Bioorganic & Medicinal Chemistry Letters, Vol.3, No.11, pp. 2277-2282, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

Inhibition of Human Leukocyte Elastase. 5.[1] Inhibition by 6-Alkyl Substituted Penem Benzyl Esters.

Paul E. Finke,[#*] Mary E. Dahlgren,[^] Hazel Weston,[^] Alan L. Maycock,[^+] James B. Doherty[#]

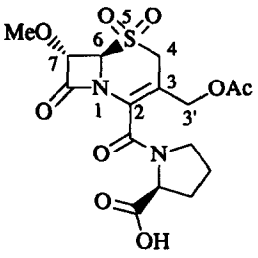
[#]Department of Medicinal Chemical Research, [^]Department of Enzymology
Merck Research Laboratories
P.O. Box 2000, Rahway, New Jersey 07065-0900 USA

(*Received* 1 *April* 1993)

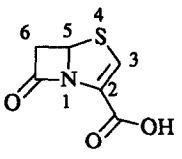
Abstract. Penem benzyl esters substituted at the 6-position with small alkyl groups and at the 3-position with a variety of carbon and heteroatom groups were prepared as inhibitors of human leukocyte elastase (HLE). The structure activity relations found for these positions are discussed in relation to known cephalosporin inhibitors and some important stereochemical implications are reported for inhibition of HLE by these β-lactam structures.

The search for inhibitors of human leukocyte elastase (HLE) (EC 3.4.21.37) is actively being continued with the investigation of a wide variety of synthetic structures.[2,3] HLE, a serine protease, is normally localized in the azurophilic granules of polymorphonuclear leukocytes (PMN) until it is released by the PMN for digestion of foreign proteins and other possible biological functions. However, excessive accumulation of uninhibited HLE in the lung environment has been associated with several pulmonary diseases.[4] Our current efforts to identify functionally irreversible HLE inhibitors have focused on the use of the β-lactam moiety to provide time-dependent, mechanism-based inhibition. Our interest in this area began with the cephalosporin nucleus[5] and resulted in the selection of **L-658,758**, a potent, time-dependent HLE inhibitor ($k_{obs}/[I]$ = 3,800 $M^{-1}sec^{-1}$), as a clinical candidate with properties suitable for formulation as a topical aerosol.[1] Herein we report our preliminary work to extend this rationale into the penem class of β-lactams (**1**).[6] In the accompanying papers, our effort to utilize the penicillin nucleus (**2**)[7] and the monocyclic β-lactam **3**[8] are also discussed.

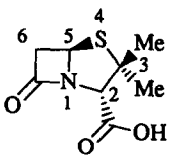
Our work in the cephalosporin area had shown that small, 7α-oriented substituents, particularly chloro and methoxy, gave the best HLE inhibition and occupied the S-1 specificity pocket.[9] The sulfone oxidation state



