Synthesis of the Cyclic Peptidic Protease Inhibitor Eurystatin A Using Acyl Cyano Phosphorane Methodology

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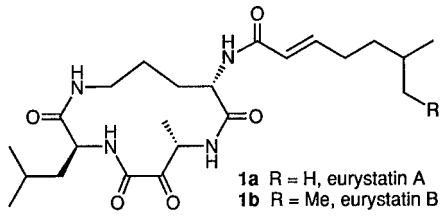
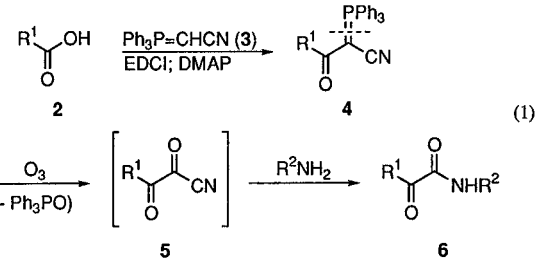
Eurystatins A (**1a**) and B (**1b**),[1] isolated from *Streptomyces eurythermus* R353-21, are potent inhibitors of the serine protease prolyl endopeptidase (PED). Their structures incorporate (*S*)-3-amino-2-oxobutanoic acid, leucine, and ornithine residues in a cyclic framework. Like the thrombin inhibitors, cyclotheonamide A and B,[2] and the PED inhibitor, poststatin,[3] the eurystatins contain an α-keto amide residue considered to be the active center for enzyme inhibition.[4] In an earlier synthesis of **1a**,[5] an intermediate α-hydroxy amide was oxidized to the α-keto grouping at a late stage of the synthesis.



We have developed a novel method for forming α-keto amide linkages from carboxylic acids **2**, as reported in a synthesis of the linear pentapeptide, poststatin.[6] The transformation involves the condensation of **2** with the cyano phosphorane **3** to form the acyl cyano ylide **4**. Oxidation of **4** yields the labile α,*β*-diketo nitrile **5**, which then undergoes aminolysis to yield an α-keto amide **6** (eq 1).[7−9] A significant feature of the process is its applicability to the formation of cyclic peptides containing α-keto amide linkages.[2,10] In this report, we demonstrate the use of this methodology in a synthesis of eurystatin A (**1a**).



2 **4** **5** **6** (1)

Of several possible pathways to the cyclic target, a lactam-forming ring closure between the leucine and ornithine residues was chosen. This route provided an opportunity to explore the scope of the acyl cyano phosphorane chemistry in two approaches to the desired secopeptide **14**, as depicted in Schemes 1 and 2.

The first synthesis (Scheme 1) began with Cbz-protected alanine (**7**), which was converted to the acyl cyano phosphorane **8** with (cyanomethylene)triphenylphosphorane (**3**) and EDCI. Ozonolysis generated the corresponding diketo nitrile **9**, which was reacted in situ with leucine *tert*-butyl ester (**10**) to form the dipeptide **11** after treatment with silver nitrate to decompose any cyanohydrin formed. Removal of the Cbz-protecting group by catalytic hydrogenolysis yielded α-amino ketone **12**,[11] which was coupled with the carboxyl group of di-*N*-protected ornithine **13** to furnish the carbonyl-extended tripeptide **14**. Considering the potential for reactivity of intermediate **12**, this one-pot chain elongation of **11** worked remarkably well, particularly on a small scale (0.46 mmol, 61%).

The second route (Scheme 2), featuring an ylide-stabilized α-amino ketone (**17**),[12] called for a different timing of the ozonolysis. The required Fmoc-protected acyl cyano phosphorane **16** was prepared from Fmoc alanine (**15**) by the same procedure as described for compound **8**. The Fmoc protecting group was eliminated smoothly with piperidine,[13] and the crude amine **17** was coupled with the ornithine derivative **13** to afford acyl cyano phosphorane **18**. Ozonolysis yielded **19** which was trapped with leucine *tert*-butyl ester (**10**) to form the tripeptide **14**.

