Tetrahedron Letters,Vol.29,No.47,pp 6109-6112,1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Press plc

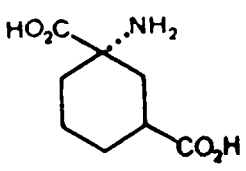
CHEMOENZYMATIC SYNTHESIS OF CONFORMATIONALLY RIGID GLUTAMIC ACID ANALOGUES[1]

F. Trigalo, D. Buisson and R. Azerad*

Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques associé au CNRS, Université R. Descartes, 45, rue des Saints-Pères, 75270- Paris Cedex 06, France

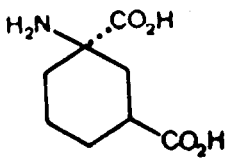
Abstract: All stereomers of cyclohexane and cyclopentane-derived analogues of glutamic acid have been synthesized from the corresponding 3-keto-cycloalkyl carboxylic acid esters by a combination of microbial steps and standard chemical methods.

Unnatural amino acid analogues begin to be introduced into particular peptides in place of the corresponding amino acid residues in order to be used as stereochemical and structural probes of selective interactions with physiologically active proteins (enzymes or receptors). In a study of rat liver vitamin K-dependent carboxylase, several peptides containing some gamma-substituted analogues of glutamic acid in an adequate position were found to be strong competitive inhibitors of the carboxylation[2-3] and associated reactions[4]. However, no obvious correlation with their stereochemical structure could be found, as no data about the conformation of the Glu side chain and its interaction with the carboxylase active site was available. Some years ago, a similar problem was encountered about the glutamine synthetase mechanism and partly resolved through the use of conformationally rigid cycloglutamic acid substrates[5]. We have thus undertaken the synthesis of the eight geometrical and optical isomers of the glutamic acid cyclic analogues **1-4**, in order to incorporate them into peptidic substrates or inhibitors of the carboxylase.



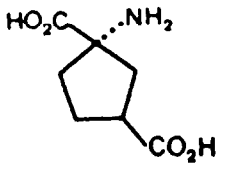
cis

1



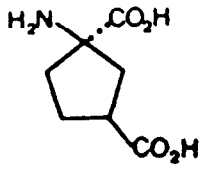
trans

2



cis

3

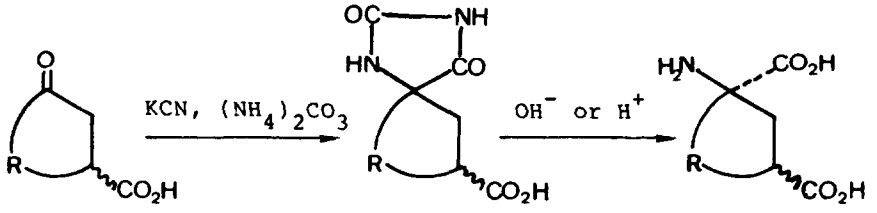


trans

4

Racemic cyclic amino acids **1-4** were easily prepared from the corresponding racemic 3-carboxycyclanones **6a-b** via a Bucherer-Berg reaction with KCN and ammonium carbonate[5-7] (Scheme 1). The resulting spirohydantoins **7** (65-70 % yield) were hydrolyzed either in alkaline (excess barium oxide in water, 140°C) or acidic (60% H_2SO_4, 140°C) conditions. The alkaline hydrolysis of the mixture of diastereomeric hydantoins **7a** led to a 9:1 mixture of the cis/trans cycloglutamic acids **1-2**. The acidic hydrolysis of the same hydantoins led to a

3:7 mixture, as a result of a simultaneous epimerisation at the carboxyl-bearing carbon atom: an identical result was obtained by acidic treatment of separated cis or trans cyclic amino acids. Isomeric spirohydantoins **7b**, obtained in a 1:1 ratio, could be separated by fractional crystallization in water; their alkaline hydrolysis gave either cis or trans cycloglutamic acids **3-4**. Diastereomeric five or six-membered ring cycloglutamic acids were most easily separated by ion exchange chromatography on a Dowex AG-1X4 (AcO^- form) column, eluted with 0.25 to 0.5 M AcOH. The relative configuration of both asymmetric centers was established by comparison with available data from the literature[5], formation of anhydrides from the cis isomers, or crystallographic analysis of the trans hydantoin **7b**[8].