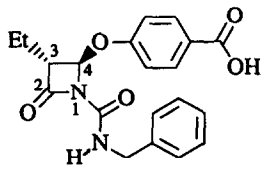
L-658,758



1



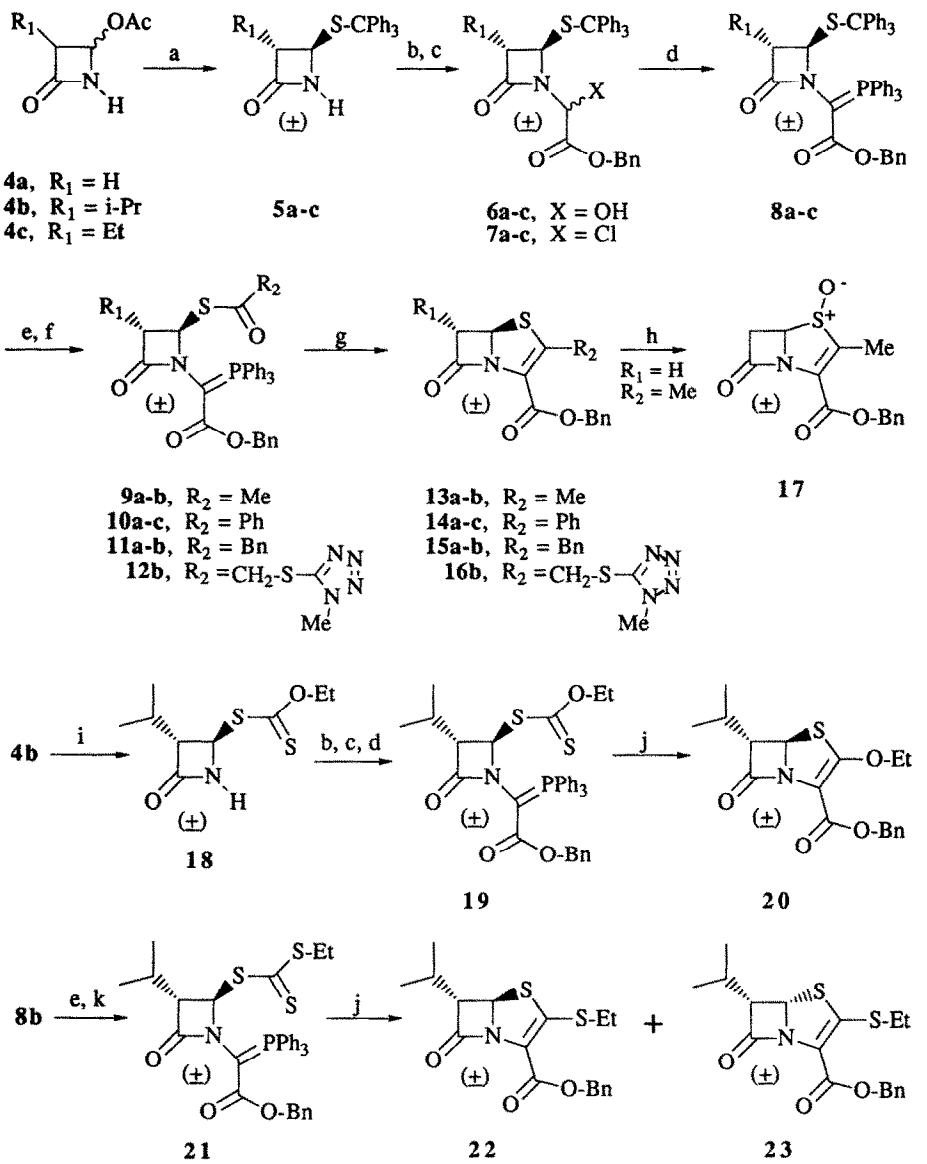
2



3

[+]Present address: Sterling Research Group, Malvern, PA 19355.

Scheme I



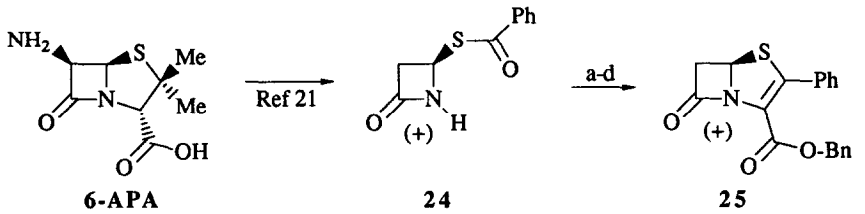
Reagents: (a) Ph_3CSH, NaH, DMF (85-95%); (b) $HCOCO_2Bn$, PhH, 3Å sieves, 25-75 °C (35-65%); (c) $SOCl_2$, pyridine, THF, 0 °C; (d) Ph_3P, 2,6-lutidine, dioxane, 65 °C; (e) $AgNO_3$, pyridine, MeOH; (f) R_2COCl, pyridine, CH_2Cl_2 (50-80%); (g) PhMe, reflux; (h) *m* -CPBA, CH_2Cl_2, -20 °C (58%); (i) KSCSOEt, 1:1 CH_2Cl_2:H_2O (98%); (j) Xylenes, trace hydroquinone, reflux; (k) $ClCS_2Et$, pyridine, CH_2Cl_2, 0 °C.

