Synthesis of (1*S*)-*N*-(1-carboxy-5-aminopentyl)-glycylglycine – a prospective competitive inhibitor for angiotensin-converting enzyme

Mario R.W. Ehlers and Ralph E. Kirsch
MRC Liver Research Group, Department of Medicine, University of Cape Town Medical School, Observatory

Robin G.F. Giles and Selwyn C. Yorke
Department of Organic Chemistry, School of Chemical Sciences, University of Cape Town, Rondebosch

The synthesis of a prospective competitive inhibitor of angiotensin-converting enzyme, (1*S*)-*N*-(1-carboxy-5-aminopentyl)glycylglycine (**4**), was accomplished in four steps in 72% overall yield starting from glycine. Hydrogenolysis of the benzyl and benzyloxycarbonyl protective groups in the last step was enhanced by the use of ultrasound.

S. Afr. J. Chem., 1986, 39, 134—136

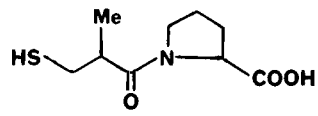
Die sintese van 'n waarskynlike kompeterende inhibeerder van angiotensien-omskakelingsensiem (1*S*)-*N*-(1-karboksi-5-aminopentiel)glisielglisien (**4**) is in vier stappe bewerkstellig met glisien as uitgangstof, in 'n totale opbrengs van 72%. Hidrogenolise van die bensiel- en bensieloksikarboniel-beskermende groepe is in die laaste stap vergemaklik deur gebruik te maak van ultraklank.

S.-Afr. Tydskr. Chem., 1986, 39, 134—136

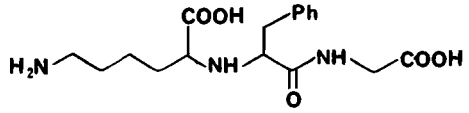
Angiotensin-converting enzyme (ACE) is a multifunctional enzyme which plays an important role in blood pressure regulation by catalysing the cleavage of dipeptide residues from the COOH terminus of peptide substrates.[1,2] Human ACE has also been implicated in a number of diseases, and thus it has aroused a great deal of interest in pathology.[3]

Studies on human ACE are incomplete, partly because purification has hitherto been difficult to achieve. However, oligopeptides from the venom of the snake *Bothrops jararaca* were noted to inhibit ACE.[4] Accordingly, a number of amino acid and peptide derivatives analogous to the venom component, which are also competitive inhibitors of ACE, have been synthesized[5,6] and are now in clinical use. [For example, D-3-mercapto-2-methylpropanoyl-L-proline (captopril) (**1**)[7] is very useful in the management of hypertension.[8]]

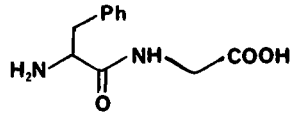
A competitive inhibitor that binds reversibly to human ACE could also be used for purification of human ACE. Such inhibitors have already been synthesized and have proved useful as solid-phase ligands in affinity chromatographic purification of ACE.[9,10] The ligand (1*S*)-*N*-(1-carboxy-5-aminopentyl)-L-phenylalanylglycine (CA–Phe–Gly) (**2**), which is claimed to possess the important properties of high specificity and capacity for human ACE, coupled with ready reversibility, has recently been reported,[11] although no chemical data were presented to support the assigned structure. The ligand CA–Phe–Gly (**2**) is essentially the dipeptide Phe–Gly (**3**) with a five-carbon spacer arm



1



2



3

M.R.W. Ehlers and R.E. Kirsch
MRC Liver Research Group,
Department of Medicine, UCT Medical School,
Observatory 7925, Republic of South Africa

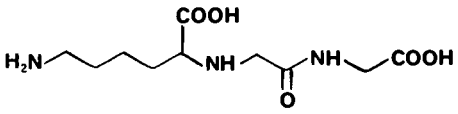
R.G.F. Giles* and S.C. Yorke
Department of Organic Chemistry,
School of Chemical Sciences, University of Cape Town,
Rondebosch 7700, Republic of South Africa

*To whom correspondence should be addressed

Received 22 November 1985

and terminal amine available for coupling to a polymer support.

As we were unable to repeat the synthesis of CA–Phe–Gly (**2**) by the method described (confirmed by the Harvard group[12]) and, in any event, as it was considered desirable to prepare an alternative competitive inhibitor of human ACE, a new ligand, (1*S*)-*N*-(1-carboxy-5-aminopentyl)-glycylglycine (CA–Gly–Gly) (**4**), the subject of this paper, was designed and synthesized. It is considered that CA–Gly–Gly (**4**) will prove to be an effective affinity chromatography ligand and therefore play an important role in the purification of human ACE.



