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INCORPORATION OF CONFORMATIONALLY CONSTRAINED PHENYLALANINE DERIVATIVES TIC, SIC, HIC AND NIC INTO A CHOLECYSTOKININ-B/GASTRIN RECEPTOR ANTAGONIST

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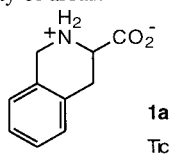
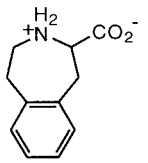
Abstract: The preparation and biological properties of a conformationally constrained series of cholecystokinin-B/gastrin receptor ligands are described. The 9-membered ring (Nic) derivative was found to be as active at these receptors as an unconstrained phenylalanine analogue. © 1997 Elsevier Science Ltd.

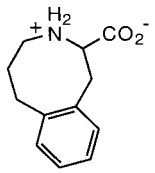
The use of conformationally constrained amino acids to explore the side-chain conformational preferences of bioactive molecules in binding to their active sites is a commonly used approach to the design of highly selective and active compounds of potential medicinal interest.[1] Focussing on phenylalanine, the synthesis and use of several conformationally constrained analogues of this amino acid have been reported including 3-phenylproline,[2] β,β-diphenylalanine,[3] α,β-dimethylphenylalanine,[4] β-methylphenylalanine[5] and 1,2,3,4-tetraisoquinoline-3-carboxylic acid (Tic) **1a**.[6] Such is the interest in the latter compound that analogues of Tic including α,β-dimethyl-Tic,[4] and benzo[f]Tic, benzo[g]Tic and benzo[h]Tic[7] are now under investigation in a variety of areas.



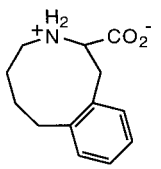
1a
Tic



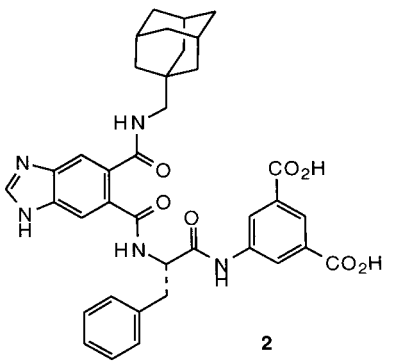
1b
Sic



1c
Hic



1d
Nic



2

We recently synthesised the novel 7-, 8- and 9-membered analogues of Tic, *i.e.* Sic **1b**, Hic **1c** and Nic **1d** in the belief that incorporation of this series of compounds into biologically active peptides or non-peptides would lead to a gradual and controlled change of conformational freedom of the aromatic ring. This should

