H, CH_3), 0.83 (dd, J = 4.2, 6.8 Hz, 6 H, CH_3), 0.11 (s, 6 H, CH_3).

***N*-[5(*S*)-[(*tert*-Butoxycarbonyl)amino]-4(*S*)-(*tert*-butyldimethylsiloxy)-6-phenyl-2(*R*)-(phenylmethyl)hexanoyl]valine (5).** Compound **4** (0.58 g, 0.95 mmol) was dissolved in 1,2-dimethoxyethane (DME; 8 mL) and treated with a 1 M LiOH solution (4 mL) with stirring at ambient temperature. After 1 h solvent was removed on a rotary evaporator, and the residue was treated with 10% citric acid solution and extracted with EtOAc (3 × 30 mL). The organic layers were combined, washed with brine, and dried (Na_2SO_4). Filtration and concentration to dryness gave 0.6 g (100%) of **5** as a white foam: ^1H NMR ($CDCl_3$) δ 7.30–6.90 (m, 11 H, ArH, NH), 4.86 (d, J = 9.6 Hz, 1 H, NH), 4.41–4.38 (m, 1 H, CH), 3.82 (q, J = 8 Hz, 1 H, CH), 3.66–3.58 (m, 1 H, CH), 2.82–2.37 (m, 5 H, CH_2), 2.24–2.11 (m, 1 H, CH), 1.79–1.70 (m, 2 H, CH_2), 1.28 (s, 9 H, CH_3), 0.93 (s, 9 H, CH_3), 0.88 (d, J = 6.8 Hz, 3 H, CH_3), 0.76 (d, J = 6.8 Hz, 3 H, CH_3), 0.10 (s, 3 H, CH_3), 0.06 (s, 3 H, CH_3).

***N*-[5(*S*)-[(*tert*-Butoxycarbonyl)amino]-4(*S*)-hydroxy-6-phenyl-2(*R*)-(phenylmethyl)hexanoyl]valine Methyl Ester (6).** Compound **4** (0.16 g, 0.25 mmol) was dissolved in THF (1 mL) and treated with TBAF (1 M in THF, 1 mL) with stirring at ambient temperature for 3 h. The reaction mixture was concentrated on a rotary evaporator then treated with H_2O (10 mL)–10% citric acid (10 mL) with stirring to precipitate 0.12 g (92%) of **6** as a white solid: mp 147–149 °C; ^1H NMR ($CDCl_3$) δ 7.96 (d, J = 9 Hz, 1 H, NH), 7.30–7.04 (m, 10 H, ArH), 6.40 (d, J = 9 Hz, 1 H, NH), 4.48 (d, J = 6 Hz, 1 H, CH), 4.10 (t, J = 7 Hz, 1 H, CH), 3.55 (s, 3 H, CH_3), 2.94–2.40 (m, 6 H, CH_2), 2.00–1.95 (m, 1 H, CH), 1.70–1.20 (m, 2 H, CH_2), 1.30 (s, 9 H, CH_3), 0.90–0.70 (m, 6 H, CH_3). Anal. ($C_{30}H_{42}N_2O_6$) C, H, N.

***N*-[5(*S*)-[(*tert*-Butoxycarbonyl)amino]-4(*S*)-hydroxy-6-phenyl-2(*R*)-(phenylmethyl)hexanoyl]valine (7).** Compound **6** (0.02 g, 0.04 mmol) was dissolved in DME (0.5 mL) and treated with 1 M LiOH solution (0.16 mL) at ambient temperature and stirred for 6 h. The reaction mixture was concentrated to remove DME, the residue dissolved in H_2O, filtered, then acidified with 10% citric acid to precipitate 0.012 g (61%) of **7** as a white solid: mp 155–158 °C; ^1H NMR (DMSO-d_6) δ 7.83 (d, J = 8.7 Hz, 1 H, NH), 7.3–7.1 (m, 10 H, ArH), 6.39 (d, J = 9.0 Hz, 1 H, NH), 4.50 (d, J = 5.9 Hz, 1 H, CH), 4.13–4.10 (m, 1 H, CH), 3.6–3.5 (m, 1 H, CH), 3.4–3.3 (m, 1 H, CH), 2.9–2.4 (m, 5 H, CH_2), 2.0–1.9 (m, 1 H, CH), 1.7–1.5 (m, 1 H, CH_2), 1.30 (s, 9 H, CH_3), 0.85–0.70 (m, 6 H, CH_3). Anal. ($C_{29}H_{40}N_2O_6$) C, H, N.

