Synthesis of N^{α}-Fmoc *N*,*N*′-bis-Boc-5-, 6- and 8-guanyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (5-GTIC, 6-GTIC and 8-GTIC)

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Received 13 July 2004; revised 1 September 2004

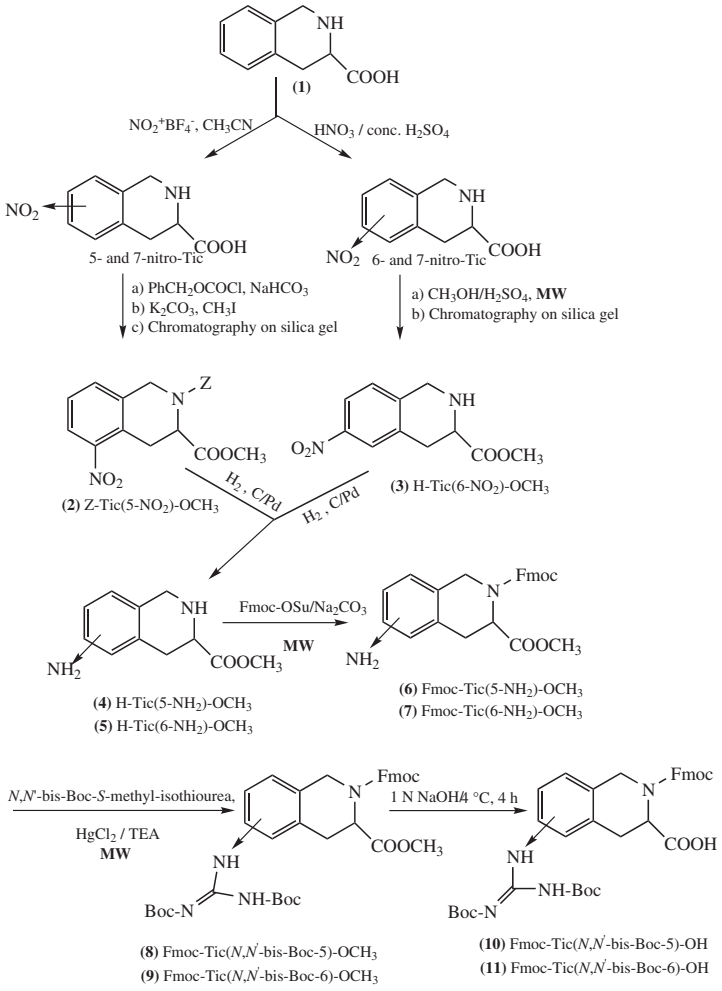
Abstract: N^{α}-Fmoc *N*,*N*′-bis-Boc-5-, 6- and 8-guanyl-Tic-OH (5-, 6- and 8-GTIC), three conformationally constrained amino acids with basic properties, have been synthesized by microwave irradiation. These amino acids combine the basic features of arginine with the aromatic features of phenylalanine and can be utilized as functional tools in peptide-based structure–activity studies.

Key words: unconventional amino acid, microwave, synthesis, GTIC

There is a continuing need to develop 'novel' amino acids that can provide specific three-dimensional properties to a peptide. Among the variety of Phe analogues which provide conformational restriction[1] of the peptide backbone and/or the lateral chain, the most extensively employed amino acids in structure–activity studies have been the α-methyl-L-phenylalanine (H-α-Me-Phe-OH) and L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (H-Tic-OH) residues. Tic and Tic(OH) have been used in medicinal chemistry as a phenylalanine replacement in many biologically active peptides (e.g., opioid,[2] substance P,[3] FTase inhibitors,[4] Bradykinin,[5] Kallikrein[6]). These amino acids are now commercially available. The continuing level of interest in Tic is reflected by the fact that several analogues of Tic have been the targets of many synthetic studies.[7] In a previous paper [6,8] we described a facile synthesis of the 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. We synthesized this amino acid to evaluate if a guanidino function in the 7 position of H-Tic-OH, either in the delta selective opioid dipeptide antagonists, H-Tyr/Dmt-Tic-R (R = OH, NH_2) [8] or as fluorogenic substrates for human tissue kallikrein,[6] could modify the receptor affinity and activity. The results of the binding assay and Ki values (opioid [8] and inhibition of human tissue kallikrein[6]), respectively, showed that 7-GTIC instead of Tic drastically modified the receptor affinity and selectivity.[6,8]