6 a: R= $-(CH_2)_3-$
b: R= $-(CH_2)_2-$

7

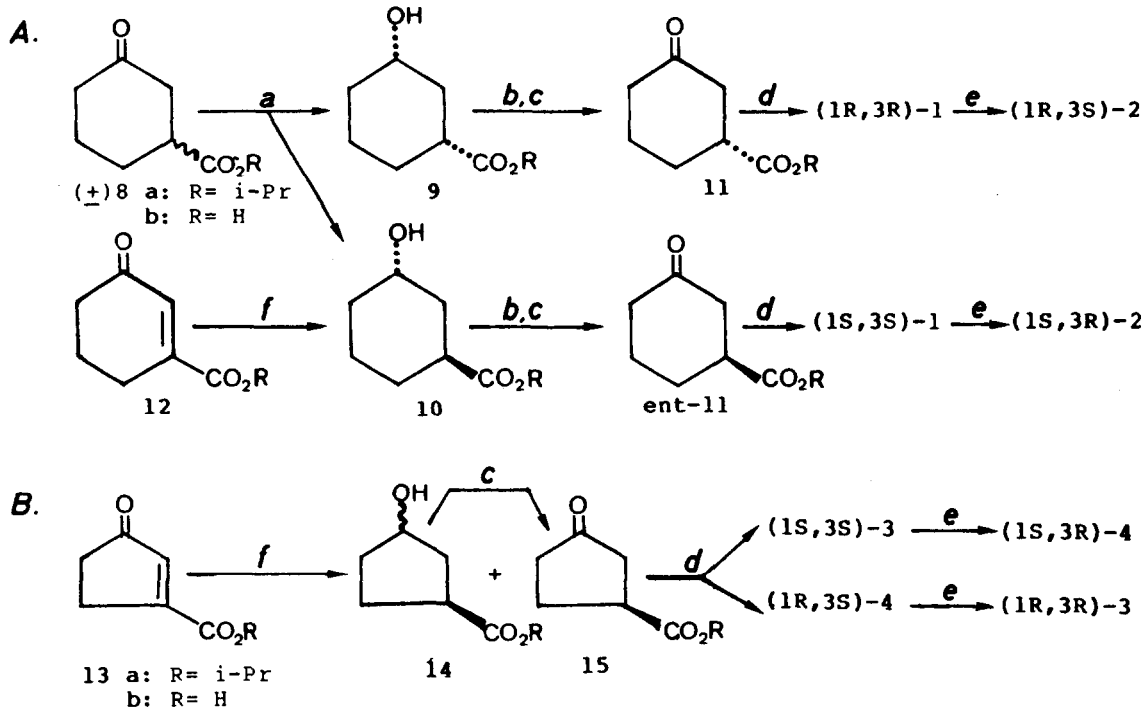
Scheme 1

Resolution of the racemic cycloglutamic acids was not preparatively feasible by usual enzymatic methods, like hydrolysis of various derivatives by aminoacylase[9] or any other peptidase, presumably because of the presence of an alpha-disubstituted carbon atom. An asymmetric synthesis of each cycloglutamic acid was thus undertaken, starting from optically pure (or enriched) 3-carboxycyclanones, and using the same spirohydantoin synthesis.