***N'*-(3-Morpholinopropyl)-*N*-[5(*S*)-[(*tert*-Butoxycarbonyl)amino]-4(*S*)-hydroxy-6-phenyl-2(*R*)-(phenylmethyl)hexanoyl]valine Amide (8).** Compound **5** (0.05 g, 0.08 mmol) was dissolved in DMF (10 mL) and treated with EDC (0.03 g, 0.16 mmol), HOBT (0.023 g, 0.16 mmol), and 4-(3-aminopropyl)morpholine (0.23 mL, 0.16 mmol) with stirring at ambient temperature. Et_3N was added to adjust the pH to 8.5. After stirring for 18 h the reaction was concentrated on a rotary evaporator to remove the DMF and partitioned between EtOAc and H_2O. The organic layer was separated, washed with 10% citric acid solution, aqueous saturated $NaHCO_3$ solution, and brine, and dried (Na_2SO_4). Filtration and concentration to dryness gave 0.045 g (75%) of silylated **8**, which was used without further purification. Filtration and concentration to dryness gave 0.045 g (75%) of silylated **8**, which was used without further purification: ^1H NMR ($CDCl_3$) δ 7.32–7.0 (m, 10 H, ArH), 6.35 (t, J = 5 Hz, 1 H, NH), 6.08 (d, J = 10 Hz, 1 H, NH), 4.70 (d, J = 10 Hz, 1 H, NH), 4.02–3.85 (m, 2 H, CH), 3.76–3.62 (m, 5 H, CH, CH_2), 3.3–3.0 (m, 2 H, CH_2), 2.82–2.28 (m, 11 H, CH_2), 2.12–2.0 (m, 1 H, CH), 1.88–1.50 (m, 4 H, CH_2), 1.32 (s, 9 H, CH_3), 0.91 (s, 9 H, CH_3), 0.78 (dd, J = 7, 15 Hz, 6 H, CH_3), 0.10 (s, 6 H, CH_3). This product was dissolved in 1 N TBAF in THF (2 mL), stirred for 18 h at ambient temperature, then partitioned between EtOAc and H_2O. The organic layer was separated, washed with brine, and dried (Na_2SO_4). Filtration and concentration to dryness gave 0.03 g (81%) of **8** after purification on a 2-mm SiO_2 preparative TLC plate eluting with 10% MeOH/$CHCl_3$: ^1H NMR ($CDCl_3$) δ 7.3–7.0 (m, 10 H, ArH), 6.75–6.65 (m, 1 H, NH), 6.32–6.10 (m, 1 H, NH), 4.82 (d, J = 9 Hz, 1 H, NH), 4.05 (t, J = 9 Hz, 1 H, CH), 3.94–3.5 (m, 7 H, CH, CH_2), 3.38–3.1 (m, 2 H, CH_2), 3.0–2.3 (m, 10 H, CH_2), 2.12–2.0 (m, 1 H, CH), 1.82–1.5 (m, 4 H, CH_2), 1.39 (s, 9 H, CH_3), 0.9–0.75 (m, 6 H, CH_3); mp 133–136°C (maleate salt). Anal. ($C_{36}H_{54}N_4O_6 \cdot C_4H_4O_4$) C, H, N.