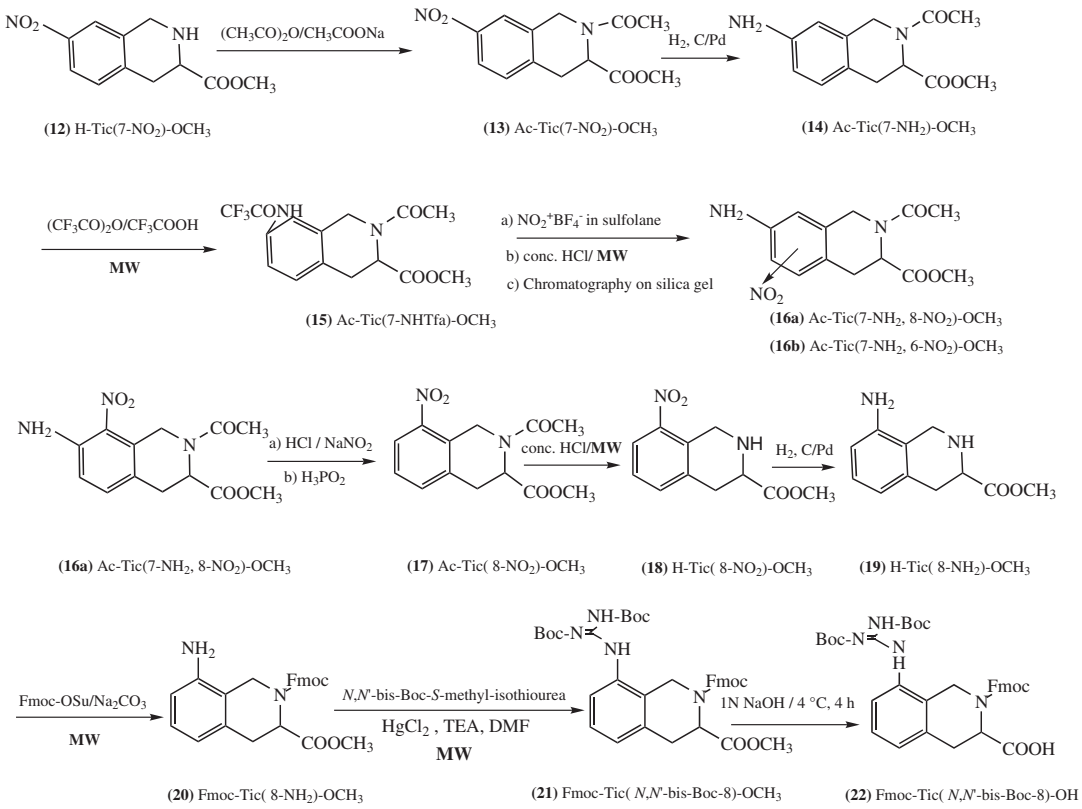
In order to refine the evaluation of the guanidine group on the aromatic ring of the Tic residue, we report the synthesis of the unknown 5-, 6- and 8-guanidine isomers. The application of microwave energy to organic compounds for conducting synthetic reactions at highly accelerated rates is an emerging technique.[9] The synthetic procedure for 5- and 6-guanidine isomers **10** and **11** is summarized in Scheme 1, while the synthetic procedure for 8-guanidine isomer **22** is reported in Scheme 2. In several steps, the synthetic procedure was performed using a microwave oven (ETHOS 1600, Milestone) especially designed for organic synthesis with superior results. The key intermediates for the synthesis of the two unconventional amino acids **10** and **11** were compounds **4** and **5**, H-Tic(5 and 6-NH_2)-OMe. In particular, H-Tic(5-NH_2)-OMe (**4**) was obtained starting from commercially available L-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid (H-Tic-OH, **1**) which was submitted to an unconventional nitration with commercially available nitronium tetrafluoroborate.[10] The obtained mixture of 5- and 7-nitro derivatives could not be separated by column chromatography and was converted into the corresponding *N*-benzyloxycarbonyl derivatives, and then esterified to the resultant methyl esters (**2**) which were separated by silica gel column chromatography.[10] Hydrogenation of intermediate **2** afforded H-Tic(5-NH_2)-OCH_3 (**4**). H-Tic(6-NH_2)-OCH_3 (**5**), instead, was obtained starting from L-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid (H-Tic-OH, **1**) which was then treated with fuming nitric acid and concentrated sulfuric acid at –10 °C to obtain the 6-nitro derivative in a mixture with the corresponding 7-nitro isomer.[8] The mixture of two regioisomers was converted, by methylation with a mixture of methanol and sulfuric acid, into the corresponding methyl ester derivatives which were separated by chromatography on silica gel column obtaining H-Tic(6-NO_2)-OCH_3 (Scheme 1, **3**). Intermediate **3** was then hydrogenated affording the desired H-Tic(6-NH_2)-OCH_3 (**5**). Successively, the Fmoc-protection on the α-amino group of H-Tic(5- or 6-NH_2)-OCH_3 (**4** and **5**) was introduced by treatment with Fmoc-OSu in aqueous sodium carbonate obtaining derivatives **6** and **7**. Under these conditions, the amino group on the ring did not react. The amino group was successively converted into the corresponding guanidine moiety by reaction with *N*,*N*′-bis-Boc-*S*-methyl-isothiourea, $HgCl_2$ and triethylamine in DMF. The obtained two products **8** and **9** were purified

