Pergamon

Tetrahedron: Asymmetry Vol. 5, No. 2, pp. 195-198, 1994
Elsevier Science Ltd
Printed in Great Britain
0957-4166/94 $6.00+0.00

0957-4166(94)E0013-Z

First Synthesis of Enantiomerically Pure N-Protected β-Amino-α-Keto Esters from α-Amino Acids and Dipeptides

Paul Darkins, Noreen McCarthy, M. Anthony McKervey* Kevin O'Donnell and Tao Ye

School of Chemistry, The Queen's University, Belfast BT9 5AG, N. Ireland

Brian Walker*

School of Biology and Biochemistry, The Queen's University, Belfast BT7 7BL, N. Ireland

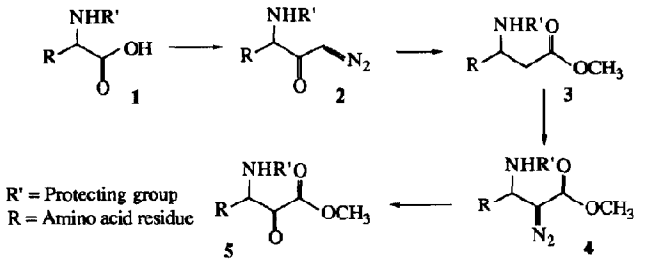
Abstract: A racemization-free route from N-protected α-amino acids and dipeptides to N-protected β-amino-α-keto esters is described, involving the sequence: diazoketone formation, Wolff rearrangement in methanol, diazo transfer, and oxidation with dimethyldioxirane.

α-Keto acids and ester derivatives thereof have found widespread application as competitive reversible inhibitors of the proteinase family of enzymes. For example, amino acid-derived α-keto acids have been synthesised as inhibitors of the serine proteinase chymotrypsin,[1] whilst their incorporation into appropriate peptide recognition sequences has furnished potent and selective inhibitors of cysteine proteinases such as calpain and cathepsin B,[1] the serine proteinases neutrophil elastase and cathepsin G,[2,3] and the aspartyl proteinase, pepsin.[3]

By far the most widely employed route to α-keto esters derived from α-amino acids and peptides has been oxidation of N-protected β-amino-α-hydroxy esters prepared by elaboration of N-protected α-amino aldehydes. Of the several oxidation procedures available, those involving the Swern or Dess Martin reagents are considered the most successful.[4] However, there is substantial evidence that both these reagents cause extensive racemization at the β-position of the α-keto esters.[2,4] Consequently, studies of proteinase inhibitory activity have the disadvantage of referring to enantiomeric or diastereoisomeric mixtures.

We have developed an alternative approach to these keto esters which completely circumvents the problem of racemization encountered with earlier syntheses and which is equally applicable to N-protected amino acids and peptides. The sequence (Scheme 1) commences with conversion of the N-protected amino acid **1** into the corresponding α-diazoketone **2** *via* standard procedures which are known not to cause racemization.[5] Groups suitable for N-protection include BOC, Cbz, phthaloyl and ethoxycarbonyl. In the second stage the diazoketone was subjected to Wolff rearrangement in methanol with silver benzoate catalysis to afford in high yield the β-amino acid methyl ester **3**. This route to β-amino acid derivatives is known to proceed with complete retention of configuration.[6] A diazo group was reintroduced in the third stage using the Danheiser procedure[7] in

which the α-position is acylated with 2,2,2-trifluoroethyl trifluoroacetate prior to diazo transfer from mesyl azide to form the β-amino-α-diazo ester **4**. To complete the synthesis the diazo function of **4** was oxidatively cleaved under strictly neutral conditions by exposure to dimethyldioxirane (DMD) in acetone to furnish in essentially quantitative yield the α-keto methyl ester **5**. This is the first example of formation of α-keto esters by DMD oxidation. Recently we demonstrated that DMD oxidation of α-diazoketones can be used to synthesise homochiral N-protected α-amino glyoxal from amino acids.[8] That the last two stages did not involve detectable amounts of racemization was readily established by comparing the ^{13}C NMR spectrum of the product **5f** derived from (3S,4S)-isoleucine with that derived from its racemic counterpart **5i**. Whereas the latter contained signals attributable to both diastereoisomers the former was clearly a single diastereoisomer. The sequence in Scheme 1 has been applied to the synthesis of a range of enantiomerically pure N-protected β-amino α-keto methyl esters, a representative selection of which are collected in Table 1.[9] Simple amino acids with R or S configurations lead efficiently to products **5a-5h**. Application to dipeptide-derived products is illustrated by structures **5j** and **5k**.



