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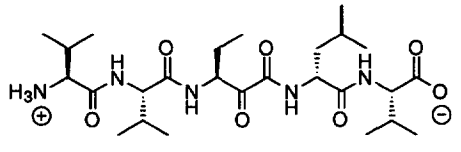
A Convergent Synthesis of Poststatin: Application of the Acyl Cyanophosphorane Coupling Procedure in the Formation of a Peptidic α-Keto Amide.

Harry H. Wasserman* and Anders K. Petersen

Department of Chemistry, Yale University, New Haven, CT 06520-8107, U.S.A.

Abstract: A convergent synthesis of the pentapeptide poststatin has been developed. The key step involves oxidative cleavage of an acyl cyanophosphorane. The resulting α,β-diketo nitrile is then coupled to the free amine of a *C*-terminal-dipeptidyl component to generate the protected natural product. Deprotection by hydrogenolysis furnishes poststatin. Copyright © 1997 Elsevier Science Ltd. © 1997, Elsevier Science Ltd. All rights reserved.

Poststatin[1] (**1**), an inhibitor of prolyl endopeptidase, is a naturally occurring pentapeptide isolated from *Streptomyces viridochromogenes*. The amino acid sequence is: H-Val-Val-Pos-D-Leu-Val-OH, where Pos is the abbreviation for the unusual (*S*)-3-amino-2-oxopentanoic acid named L-postine. The α-keto-amide group, which is present in a number of cyclic peptides,[2] appears to be necessary for the biological activity of poststatin.[1b] The cyclotheonamides[3] contain the α-keto homologue of arginine, whereas the structurally related orbiculamides,[4] keramamides,[5] and discobahamins[6] incorporate leucine extended with an α-carbonyl group. In the eurystatins,[7] (*S*)-3-amino-2-oxobutanoic acid is believed to be responsible for the prolyl endopeptidase inhibitory effect, while the tricarbonyl amino acid 4-amino-2,3-dioxo-6-methylheptanoic acid comprises the electrophilic binding site in the depsipeptides YM-47141 and YM-47142.[8] Examples of peptide alkaloids derived from α-keto amides include verongamine,[9] eusynstyelamide,[10] and the anchinopeptolides.[11] Other studies have shown, that rationally designed derivatives of α-keto acids represent an important class of enzyme inhibitors.[12]



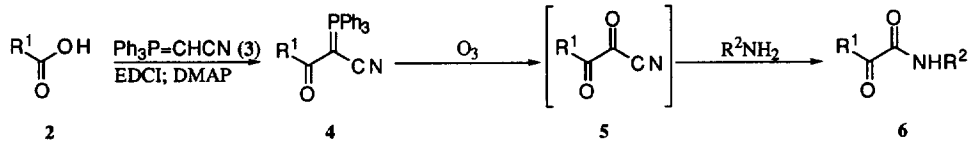
1 Poststatin: H-Val-Val-Pos-D-Leu-Val-OH; Pos = Postine

In a previous synthesis of poststatin,[1d,f] a linear approach employed (2*R*,3*S*)-3-amino-2-hydroxypentanoic acid as a precursor of postine. This procedure followed traditional lines, whereby 2-keto carboxylates are prepared through oxidation of the intermediate hydroxy compounds, which may be obtained

from the appropriate 2-hydroxy trithioorthoformate,[3b,13] cyanohydrin,[14] 2-(hydroxymethyl)furan,[15] or other 2-hydroxy carboxylate precursors.[12,16,17] In general, these syntheses have disadvantages associated with many steps or racemization.[18]

As part of a research program focused on the synthesis of peptidyl enzyme inhibitors,[19a] we have developed a novel method for the efficient construction of α-keto amides by acylation of amines with α,β-diketo nitriles (cf. Scheme I).[19b] This procedure has now been applied in a total synthesis of poststatin.

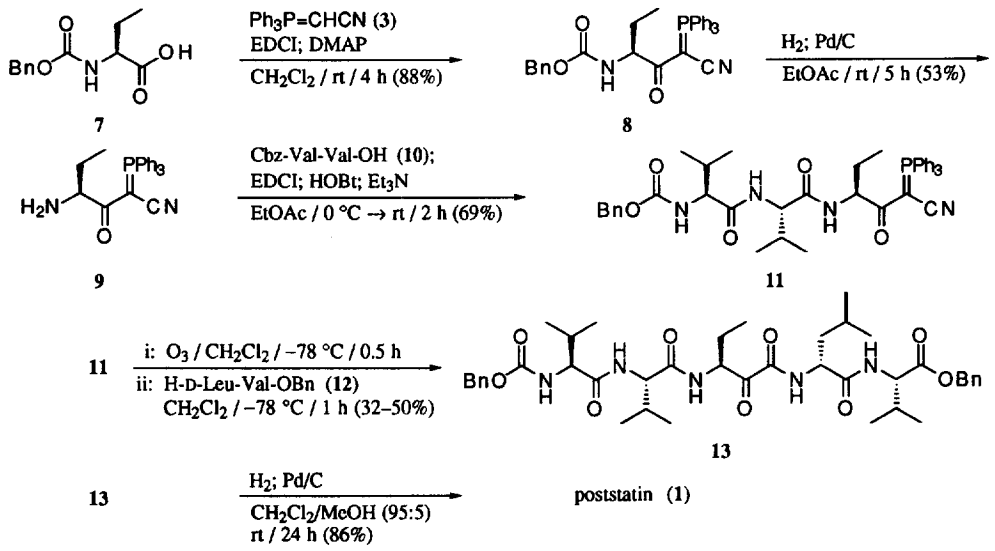
Scheme I. Preparation of α-Keto Amides.