SYNTHESIS 2004, No. 18, pp 3011–3016
Advanced online publication: 21.10.2004
DOI: 10.1055/s-2004-834875; Art ID: P07704SS



Scheme 1 Synthetic procedure of 5- and 6-GTIC

using silica gel chromatography and diethyl ether–hexane as eluents. The final products **10** and **11**, Fmoc-Tic(*N*,*N*′-bis-Boc-5- or 6-)-OH, were obtained by saponification of the methyl ester derivatives with 1 N NaOH. The synthetic route utilized to obtain 5- and 6-isomers (Scheme 1) was not suitable to achieve 8-isomer **22**. Therefore, in order to obtain the derivative with the guanidine moiety in 8-position, we optimized the following procedure as reported in Scheme 2. In fact, the synthetic procedure for 8-guanidine isomer **22** started from H-Tic(7-NO_2)-OCH_3 (**12**) which was synthesized as previously described[8] and converted into the corresponding *N*-acetyl derivative **13** by treatment with acetic anhydride/sodium acetate. Reduction of the nitro group to the corresponding amine Ac-Tic(7-NH_2)-OCH_3 (**14**) was accomplished using H_2 and C/Pd (yield 95%), followed by N-protection with trifluoroacetic anhydride/trifluoroacetic acid (TFAA/TFA) (**15**, yield 85%). An unconventional nitration of Ac-Tic(7-NHTfa)-OCH_3 (**15**) using NO_2^+ BF_4^- in sulfolane gave a mixture of 6- and 8-NO_2 isomer derivatives (the anhydrous environment was necessary to avoid the partial hydrolysis of the methyl ester moiety). Subsequent hydrolysis of the trifluoroacetamide derivatives using concentrated hydrochloric acid afforded a mixture of the corresponding amines Ac-Tic(7-NH_2, 6- or 8-NO_2)-OCH_3 (**16a** and **16b**), respectively, which were separated by column chromatography on silica gel (Scheme 2, yield 25% and 32%, respectively for the 8- and 6-isomers). Deamination of Ac-Tic(7-NH_2, 8-NO_2)-OCH_3 (**16a**) with hypophosphorous acid gave Ac-Tic(8-NO_2)-OCH_3 (**17**), the acetamide moiety of which was hydrolysed to give the required H-Tic(8-NO_2)-OCH_3 (**18**). Reduction of the nitro compound to the corresponding amine H-Tic(8-NH_2)-OCH_3 (**19**) was straightforward (yield 98%), and Fmoc-protection of the α-amino group was carried out with Fmoc-OSu in aqueous sodium carbonate to give Fmoc-Tic(8-NH_2)-OCH_3 (**20**). Under these conditions, the amino group on the ring did not react. The yield after this procedure was modest (55%), 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. The 8-amino group was converted into the corresponding guanidine moiety (**21**) by reaction with *N*,*N*′-bis-Boc-*S*-methyl-isothiourea, $HgCl_2$ and TEA in DMF. Hydrolysis of the methyl ester with 1 N NaOH afforded the final compound



(12) H-Tic(7-NO_2)-OCH_3 **(13)** Ac-Tic(7-NO_2)-OCH_3 **(14)** Ac-Tic(7-NH_2)-OCH_3

(15) Ac-Tic(7-NHTfa)-OCH_3 **(16a)** Ac-Tic(7-NH_2, 8-NO_2)-OCH_3 **(16b)** Ac-Tic(7-NH_2, 6-NO_2)-OCH_3

(16a) Ac-Tic(7-NH_2, 8-NO_2)-OCH_3 **(17)** Ac-Tic(8-NO_2)-OCH_3 **(18)** H-Tic(8-NO_2)-OCH_3 **(19)** H-Tic(8-NH_2)-OCH_3

(20) Fmoc-Tic(8-NH_2)-OCH_3 **(21)** Fmoc-Tic(*N*,*N*′-bis-Boc-8)-OCH_3 **(22)** Fmoc-Tic(*N*,*N*′-bis-Boc-8)-OH

Scheme 2 Synthetic procedure of 8-GTIC

Fmoc-Tic(*N*,*N*′-bis-Boc-8)-OH (**22**) which was purified by column chromatography on silica gel using a mixture of diethyl ether–hexane as eluent (yield 60%). Under these conditions the Fmoc group was poorly cleaved. The final compounds and all intermediates were characterized by ^{1}H NMR and mass spectroscopy.

