

Stereospecific Synthesis of α -Aminoacyl Aldehydes and their Interaction with α -Chymotrypsin

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Summary A general procedure for the synthesis of optically pure α -aminoacyl aldehydes is described; these aldehyde analogues have been used to explore the nature of the various P-S interactions in α -chymotrypsin (from bovine pancreas).

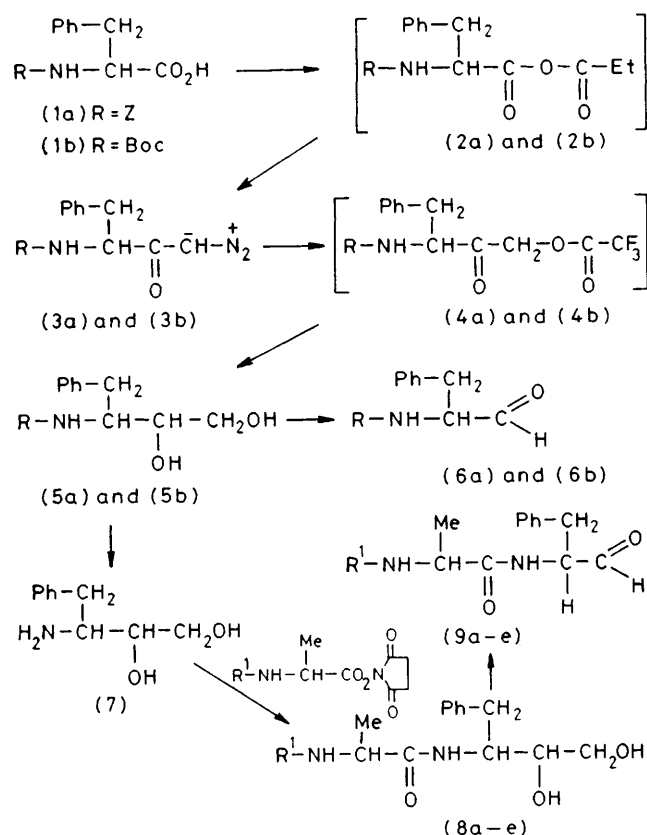
AFTER continuing studies on the development of methods for the preparation of new analogues of amino acids and peptides in which the carboxy-function is replaced by

electrophilic substituents, we now report on the synthesis of α -aminoacyl aldehydes. Although the enzymological and medical potentials¹ of these compounds have been recognized for some time, approaches to their synthesis have attracted attention only recently.^{1b,2} The procedure described in this communication offers two major advantages hitherto not realised in previous endeavours.^{1b,2} These are (a) the maintenance of a high degree of stereospecificity at the sensitive α -carbon atom of the aminoacyl

aldehyde and (b) the convenience of introducing the terminal aldehyde residue during the final stages of the synthesis of polypeptide analogues.

The aldehyde analogues synthesized in this study have found use in defining the contours of the S_3 subsite in chymotrypsin.

The synthetic protocol involved the conversion of benzyloxy- and butyloxy-carbonylphenylalanine derivatives (**1a**) and (**1b**), via their mixed anhydrides (**2**), into the diazoketones (**3**).[†] The latter were decomposed with trifluoroacetic acid[‡] to yield the trifluoroacetyl ketones (**4**) which were treated with NaBH_4 without any purification. The treatment resulted not only in the reduction of the carbonyl group but also the hydrolysis of the trifluoroacetyl function to furnish the *N*- α -aminoacyl diols (**5**) which were

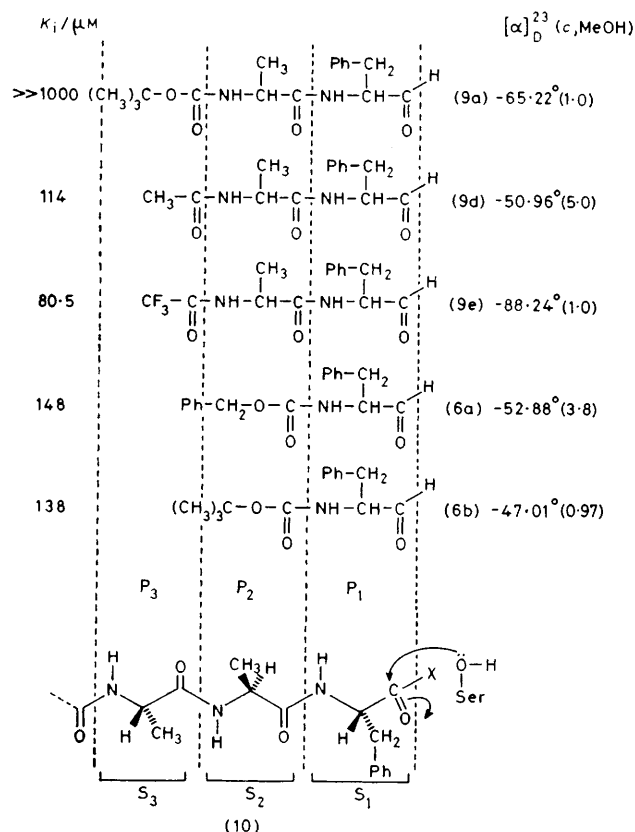


SCHEME

- a; $R^1 = \text{Boc} [= \text{Bu}^t\text{OC}(:\text{O})-]$
 b; $R^1 = \text{Z} [= \text{PhCH}_2\text{OC}(:\text{O})-]$
 c; $R^1 = \text{Bu}^t\text{OC}(:\text{O})\text{NHCHMeC}(:\text{O})-$
 d; $R^1 = \text{CH}_3\text{C}(:\text{O})-$
 e; $R^1 = \text{CF}_3\text{C}(:\text{O})-$

smoothly oxidized with NaIO_4 to give the α -aminoacyl aldehydes (**6**) in high yield. Our results show that the α -aminoacyl aldehydes (**6**) have >95% optical purity. The α -aminoacyl diol (**5b**) on treatment with hydrogen chloride in diethyl ether gave the hydroxymethylphenylalaninol (**7**) which smoothly condensed with *N*-hydroxy-succinimido esters of variously protected alanine and alanylalanine derivatives to afford the diol analogues of the peptides (**8a-e**). The latter diols on NaIO_4 cleavage furnished the aldehyde analogues (**9a-e**) which were obtained pure by direct recrystallization.