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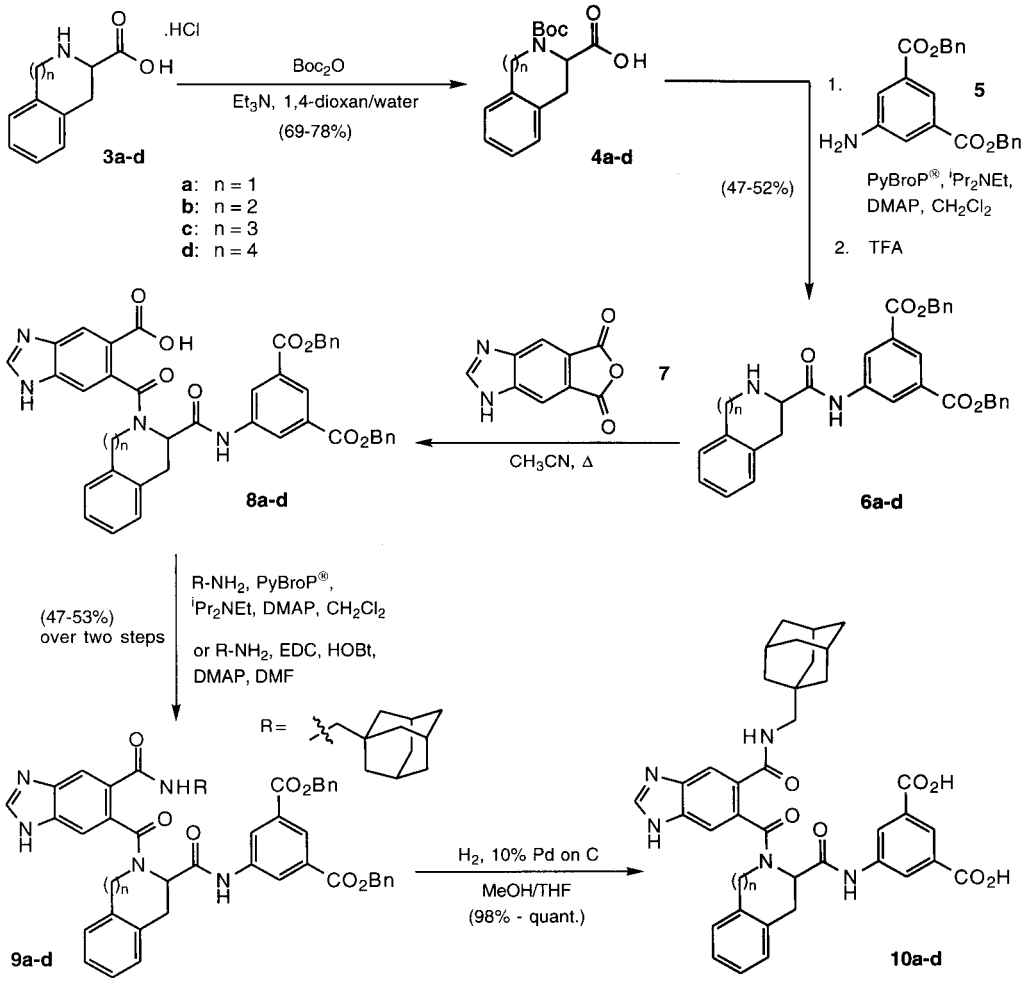
ultimately lead to greater insight into the conformational preferences of the ligand under investigation and the nature of its interaction with the active site.[8] We now wish to report the synthesis of racemic Tic, Sic, Hic and Nic analogues of the recently reported phenylalanine-containing cholecystokinin-B (CCK_B)/gastrin receptor antagonist **2**[9] and the receptor affinity values obtained for this series of compounds. Our study reveals that the the size of the saturated ring is a determining factor for CCK_B/gastrin receptor activity and represents the first application of the conformationally constrained amino acids Sic, Hic and Nic.



The Tic, Sic, Hic and Nic analogues of the CCK_B/gastrin receptor antagonist **2** were synthesised by appropriate modification of the synthetic route developed for **2** itself.[9] *N*-Protection of the hydrochloride salts **3a-d**[8] gave acids **4a-d**, which were coupled with amine **5** using bromotris(pyrrolidine)phosphonium hexafluorophosphate (PyBroP) to give, after deprotection, amides **6a-d**. Reaction of amines **6a-d** with the anhydride **7** gave acids **8a-d**, which were subsequently coupled with 1-adamantanemethylamine using either PyBroP or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC)/1-hydroxybenzotriazole

(HOBt) to give amides **9a-d**. Subsequent hydrogenolysis revealed the diacids **10a-d**, which were characterised by ^{1}H NMR and mass spectroscopy, and by microanalysis of their bis(*N*-methyl-D-glucamine) salts. Thus, compounds **10a-d** represent molecules in which the phenylalanine moiety of compound **2** has been restricted using Tic and the newly available Sic, Hic and Nic synthons.