In conclusion, the Fmoc-*N*,*N*′-bis-Boc-5-, 6- and 8-guanyl-Tic-OH (**GTIC**), three conformationally constrained amino acids, will serve as useful tools in peptide-based structure–activity studies. Furthermore, the application of microwave irradiation significantly reduces reaction times and improves the yields compared with conventional procedures.

Microwave Equipment and Conditions

The synthetic steps performed by microwave irradiation were carried out using a microwave oven (ETHOS 1600, Milestone) especially designed for organic synthesis. The experimental conditions used during microwave application were similar, with the same concentration of starting material and volume of solvent, to those used by conventional heating. All reactions performed by microwave heating were conducted in standard Pyrex glassware and were performed by a microwave program which was composed of appropriate ramping and holding steps. The temperature of the stirred reaction mixture was monitored directly by a microwave-transparent fluoroptic probe inserted into the solution.

Methyl 5-Amino-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (4)

Methyl 5-nitro-2-(benzyloxy)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**2**, 0.70 g, 1.9 mmol) was dissolved in MeOH (100 mL). The solution was degassed with N_2 and 10% Pd/C (0.2 g) was added. The reaction mixture was maintained under H_2 atmosphere for 15 min at r.t. The mixture was then filtered to remove the Pd/C and, finally, the solvent was removed to give **4** as a white solid (0.36 g, 93%).

Mp 124–126 °C; R_f 0.40 (Et_2O–EtOH, 9:1).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.54–2.60 (m, 2 H), 2.76–2.80 (m, 1 H), 3.73–3.75 (dd, 1 H, NH), 3.77 (s, 3 H), 4.04–4.07 (d, 2 H), 6.47–6.49 (d, 1 H), 6.52–6.54 (d, 1 H), 6.95–6.98 (t, 1 H).

ESI-MS: *m*/*z* (%) = 207 (MH^+).

Methyl 6-Amino-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (5)

(3*S*)-1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid **1** (2.5 g,14.1 mmol) was added slowly to conc. H_2SO_4 (7.5 mL) maintained at a temperature of –10 °C. Conc. HNO_3 (1.8 mL) was then added drop-wise. The reaction mixture was stirred at this temperature for 3.5 h and then poured into ice water (75 mL). The product was precipitated by neutralization with NH_4OH, filtered, washed with H_2O, then dried to give a brown solid. The mixture of 6- and 7-regioisomers was dissolved in MeOH (50 mL), conc. H_2SO_4 (1.1 mL) was added and the mixture was stirred under microwave irradiation at reflux for 30 min, followed by evaporation. The following extraction with CH_2Cl_2 and washing with 5% $NaHCO_3$ and brine gave a mixture of 6- and 7-nitro-Tic-OMe which was separated by silica gel column chromatography (Et_2O–EtOH, 9:1) to give methyl 6-nitro-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**3**, 1 g, 30%).

Mp 82–84 °C; R_f 0.60 (Et_2O–EtOH, 9:1).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.07–3.09 (m, 2 H), 3.18–3.22 (m, 1 H), 3.77 (s, 3 H), 4.11–4.25 (d, 2 H), 7.18–7.19 (d, 1 H), 7.99–8.00 (d, 1 H), 8.01(s, 1 H).

ESI-MS: *m*/*z* (%) = 237 (MH^+).

The obtained product **3** (1 g, 4.2 mmol) was dissolved in MeOH (50 mL). The solution was degassed with N_2 and 10% Pd/C (0.44 g) was added. The reaction mixture was maintained under H_2 atmosphere for 1 h at r.t. The mixture was then filtered to remove the Pd/C and, finally, the solvent was removed to give **5** a white solid (0.8 g, 93%).

