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A convenient synthesis of *N*-Fmoc-*N*,*N*′-bis-Boc-7-guanyl-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid (Fmoc-*N*,*N*′-bis-Boc-7-guanyl-Tic-OH, GTIC)

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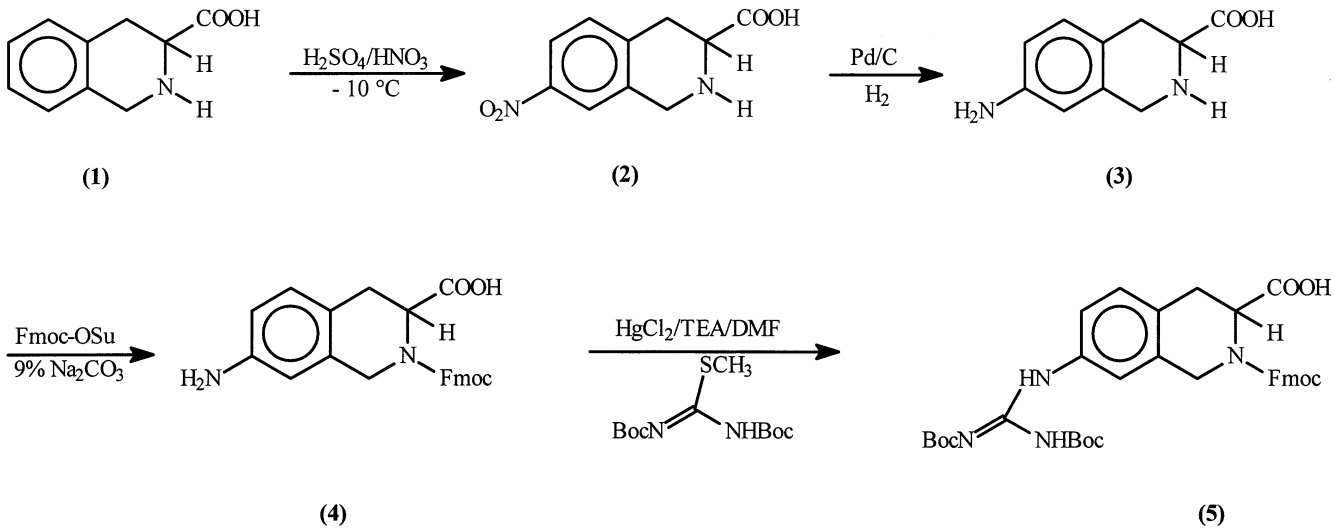
Abstract—Fmoc-*N*,*N*′-Bis-Boc-7-guanyl-Tic-OH (GTIC), a conformationally constrained amino acid with basic properties, has been synthesized in four steps. This amino acid can be incorporated into peptides using standard Fmoc solid phase synthesis, and to test its potential for biological activity applications, we prepared an analog of H-Dmt-Tic-NH_2.

Providing conformational constraints is a major approach to the modification of the chemical and biological properties of endogenous bioactive peptides, hormones and neurotransmitters. Much evidence indicates that such modifications can improve significantly the physical and pharmacological properties of bioactive peptides and peptidomimetics including improvement in their affinities and selectivities for biological receptors/acceptors.[1] Constrained amino acids have been introduced into the sequences of bioactive pep-



Scheme 1. Synthesis of GTIC.

Keywords: unconventional amino acid; synthesis; GTIC.

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tides in order to provide local constraints. Incorporation of these amino acids specifically restricts the rotation of the N^{α}–C^{α}, C^{α}–C(O), C(O)–NH bonds, and side chain conformations by covalent or noncovalent steric interactions. Therefore, the design and synthesis of conformationally constrained amino acids, bringing selective chemical functions into their structure, may provide a unique approach to obtain new insights into the stereochemical, conformational and topographical requirements of peptide ligand–receptor interactions and for signal transduction that are not possible with the 20 genetically coded (natural) residues. The cyclization between N and C^{δ} of Phe and Tyr has led to highly constrained amino acids such as 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) and 7-hydroxy - 1,2,3,4 - tetrahydroisoquinoline - 3 - carboxylic acid (HO–Tic). These structural analogues of the naturally occurring amino acids phenylalanine and tyrosine exhibit a diverse range of effects when introduced into biological systems. In fact, Tic has been incorporated as a phenylalanine replacement in many biologically active peptides (e.g. opioid,[2] substance P,[3] FTase inhibitors,[4] bradykinin,[5] etc.).

