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CARBOXYL-MODIFIED AMINO ACIDS AND PEPTIDES: II) A CONVENIENT ROUTE FOR THE SYNTHESIS OF DIFUNCTIONALIZED ENAMINES FROM THIOAMIDES VIA THIOIMINIUM SALTS

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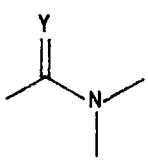
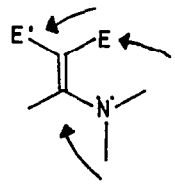
ABSTRACT: From thioamides **4**, via thioiminium salts **5**, various difunctionalized enamines **6** with different electron-withdrawing groups E, E' (E: CN, CO_2Me, COR, SO_2R, etc; E': CN, CO_2Me) are prepared. Application of the methodology, the determination of stereochemistry and the degree of racemization of some amino acids are discussed.

Thioamide **2a** and amidoxime **2b** are peptide analogues in which the oxygen atom of the amide bond **1** is replaced by a sulfur or nitrogen atom. Although these modified links resemble the peptide bond in some ways, they display increased resistance to enzyme-catalyzed hydrolysis[2], thus providing potentially useful probes of protease mechanisms and active-site chemistry. In the course of our ongoing studies on modifications[3] of the peptide bond, we synthesized monofunctionalized enamine analogues **2c** (see preceeding communication[4]) as potential protease inhibitors. Here we describe a simple, general procedure for the preparation of new derivatives as potential modifiers of α-chymotrypsin. More specifically, we report on the difunctionalized enamine analogues **3** (with different electron-withdrawing groups) which have the potential of acting as effective active site-directed inhibitors. The synthesis of these difunctionalized enamine analogues involves condensation of the appropriate activated methylene reactant with an electrophilic thioiminium[5] intermediate.



1, 2



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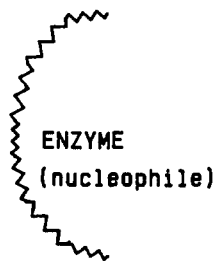
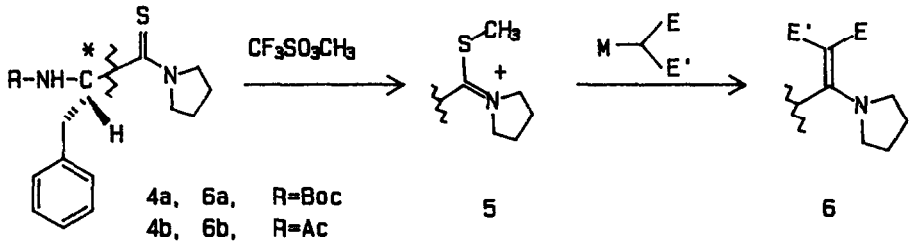


1 Y=O
2a Y=S
2b Y=NR
2c Y=CHE

Treatment of Boc-thioamide **4a** with methyl triflate produced the thioiminium salt **5** which was reacted with difunctionalized methylene carbanions to give the corresponding enamines **6a** where one substituent, E', is a cyano group and the other, E, selected from a group of different electron-attracting functions (Table 1, entries 1-9: E= cyano, alkoxy carbonyl, benzoyl, p-nitrophenyl, phenylsulfone, alkyl phosphonate, amide, thiophene and thiophenyl).

These latter acetonitrile derivatives were chosen as reactants because of their ready accessibility or the ease with which they can be prepared and also because the nitrile function minimizes steric repulsions apart from its special reactivity towards certain enzyme nucleophiles such as thiol groups (e.g. papain)[6]. Similar results were obtained by replacing the nitrile function with other electron-withdrawing groups as examplified by the use of the malonate anion (entries 10,11).



4a, 6a, R=Boc
4b, 6b, R=Ac
4c, 6c, R=Boc-L-Leu (L-Phe)
4d, 6d, R=Boc-L-Leu (D-Phe)

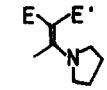
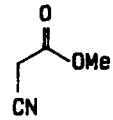
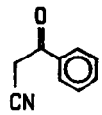
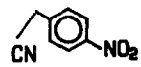
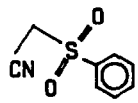
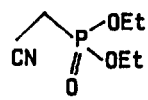
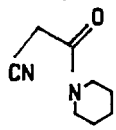
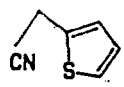
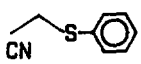
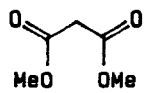
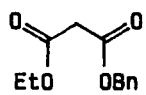
4 5 6