4

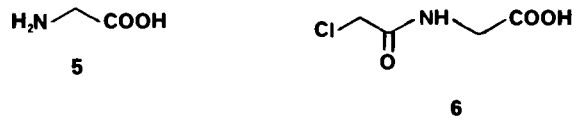
Excluding the protection and deprotection of functional groups, the synthesis of CA–Gly–Gly (**4**) is essentially a two-step process; however, as with all syntheses of peptides, careful choice of *N*- and *O*-protective groups can greatly simplify the total synthesis. The benzyl group was useful in this case as it is conveniently non-polar, but especially it was envisaged that both the benzyl ester groups and the benzyloxycarbonyl group could be removed simultaneously by hydrogenolysis.

Hydrogenolysis was deemed to be a useful method that avoided the need for saponification of non-benzyl ester protective groups as a separate step. Saponification has the attendant problems of low yield, base-induced racemization at the chiral carbon and difficult purification, as is the case with other esters.[13]

Discussion

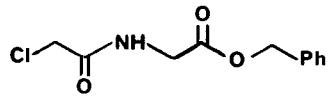
Glycine (**5**) in 4M-sodium hydroxide was treated with chloroacetic anhydride[14] to form an intractable oily–crystalline mixture of chloroacetylglycine (**6**) and chloroacetic acid. As both compounds possessed the same t.l.c. R_f values, isolation of the acid (**6**) was not attempted as purification was simplified by protection of the carboxyl group. Esterification of the mixed acids in benzene with benzyl alcohol, assisted by azeotropic removal of water, afforded a mixture from which the requisite ester (**7**) was isolated by chromatography, in 86% yield from glycine.

Nucleophilic substitution of chlorine in compound (**7**) by the α-amino group of (2*S*)-*N*-ε-benzyloxycarbonyl-2-lysine benzyl ester (**8**) was achieved by boiling for 18 h under reflux in toluene with one mol equivalent of triethylamine. The secondary amine was formed cleanly, and chromatographic purification afforded the product (**9**) in 84% yield. The i.r. spectrum was diagnostic, as ester, carbamate, and amide carbonyl absorptions were observed (ν_{max} 1727, 1705, and 1676 and 1529 cm^{-1} respectively). Also, the ^1H n.m.r. spectrum of the amide (**9**) shows *inter alia*, the diastereotopic nature of the two methylene groups of the glycylglycine moiety of the chiral molecule; those adjacent to the amine nitrogen appear as a pair of doublets (J 17,3 Hz) at δ 3,01 and 3,44, whereas those adjacent to the amide nitrogen appear as a pair of doublets of doublets at δ 3,90 (J_{gem} 18,5 Hz, J_{HNCH} 5,0 Hz) and 4,18 (J_{gem} 18,5 Hz, J_{HNCH} 5,9 Hz).

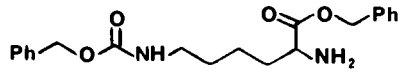


5

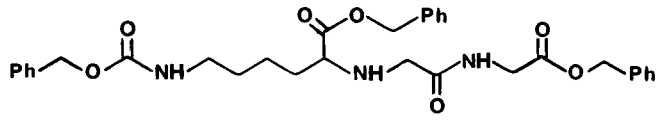
6



7



8



9

The final step of the synthesis entailed removal of the protective groups. Although several methods of hydrogenolysis of the benzyloxycarbonyl and benzyl ester groups were attempted, the preferred technique was by a modified catalytic transfer hydrogenation. The reported method[15] was improved by the use of ultrasound, which encouraged cleavage using much less catalyst for a similar reaction time. Thus, compound (**9**), dissolved in formic acid and methanol under sonication with 10% palladium on carbon, was cleanly and quantitatively deprotected after 1 h to afford the title compound (**4**), which was isolated as the zwitterion simply by crystallization.

In summary, a concise and efficient synthesis of CA–Gly–Gly (**4**) was completed in four steps in 72% overall yield.