Affinity estimates for these compounds at CCK_B/gastrin receptors were determined in two assays: the isolated immature rat stomach, a functional bioassay;[10] and mouse cortical homogenate, a radioligand binding assay.[11] This choice was made because the CCK_B/gastrin receptor types have been shown to be homogeneous within each assay, and because L-365,260, a well established reference competitive antagonist,[12] displayed different affinity estimates (rat stomach $pK_B = 7.54 \pm 0.03$;[10] mouse cortex $pK_i = 8.42 \pm 0.03$[11]) between the two assays, indicating that there are different receptors in the two tissues.[10,11] An important characteristic of the parent ligand **2** is its reversed selectivity, relative to L-365,260, in the two assays (**Table**). Relative to compound **2**, the 6-, 7- and 8-membered ring analogues **10a-c**, whilst maintaining the same sense of selectivity, lost affinity in both assays. However, the affinities and selectivity of the 9-membered ring analogue **10d** were indistinguishable from those of compound **2**. From these results it may be suggested that the selectivity ratio is dependent on other parts of these molecules.

Table. Receptor Affinity Values for CCK_B/Gastrin Antagonists

Compound No.[a]	n	Rat stomach[b]	Mouse cortex[c]
2		9.08 ± 0.10[d]	8.28 ± 0.13[e,f]
10a	1	7.64 ± 0.46	6.75 ± 0.10
10b	2	7.66 ± 0.23	6.63 ± 0.14[f]
10c	3	7.93 ± 0.21	6.65 ± 0.10
10d	4	9.13 ± 0.16	8.30 ± 0.02[f]

[a] Compounds **2** and **10b-d** were tested as their bis(*N*-methyl-D-glucamine) salts. [b] $pK_B' \pm$ SEM values were estimated from single shifts of pentagastrin concentration–effect curves in the isolated lumen–perfused immature rat stomach in at least six separate tissues, in which the compounds behaved as surmountable antagonists. [c] $pIC_{50} \pm$ SEM values were estimated from at least three separate competition experiments in which 20 pM [^{125}I]BH-CCK-8S for CCK_B was used to label CCK_B/gastrin binding sites in mouse cortical homogenates. The $pIC_{50} \approx pK_i$ because $(1 + [L]/K_L)$ approximated to unity. [d] Value is taken from reference 13. [e] Value is taken from reference 9. [f] The slope of these competition curves was significantly different from unity. Therefore, a strict comparison of the pIC_{50} values is potentially unreliable.

It can be seen that the size of the restricting ring is of importance in the biological activity of these compounds. It may be that the Nic analogue **10d** confers a favourable disposition of the aromatic ring, comparable to that of the aromatic ring of phenylalanine in compound **2**. However, it is recognised that the transposition of Tic, Sic, Hic and Nic analogues for phenylalanine will affect a number of factors beyond the simple spatial orientation of the aromatic ring, such as the potential for intramolecular hydrogen bonds, which

may be altered by replacing the secondary amide of **2** with a tertiary amide. Further details about the conformational preferences of these compounds will be reported in due course.

In conclusion, we have shown the utility of Tic, Sic, Hic and Nic in constraining a portion of a biologically active, non-peptide ligand and have observed that one compound in particular, the 9-membered ring derivative, **10d**, is better able to interact with CCK_B/gastrin receptors than smaller ring analogues. It is evident that these constrained amino acids have a clear role to play, complementary to established phenylalanine analogues, in providing structural information about ligands and their corresponding sites of action.