***N'*-(2-Benzimidazolylmethyl)-*N*-(*tert*-butoxycarbonyl)-isoleucine Amide (10).** *tert*-(Butoxycarbonyl)isoleucine (15 g, 0.065 mol) and *N*-hydroxysuccinimde (8.2 g, 0.071 mol) were dissolved in DMF (60 mL), treated with EDC (13.7 g, 0.071 mol), and stirred at ambient temperature for 18 h. The reaction mixture was partitioned between EtOAc (200 mL) and H_2O (200 mL), the organic layer separated, washed with H_2O (4 × 100 mL) and brine (1 × 100 mL), and dried (Na_2SO_4). Filtration and concentration to dryness gave 18.9 g (96%) of *N*-(*tert*-butoxycarbonyl)isoleucine succinimidyl ester, which was used without further purification. This intermediate (4.6 g, 0.014 mol) was dissolved in DME (70 mL) and treated with 2-(aminomethyl)benzimidazole dihydrochloride (5.9 g, 0.028 mol) with stirring at ambient temperature. The pH of the solution was adjusted to 7 with Et_3N (7.8 mL, 0.056 mol) and stirring was continued for 18 h. The DME was removed under reduced pressure, and the residue was partitioned between EtOAc and H_2O. The organic layer was washed with H_2O (3 × 50 mL) and brine (1 × 50 mL), and dried (Na_2SO_4). Filtration and concentration to dryness gave 3.8 g (75%) of **10** after chromatography on SiO_2 (3–5% MeOH–$CHCl_3$): mp 206–209 °C (after crystallization from EtOAc); ^1H NMR ($CDCl_3$) δ 7.80–7.68 (m, 1 H, NH), 7.61–7.25 (m, 4 H, ArH), 5.28–5.20 (m, 1 H, NH), 4.97–4.69 (m, 3 H, CH, CH_2), 3.96 (t, J = 6 Hz, 1 H, CH), 1.96–1.84 (m, 1 H, CH), 1.6–1.4 (m, 1 H, CH_2), 1.45 (s, 9 H, CH_3), 1.28–1.14 (m, 1 H, CH_2), 1.0–0.8 (m, 6 H, CH_3). Anal. ($C_{19}H_{28}N_4O_3$) C, H, N.

***N'*-(2-Benzimidazolylmethyl)isoleucine Amide (11).** A stream of HCl was bubbled through a solution of **10** (2.0 g, 5.5 mmol) in EtOAc (20 mL) at −25 °C in a dry ice–acetone bath with stirring for 20 min. The system was purged with N_2 to remove excess HCl, and the mixture was concentrated to dryness to give 1.6 g (86%) of **11**, which was used without further purification.

***N'*-(2-Benzimidazolylmethyl)-*N*-[5(*S*)-[(*tert*-butoxycarbonyl)amino]-4(*S*)-hydroxy-6-phenyl-2(*R*)-(phenylmethyl)hexanoyl]isoleucine Amide (12).** Acid **2** (0.5 g, 0.95 mmol) was dissolved in DMF (10 mL) and treated with EDC (0.2 g, 1.04 mmol), HOBT (0.14 g, 1.04 mmol), and **11** (0.38 g, 1.14 mmol) with stirring at ambient temperature. The pH of the solution was adjusted to 8.0 with Et_3N (0.44 mL, 3.13 mmol), and the mixture was stirred for 20 h. The DMF was removed under reduced pressure, and the residue was partitioned between EtOAc and H_2O. The organic layer was washed with H_2O (3 × 50 mL) and brine (1 × 50 mL) and dried (Na_2SO_4). Filtration and concentration to dryness gave 0.6 g (82%) of silylated **12**: ^1H NMR ($CDCl_3$) δ 7.78–7.68 (m, 1 H, NH), 7.50–7.44 (m, 1 H, NH), 7.35–7.07 (m, 13 H, ArH), 6.89 (d, J = 6 Hz, 1 H, ArH), 6.10 (d, J = 6 Hz, 1 H, NH), 6.69 (t, J = 6 Hz, 1 H, NH), 4.78 (d, J = 10 Hz, 1 H, CH), 4.66 (dd, J = 7, 15 Hz, 1 H, CH), 4.23–3.97 (m, 3 H, CH), 3.84–3.77 (m, 1 H, CH), 2.89–2.5 (m, 6 H, CH_2), 1.92–1.62 (m, 2 H, CH_2), 1.36 (s, 9 H, CH_3), 0.97 (s, 9 H, CH_3), 0.87–0.8 (m, 6 H, CH_3), 0.14 (s, 6 H, CH_3). This material (0.6 g, 0.78 mmol)