Experimental

All ^1H n.m.r. spectra were recorded for solutions in [^2H]-chloroform with tetramethylsilane as internal reference unless otherwise stated, and i.r. spectra were recorded for Nujol mulls. Column chromatography refers to dry-packed columns using Merck Kieselgel 60 (70—230 mesh), and thin-layer chromatography (t.l.c.) was performed on plates coated with Merck Kieselgel 60 F_{254}. Sonication was performed in a Decon FS 100 ultrasonic bath with a water medium. Light petroleum refers to the fraction of b.p. 60—80°C. The phrase 'residue upon work-up' refers to the residue when the organic layer was separated, dried ($MgSO_4$), and the solvent evaporated under reduced pressure.

Benzyl chloroacetylglycinate (7)

Chloroacetic anhydride (18,20 g) and 4M-sodium hydroxide (27 ml) were added alternately in small portions over 15 min to a stirred, ice-cold solution of glycine (4,00 g) in 4M-sodium hydroxide (13 ml). The mixture was allowed to stir a further 15 min over ice, at ambient temperature for 30 min, and finally at 40°C for 30 min. The reaction was quenched by the addition of 10M-hydrochloric acid until pH 1 was reached, then saturated with solid sodium chloride and extracted with ethyl acetate (8 × 100 ml). The residue obtained upon work-

up was an intractable semi-crystalline oil (22,30 g).

The crude product was not purified but was immediately dissolved in benzene (210 ml) to which benzyl alcohol (22,73 g) and toluene-*p*-sulphonic acid (2,13 g) had been added. The mixture was heated at reflux for 3 h in a Dean–Stark apparatus. The solution was then cooled to ambient temperature and washed with aqueous 5% sodium hydrogencarbonate (2 × 100 ml). The residue obtained upon work-up was chromatographed (eluent, 20—50% ethyl acetate in light petroleum) to afford the *product* (11,02 g; 86%) as colourless needles, m.p., 76—77°C (from hexane–dichloromethane); ν_{max} 3388sh (NH), 1727 (ester CO), and 1676 and 1526 (amide CO) cm^{-1}; δ 4,03 (2H, s, CH_2Cl), 4,07 (2H, d, *J* 4 Hz, NCH_2), 5,17 (2H, s, CH_2Ph), 7,27 (1H, br. s, NH), and 7,38 (5H, s, C_6H_5) (Found: C, 54,7; H, 4,9; N, 5,9. Calc. for $C_{11}H_{12}ClNO_3$: C, 54,7; H, 5,0; N, 5,8%).

Benzyl (1S)-5-(N-*benzyloxycarbonylamino*)-1-(*benzyloxycarbonyl*)*pentylglycylglycinate* (**9**)

(2*S*)-*N*-ε-Benzyloxycarbonyl-2-lysine benzyl ester hydrochloride (8HCl) (Sigma) (6,74 g) was dissolved in a warm solution of triethylamine (1,70 g; 1 mol equiv.) in anhydrous methanol (20 ml). The methanol was removed by evaporation under reduced pressure and toluene (100 ml) was added, followed by chloroacetylglycine benzyl ester (**7**) (4,00 g; 1 mol equiv.) followed by more triethylamine (1,70 g). The solution was refluxed in a Dean–Stark condenser. After 18 h, the toluene was removed under reduced pressure, and saturated brine was added to the residual oil. The solution was partitioned with ethyl acetate (3 × 200 ml) and the residue obtained upon work-up was chromatographed (eluent, 60—70% ethyl acetate in light petroleum) to afford starting materials and the *product* [5,16 g; 67%, or 84% based on unrecovered amine (**8**)] as a viscous oil; $[\alpha]_D$ −25,8° (*c* 1,0; $CHCl_3$); ν_{max} 3325br (NH), 1727 (ester CO), 1705 (carbamate CO), and 1676 and 1529 (amide CO) cm^{-1}; δ 1,48 [6H, m, $NHCH_2(CH_2)_3$], 1,90 (1H, s, D_2O exchangeable, amine NH), 3,01 and 3,44 (each 1H, d, *J* 17,3 Hz, amine $NHCH_2$), 3,21 (3H, m, carbamate NCH_2, chiral *CH*), 3,90 (1H, dd, *J* 5,0 and 18,5 Hz, collapsed to d, *J* 18,5 Hz, upon D_2O exchange, amide $NHCH_2$), 4,18 (1H, dd *J* 5,9 and 18,5 Hz, collapsed to d, *J* 18,5 Hz, upon D_2O exchange, amide $NHCH_2$), 5,0br (1H, D_2O exchangeable, carbamate NH), 5,08 (2H, s, CH_2Ph), 5,13 (4H, s, 2 × CH_2Ph), 7,30 (15H, m, 3 × C_6H_5), and 7,71br (1H, t, D_2O exchangeable, amide NH) (Found: C, 66,5; H, 6,5; N, 7,3. Calc. for $C_{32}H_{37}N_3O_7$: C, 66,8; H, 6,4; N, 7,3%).