of the ring sulfur afforded the highest activity[9] and benzyl esters were the most potent binding derivatives of the C-2 carboxylic acid, the free acids being totally inactive.[10] A leaving group at C-3' afforded much enhanced time-dependent inhibition[11] and its role in the mechanism of inhibition of HLE has been investigated.[12,13] However, while these features activated the β-lactam carbonyl for reaction with the hydroxyl of serine 195 to provide very potent inhibitors, reaction of the β-lactam with other nucleophiles was also enhanced, and therefore these compounds were not systemically active. The best compromise between activity and stability was found with the L-proline amide **L-658,758**, which has now shown activity in a variety of HLE mediated disease models.[14,15] Since the penem structure is intrinsically even more reactive and the 7α-alkyl cephalosporins were known to have much improved stability,[1] a series of 6-alkyl penem benzyl ester derivatives were initially investigated and a variety of functionalities were incorporated at the C-3 position.

Chemistry. The preparation of the compounds[16] listed in Table 1 was based on previously reported penem antibiotic work[17,18] and is summarized in Scheme I. The synthetic sequence started with the commercial 4-acetoxy-2-azetidinone (**4a**) or the readily available 3-isopropyl or 3-ethyl azetidinones **4b** and **4c**.[19] The acetoxy was displaced with sodium triphenylmethyl mercaptide affording the protected sulfides **5**, giving almost exclusively *trans* substitution in **5b** and **5c** (>85% isolated yield). Reaction with benzyl glyoxylate in the presence of 3Å sieves gave the stable hydroxymethyllactams **6** as a mixture of diastereomers. These were converted to the ylides **8** *via* the chlorides **7** in 40-75% yields. The trityl protecting group was removed with silver nitrate and the thiolate was immediately reacted with a variety of acid chlorides to yield the thiol esters **9-12**. Thermal cyclization in refluxing toluene afforded the desired racemic penems **13-16** in variable yields. The 3-ethoxy derivative **20** was obtained through the xanthate **18**, prepared directly from **4b** with potassium ethyl xanthate,[20] and cyclization of the ylide **19** in refluxing xylenes. Acylation of the silver thiolate derived from **8b** with ethyl chlorodithioformate afforded the trithiocarbonate **21**, which on cyclization in refluxing xylenes yielded a separable mixture of the *trans* and *cis* products **22** and **23** resulting from epimerization at C-5.

The synthesis of a chiral analogue, **25**, is shown in Scheme II and utilized optically pure **24** derived from 6-aminopenicillanic acid (6-APA) by known methods.[21] Reaction to give the ylide as above and its thermal cyclization in refluxing toluene afforded **25** without racemization, $[\alpha]_D = +136^o$ ($CHCl_3$, c = 1.0) (lit.[21] for chiral **13a** *p*-nitrobenzyl ester, $[\alpha]_D = +136 \pm 1^o$). Finally, the sulfoxide **17** (Scheme I) was prepared by oxidation at -20 °C with 1 equivalent of *m*-chloroperbenzoic acid (*m*-CPBA). Attempts to prepare a sulfone analogue were unsuccessful.

Scheme II



6-APA **24** **25**

Reagents: (a) $HCOCO_2Bn$, PhH, 3Å sieves, 65 °C, 24 h; (b) $SOCl_2$, pyridine, THF, 0 °C; (c) Ph_3P, 2,6-lutidine, dioxane, 65 °C, 24 h (39% from **24**); (d) PhMe, reflux, 4 h (71%).