The (*E*)-6-methyl-2-heptenoic acid side chain (**24**)[1b] was prepared from 4-methyl-1-pentanol (**20**) by oxidation to the aldehyde **21**.[14] Wittig olefination with [(*tert*-butoxycarbonyl)methylene]triphenylphosphorane (**22**) afforded

(1) (a) Toda, S.; Obi, Y.; Numata, K.-i.; Hamagishi, Y.; Tomita, K.; Komiyama, N.; Kotake, C.; Furumai, T.; Oki, T. *J. Antibiot.* **1992**, *45*, 1573. (b) Toda, S.; Kotake, C.; Tsuno, T.; Narita, Y.; Yamasaki, T.; Konishi, M. *J. Antibiot.* **1992**, *45*, 1580. (c) Suzuki, K.; Toda, S.; Furumai, T.; Fukagawa, Y.; Oki, T. *J. Antibiot.* **1994**, *47*, 982.

(2) (a) Fusetani, N.; Matsunaga, S.; Matsumoto, H.; Takebayashi, Y. *J. Am. Chem. Soc.* **1990**, *112*, 7053. (b) Hagihara, M.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 6570.

(3) (a) Aoyagi, T.; Nagai, M.; Ogawa, K.; Kojima, F.; Okada, M.; Ikeda, T.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1991**, *44*, 949. (b) Nagai, M.; Ogawa, K.; Muraoka, Y.; Naganawa, H.; Aoyagi, T.; Takeuchi, T. *J. Antibiot.* **1991**, *44*, 956. (c) Tsuda, M.; Muraoka, Y.; Nagai, M.; Aoyagi, T.; Takeuchi, T. *J. Antibiot.* **1996**, *49*, 281.

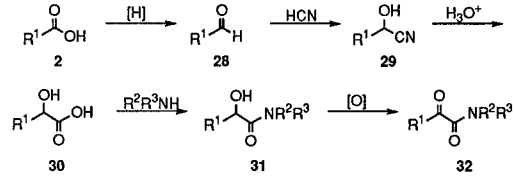
(4) Kamei, H.; Ueki, T.; Obi, Y.; Fukagawa, Y.; Oki, T. *Japan. J. Pharmacol.* **1992**, *60*, 377.

(5) Schmidt, U.; Weinbrenner, S. *J. Chem. Soc., Chem. Commun.* **1994**, 1003.

(6) Wasserman, H. H.; Petersen, A. K. *Tetrahedron Lett.* **1997**, *38*, 953.

(7) Wasserman, H. H.; Ho, W.-B. *J. Org. Chem.* **1994**, *59*, 4364.

(8) This method[7] has considerable advantages over conventional multistep procedures for converting a carboxylic acid to the corresponding carbonyl-extended α-keto amide. An example is outlined below. (a) Wipf, P.; Kim, H.-Y. *Tetrahedron Lett.* **1992**, *33*, 4275. (b) Wipf, P.; Kim, H. *J. Org. Chem.* **1993**, *58*, 5592.



2 **28** **29** **30** **31** **32**

(9) *Ylides and Imines of Phosphorus*; Johnson, A. W., Ed.; John Wiley and Sons: New York, 1993; Chapter 5.

(10) (a) Fusetani, N.; Sugawara, T.; Matsunaga, S.; Hirota, H. *J. Am. Chem. Soc.* **1991**, *113*, 7811. (b) Kobayashi, J.; Itagaki, F.; Shigemori, H.; Ishibashi, M.; Takahashi, K.; Ogura, M.; Nagasawa, S.; Nakamura, T.; Hirota, H.; Ohta, T.; Nozoe, S. *J. Am. Chem. Soc.* **1991**, *113*, 7812. (c) Gunasekera, S. P.; Pomponi, S. A.; McCarthy, P. J. *J. Nat. Prod.* **1994**, *57*, 79. (d) Greco, M. N.; Powell, E. T.; Hecker, L. R.; Andrade-Gordon, P.; Kauffman, J. A.; Lewis, J. M.; Ganesh, V.; Tulinsky, A.; Maryanoff, B. E. *BioMed. Chem. Lett.* **1996**, *6*, 2947.