was dissolved in THF (9 mL) and treated with 1 M TBAF in THF (6 mL) with stirring at ambient temperature. After stirring for 20 h the THF was removed and the residue treated with H_2O to precipitate 0.5 g (98%) of 12 as a white solid: mp 233–235 °C (after chromatography) (SiO_2, 3% MeOH–$CHCl_3$ saturated NH_3); ^1H NMR (DMSO-d_6) δ 8.34 (t, J = 6 Hz, 1 H, NH), 7.83 (d, J = 9 Hz, 1 H, NH), 7.54–7.44 (m, 2 H, NH), 7.26–7.03 (m, 13 H, ArH), 6.41 (d, J = 9 Hz, 1 H, ArH), 4.54 (d, J = 6 Hz, 1 H, OH), 4.43 (qd, J = 6, 15 Hz, 2 H, CH_2), 4.20 (t, J = 8 Hz, 1 H, CH), 3.58–3.53 (m, 1 H, CH), 3.44–3.40 (m, 1 H, CH), 2.91–2.43 (m, 5 H, CH_2), 1.75–1.61 (m, 2 H, CH_2), 1.45–1.02 (m, 3 H, CH_2), 1.29 (s, 9 H, CH_3), 0.84–0.73 (m, 6 H, CH_3). Anal. ($C_{38}H_{49}N_5O_5$) C, H, N.

Acknowledgment. We thank Mr. J. P. Moreau and Mr. C. Homnick for analytical support and Ms. J. Kaysen for manuscript preparation.

Dual-Action Cephalosporins: Cephalosporin 3′-Quinolone Carbamates

Harry A. Albrecht,* George Beskid, James G. Christenson, Nafsika H. Georgopapadakou, Dennis D. Keith, Frederick M. Konzelmann, David L. Pruess, Pamela L. Rossman, and Chung-Chen Wei

Roche Research Center, Hoffmann-La Roche Inc., Nutley, New Jersey 07110. *Received February 14, 1991*

A series of cephalosporins has been prepared in which the 3′-position was linked to the nitrogen of the antibacterial quinolone ciprofloxacin through a carbamate function. Like the ester-linked and quaternary-linked dual-action cephalosporins reported earlier, these carbamate-linked compounds exhibited a broad antibacterial spectrum derived from both cephalosporin-like and quinolone-like activities, suggesting a dual mode of action. Studies to elucidate details of the mechanism of action have been inconclusive. Ciprofloxacin liberated as a consequence of bacterial enzyme-mediated reactions may contribute to the second mode of action, although some evidence indicates that the intact carbamate-linked bifunctional molecules may possess intrinsically both β-lactam and quinolone activities.

Introduction

When cephalosporins exert their biological activity by covalently binding to bacterial enzymes, opening of the β-lactam ring is accompanied by liberation of the 3′-substituent, if that substituent can function as a leaving group.[1-7] When the eliminated substance possesses antibacterial activity of its own, the cephalosporin should exhibit a dual mode of action.[8-10] As a rationale for drug design, this mechanism has been discussed in some detail, and we have reported the synthesis and biological activity of two classes of dual-action cephalosporins.[10-21] In these