Mp 121–123 °C; R_f 0.40 (Et_2O–EtOH, 9:1).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.84–2.9 (m, 2 H), 2.95–2.99 (m, 1 H), 3.69–3.72 (dd, 1 H, NH), 3.77 (s, 3 H), 3.99–4.03 (d, 2 H), 6.44 (s, 1 H), 6.50–6.52 (d, 1 H), 6.81–6.83 (d, 1 H).

ESI-MS: *m*/*z* (%) = 207 (MH^+).

N-Fmoc 3-Methyl 5-Amino-1,2,3,4-tetrahydroisoquinoline-3-carboxylate and *N*-Fmoc 3-Methyl 6-Amino-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (6 and 7)

5- or 6-Amino-Tic-OMe (**4** or **5**, 0.9 g, 4.4 mmol) was suspended in 9% Na_2CO_3 (9.8 mL) and cooled in ice water. A solution of Fmoc-OSu (1.18 g, 3.5 mmol) in dioxane (10.5 mL) was then added dropwise and the mixture was stirred and heated by microwave at 40 °C for 20 min. The solvent was evaporated, EtOAc was then added and the phases were separated. The organic phase was evaporated and the residue, loaded onto a silica gel column, was purified (Et_2O–hexane, 8:2). Product-containing fractions were pooled and concentrated to obtain a white solid (**6**: 1.67 g, 90%; **7**, 1.73 g, 92%).

6

Mp 77–79 °C; R_f 0.50 (Et_2O–hexane, 8:2).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.11–3.19 (m, 2 H), 3.65 and 3.68 (2 s, 3 H, OCH_3, rotamers), 4.22–4.70 (m, 6 H), 6.58–7.85 (m, 11 H, Ar).

ESI-MS: *m*/*z* (%) = 428 (MH^+).

7

Mp 80–82 °C; R_f 0.50 (Et_2O–hexane, 8:2).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.19–3.25 (m, 2 H), 3.66 and 3.69 (2 s, 3 H, OCH_3, rotamers), 4.22–4.70 (m, 6 H), 6.61–7.84 (m, 11 H, Ar).

ESI-MS: *m*/*z* (%) = 428 (MH^+).

N-Fmoc 3-Methyl *N*,*N*′-Bis-Boc-5-guanyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate and *N*-Fmoc 3-Methyl *N*,*N*′-Bis-Boc-6-guanyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (8 and 9)

Fmoc-5- or 6-amino-Tic-OMe (**6** or **7**, 1.1 g 2.7 mmol) was dissolved in DMF (40 mL).The mixture was cooled to 0 °C and then added *N*,*N*′-bis-Boc-*S*-methyl-isothiourea (0.9 g 3.0 mmol), $HgCl_2$ (1.36 g 5.0 mmol) and after 10 min Et_3N (1.0 mL). The reaction mixture was warmed to r.t., heated by microwave to 40 °C for 20 min, and then was filtered. The organic phase was concentrated and purified on by silica gel column chromatography (Et_2O–hexane, 6:4) (**8**: 1.23 g, 68%; **9**: 1.27 g, 70%).

8

Mp 120–122 °C;) R_f 0.40 (Et_2O–hexane, 6:4).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.45 (s, 9 H), 1.58 (s, 9 H), 3.20–3.30 (m, 2 H), 3.55 and 3.65 (2 s, 3 H, OCH_3, rotamers), 4.30–4.50 (m, 6 H), 7.17–7.80 (m, 11 H, Ar).

ESI-MS: *m*/*z* (%) = 671 (MH^+);

9

Mp 113–115 °C; R_f 0.40 (Et_2O–hexane, 6:4).

^{1}H NMR (500 MHz, $CDCl_3$): δ =1.43 (s, 9 H), 1.50 (s, 9 H), 3.18–3.25 (m, 2 H), 3.61 and 3.68 (2 s, 3 H, OCH_3, rotamers), 4.32–4.65 (m, 6 H), 7.17–7.88 (m, 11 H, Ar).

ESI-MS: *m*/*z* (%) = 671 (MH^+).

N-Fmoc *N*,*N*′-Bis-Boc-5-guanyl-1,2,3,4- tetrahydroisoquinoline-3-carboxylic Acid and *N*-Fmoc- *N*,*N*′-Bis-Boc-6-guanyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (10 and 11)

Methyl ester groups were removed by treating amino acids **8** or **9** (1 mmol) in MeOH (8 mL) with 1 N NaOH (1.2 equiv) for 4 h at 4 °C. The solution was then diluted with H_2O, concentrated in vacuo to remove the MeOH, and washed with EtOAc. After cooling to 0 °C, the aqueous solution was acidified with citric acid (10%) and the product extracted with EtOAc. The organic layer was washed with H_2O, dried (Na_2SO_4) and evaporated. The residue was purified by silica gel column chromatography (Et_2O–hexane, 10:1) (**10**: 0.41g, 63%; **11**: 0.43 g, 65%).