(11) Amino ketone **12** proved to be sufficiently stable to undergo peptide coupling successfully. In addition, it was found that the precursor **11** kept its stereochemical integrity upon treatment with weak bases such as sodium hydrogen carbonate and pyridine. However, the stronger base triethylamine epimerized the alanine-like residue completely within a few minutes.

(12) The ylide-stabilized α-amino ketone **17** was both chemically and stereochemically stable. As reported previously,[6] the cyano group may undergo intramolecular addition of the amine during hydrogenolysis of the Cbz-protected amine. In the present case, the problem was circumvented by employing Fmoc-protected alanine.

(13) Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404.

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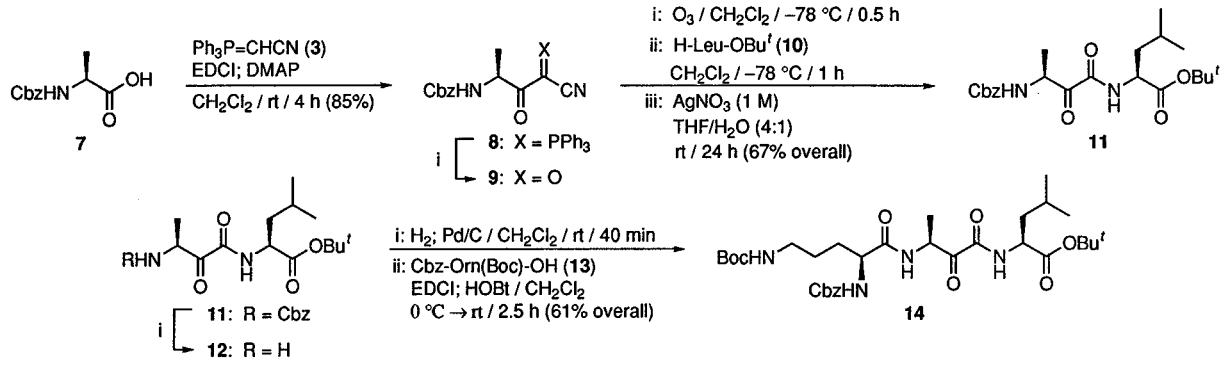
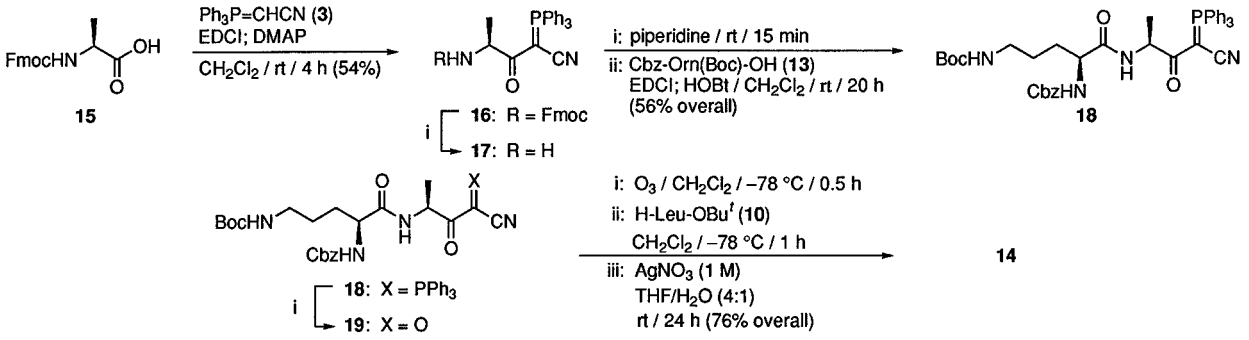
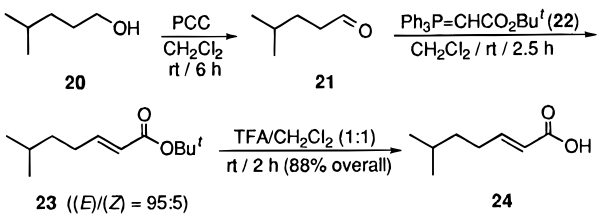
Scheme 1