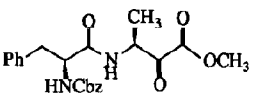
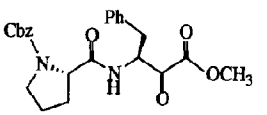
Scheme 1

A few of the α-keto ester derivatives described above were examined for their inhibitory activity against bovine α-chymotrypsin and porcine pancreatic elastase (P.P.E.).[10] Thus, the phenylalanyl derivatives **5a** and **5b** were tested against the former, whereas **5d** and **5e** were examined as inhibitors of the latter. As expected, the L-phenylalanine-derived α-keto ester **5a**, functioned as a superb competitive reversible inhibitor of chymotrypsin with a determined K_i of ~ 0.01μM, whereas the D-phenylalanine-derived analogue **5b** exhibited no inhibition whatsoever, even when tested at concentrations as high as 400 μM. These observations are in keeping with the known absolute specificity of chymotrypsin for aromatic amino acids of the L-configuration at the P_1 position of substrates and inhibitors (nomenclature of Schecter and Berger).[11]

The L-valine and L-alanine derivatives **5d** and **5e** functioned as competitive reversible inhibitors of P.P.E., the former being only a very modest inhibitor (K~480μM) whilst the latter exhibits moderate affinity for

the protease (K 30 μM). These observations, coupled with the fact that **5a** exhibits no inhibitory activity against P.P.E. even when used at concentrations as high as 500 μM, are entirely in keeping with the known specificity of the protease which, due to the presence of bulky amino acids at positions 216 and 226 in the active-site-cleft, can only accommodate small aliphatic residues at the P_1 position.

Table 1. N-Protected β-amino-α-keto esters

Entry	β-Amino-α-keto ester	$[\alpha]_D^{20}$ [a]	Configuration
5a	R = $PhCH_2$; R' = Cbz	+ 53.0 (c, 2.3)	3S
5b	R = $PhCH_2$; R' = Cbz	- 53.1 (c, 1.0)	3R
5c	R = Ph; R' = Cbz	+ 9.6 (c, 1.3)	3S
5d	R = $(CH_3)_2CH$; R' = Cbz	+ 71.6 (c, 13.7)	3S
5e	R = CH_3; R' = Cbz	+ 21.0 (c, 2.6)	3S
5f	R = $CH_3CH_2CH(CH_3)$; R' = Boc	+ 23.7 (c, 4.3)	3S, 4S
5g	R = $PhCH_2$; R' = Boc	+ 40.7 (c, 7.7)	3S
5h	R = Cbz-$NH(CH_2)_4$; R' = Boc	+ 20.1 (c, 1.3)	3S
5i	R = $CH_3CH_2CH(CH_3)$; R' = Boc	-	-
5j		+ 11.5 (c, 1.0)	3S, 6S
5k		- 42.4 (c, 1.0)	3S, 6S

[a] Measured in dichloromethane

The inhibitory activity of the remaining α-keto esters are presently being examined, and the results of these studies will be presented elsewhere.

Acknowledgement: We thank the Department of Education of N. Ireland for a postgraduate award to P.D.

References and footnotes:

1 Angelastro, M. R.; Mehdi, S.; Burkhart, J. P.; Peet, N. P. and Bey, P. *J. Med. Chem.*, **1990**, *33*, 13

2 Peet, N. P.; Burkhart, J. P.; Angelastro, M. R.; Giroux, E. L.; Mehdi, S.; Bey, P.; Kolb, M.; Neises, B. and Schirlin, D. *J. Med. Chem.*, **1990**, *33*, 394

3 Hori, H.; Tasutake, A.; Minemarsu, Y. and Powers, J. C. *Peptides, Structure and Function* (Proceedings of the Ninth American Peptide Symposium), Eds. Deber, C. M.; Hruby, V. J. and

Kopple, K. D.; Pierce Chemical Co., Rockford, IL., **1985**, p. 819

4 Burkhart, J. P.; Peet, N. P. and Bey, P. *Tetrahedron Lett.*, **1990**, *31*, 1385

5 Ye, Tao. and McKervey, M. A. *Tetrahedron*, 1992, *48*, 8007

6 Nishi, T. and Morisawa, Y. *Heterocycles* **1989**, *19*, 1835.; Ondetti, M. A. and Engel, S. L. *J. Med. Chem.*, **1975**, *18*, 761

