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

By RAM P. SHARMA, MICHAEL G. GORE, and MUHAMMED AKHTAR

(*Department of Biochemistry, School of Biochemical and Physiological Sciences, University of Southampton, Southampton* SO9 3TU)

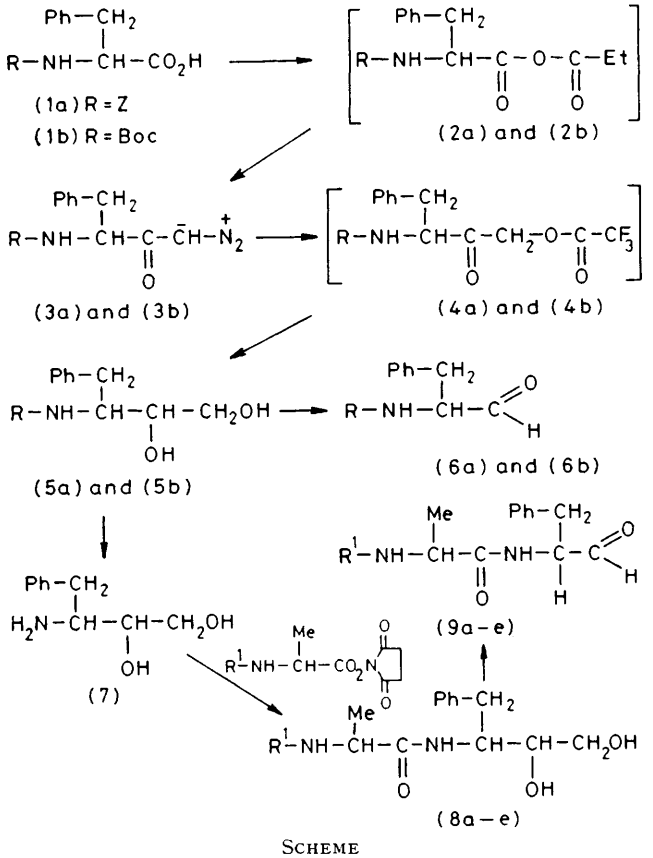
Summary A general procedure for the synthesis of optically pure α-aminoacyl aldehydes is described; these aldehyde analogues have been used to explore the nature of the various P–S interactions in α-chymotrypsin (from bovine pancreas).

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 potentials[1] 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**).[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 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*-hydroxysuccinimido 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.



SCHEME

a; R^1 = Boc [= $Bu^tOC(:O)$–]
b; R^1 = Z [= $PhCH_2OC(:O)$–]
c; R^1 = $Bu^tOC(:O)NHCHMeC(:O)$–
d; R^1 = $CH_3C(:O)$–
e; R^1 = $CF_3C(:O)$–

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 mechanism[4] for the hydrolysis involves the participation of a tetrahedral intermediate[5] formed by the attack of the –OH group of Ser 195 on the sensitive carbonyl group (see Figure, structure **10**). Parts of the substrate molecules