The above methodology was also applied successfully to the synthesis of N-acetyl enamine derivatives **6b** (N-Ac-Phe, entries 1-11) from the corresponding N-Ac-thioamide **4b**. In general, our results (Table 1) indicate that enamine compounds can be formed but in moderate yields (40-65%). When sterically hindered anions were used low yields resulted (≈ 20%) (compare, for example, entries 2 and 7 or entries 10 and 11). In order to demonstrate that racemization did not occur under the reaction conditions, the enamines **6c** and **6d** (E=COOMe, E'= CN; Y=50%) were prepared from the corresponding thiodipeptides **4c** and **4d** respectively. The ^{1}H NMR (400 MHz) showed the absence of diastereoisomers. The present synthetic approach is also applicable to other protected amino acids. For example, condensation of methyl cyanoacetate with either Boc-Leu-thioamide or Boc-Met-thioamide furnished the corresponding enamine derivatives in 45 and 31% yield respectively.

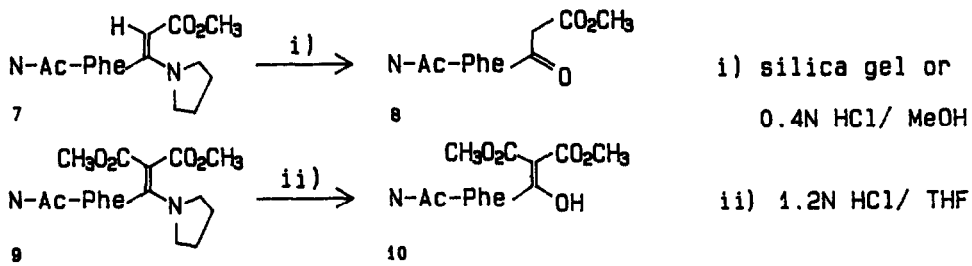
At room temperature the enamine analogues exhibited only one set of relevant signals (^{1}H NMR 400 MHz, $CDCl_3$), indicating the existence of either a single geometric form or a rapid equilibrium between the two isomeric forms (E and Z). The ^{1}H NMR spectra of the enamines **6b** (entries 2 and 11) at low temperature (-60°C and -100°C respectively) clearly indicated the existence of these two forms. A similar equilibrium involving electron-withdrawing substituents at the β position to the amino group has been reported previously[7]. These functions reduce the tautomerization energy barrier about the carbon-carbon double bond so that rotation occurs at a rate comparable to that of the NMR time scale.

There are differences in the stability and reactivity of monofunctionalized and difunctionalized enamines. For example, the enamino ester **7** is unstable on silica gel and rapidly hydrolyzed to β-keto ester **8**, whereas the enamine dimethyl ester **9** is stable under the same conditions but yields enol **10** under strong acidic conditions (1.2N HCl). These differences in reactivity should influence their mode of interaction with relevant enzymes.

Table 1: Synthesis of difunctionalized enamines of Boc- and N-Ac-phenylalanine[a].

Entry	Carbanion Precursor 	YIELD[a] (%)	
		Intermediate	Analogues of α-chymotrypsin substrate
		Boc-Phe  6a	N-Ac-Phe  6b
1[b]		62	59
2		52	48
3		35	22
4		49	38
5		47	49
6		38	32
7		19	17
8[c]		42	20
9[c]		55	28[d]
10		43	38
11		22	21

The methodology is currently being applied to the incorporation of difunctionalized enamine bond isosteres at the C-terminal function in some biologically important peptides. We are also in the process of extending this synthetic approach to modify the endo amide bonds of peptides. Full experimental details and the results of enzymatic studies using these analogues will be reported elsewhere.



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8. **a)** isolated yields; all products exhibited physical and spectral characteristics in accordance with the assigned structures. **b)** general experimental conditions (unless otherwise stated): 1° substrate **4** (1 eq), methyl triflate (1.05 eq) DME, 15 min, 0°C; 2° at - 78°C a mixture of the enolized reagent (1.05 eq) with base at 0°C in DME was added dropwise, 20 min, -78°C, 30 min, 0°C; 3° quench at 0°C with saturated ammonium chloride solution: entries 1-7, 10, 11, base NaH. **c)** following the general conditions except for entries 8 and 9: 2° at -78°C a mixture of the enolized reagent with LDA (1.05 eq) at -78°C in DME was added dropwise, 30 min -78°C and 30 min 0°C. **d)** obtained by deblocking the Boc group with HCl in ether and acylation with Ac_2O and pyridine.

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