7 Danheiser, R.L.; Miller, R.F.; Brisbois, R.G.and Park, S.Z. *J. Org. Chem.* **1990**, *55*, 1959

8 Darkins, P.; McCarthy, N.; McKervey, M. A. and Ye, Tao *J. Chem. Soc., Chem. Commun.* **1993**, 1222.

9 All new compounds gave satisfactory analytical and/or high resolution mass spectra; and were fully characterised spectroscrpically. **Selected data**: **5a**, **5b**, ^{1}H-NMR (300MHz, $CDCl_3$) 3.07 (1H, m, PhC$\underline{H}_2$), 3.22 (1H, m, PhC$\underline{H}_2$), 3.85 (3H, s, OC$\underline{H}_3$), 5.08 (2H, s, OC$\underline{H}_2$Ph), 5.25 (2H, m, C$\underline{H}$(N)CO and N$\underline{H}$), 7.08-7.15 (10H, m, Ar-$\underline{H}$); ^{13}C-NMR (75MHz, $CDCl_3$) 36.79, 52.86, 57.90, 66.91, 127.08, 127.86, 128.00, 128.29, 128.51, 129.03, 129.19, 134.70, 135.82, 155.39, 160.48, 191.65; **5f** ^{1}H-NMR (300MHz, $CDCl_3$), 0.89 (3H, t, J = 7.3Hz, $CH_2C\underline{H}_3$), 1.01 (3H, d, J = 6.8Hz, C$\underline{H}_3$CH), 1.44 (9H, s, (C$\underline{H}_3$)$_3$C), 1.00-1.60 (2H, m, $CH_3C\underline{H}_2$), 1.97 (1H, m, $CH_3C\underline{H}$), 3.90 (3H, s, OC$\underline{H}_3$), 4.89 (1H, m, C$\underline{H}$(N)CO), 5.08 (1H, br d, N$\underline{H}$).; ^{13}C-NMR (75MHz, $CDCl_3$) 11.26, 15.83, 24.13, 28.03, 36.46, 52.87, 61.09, 79.95, 155.39, 161.21, 193.47; **5i** ^{1}H-NMR (300MHz, $CDCl_3$), 0.90 (6H, m, $CH_2C\underline{H}_3$ & C$\underline{H}_3$CH), 1.44 (9H, s, (C$\underline{H}_3$)$_3$C), 1.00-1.65 (2H, m, $CH_3C\underline{H}_2$), 1.90-2.05 (1H, br m, $CH_3C\underline{H}$), 3.90 (3H, s, OC$\underline{H}_3$), 4.74, 4.89 (1H, 2 x m, C$\underline{H}$(N)CO), 4.95, 5.07 (1H, 2 x br m, N$\underline{H}$); ^{13}C-NMR (75MHz, $CDCl_3$) 11.13, 11.26, 15.02, 15.83, 24.13, 24.79, 28.03, 36.32, 36.46, 52.87, 60.56, 61.09, 79.97, 155.31, 155.39, 161.02, 161.21, 193.15, 193.47

10 **Inhibition studies on chymotrypsin**: Chymotrypsin (10 µl of a ~ 0.1µM stock solution in 1 mM-HCl) was added to a solution (1ml) of succinyl-Ala-Ala-Pro-Phe-NH-Mec (50 µM) and inhibitor under study (0.005-400 µM) in 50 mM-sodium phosphate buffer, pH 7.4, containing 100 mM-NaCl, maintained at 37 °C. The rate of hydrolysis of substrate was monitored continuously by measuring the rate of increase in fluorescence at 455 nm (excitation wavelenth 383 nm) in a Perkin-Elmer MPF 44B spectrofluorimeter. **Inhibition studies with pancreatic elastate**: Pancreatic elastase (10 µl of a ~ 0.1µM stock solution in 1 mM-HCl) was assayed in the presence of methoxysuccinyl-Ala-Ala-Pro-val-NH-Mec (50 mM) and inhibitor under study (10-500 µM), exactly as described for chymotrypsin. **Determination of K_m and V_{max} for the fluorogenic substrates**: To determine the K_m and V_{max} for the substrates used in the present study, substrate concentrations spanning a range 0.2-5 times the K_m were used. For each enzyme/substrate pair studied, it was ensured that the determination of the kinetic constants were carried out under the exact conditions used to monitor the inhibition processes described above. Km and Vmax were determined by using the least-squares method of Roberts. (Roberts, D. V. *Enzyme Kinetics* pp. 299-306, Cambridge University Press, Cambridge, **1977**)

11 Schecter, I. and Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157

(*Received in UK* 16 *December* 1993)