2 4 5 6

In a retrosynthetic sense, poststatin may be regarded as postine substituted with a dipeptide in each direction. Two ways were considered for assembling these fragments. In one route, the *N*-terminal may be initially coupled to *N*-protected valylvaline, followed by *C*-terminal chain elongation with *O*-protected D-leucylvaline. Alternatively, the order of the peptide couplings may be reversed. We chose the former strategy, because it has the advantage of generating the α-keto-amide functionality at a late stage in the synthesis, thereby minimizing epimerization of the postine residue. As outlined in Scheme II, the key bond-forming step is the condensation of Cbz-Val-Val-Pos-CN and H-D-Leu-Val-OBn (**12**). The activated acylating agent is generated in situ by ozonolysis of the corresponding acyl cyanophosphorane **11**. Thus, the unmasking of the α-keto-carboxylate and the peptide coupling reaction take place in one synthetic operation.

Scheme II. Synthesis of Poststatin.[20]



7 8 9 11 13

The acyl cyanophosphorane **8** was formed (88%) by the coupling of commercially available Cbz-protected (*S*)-(+)-2-aminobutanoic acid (**7**) with (cyanomethylene)triphenylphosphorane (**3**)[21,22] using EDCI and DMAP. Removal of the protecting group was accomplished by hydrogenolysis over Pd/C.[23] The crude amine was coupled with Cbz-protected valylvaline[24] under standard peptide-coupling conditions to yield the crystalline tripeptide **11** (69%) as the sole epimer. Oxidative cleavage of the carbon-phosphorus double bond, by reaction with ozone, generated the highly electrophilic α,β-diketo nitrile intermediate corresponding to **5**. Immediate addition of the free amine[25] of D-leucylvaline *O*-benzyl ester (**12**)[1d,f] produced the protected pentapeptide (**13**).[26] Essentially no epimerization of the postine unit was observed at this stage. Hydrogenolysis of the benzylic protecting groups furnished poststatin (**1**)[27] (86%). NMR examination of the final product suggested the presence of ca 15% of a poststatin epimer.[1d,f]

The possibility of establishing the α-keto-amide linkage between simple substrates followed by bidirectional synthetic manipulations is currently being investigated. If successful, this versatile methodology may be applied to peptides such as the orbiculamides,[4] keramamides,[5] discobahamins,[6] and other target molecules containing substructural elements which are sensitive to the oxidative conditions.

Acknowledgments

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18. In the Dakin–West reaction, for example, a 3-*N*-acylamino-2-keto ester is formed directly from a 2-*N*-acylamino acid. However, the product is completely racemized due to the intermediacy of an enamide. Charles, I.; Latham, D. W. S.; Hartley, D.; Oxford, A. W.; Scopes, D. I. C. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1139–1146.
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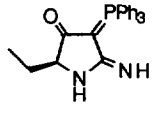
22. Improved procedure for the preparation of (cyanomethylene)triphenylphosphorane **3**:[21] (Cyanomethyl)triphenylphosphonium chloride (Lancaster Synthesis Inc.) (123.6 g, 0.37 mol) was suspended in dry dichloromethane (1.8 L). Triethylamine (127 mL, 92.2 g, 0.91 mol) was added over a period of 15 min. The clear yellow solution was stirred for a further 25 min, before it was washed with prechilled water (2 × 500 mL) and dried with magnesium sulfate (1 h). Filtration and concentration afforded a pale yellow solid (108.1 g, 0.36 mol, 98% yield). The crude ylide was contaminated with less than 5% triphenylphosphine oxide and could be condensed successfully with carboxylic acids. The product was purified by recrystallization from benzene (1.5 L, 14 mL/g) to give an off-white product (87.0 g, 0.29 mol, 79%).
23. Along with amine **9** (53%), the cyclic amidine **9a** (21%) formed by intramolecular aminolysis of the cyano group (26% unconverted **8**).



9a

24. Prepared from valylvaline (85%) by reaction with benzyl chloroformate.
25. The dipeptidyl amine **12** was obtained (85%) from the hydrotrifluoroacetate of D-leucylvaline *O*-benzyl ester[1d,f] by treatment with saturated aqueous sodium hydrogen carbonate.
26. When conducted on a 5.5 mmol scale, a 32% yield of the pentapeptide **13**[1d,f] was obtained in impure state after chromatography. At 0.1 mmol scale the yield was consistently in the 50% range, and the product quality was significantly higher.
27. The crude poststatin was purified by reversed-phase-silica-gel flash chromatography using methanol/water = 60:40 as the eluent. The synthetic product was identical in all respects to a sample of synthetic poststatin, kindly provided by Dr. Muraoka. Due to the pronounced concentration dependence of the ^{1}H NMR and ^{13}C NMR spectra, the final comparison was achieved by mixing equal amounts of the two samples. Under these circumstances, a single set of signals was observed.

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