Biological Results and Discussion. Table 1 contains the HLE inhibitory activity of these penem derivatives. Initially the potency was measured as an IC_{50} value (μM), the concentration of the inhibitor required to give 50% reduction in the activity of 1 μg/mL of HLE at a nominal 2 minute time point, which gives an estimation of the initial K_i.[5] Upon longer incubation, some of these compounds also showed time-dependent inhibition and their second-order rate constants ($k_{obs}/[I]$, $M^{-1}sec^{-1}$) were determined, which afforded a better measure of the inactivation process.[12] The C-6 unsubstituted analogues, except for **25** at high concentrations, did not show time-dependent inhibition and thus K_i values were calculated from their initial velocities. From our cephalosporin work,[1,12,13] it would be expected that the C-6 substituent fits into the S-1 specificity pocket of HLE and that, after binding occurs, the serine 195 reacts with and opens the β-lactam to give an acyl-enzyme intermediate as shown in Figure 1. This intermediate can either be hydrolyzed, as occurs with normal substrates, to give turnover of the inhibitor and regeneration of active HLE or react further to afford time-depentent inhibition. Opening of the thiazolidine ring (Route A) has been demonstrated with penicillin sulfone β-lactamase inhibitors[22] and other rearrangement products (such as Route B) have been reported for the penem antibiotics.[23] When there is a leaving group at C-3' (X = CH_2L), the β-lactam carbonyl reactivity is enhanced through generation of the exomethylene product[1,23] (Routes C) which can also lead to further reaction with the enzyme.[13] Eventual hydrolysis of these latter intermediates would lead to reactivation of the enzyme.

As expected,[9] the C-6 unsubstituted penems **13a, 14a and 15a** were only weak, competitive inhibitors, although significant activity (IC_{50} = 3 μM) was observed with the 3-phenyl derivative **14a** consistent with the known enhanced activity of more lipophilic inhibitors.[11] On longer incubation, no time-dependent inhibition was evident and the calculated K_i values were in good agreement with their initial IC_{50}'s. It was known from active site mapping[24] that HLE binds a valine residue best in the S-1 pocket and thus the first series of C-6 substituted penems incorporated an isopropyl group at C-6. The isopropyl would be expected to both increase the binding to HLE and stabilize the acyl-enzyme intermediate, thus enhancing the possibility for time-dependent inactivation. Indeed, with the same series of C-3 derivatives as above (**13b, 14b** and **15b**), substantial improvement in activity was obtained, with the phenyl and benzyl compounds affording IC_{50} values of 1 μM or less. In addition, time-dependent inhibition was now observed, having the same relative order of activity as their IC_{50}'s ($k_{obs}/[I]$ = 40, 530, 630 M^{-1} sec^{-1}, respectively). The 3-ethoxy and *trans*-3-thioethoxy derivatives **20** and **22** also gave some improved potency

Figure 1. Possible mechanisms of inhibition.

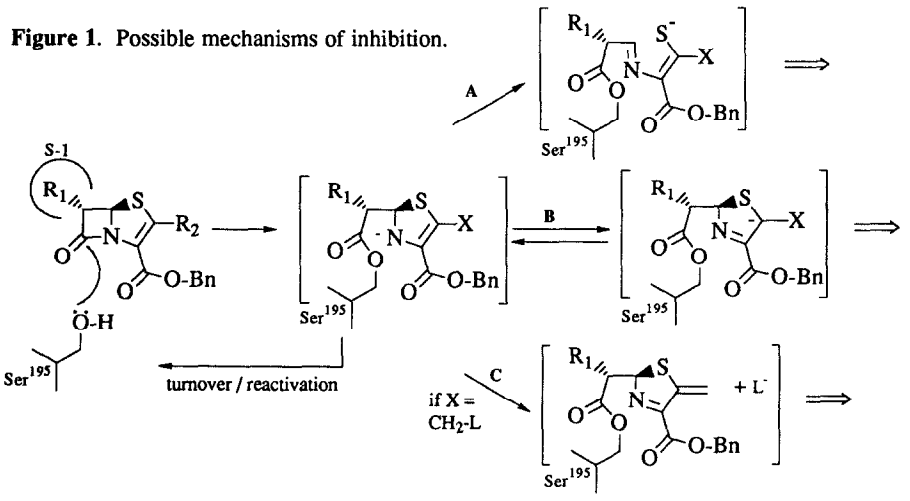
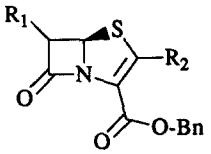


Table 1. HLE inhibitory activity of penem benzyl esters.