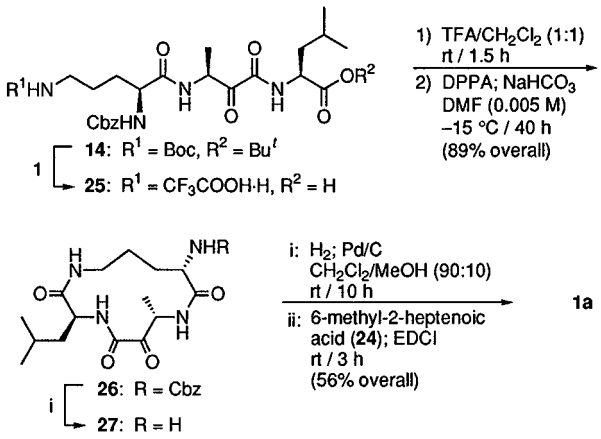
Scheme 2



Scheme 3



Scheme 4



tert-butyl (*E*)-6-methyl-2-heptenoate (**23**) as a 95:5 mixture of geometric isomers. Deprotection with TFA gave the free acid **24**. Both **23** and **24** were obtained isomerically pure by fractional distillation. Using the three-step sequence shown in Scheme 3, without isolation of intermediates, an 88% overall yield was obtained for the (*E*)-[1b] and (*Z*) alkenes[15] combined.

Simultaneous removal of the protecting groups at the δ-amino and the carboxyl termini of tripeptide **14** was accomplished with TFA.[16] Cyclization of **25** was performed in DMF under conditions of high dilution using diphenyl phosphorazidate (DPPA) and sodium hydrogen carbonate[17] (cf. Scheme 4). Following hydrogenolysis of the cyclic tripeptide **26**, the amine **27** was coupled with the α,β-unsaturated acid **24** to yield eurystatin A (**1a**), identical to the natural material.[18]

A noteworthy feature of this synthesis pertains to the preservation of the stereochemical integrity throughout, as shown by the appearance of a single set of signals in the ^{1}H and ^{13}C NMR spectra of all intermediates. Additional evidence was obtained from a 1:1 mixture of **14** and its alanine epimer prepared by equilibration with triethylamine. Cyclization, according to the protocol shown in Scheme 4, furnished **26** and its alanine epimer in a ratio of 1:1. This control experiment demonstrated unambiguously that the stereochemistry of **26** was intact.

In summary, the versatility of the acyl cyano phosphorane methodology has been demonstrated by diverse syntheses of tripeptide **14**. In particular, it was shown that the *N*-deprotection of the alanine-like residue could be accomplished both before and after the oxidative cleavage of the ylide; i.e., both of the α-amino ketones **12** and **17** were viable synthetic intermediates.

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Supporting Information Available: Experimental procedures and NMR spectra of obtained compounds (40 pages).

JO9718253

(17) (a) Shioiri, T.; Ninomiya, K.; Yamada, S.-i. *J. Am. Chem. Soc.* **1972**, *94*, 6203. (b) Brady, S. F.; Freidinger, R. M.; Paleveda, W. J.; Colton, C. D.; Homnick, C. F.; Whitter, W. L.; Curley, P.; Nutt, R. F.; Veber, D. F. *J. Org. Chem.* **1987**, *52*, 764.

(18) In the absence of an authentic sample of eurystatin A, we relied on the identity of the ^{1}H NMR spectrum of our synthetic product with the corresponding spectrum of the natural material supplied by Bristol-Myers Squibb Pharmaceutical Research Institute. In addition, the ^{13}C NMR, IR, MS, HRMS, mp, optical rotation, elemental analysis, and TLC were in agreement with the published data.[1b]