The preparation of enantiomeric 3-carboxycyclohexanones could be effected by two methods: i) resolution of an ester of the racemic ketoacid[5,10,11] **8b** (Scheme 2A) through a highly stereospecific microbiological reduction catalyzed by a washed mycelium of Rhizopus arrhizus , followed by diastereomer separation by silicagel chromatography and alkaline hydrolysis to optically pure enantiomeric hydroxyacids **9-10**[12]: (1S,3R)-cis, mp. 129-130° (AcOEt-ether), $[\alpha]_D^{21}$ -10° (c 1.1,MeOH) (lit.[15]: $[\alpha]_D^{21}$ -9.6°, c 5.2,MeOH); (1S,3S)-trans, mp. 102-3°, $[\alpha]_D^{21}$ +12° (c 1.3,MeOH). The hydroxyacids were easily oxidized with Jones' reagent to the known enantiomeric ketoacids **11b**[15]. Both enantiomers were obtained by such a method, but the yield of the (S)-ketoacid was lower, owing to a selective consumption of the trans-alcohol by the mould during the reduction step; ii) asymmetric microbiological reduction of the 3-carboxy-2-cyclohexenone ester **12a** by Geotrichum candidum, leading to a single optically pure (1S,3S)-hydroxyester **10a**[16], followed by oxidation to the (S)-ketoester **ent-11a** (Scheme 2A).

Similar methods were applied to the synthesis of the corresponding enantiomeric 3-carboxycyclopentanones[17]; starting from an ester of the racemic ketoacid **6b**[18], the former method failed to effect the desired resolution,

leading to nearly racemic cis and trans hydroxyesters. On the other hand, use of the latter method, starting from 3-carboxy-2-cyclopentenone[19] isopropyl ester **13a**, afforded[20] highly enriched (85%-90% ee[21]) (S)-**15b**, mp. 64-65° (AcOEt- hexane), $[\alpha]_D^{21}$ -21° (c 0.75,MeOH) (lit.[22,23]: mp.60-61, 63-65°; $[\alpha]_D^{21}$ +22.2°, MeOH; $[\alpha]_D^{20}$ -17.5°, c 2, MeOH) (Scheme 2B).



a) reduction by R.arrhizus; silicagel chromatography. b) OH^-. c) CrO_3 oxidation. d) KCN-$(NH_4)_2CO_3$/50°/5 hours; $Ba(OH)_2$/140°/30 min.; ion exchange chromatography.[3] e) 10 N HCl /120°/10 days.[2] f) reduction by G.candidum

Scheme 2

Using the hydantoin reaction, optically pure or enantiomerically-enriched cis and trans cycloglutamic acids diastereomers were prepared from the corresponding enantiomeric ketoesters (or acids) and separated by ion exchange chromatography as described for the racemic compounds; in each case, their 3-epimers could be easily obtained by acidic treatment, followed by ion-exchange chromatography (Scheme 2).

The absolute configuration of all stereomers was determined from the knowledge of the absolute configuration of starting ketones and the relative configuration of both asymmetric centers. The optical purity of each isomer was ultimately determined by a chromatographic method[24].The enantiomeric excess was found to be better than 98% for each of the **1** and **2** enantiomers, and about 85% for **3-4**; however, in the latter case, a single crystallization of the intermediate ketoacid **15b** allowed the recovery of optically pure compounds.

Work is in progress to effect the selective protection of the cyclic amino acids **1-4**, in order to incorporate them into synthetic active peptides.