Compound no.	R_1	R_2	Stereochemistry	IC_{50}, μM[a]	$k_{obs}/[I]$, $M^{-1}sec^{-1} \pm SD$[b] $[K_i, \mu M \pm SD^b]$[c]
13a	H-	-Me	±	>20	$[K_i = 310 \pm 16]$[c]
14a	H-	-Ph	±	3	$[K_i = 4.8 \pm 0.8]$[c]
15a	H-	-Bn	±	20	$[K_i = 10 \pm 0.5]$[c]
13b	i-Pr-	-Me	*trans*, ±	20	40 ± 1
14b	i-Pr-	-Ph	*trans*, ±	1.0	530 ± 30
15b	i-Pr-	-Bn	*trans*, ±	0.6	630 ± 35
16b	i-Pr-	$-CH_2S(CN_4Me)$	*trans*, ±	0.7	nd[d]
14c	Et-	-Ph	*trans*, ±	0.3	2,300 ± 100
17 (Sulfoxide)	H-	-Me	±	15	$[K_i = 40 \pm 1]$[c]
20	i-Pr-	-OEt	*trans*, ±	2	nd
22	i-Pr-	-SEt	*trans*, ±	3	170 ± 6
23	i-Pr-	-SEt	*cis*, ±	0.6	nd
25	H-	-Ph	(R), +	>20	137 ± 6
26[e]	MeCHOH-	-SEt	*threo-trans*, ±	2	nd

[a]See ref 5 for methodology. The values are a result of a single experiment [b]See ref 12 for methodology. The mean and standard deviation from two or more determinations at different inhibitor concentrations are listed. [c]No time-dependent inhibition was observed and the K_i was determined from the initial velocity. [d]Not determined. [e]Tested as the *p*-nitrobenzyl ester.

(IC_{50} = 2 and 3 μM), which may be due to stabilization of the developing negative charge (Route B). Incorporation of a (1-methyltetrazol-5-yl)thio moiety (**16b**) at the C-3' position as a leaving group (Route C) gave a 20-fold enhancement of activity (IC_{50} = 0.7 μM) consistent with that seen in our cephalosporin studies.[5,11] This enhancement was most likely due to activation of the β-lactam carbonyl for attack by serine 195 of HLE.[1] The sulfoxide **17** (IC_{50} = 15 μM) also gave improved activity compared to the sulfide **13a**, possibly resulting from the sulfur being a better leaving group (Route A), but still no time-dependent inhibition was observed. This increased reactivity of the sulfoxide **17** is consistent with the observed instability of the sulfones. Interestingly, the smaller 6-ethyl analogue **14c** showed a 3-fold improvement in HLE binding activity (IC_{50} = 0.3 μM) over the isopropyl compound **14b** and a 4-fold increase in its inactivation rate ($k_{obs}/[I]$ = 2,300 $M^{-1}sec^{-1}$). Compound **26**,[25] which contains a 6α-hydroxyethyl substituent prominent in penem and carbapenem antibiotics, showed the same activity as the corresponding isopropyl derivative **22** (IC_{50} = 2 *vs* 3 μM).

Based on the natural R configuration at C-6 of the cephalosporins, chiral **25** was prepared to confirm that most of the activity of the racemic **14a** was retained in the corresponding R configuration of the penems. However, this turned out not to be the case (IC_{50} >20 μM) and thus the S configuration must be the more active species in the penem series. This finding and the observation that the *cis* compound **23** is more active than the *trans* **22** (IC_{50} = 0.6 *vs* 3 μM) implies that the 6R, 7α configuration of **L-658,758** may not be optimal for HLE activity. In as much as chiral 7β-aminocephalosporanic acid was always used as the starting material in those studies, it is interesting to speculate whether the unnatural C-6 S configuration would also be more potent.

Conclusion. Modification of the penem structure incorporating small alkyl groups at C-6 and either a large lipophilic or an activating group at the C-3 position can lead to potent, time-dependent inhibition of HLE for this class of β-lactams. The observation that the stereochemistry at C-6 and the ring junction need not be that of the cephalosporins proved to be pivotal for our subsequent development of structure **3** into 3,3-dialkyl-2-azetidinone structures, which to date are the only reported biologically potent, orally active HLE inhibitors.[26]

Acknowledgments. The authors wish to thank Dr. Shrenik K. Shah and Dr. Frank P. Di Ninno for many helpful discussions during the course of this work and for reviewing this manuscript.