In this paper we describe a facile synthesis of Fmoc-*N*,*N*′-bis-Boc-7-guanyl-Tic-OH, (GTIC), a new Tic derivative substituted on the aromatic ring with a basic guanidine group. This amino acid combines the basic features of arginine with the aromatic features of phenylalanine.

The synthetic procedure, summarized in Scheme 1, started from commercially available L-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid (Tic, **1**) which was treated with fuming nitric acid and concentrated sulfuric acid at −10°C to obtain the 7-nitro derivative (**2**) in a mixture with the corresponding 6-nitro isomer in a yield of over 90%.[6] The direct nitration was very regioselective, giving the 7-nitro-Tic isomer in over 90% yield. The position of the nitro group was established using NMR methods.

Reduction of the nitro compound to the amine (**3**) was straightforward (yield 98%), although the final product had to be protected from air to prevent oxidation.[7] Next, the Fmoc- protection on the α-amino group of the 7-amino Tic-OH (**4**),[8] was introduced by treatment with Fmoc–OSu in aqueous sodium carbonate. Under these conditions, the amino group on the ring did not react. The yield of this procedure was modest (63%), presumably because of competition between the protection reaction and breakdown of the Fmoc- group by the secondary amine followed by trapping of the amine by dibenzofulvene.[9] The amino group was converted into the corresponding guanidine moiety (**5**) by reaction with *N*,*N*′-bis-Boc-*S*-methyl-isothiourea, $HgCl_2$ and TEA in DMF.[10] The product was purified using silica gel chromatography and diethyl ether/hexane (yield 58%).[11] All new compounds were characterized by ^1H NMR and MS.

Recently, we reported that the di- and tripeptides of the structure Tyr/Dmt-Tic-R (R = H, NH_2, Ala) were endowed with potent and selective δ opioid antagonist activity.[2c] We were interested to test if a guanidino function in the 7-position of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) could modify the opioid receptor occupation and activation. We therefore prepared the dipeptide, H-Dmt-GTIC-NH_2[12] and tested it for μ and δ receptor binding.[2c]

The result of the binding assay showed that GTIC instead of Tic drastically modified the receptor affinity and selectivity. The affinity to the δ opioid receptor (Ki, nM = 26.0) is 213-fold lower than the reference compound H-Dmt-Tic-NH_2,[2c] while binding to the μ site (Ki, nM = 10.8) is 26-fold better than the same reference compound.

This result means that a guanidino function, as a substituent in the 7 position of the aromatic ring of Tic, induces a change of receptor affinity and selectivity.

In conclusion the Fmoc-*N*,*N*′-bis-Boc-7-guanyl-Tic-OH (GTIC), a conformationally constrained amino acid, will serve as a useful tool in peptide-based structure–activity studies.

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6. Procedure for the synthesis of **2**: (*S*)-(−)-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid (2.5 g, 14.1 mmol) was added slowly to 7.5 ml of concentrated sulfuric acid maintained at a temperature of −10°C; 1.8 ml of concentrated nitric acid was then added dropwise. The reaction mixture was stirred at this temperature for 3.5 h then poured into 75 ml ice water. The product was precipitated by neutralization with ammonium hydroxide, filtered, washed with water, then dried to give a brown solid (3 g). ^1H NMR (D_2O+DCl, 500 MHz): 3.10 (m,

1H), 3.3 (m, 1H), 4.30 (m, 3H), 7.4 (m, 1H), 7.90 (m, 2H); ESI: 223 (MH^+); TLC ($CHCl_3$/MeOH/H_2O/HOAc: 6/4/2/2) R_f = 0.70; mp = 277–278°C; yield: 90%.