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References and Notes

1. See, for example, (a) Toniolo, C. *Int. J. Peptide. Protein Res.* **1990**, *35*, 287; (b) Giannis, A.; Kolter, T.; *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244; (c) Qian, X.; Shenderovich, M.D.; Kover, K.E.; Davis, P.; Horvath, R.; Zalewska, T.; Yamamura, H. I.; Porreca, F.; Hruby, V.J. *J. Am. Chem. Soc.* **1996**, *118*, 7280.
2. Chung, J.Y.L.; Wasicak, J.T.; Arnold, W.A.; May, C.S.; Nadzan, A.M.; Holladay, M.W. *J. Org. Chem.* **1990**, *55*, 270.
3. Hsieh, K.; LaHann, T.R.; Speth, R.C. *J. Med. Chem.* **1989**, *32*, 898.
4. Kazmierski, W.M.; Urbanczyk-Likowska, Z.; Hruby, V.J. *J. Org. Chem.* **1994**, *59*, 1789.
5. Azizeh, B.Y.; Shenderovich, M.D.; Trivedi, D.; Li, G.; Sturm, N.S.; Hruby, V.J. *J. Med. Chem.* **1996**, *39*, 2449.
6. See, for example, (a) Kazmierski, W.M.; Yamamura, H.I.; Hruby, V.J. *J. Am. Chem. Soc.* **1991**, *113*, 2275; (b) Schiller, P.W.; Weltrowska, G.; Nguyen, T.M.D.; Lemieux, C.; Chung, N.N.; Marsden, B.J.; Wilkes, B.C. *J. Med Chem.* **1991**, *34*, 3125; (c) Kyle, D.J.; Martin, J.A.; Burch, R.M.; Carter, J.P.; Lu, S.; Meeker, S.; Prosser, J.C.; Sullivan, J.P.; Togo, J.; Noronha-Blob, L.; Sinsko, J.A.; Walters, R. F.; Whaley, L.W.; Hiner, R.N. *J. Med. Chem.* **1991**, *34*, 2649; (d) Feltou, M.; Robineau, P.; Lonchampt, M.; Bonnardel, E.; Thurieau, C.; Fauchere, J.L.; Widdowson, P.; Mahieu, J.P.; Serkiz, B.; Volland, J.P.; Martin, C.; Naline, E.; Advenier, C.; Prost, J.F.; Canet, E. *J. Pharm. Expt. Therap.* **1995**, *23*, 1071; (e) Wilkes, B.C.; Schiller, P.W. *Biopolymers* **1995**, *37*, 391; (f) Capasso, A.; Guerrini, R.; Balboni, G.; Sorrentino, L.; Temussi, P.; Lazarus, L.H.; Bryant, S.D.; Salvadori, S. *Life Sciences* **1996**, *59*, PL 93.
7. Wang, C.; Mosberg, H.I. *Tetrahedron Lett.* **1995**, *36*, 3623.
8. Gibson (née Thomas), S.E.; Guillo, N.; Middleton, R.J.; Thuilliez, A.; Tozer, M.J. *J. Chem. Soc., Perkin Trans. 1* **1997**, 447.
9. Kalindjian, S.B.; Buck, I.M.; Davies, J.M.R.; Dunstone, D.J.; Hudson, M.L.; Low, C.M.R.; McDonald, I.M.; Pether, M.J.; Steel, K.I.M.; Tozer, M.J.; Vinter, J.G. *J. Med. Chem.* **1996**, *39*, 1806.
10. Roberts, S.P.; Harper, E.A.; Watt, G.F.; Gerskowitch, V.P.; Hull, R.A.D.; Shankley, N.P.; Black, J.W. *Br. J. Pharmacol.* **1996**, *118*, 1779.
11. Harper, E.A.; Roberts, S.P.; Shankley, N.P.; Black, J.W. *Br. J. Pharmacol.* **1996**, *118*, 1717.
12. Bock, M.G.; DiPardo, R.M.; Evans, B.E.; Rittle, K.E.; Whitter, W.L.; Garsky, V.M.; Gilbert, K.F.; Leighton, J.L.; Carson, K.L.; Mellin, E.C.; Veber, D.F.; Chang, R.S.L.; Lotti, V.J.; Freedman, S.B.; Smith, A.J.; Patel, S.; Anderson, P.S.; Freidinger, R.M. *J. Med. Chem.* **1993**, *36*, 4276.
13. Hills, D.M.; Gerskowitch, V.P.; Roberts, S.P.; Welsh, N.J.; Shankley, N.P.; Black, J.W. *Br. J. Pharmacol.* **1996**, *119*, 1401.

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