References and Notes.

1. For the previous paper in this series, see: Finke, P. E.; Shah, S. K.; Ashe, B. M.; Ball, R. G.; Blacklock, T. J.; Bonney, R. J.; Brause, K. A.; Chandler, G. O.; Cotton, M.; Davies, P.; Dellea, P. S.; Dorn, C. P., Jr.; Fletcher, D. S.; O'Grady, L. A.; Hagmann, W. K.; Hand, K. M.; Knight, W. B.; Maycock, A. L.; Mumford, R. A.; Osinga, D. G.; Sohar, P.; Thompson, K. R.; Weston, H.; Doherty, J. B. *J. Med. Chem.* **1992**, *35*, 3731-3744.
2. For a review of synthetic inhibitors, see: Trainor, D. A. *Trends Pharmacol. Sci.* **1987**, *8*, 303-307.
3. Skiles, J. W.; Fuchs, V.; Miao, C.; Sorcek, R.; Grozinger, K. G.; Mauldin, S. C.; Vitous, J.; Mui, P. W.; Jacober, S.; Chow, G.; Matteo, M.; Skoog, M.; Weldon, S. M.; Possanza, G.; Keirns, J.; Letts, G.; Rosenthal, A. S. *J. Med. Chem.* **1992**, *35*, 641-662 and references therein.
4. *Pulmonary Emphysema and Proteolysis*; Taylor, J. C.; Mittman, C., Eds.; Academic Press, Inc.: New York, **1987**, pp 1-550.
5. Doherty, J. B.; Ashe, B. M.; Argenbright, L. W.; Barker, P. L.; Bonney, R. J.; Chandler, G. O.; Dahlgren, M. E.; Dorn, C. P., Jr.; Finke, P. E.; Firestone, R. A.; Fletcher, D.; Hagmann, W. K.; Mumford, R.; O'Grady, L.; Maycock, A. L.; Pisano, J. K.; Shah, S. K.; Thompson, K. R.; Zimmerman, M. *Nature* **1986**, *322*, 192-194.
6. This work comprises a part of Finke, P. E.; Zimmerman, M.; Dorn, C. P.; Doherty, J. B.; Ashe, B. M. US Patent 4,711,886, **1987**; CA. **106**:67002c. The numbering method in this paper is shown in structures **1-3**.
7. Thompson, K. R.; Finke, P. E.; Shah, S. K.; Weston, H.; Maycock, A. L.; Doherty, J. B. *BioMed. Chem. Lett.* **1993**, *3*, following paper in this issue.
8. Shah, S. K.; Finke, P. E.; Brause, K. A.; Chandler, G. O.;Ashe, B. M.; Weston, H.; Mumford, R. A.; Doherty, J. B. *BioMed. Chem. Lett.* **1993**, *3*, third following paper in this issue.
9. Doherty, J. B.; Ashe, B. M.; Barker, P. L.; Blacklock, T. J.; Butcher, J. W.; Chandler, G. O.; Dahlgren, M. E.; Davies, P.; Dorn, C. P., Jr.; Finke, P. E.; Firestone, R. A.; Hagmann, W. K.; Halgren, T.; Knight, W. B.; Maycock, A. L.; Navia, M. A.; O'Grady, L.; Pisano, J. M.; Shah, S. K.; Thompson, K. R.; Weston, H.; Zimmerman, M. *J Med. Chem.* **1990**, *33*, 2513-2521.
10. Finke, P. E.; Ashe, B. M. ; Knight, W. B.; Maycock, A. L.; Navia, M. A.; Shah, S. K.; Thompson, K. R.; Underwood, D. J.; Weston, H.; Zimmerman, M.; Doherty, J. B. *J. Med Chem.* **1990**, *33*, 2522-2528.
11. Shah, S. K.; Brause, K. A.; Chandler, G. O.; Finke, P. E.; Ashe, B. M.; Weston, H.; Knight, W. B.; Maycock, A. L.; Doherty, J. B. *J. Med. Chem.* **1990**, *33*, 2529-2535.