REFERENCES

1- Part of this report was presented at the 2nd Peptide Forum, May 1988, Nancy (France).
2- M.Gaudry, S.Bory, J.Dubois, R.Azerad and A.Marquet, Biochem.Biophys.Res.Commun., (1983) **113**, 454.
3- E.Guibé, P.Decottignies-Le Maréchal, P.Le Maréchal and R.Azerad, FEBS Lett., (1984) **177**, 265.
4- C.Ducrocq, A.Rhighini-Tapie, R.Azerad, J.F.Green, P.A.Friedman, J-P.Beaucourt and B.Rousseau, J.Chem.Soc.Perkin Trans.I, (1986) 1323.
5- J.D.Gass and A.Meister, Biochemistry, (1970) **9**, 842; R.A.Stephani, W.B.Rowe, J.D.Gass and A.Meister, Biochemistry, (1972) **11**, 4094.
6- L.Munday, J.Chem.Soc., (1961) 4372.
7- H.C.Brimelow, H.C.Carrington, C.H.Vasey and W.S.Waring, J.Chem.Soc., (1962) 2789.
8- We are indebted to Dr.C.Pascard, Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France, for X-ray diffraction data and their interpretation.
9- S.C.J.Fu and S.M.Birnbaum, J.Am.Chem.Soc., (1953) **75**, 918.
10- M.E.C.Biffin, A.G.Moritz and D.B.Paul, Aust.J.Chem., (1972) **25**, 1329.
11- A.J.Birch, P.Hextall, S.Sternhell, Aust.J.Chem,(1954) **7**, 256.
12- R.arrhizus ATCC 11145, grown for 48 hours in 1 l. of a standard medium (see ref.13) was filtered, washed and resuspended in water (0.5 l.). The isopropyl ester of the ketoacid (2-3 g) was added and incubated at 27°C with gentle shaking until complete reduction (36-48 hours). After filtration, the same mycelium could be repeatedly used in the same conditions. Pooled filtrates and washing, saturated with NaCl, were extracted with ether to afford the crude hydroxyester which was submitted to medium pressure chromatography on silicagel H60 (200 g/ 5 g of hydroxyester) (60% total yield, trans/cis 4:6); the trans product was eluted before the cis isomer (relative configurations attributed by ^{1}H and ^{13}C-NMR). Both hydroxyesters were nearly optically pure (>98% ee), as shown by chiral GC of their isopropyl carbamates (see ref.14). (1S,3R)-cis: $[\alpha]_D^{21}$ -5° (c 2,MeOH); (1S,3S)-trans: $[\alpha]_D^{21}$ +10° (c 1.4,MeOH).
13- D.Buisson and R.Azerad, Tetrahedron Lett., (1986) **27**, 2631.
14- W.A.König, I.Benecke and S.Sievers, J.Chromatogr., (1982) **238**, 427.
15- R.E.Helmchen-Zeier, Dipl.Ing-Chem.ETH, Zürich (1973) p.59.
16- G.candidum (local strain) grown in 1 liter of standard medium (see ref.13) for 48 hours was filtered, washed, resuspended in 1 l. of 0.02 M K phosphate buffer pH 7.0 and used repeatedly for the reduction of 1.5-2 g portions of isopropyl ester (total amount: 5.5 g) with occasional addition of glucose (5g). Extraction of filtrates and washing afforded 5 g of crude product mainly consisting of optically pure (1S,3S)-trans-hydroxyester with 10% unsaturated hydroxyester. Oxidation by Jones' reagent and silicagel chromatography gave 3.8 g of the (S)-ketoester (75% yield).
17- After submission of this paper, we have been aware of a report (K.Curry, M.J.Peet, D.S.K.Magnuson and H.McLennan, J.Med.Chem. (1988) **31**, 864) describing a similar preparation of isomeric 1-amino-1,3-cyclopentanedicarboxylic acids from enantiomeric 3-carboxycyclopentanones obtained by chemical resolution. No yields were given for these syntheses.
18- A.P.Arendaruk, E.I.Budovski, B.P.Gootikh, A.P.Skoldinov, N.V.Smirnova, A.Y.Khorlin and N.K.Kochetkov, Zhur.Obshchei.Khim., (1957) **27**, 1312, 1318; C.A., (1958) **52**, 4508d.
19- W.C.Agosta and W.W.Lowrance Jr., J.Org.Chem., (1970) **35**, 3851.
20- The reduction by G.candidum (see ref.16) afforded after chromatographic separation the (S)-ketoester ($[\alpha]_D^{21}$ -13.5°,c 1.7,acetone) and two diastereoisomeric hydroxyesters ($[\alpha]_D^{21}$ +6°,c 1.8,$CHCl_3$ and +7°, c 0.9, acetone) with a (3S) configuration, as shown by oxidation to the same (S)-kétoester ($[\alpha]_D^{21}$ -12°,c 0.8,acetone and -11°, c 1,acetone, respectively). Total yield: 60%.
21- determined (see ref.24) after conversion to cyclic amino acids **3-4**.
22- K.Toki, Bull.Chem.Soc.Jpn, (1958) **31**, 333.
23- R.D.Allan, G.A.R.Johnston and B.Twitchin, Aust.J.Chem., (1979) **32**, 2517.
24- M.Maurs, F.Trigalo and R.Azerad, J.Chromatogr., (1988) **440**, 209 .

(Received in France 3 August 1988)