(1S)-N-(1-*Carboxy*-5-*aminopentyl*)*glycylglycine* (**4**)

Palladium on carbon (10%; 0,32 g) was added to a solution of compound (**9**) (1,28 g) in 90% formic acid (6 ml) and methanol (89 ml), and the mixture was sonicated for 1 h, by which time the reaction was complete. The catalyst was removed by filtration through a bed of Celite and washed with methanol (50 ml) and water (100 ml). The filtrates were combined and the solvent was removed by evaporation under low pressure. Toluene was added and the residual moisture and formic acid were removed by azeotropic distillation, to leave a viscous, colourless oil. Addition of methanol to the oil promoted crystallization. Recrystallization afforded the *product* as white 'snow' flakes in quantitative yield (640 mg), m.p. 265°C (water–ethanol); $[\alpha]_D$ = +8,9° (*c* 1,0; H_2O); ν_{max} 3357 (NH), 3200—2200br (NH^+ and CO_2H), 1661, 1633, 1603, 1569, and 1523 (amide I and II and CO_2^- region) cm^{-1}; δ (D_2O) 1,60 (4H, m), 1,90 (2H, m), 3,00 (2H, t), 3,70 (1H, m), 3,79 (2H, s,) and 3,91 (2H, s) (Found: C, 45,1; H, 7,2; N, 15,9; Calc. for $C_{10}H_{19}N_3O_5.0{,}25H_2O$: C, 45,2; H, 7,4; N, 15,8%).

Other methods of hydrogenolysis that were attempted included: hydrogen in the presence of 10% palladium on carbon at 1 or 2 atmospheres pressure, 40% hydrobromic acid in glacial acetic acid, and catalytic transfer hydrogenation with vigorous stirring[15] (which requires 200 weight % of 10% palladium on carbon) instead of sonication.

Added in proof

CA–Gly–Gly has proved to be an excellent competitive inhibitor of human ACE.[13(a)]

Acknowledgements

Financial support from the Council of the University of Cape Town, the Council for Scientific and Industrial Research, and the Medical Research Council is gratefully acknowledged.

References

1 R.L. Soffer, *Annu. Rev. Biochem.*, 1976, **45**, 73.
2 K.K.F. Ng and J.R. Vane, *Nature (London)*, 1967, **215**, 762; K.K.F. Ng and J.R. Vane, *Nature (London)*, 1968, **218**, 144.
3 P.R. Studdy, R. Lapworth, and R. Bird, *J. Clin. Pathol.*, 1983, **36**, 938.
4 Y.S. Bakhle, *Nature (London)*, 1968, **220**, 919.
5 D.W. Cushman, J. Pluscek, and N.J. Williams, *Experentia*, 1973, **29**, 1032.
6 H.S. Cheung and D.W. Cushman, *Biochem. Biophys. Acta.*, 1973, **293**, 451.
7 D.W. Cushman, H.S. Cheung, E.F. Sabo, and M.A. Ondetti, *Biochemistry*, 1977, **16**, 25, 5484.
8 A.B. Atkinson and J.I.S. Robertson, *Lancet*, 1979, 836.
9 H.A. El-Dorry, H.G. Bull, K. Iwata, N. Thornberry, E. Cordes, and R.L. Soffer, *J. Biol. Chem.*, 1982, **257**, 14 128.
10 R.B. Harris, J.T. Ohlsson, and I.B. Wilson, *Anal. Biochem.*, 1981, **3**, 227.
11 M.W. Pantoliano, B. Holmquist, and J.F. Riordan, *Biochemistry*, 1984, **23**, 1037.
12 J.F. Riordan, personal communication.
13 (a) M.R.W. Ehlers, unpublished results; (b) K.D. Kopple, 'Peptides and Amino Acids', Benjamin, New York, 1966, ch.3.
14 S.M. Birnbaum, L. Levintow, R.B. Kingsley, and J.P. Greenstein, *J. Biol. Chem.*, 1952, **194**, 455.
15 B. ElAmin, G.M. Anatharamaiah, G.P. Roger, and G.E. Means, *J. Org. Chem.*, 1979, **44**, 3442.