12. Knight, W. B.; Maycock, A. L.; Green, B. G.; Ashe, B. A.; Gale, P.; Weston, H.; Finke, P. E.; Hagmann, W. K.; Shah, S. K.; Doherty, J. B. *Biochemistry* **1992**, *31*, 4980-4986.
13. Navia, M. A.; Springer, J. P.; Lin, T.-Y.; Williams, H.; Firestone, R. A.; Pisano, J. M.; Doherty, J. B.; Finke, P. E.; Hoogsteen, K. *Nature* **1987**, *327*, 79-82.
14. Fletcher, D. S.; Osinga, D. G.; Keenan, K.; Bonney, R. J.; Doherty, J. B.; Finke, P. E.; Davies, P. *J. Exp. Med.* submitted.
15. Davies, P.; Ashe, B. M.; Bonney, R. J.; Dorn, C.; Finke, P. E.; Fletcher, D.; Hanlon, W. A.; Humes, J. L.; Maycock, A.; Mumford, R. A.; Navia, M. A.; Opas, E. E.; Pacholok, S.; Shah, S.; Zimmerman, M.; Doherty, J. B. *Ann. N. Y. Acad Sci.* **1991**, *624*, 219-229.
16. All compounds gave satisfactory C, H, N analytical data and/or ^{1}H NMR spectra. For representative examples of experimental details and ^{1}H NMR spectral data, see US Patent 4,711,886.[6]
17. (a) Ernest, I; Gosteli, J.; Greengrass, C. W.; Holick, W.; Jackman, D. E.; Pfaendler, H. R.; Woodward, R. B. *J. Am Chem. Soc*, **1978**, *100*, 8214-8222. (b) Lang, M.; Prasad, K.; Holick, W.; Gosteli, J.; Ernest, I; Woodward, R. B. *J. Am. Chem. Soc.* **1979**, *101*, 6296-6301.
18. Martel, A.; Dextraze, P.; Daris, J.-P.; Saintonge, R.; Lapointe, P.; Conway, T. T.; Monkovic, I.; Kavadias, G.; Ueda, Y.; Elie, P.; Patil, S.; Caron, G.; Douglas, J. L.; Menard, M.; Belleau, B. *Can. J. Chem.* **1982**, *60*, 942-944.
19. (a) Clauss, K.; Grimm, D.; Prossel, G. *Liebigs Ann. Chem.* **1974**, 539. (b) Kametani, T.; Honda, T.; Nakayama, A.; Sasakai, Y.; Mochizuki, T.; Fukumoto, K. *J. Chem. Soc., Perkin I* **1985**, 2228-2232.
20. Brian, E. G. Great Britain Patent 2,042,508A , **1980**; CA. **95**: 132870e.
21. Ernest, I.; Gosteli, J.; Woodward, R. B. *J Am. Chem. Soc.* **1979**, *101*, 6301-6305.
22. Knowles, J. R. *Acc Chem. Res.* **1985**, *18*, 97-104.
23. Visentin, G.; Perrone, E.; Borghi, D.; Rizzo, V.; Alpegiani, M.; Bedeschi, A.; Corigli, R.; Rivola, G.; Franceschi, G. *Heterocycles* **1992**, *33*, 859-891.
24. Nakajima, K.; Powers, J. C.; Ashe, B. M.; Zimmerman, M. *J. Biol. Chem.* **1979**, *254*, 4027-4032.
25. Di Ninno, F.; Muthard, D. A.; Ratcliffe, R. W.; Christensen, B. G. *Tet. Lett.* **1982**, *23*, 3535-3528.
26. Shah, S. K.; Dorn, C. P., Jr.; Finke, P. E.; Hale, J. J.; Hagmann, W. K.; Brause, K. A.; Chandler, G. O.; Kissinger, A. L.; Ashe, B. M.; Weston, H.; Knight, W. B.; Maycock, A. L.; Dellea, P. S.; Fletcher, D. S.; Hand, K. M.; Mumford, R. A.; Underwood, D. J.; Doherty, J. B. *J. Med. Chem.* **1992**, *35*, 3745-3754.