7. Procedure for the synthesis of **3**: 7-Nitro-Tic-OH (1.12 g 0.0050 mol) was dissolved in 50 mL methanol. The solution was degassed with nitrogen and 0.45 g of 10% Pd/C was added. The reaction mixture was maintained under a hydrogen balloon for 2 hours at room temperature. The mixture was then filtered to remove the Pd/C and finally, the solvent was removed to give a white solid (0.95 g). ^{1}H NMR (D_2O, 500 MHz): 3.21 (m, 1H), 3.4 (m, 1H), 4.40 (m, 3H), 7.20 (m, 1H), 7.5 (m, 2H); ESI: 193 (MH^+); TLC ($CHCl_3$/MeOH/H_2O/HOAc: 6/4/2/2) R_f = 0.50; mp = 220–221°C; yield: 98%.
8. Procedure for the synthesis of **4**: 7-Amino-Tic-OH (0.9 g 0.0047 mol) was suspended in 10.5 mL of 9% Na_2CO_3 and cooled in ice water. A solution of 1.28 g (0.0038 mol) of Fmoc-OSu in 11.2 mL of dioxane was then added dropwise and the mixture was stirred at room temperature for 3 h. The solvent was evaporated, ethyl acetate was then added and the water phase and organic phases were separated. The organic phase was evaporated and the residue loaded onto a silica gel column. The column was eluted with diethyl ether. Fractions were checked by TLC using the same solvent mixture for development. Fractions containing the product were pooled and concentrated to obtain a white solid (1.18 g). ^{1}H NMR ($CDCl_3$ 500 MHz): 3.06–3.17 (m, 2H), 4.22–5.12 (m, 6H), 6.46–7.79 (m, 11H, aromatic); EI: 416 (MH^+); TLC ($CHCl_3$/MeOH/H_2O/HOAc: 6/4/2/2) R_f = 0.85; mp = 159–160°C; yield: 63%.
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11. Procedure for the synthesis of **5**: *N*-Fmoc-7-amino-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid (Fmoc-7-amino-Tic-OH) (1.1 g 0.0027 mol) was dissolved in DMF (40 ml). The mixture was cooled to 0°C, treated with *N*,*N*′-bis-Boc-*S*-methyl-isothiourea (0.9 g, 0.0031 mol) and $HgCl_2$ (1.41 g 0.0052 mol), then after 10 minutes, TEA (1.1 ml) was added. The reaction was warmed to room temperature and after 2 hours the mixture was filtered, the organic phase concentrated and loaded onto a silica gel column. The column was eluted with diethyl ether/hexane = 6:4 yielding 1 g of *N*-Fmoc-*N*,*N*′-bis-Boc-7 - guanyl - 1,2,3,4 - tetrahydro - 3 - isoquinolinecarboxylic acid.
^{1}H NMR ($CDCl_3$, 500 MHz), 1.50 (s, 9H), 1.54 (s, 9H), 3.10–3.23 (m, 2H), 4.34–4.80 (m, 6H), 7.10–7.79 (m, 11H aromatic); ESI: 657.6 (MH^+), TLC (hexane/diethyl ether, 9:1) R_f = 0.85, mp = 167–168°C, yield: 58%.
12. Procedure for the synthesis of H-Dmt-GTIC-NH_2: The dipeptide was obtained by standard solution methods. To a solution of Boc-L-Dmt-OH (0.31 g, 1 mmol) and H-GTIC-NH_2 (0.43, 1 mmol) in DMF (10 mL) at 0°C were added HOBt (0.18 g, 1.2 mmol), WSC (0.21 g, 1.2 mmol), and TEA (0.17 mL, 1.2 mmol). The reaction mixture was stirred for 3 h at 0°C and 24 h at room temperature. After evaporation of DMF, the residue was solubilized in EtOAc and washed with citric acid (10%), $NaHCO_3$ (5%), and brine. The organic phase was dried and evaporated to dryness. The residue was crystallized from Et_2O/petroleum ether (1:1) to obtain the protected dipeptide with a yield of 80%. The protected dipeptide (0.52 g, 0.72 mmol) was treated with TFA (2 mL) for 0.5 h at room temperature. Et_2O was added to the solution until the product precipitated: yield 80%. The crude product was purified by preparative RP-HPLC. The final compound TFA·H-Dmt-GTIC-NH_2 was characterized by FAB-MS. The amino acid H-GTIC-NH_2 was obtained from Fmoc-GTIC-OH by reaction with DCC/BtOH·NH_3 to obtain Fmoc-GTIC-NH_2 which was successively deprotected by treatment with 33% diethylamine in THF.