10

Mp 196–198 °C; R_f 0.50 (Et_2O–hexane, 10:1).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.45 (s, 9 H), 1.58 (s, 9 H), 3.20–3.30 (m, 2 H), 4.30–4.50 (m, 6 H), 7.17& ndash;7.80 (m, 11 H, aromatic).

ESI-MS: *m*/*z* (%) = 657 (MH^+).

11

Mp 185–187 °C; R_f 0.50 (Et_2O–hexane, 10:1).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.43 (s, 9 H), 1.50 (s, 9 H), 3.18–3.25 (m, 2 H), 4.32–4.65 (m, 6 H), 7.17–7.88 (m, 11 H, aromatic).

ESI-MS: *m*/*z* (%) = 657 (MH^+).

Methyl 2-Acetyl-7-nitro-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (13)

Ester **12** (4.2 g, 17.8 mmol) was dissolved in EtOH (20 mL) and conc. HCl (2 mL) was added. The precipitated hydrochloride salts (4.7 g), anhyd NaOAc (0.92 g) and acetic anhydride (9.2 mL) were heated (50–60 °C) on a steam bath for 1 h and the reaction mixture was poured into water. The product was extracted with $CHCl_3$ (3 × 25 mL) and chromatographed over silica gel (EtOAc–MeOH, 8:2). The resulting acetyl derivative **13** was obtained as a brown oil (4.6 g, 96%).

R_f 0.50 (Et_2O).

^{1}H NMR (500 MHz, $CDCl_3$) showed a mixture of rotamers doubling most signals: δ = 2.19 and 2.27 (2 s, 3 H, $COCH_3$, rotamers), 3.40 (m, 2 H), 3.46 (m, 1 H), 3.62 and 3.64 (2 s, 3 H, OCH_3, rotamers), 4.79 and 4.80 (2 d, *J* = 6.1 Hz, 2 H, rotamers), 7.35 (d, *J* = 8.2 Hz, 1 H), 8.02 (s, 1 H), 8.05 (d, *J* = 8.2 Hz, 1 H).

ESI-MS: *m*/*z* (%) = 279 (MH^+).

Methyl 2-Acetyl-7-amino-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (14)

Derivative **13** (4.6 g, 16.5 mmol), dissolved in MeOH (50 mL), was hydrogenated at 1 atm at r.t. for 30 min in the presence of 10% Pd/C (460 mg). The filtered solution was evaporated in vacuo to give an oil, **14**, which was crystallized from Et_2O (3.9 g, 95%).

Mp 78–80 °C; R_f 0.60 (Et_2O–EtOH, 9:1).

^{1}H NMR (500 MHz, $CDCl_3$) showed a mixture of rotamers doubling most signals: δ = 2.13 and 2.23 (2 s, 3 H, $COCH_3$, rotamers), 3.04 (m, 2 H), 3.10–3.14 (m, 1 H), 3.60 and 3.61 (2 s, 3 H, OCH_3, rotamers), 4.58 (d, *J* = 6.1 Hz, 2 H, rotamers), 6.43 (s, 1 H), 6.52 (d, *J* = 8.1 Hz, 1 H), 6.93 (d, *J* = 8.1 Hz, 1 H).

ESI-MS: *m*/*z* (%) = 249 (MH^+).

Methyl 2-Acetyl-7-[(trifluoroacetyl)amino]-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (15)

A mixture of **14** (3.9 g, 15.7 mmol), trifluoroacetic anhydride (3.7 mL, 26.4 mmol) and trifluoroacetic acid (4.45 mL, 26.4 mmol) in CH_2Cl_2 (60 mL) was stirred and heated at reflux under microwave conditions for 1 min, then cooled and evaporated. The residue was dissolved in CH_2Cl_2 and washed with brine. The organic phase was dried (Na_2SO_4) and evaporated to dryness. The filtered solution was evaporated in vacuo to give an oil, **15**, which was crystallized from Et_2O (5.15 g, 95%).

Mp 129–131 °C; R_f 0.75 (Et_2O–EtOH, 9:1).