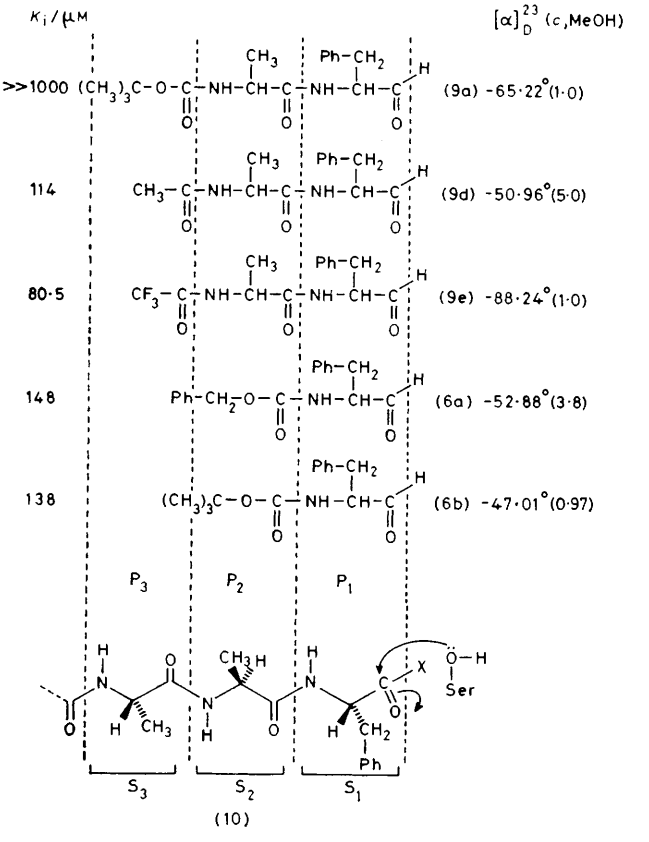


FIGURE. X = leaving group

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

‡ In a typical experiment, the diazoketone[3] (**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° (*c* 1·5, MeOH) [Z-phenylalaninol prepared from L-phenylalaninol showed $[\alpha]_D^{23}$ −40·5° (*c* 2·7, MeOH)]. Boc-phenylalaninol was converted into phenylalaninol hydrochloride, $[\alpha]_D^{23}$ −20·0 (*c* 0·5, 1 N-HCl) [phenylalaninol hydrochloride obtained from L-phenylalaninol showed $[\alpha]_D^{23}$ −18·0 (*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 Berger[7] is used which designates residues of the substrate as P_1, P_2, P_3, P_4, and complementary subsites on the enzyme as S_1, S_2, S_3, S_4 (see Figure). Thus the P_1 residue of the substrate representing the acyl portion of the scissile bonds binds to S_1; P_2 to S_2 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*-nitroanilide.¶ The results from such a study are displayed in the Figure as binding dissociation constants K_I (the lower the value of K_I 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 phenylalaninal unit of all the inhibitors constitutes the P_1 residue and always binds to the S_1 subsite. The relatively low K_I values obtained for the analogues (**6a**), (**6b**), (**9d**), and (**9e**) then suggest that the S_2 subsite may favourably accommodate a diverse nature of apolar side chains; for example, the CH_3–$CH<$ side chain of (**9d**) and (**9e**), C_6H_5–CH_2O– of (**6a**), and $(CH_3)_3$–C–O– of (**6b**) are all tolerated well at the S_2 subsite. The results showing the broad specificity of S_2 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 S_2 'hole' accommodating the P_2 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 S_2 subsite with the P_2 side chain the S_3 subsite is much more discriminating with respect to P_3 side chains. The acetyl side chain as in (**9d**) and trifluoroacetyl as in (**9e**) interact favourably with S_3 while the corresponding analogue (**9a**) containing the t-butyloxy-group showed considerably reduced binding. Thus the 'short and fat' t-butyloxy-group at the P_3 residue must interact with chymotrypsin so adversely as to wipe out the combined P_1–S_1 + P_2–S_2 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 P_3 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 P_3 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 S_3 subsite surrounding the C_α and C_β atoms of the P_3 residue.

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

¶ All the enzymic assays were carried out in the presence of 7·5% dimethyl sulphoxide.

[1] (a) A. Ito, K. Tokawa, and B. Shimizu, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 343; (b) R. C. Thompson, *Biochemistry*, 1973, **12**, 47 and references cited therein; (c) E. J. Breaux and M. L. Bender, *FEBS Letters*, 1975, **56**, 81; (d) W. P. Kennedy and R. M. Schultz, *Biochemistry*, 1979, **18**, 349; (e) G. D. Brayer, L. T. J. Delbaere, M. N. G. James, C. A. Bauer, and R. C. Thompson, *Proc. Nat. Acad. Sci., U.S.A.*, 1979, **76**, 96, and references cited therein.

[2] H. Seki, K. Koga, and S. Yamada, *Chem. Pharm. Bull. (Japan)*, 1972, **20**, 361; H. Saeki, Y. Shimada, N. Kawakita, B. Shimizu, E. Ohki, K. Maeda, and H. Umezawa, *ibid.*, 1973, **21**, 163; A. Ito, R. Takahashi, and Y. Baba, *ibid.*, 1975, **23**, 3081; B. Shimizu, A. Saito, A. Ito, K. Tokawa, K. Maeda, and H. Umezawa, *J. Antibiotics*, 1972, **25**, 515; H. Khatri and C. H. Stammer, *J.C.S. Chem. Comm.*, 1979, 79; S. Bajusz, E. Barabas, P. Tolnay, E. Szell, and D. Bagdy, *Internat. J. Peptide Protein Res.*, 1978, **12**, 217.

[3] P. L. Birch, H. A. El-Obeid, and M. Akhtar, *Arch. Biochem. Biophys.*, 1972, **148**, 447; C. G. Rasool, S. Nicolaidis, and M. Akhtar, *Biochem. J.*, 1976, **157**, 675.

[4] D. M. Blow, J. J. Birktoft, and B. S. Hartley, *Nature*, 1969, **221**, 337.

[5] G. Lowe and D. Nurse, *J.C.S. Chem. Comm.*, 1977, 815, and references cited therein.

[6] D. M. Segal, G. H. Cohen, D. R. Davies, J. C. Powers, and P. E. Wilcox, *Cold Spring Harbor Symposia on Quantitative Biology*, 1971, **36**, 85.

[7] I. Schechter and A. Berger, *Biochem. Biophys. Res. Comm.*, 1967, **27**, 157.