These analogues were then used to study the specificity aspect of α -chymotrypsin (from bovine pancreas) which catalyses the hydrolysis of ester and amide linkages of derivatives of the type (**10**). The postulated mechanism⁴ for the hydrolysis involves the participation of a tetrahedral intermediate⁵ formed by the attack of the $-\text{OH}$ group of Ser 195 on the sensitive carbonyl group (see Figure, structure **10**). Parts of the substrate molecules

FIGURE. $X = \text{leaving group}$

† All new compounds reported herein are crystalline solids and have been characterized by their analytical and spectral data.

‡ In a typical experiment, the diazoketone³ (**3b**) (5.0 g) was decomposed at room temperature (2 h) in dry diethyl ether (25 ml), glacial acetic acid (5 ml), and trifluoroacetic acid (4 ml) to give (**4b**) (55%). The latter was dissolved in methanol (20 ml) and then treated with NaBH_4 (0.4 g, excess) at room temperature to give after the usual work-up and chromatography (**5b**) [in 40% overall yield from (**3b**)], m.p. 97–98 °C. The reaction of the diol (**5b**) (0.5 mmol) in methanol (5 ml) with NaIO_4 (1 mmol) at room temperature for 1 h gave after recrystallization from light petroleum (**6b**) (ca. 85%).

§ The aldehyde derivatives (**6a**) and (**6b**) were reduced with NaBH_4 in methanol to give the corresponding *N*-acylphenylalaninols. Z-Phenylalaninol, $[\alpha]_D^{23} -39.6^\circ$ (c 1.5, MeOH) [Z-phenylalaninol prepared from L-phenylalaninol showed $[\alpha]_D^{23} -40.5^\circ$ (c 2.7, MeOH)]. Boc-phenylalaninol was converted into phenylalaninol hydrochloride, $[\alpha]_D^{23} -20.0^\circ$ (c 0.5, 1 N-HCl) [phenylalaninol hydrochloride obtained from L-phenylalaninol showed $[\alpha]_D^{23} -18.0^\circ$ (c 0.98, 1 N-HCl)].

away from the scissile bond are known to interact favourably with specific sites on the enzyme and this type of interaction makes a significant contribution to the free-energy of binding.⁶ In order to specify these interactions the notation of Schechter and Berger⁷ is used which designates residues of the substrate as P₁, P₂, P₃, P₄, and complementary subsites on the enzyme as S₁, S₂, S₃, S₄ (see Figure). Thus the P₁ residue of the substrate representing the acyl portion of the scissile bonds binds to S₁; P₂ to S₂ and so on. The nature of the various P-S interactions was analysed by studying the inhibitory effect of the aldehyde analogues on the α -chymotrypsin-catalysed hydrolysis of a synthetic substrate, glutaryl-L-phenylalanine-*p*-nitro-anilide.[¶] The results from such a study are displayed in the Figure as binding dissociation constants K_1 (the lower the value of K_1 the better the binding) and may be interpreted as follows. From our current knowledge of the specificity of chymotrypsin-catalysed reactions, it is reasonable to assume that the C-terminal phenylalanine unit of all the inhibitors constitutes the P₁ residue and always binds to the S₁ subsite. The relatively low K_1 values obtained for the analogues (6a), (6b), (9d), and (9e) then suggest that the S₂ subsite may favourably accommodate a diverse nature of apolar side chains; for example, the CH₃-CH< side chain of (9d) and (9e), C₆H₅-CH₂O- of (6a), and (CH₃)₃-C-O- of (6b) are all tolerated well at the S₂ subsite. The results showing the broad specificity of S₂ with respect to apolar side chains are in general agreement with the

interpretation of X-ray crystallographic and kinetic data by Segal *et al.*⁶ but suggest that the S₂ 'hole' accommodating the P₂ side chain may be somewhat larger than the 3-4 Å estimated previously. In sharp contrast to the broad specificity of the interaction of the S₂ subsite with the P₂ side chain the S₃ subsite is much more discriminating with respect to P₃ side chains. The acetyl side chain as in (9d) and trifluoroacetyl as in (9e) interact favourably with S₃ while the corresponding analogue (9a) containing the *t*-butoxy-group showed considerably reduced binding. Thus the 'short and fat' *t*-butoxy-group at the P₃ residue must interact with chymotrypsin so adversely as to wipe out the combined P₁-S₁ + P₂-S₂ interactions, with an energy of several kcal mol⁻¹. In a critical analysis of the X-ray and kinetic data Segal *et al.*⁶ considered the possibility that the side chain of P₃ may point to the exterior of chymotrypsin without interacting with the enzyme, but then signalled caution and drew attention to the fact that an ϵ -amino-group of Lys 175 which was not too far from the P₃ peptide binding site could interact with the substrate and confer additional specificity. Without specifying the nature of interacting residue(s), our results may support the latter adumbration and point to a sterically crowded region at the S₃ subsite surrounding the C $_{\alpha}$ and C $_{\beta}$ atoms of the P₃ residue.

(Received, 12th June 1979; Com. 617.)

¶ All the enzymic assays were carried out in the presence of 7.5% dimethyl sulphoxide.

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