^1H NMR (500 MHz, $CDCl_3$) showed a mixture of rotamers doubling most signals: δ = 2.18 and 2.25 (2 s, 3 H, $COCH_3$, rotamers), 3.08 (m, 2 H), 3.25 (m, 1 H), 3.60 and 3.64 (2 s, 3 H, OCH_3, rotamers), 4.70 (d, *J* = 6.2 Hz, 2 H, rotamers), 7.18 (d, *J* = 8.1 Hz, 1 H,), 7.26 (d, *J* = 8.1 Hz, 1 H,) 7.44 (s, 1 H).

ESI-MS: *m*/*z* (%) = 345 (MH^+).

Methyl 2-Acetyl-7-amino-8-nitro-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (16a)

To an ice-cold solution of **15** (4.6 g, 13.4 mmol) in tetramethylene sulfone (5 mL) was added drop-wise nitronium tetrafluoroborate (5.2 g of 85% $NO_2^+BF_4^-$, 33.5 mmol) dissolved in tetramethylene sulfone (10 mL). After the addition was completed, the reaction mixture was stirred at r.t. for another 10 min, then the solution was diluted with H_2O and extracted with Et_2O. The organic phase was dried (Na_2SO_4) and evaporated to dryness. This product contained a mixture of 6-NO_2 and 8-NO_2 derivatives. Hydrolysis of the trifluoroacetamides in MeOH (50 mL) and conc. HCl (15 mL) under microwave heating at reflux for 6 min, followed by evaporation, extraction with CH_2Cl_2 and washing with H_2O gave a mixture of the nitroamines which were chromatographed on silica gel (Et_2O–EtOH, 9:1). The elution firstly gave methyl 2-acetyl-7-amino-8-nitro-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**16a**, 1 g, 32%).

Mp 180–183 °C; R_f 0.70 (Et_2O–EtOH, 9:1).

^1H NMR (500 MHz, $CDCl_3$) showed a mixture of rotamers doubling most signals: δ = 2.10 and 2.20 (2 s, 3 H, rotamers), 3.08 (m, 2 H), 3.24–3.28 (m, 1 H), 3.60 and 3.64 (2 s, 3 H, OCH_3, rotamers), 4.65 (d, *J* = 6.1 Hz, 2 H, rotamers), 6.12 (d, *J* = 7.8 Hz, 1 H), 6.68 (d, *J* = 8.1 Hz, 1 H).

ESI-MS: *m*/*z* (%) = 294 (MH^+).

Continued elution gave methyl 2-acetyl-7-amino-6-nitro-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**16a**, 1.57 g, 43%).

Mp 182–184 °C; R_f 0.40 (Et_2O–EtOH, 9:1).

^1H NMR (500 MHz, $CDCl_3$) showed a mixture of rotamers doubling most signals: δ = 2.07 and 2.08 (2 s, 3 H, rotamers), 3.08 (m, 2 H), 3.25 (m, 1 H), 3.60 and 3.64 (2 s, 3 H, OCH_3, rotamers), 4.55 (d, *J* = 6.1 Hz, 2 H, rotamers), 6.81 (s, 1 H), 7.81 (s, 1 H).

ESI-MS: *m*/*z* (%) = 294 (MH^+).

Methyl 2-Acetyl-8-nitro-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (17)

A stirred solution of **16a** (0.78 g, 2.66 mmol) in HCl (6 N, 8 mL) was treated drop-wise with a solution of $NaNO_2$ (0.22 g, 3.2 mmol) in H_2O (1 mL) at 0 °C. After 100 min at 0 °C, hypophosphorous acid (50% aq soln, 2.7 mL) was added drop-wise and the mixture was kept at 4 °C for 18 h, then poured into H_2O and extracted with CH_2Cl_2. The organic phase was dried (Na_2SO_4) and evaporated to dryness giving methyl 2-acetyl-8-nitro-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**17**, 0.59 g, 80%).

Mp 138–140 °C; R_f 0.60 (Et_2O–EtOH, 9:1).

^1H NMR (500 MHz, $CDCl_3$) showed a mixture of rotamers doubling most signals: δ = 2.19 and 2.29 (2 s, 3 H, rotamers), 3.18 (m, 2 H), 3.38 (m, 1 H), 3.65 and 3.68 (2 s, 3 H, OCH_3, rotamers), 4.93 and 5.18 (dd, *J* = 6.2 Hz, 2 H, rotamers), 7.40 (t, *J* = 8.1 Hz, 1 H), 7.48 (d, *J* = 7.8 Hz, 1 H), 8.02 (d, *J* = 7.8 Hz, 1 H).

ESI-MS: *m*/*z* (%) = 279 (MH^+).