(1) Hamilton-Miller, J. M. T.; Newton, G. G. F.; Abraham, E. P. Products of Aminolysis and Enzymic Hydrolysis of Cephalosporins. *Biochem. J.* **1970**, *116*, 371–384.
(2) O'Callaghan, C. H.; Kirby, S. M.; Morris, A.; Waller, R. E.; Duncombe, R. E. Correlation Between Hydrolysis of the β-Lactam of the Cephalosporin Nucleus and Expulsion of the 3-Substituent. *J. Bacteriology* **1972**, *110*, 988–991.
(3) Faraci, W. S.; Pratt, R. F. Elimination of a Good Leaving Group from the 3′-Position of a Cephalosporin Need Not Be Concerted with β-Lactam Ring Opening. *J. Am. Chem. Soc.* **1984**, *106*, 1489–1490.
(4) Russell, A. D.; Fountain, R. H. Aspects of the Mechanism of Action of Some Cephalosporins. *J. Bacteriology* **1971**, *106*, 65–69.
(5) Boyd, D. B. Elucidating the Leaving Group Effect in the β-Lactam Ring Opening Mechanism of Cephalosporins. *J. Org. Chem.* **1985**, *50*, 886–888.
(6) Page, M. I.; Proctor, P. Mechanism of β-Lactam Opening in Cephalosporins. *J. Am. Chem. Soc.* **1984**, *106*, 3820–3825.
(7) Grabowski, E. J.; Douglas, A. W.; Smith, G. B. Ammonolysis of Cephamycins: ^{13}C NMR Characterization of the Intermediates from β-Lactam Ring Cleavage Prior to Loss of the 3′-Group. *J. Am. Chem. Soc.* **1985**, *107*, 267–268.
(8) O'Callaghan, C. H.; Sykes, R. B.; Staniforth, S. E. A New Cephalosporin with a Dual Mode of Action. *Antimicrob. Agents Chemother.* **1976**, *10*, 245–248.
(9) Greenwood, D.; O'Grady, F. Dual-Action Cephalosporin Utilizing a New Therapeutic Principle. *Antimicrob. Agents Chemother.* **1976**, *10*, 249–252.
(10) Albrecht, H. A.; Beskid, G.; Chan, K.-K.; Christenson, J. G.; Cleeland, R.; Deitcher, K. H.; Georgopapadakou, N. H.; Keith, D. D.; Pruess, D. L.; Sepinwall, J.; Specian, A. C.; Then, R. L.; Weigele, M.; West, K. F.; Yang, R. Cephalosporin 3′-Quinolone Esters with a Dual Mode of Action. *J. Med. Chem.* **1990**, *33*, 77–86.
(11) Beskid, G.; Fallat, V.; Lipschitz, E. R.; McGarry, D. H.; Cleeland, R.; Chan, K.-K.; Keith, D. D.; Unowsky, J. In Vitro Activities of a Dual-Action Antibacterial Agent, Ro 23-9424, and Comparative Agents. *Antimicrob. Agents Chemother.* **1989**, *33*, 1072–1077.
(12) Beskid, G.; Siebelist, J.; McGarry, C. M.; Cleeland, R.; Chan, K.-K.; Keith, D. D. In Vivo Evaluation of a Dual-Action Antibacterial, Ro 23-9424, Compared to Cefotaxime and Fleroxacin. *Chemotherapy* **1990**, *36*, 109–116.
(13) Georgopapadakou, N. H.; Bertasso, A.; Chan, K.-K.; Chapman, J. S.; Cleeland, R.; Cummings, L. M.; Dix, B. A.; Keith, D. D. Mode of Action of the Dual-Action Cephalosporin Ro 23-9424. *Antimicrob. Agents Chemother.* **1989**, *33*, 1067–1071.
(14) Jones, R. N.; Barry, A. L.; Thornsberry, C. Antimirobial Activity of Ro 23-9424, a Novel Ester-Llnked Codrug of Fleroxacin and Desacetylcefotaxime. *Antimicrob. Agents Chemother.* **1989**, *33*, 944–950.
(15) Georgopapadakou, N. H.; Albrecht, H. A.; Bertasso, A.; Cummings, L. M.; Keith, D. D.; Russo, D. A. Cephalosporin-Quinolone Esters: Biological Properties. 28th Intersci. Conf. Antimicrob. Agents Chemother., 1988, Abstract No. 442.
(16) Christenson, J. G.; Pruess, D. L.; Robertson, T. L. Hydrolysis of Ro 23-9424 by β-Lactamases. 28th Intersci. Conf. Antimicrob. Agents Chemother., 1988, Abstract No. 443.
(17) Christenson, J. G.; Brocks, V.; Chan, K.-K.; Keith, D. D.; Pruess, D. L.; Schaefer, F. F.; Talbot, M. K. Mode of Action of Ro 23-9424, a Dual-Action Cephalosporin. 28th Intersci. Conf. Antimicrob. Agents Chemother., 1988, Abstract No. 444.
(18) Christenson, J. G.; Chan, K.-K.; Farrish, H. H.; Patel, I. H.; Specian, A. Pharmacokinetics of Ro 23-9424, a Dual-Action Cephalosporin, in Animals. 28th Intersci. Conf. Antimicrob. Agents Chemother., 1988, Abstract No. 449.
(19) Christenson, J.; Albrecht, H.; Georgopapadakou, N.; Keith, D.; Pruess, D.; Talbot, M.; Then, R. Mode of Action of Ro 23-5068, a Dual-Action Cephalosporin. 28th Intersci. Conf. Antimicrob. Agents Chemother., 1988, Abstract No. 450.
(20) Albrecht, H. A.; Beskid, G.; Christenson, J.; Durkin, J.; Fallat, V.; Keith, D. D.; Konzelmann, F. M.; Lipschitz, E. R.; McGarry, D. H.; Siebelist, J.; Wei, C.-C.; Weigele, M.; Yang, R. Dual-Action Cephalosporins: Cephalosporin 3′-Quaternary Quinolones. 29th Intersci. Conf. Antimicrob. Agents Chemother., 1989, Abstract No. 360.

0022-2623/91/1834-2857$02.50/0