Methyl 8-Amino-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (19)

Hydrolysis of **17** (0.59 g, 2.1 mmol) in MeOH (15 mL) and conc. HCl (7.5 mL) under microwave irradiation at reflux for 20 min gave methyl 8-nitro-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**18**, 0.49 g, 99%) which was dissolved in MeOH (50 mL). The solution was degassed with N_2 and 10% Pd/C (0.2 g) was added. The reaction mixture was maintained under a H_2 atmosphere for 30 min at r.t. The mixture was then filtered to remove the Pd/C and, finally, the solvent was removed to give **19** as a white solid (0.38 g, 98%).

Mp 124–126 °C; R_f 0.40 (Et_2O–EtOH, 7:3).

^1H NMR (500 MHz, $CDCl_3$): δ = 2.84 (m, 2 H), 2.97 (m, 1 H), 3.69 (dd, *J* = 6.2 Hz, 1 H), 3.77 (s, 3 H), 3.99 (d, *J* = 6.1 Hz, 2 H), 6.20 (d, *J* = 7.8 Hz 1 H), 6.35 (d, *J* = 8.2 Hz, 1 H), 6.80 (t, *J* = 8.2 Hz, 1 H).

ESI-MS: *m*/*z* (%) = 207 (MH^+).

N^{α}-Fmoc 3-Methyl 8-Amino-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (20)

Intermediate **19** (0.90 g, 4.4 mmol) was suspended in 9% Na_2CO_3 (9.8 mL) and cooled in ice water. A solution of Fmoc-OSu (1.18 g, 3.5 mmol) in dioxane (10.5 mL) was then added drop-wise and the mixture was heated under microwave irradiation to 40 °C for 20 min. The solvent was evaporated, EtOAc was then added and the phases were separated. The organic phase was evaporated and the residue, loaded onto a silica gel column, was chromatographically purified (Et_2O–hexane, 8:2). Product-containing fractions were pooled and concentrated to give **20** as a white solid (1.23g, 68%).

Mp 80–82 °C; R_f 0.5 (Et_2O–hexane, 8:2).

^1H NMR (500 MHz, $CDCl_3$): δ = 3.10 (m, 2 H), 3.60 and 3.61 (2 s, 3 H, OCH_3, rotamers), 4.19 (m, 6 H), 6.56 (m, 11 H).

ESI-MS: *m*/*z* (%) = 428 (MH^+).

N^{α}-Fmoc 3-Methyl *N*,*N*′-Bis-Boc-8-guanyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (21)

Intermediate **20** (1.1 g, 0.0027 mol) was dissolved in DMF (40 mL). The mixture was cooled to 0 °C, then *N*,*N*′-bis-Boc-*S*-methylisothiourea (0.9 g, 3.0 mmol), $HgCl_2$ (1.36 g, 5.0 mmol) were added followed by Et_3N (1.0 mL) after 10 min. The reaction was heated under microwave irradiation at 40 °C for 20 min. Then the mixture was filtered, the organic phase was concentrated and purified by silica gel column chromatography (Et_2O–hexane, 6:4) to give **21** (1.19 g, 66%).

Mp 121–123 °C; R_f 0.40 (Et_2O–hexane, 6:4).

^1H NMR (500 MHz, $CDCl_3$): δ = 1.34 (s, 9 H), 1.46 (s, 9 H), 3.24 (m, 2 H), 3.60 and 3.66 (s, 3 H, rotamers), 4.45 (m, 6 H), 7.62 (m, 11 H).

ESI-MS: *m*/*z* (%) = 671 (MH^+).

N^{α}-Fmoc *N*,*N*′-Bis-Boc-8-guanyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (22)

The methyl ester group was removed by treating intermediate **21** (1 mmol) in MeOH (8 mL) with 1 N NaOH (1.2 equiv) for 4 h at 4 °C. The solution was then diluted with H_2O, concentrated in vacuo to remove the MeOH, and washed with EtOAc. After cooling to 0 °C, the aqueous solution was acidified with citric acid (10%) and the

product extracted with EtOAc. The organic layer was washed with H_2O, dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography on silica gel (Et_2O–hexane, 10:1) to give compound **22** (0.39 g, 60%).

Mp 182–184 °C; R_f 0.50 (Et_2O–hexane, 10:1).

^1H NMR (500 MHz, $CDCl_3$): δ = 1.34 (s, 9 H), 1.46 (s, 9 H), 3.22–3.26 (m, 2 H), 4.34–4.70 (m, 6 H), 7.65 (m, 11 H).

ESI-MS: *m/z* (%) = 657 (MH^+).

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