Supplemental Information

Conversion of the Potent δ-Opioid Agonist H-Dmt-Tic-NH-CH_2-Bid into δ-Opioid Antagonists by N^1-Benzimidazole Alkylation

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Experimental

General Methods Crude peptides were purified by preparative reversed-phase HPLC [Waters Delta Prep 4000 system with Waters Prep LC 40 mm Assembly column C18 (30 cm x 4 cm, 15 μm particle)] and eluted at a flow rate of 40 mL/min with mobile phase A (10% acetonitrile + 0.1% TFA in H_2O, v/v), and a linear gradient from 25 to 75% B (60%, acetonitrile + 0.1% TFA in H_2O, v/v) in 25 min. Analytical HPLC analyses were performed with a Beckman System Gold (Beckman ultrasphere ODS column, 250 mm x 4.6 mm, 5 μm particle). Analytical determinations and capacity factor (K') of the products used HPLC in solvents A and B programmed at flow rate of 1 mL/min with linear gradients from 0 to 100% B in 25 min. Analogues had less than 1% impurities at 220 and 254 nm.

TLC was performed on precoated plates of silica gel F254 (Merck, Darmstadt, Germany): (A) 1-butanol/AcOH/H_2O (3:1:1, v/v/v); (B) CH_2Cl_2/toluene/methanol (17:1:2). Ninhydrin (1% ethanol, Merck), fluorescamine (Hoffman-La Roche) and chlorine spray reagents. Melting points were determined on a Kofler apparatus and are uncorrected. Optical rotations were assessed at 10 mg/mL in methanol with a Perkin-Elmer 241 polarimeter in a 10 cm water-jacketed cell. Molecular weights of the compounds were determined by a MALDI-TOF analysis (Hewlett Packard G2025A LD-TOF system mass spectrometer) and α-cyano-4-hydroxycinnamic acid as a matrix. ^1H NMR (δ) spectra were measured, when not specified, in DMSO solution using a Bruker AC-200 spectrometer, and peak positions are given in parts per million downfield from tetramethylsilane as internal standard. Elemental Analysis is in the accompanying Table.

Peptide Synthesis

***Tert*-butyl-3-((1-methyl-1*H*-benzo[*d*]imidazol-2-yl)methylcarbamoyl)-3,4-dihydroiso-quinoline-2(1*H*)-carboxylate [Boc-Tic-NH-CH_2-Bid(N^1-CH_3)].** To a solution of Boc-Tic-NH-CH_2-Bid.[9] (0.20 g, 0.49 mmol) in DMF (10 mL) at room temperature, K_2CO_3 (0.24 g, 1.72 mmol) and, after 1 h, iodomethane, (0.03 mL, 0.52 mmol) were added. The reaction mixture was stirred for 24 h at room temperature. After DMF was evaporated, the residue was dissolved in EtOAc and washed with $NaHCO_3$ (5% in H_2O) and brine. The organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was precipitated from Et_2O/Pe (1:9, v/v): yield 0.18 g (88%); R_f (B) 0.84; HPLC K' 5.86; mp 145-147 °C; $[\alpha]^{20}_D$ -13.5; MH^+ 421 ^{1}H NMR (DMSO-d_6) δ 1.30-1.40 (m, 9H), 2.92-3.17 (m, 2H), 3.63 (s, 3H), 4.17-4.27 (m, 2H), 4.46 (m, 1H), 4.92 (m, 1H), 6.96-7.70 (m, 8H).

***Tert*-butyl-3-((1-benzyl-1*H*-benzo[*d*]imidazol-2-yl)methylcarbamoyl)-3,4-dihydroiso-quinoline-2(1*H*)-carboxylate [Boc-Tic-NH-CH_2-Bid(N^1-CH_2-C_6H_5)].** This compound was obtained by alkylation of Boc-Tic-NH-CH_2-Bid with K_2CO_3 and benzyl bromide as reported for Boc-Tic-NH-CH_2-Bid(N^1-CH_3): yield 0.19 g (89%); R_f (B) 0.88; HPLC K' 7.92; mp 148-150 °C; $[\alpha]^{20}_D$ -12.1; MH^+ 498; ^{1}H NMR (DMSO-d_6) δ 1.30-1.40 (m, 9H), 2.92-3.17 (m, 2H), 4.17-4.27 (m, 2H), 4.46 (m, 1H), 4.92-4.99 (m, 3H), 6.96-7.70 (m, 13H).

***Tert*-butyl-3-((1-allyl-1*H*-benzo[*d*]imidazol-2-yl)methylcarbamoyl)-3,4-dihydroiso-quinoline-2(1*H*)-carboxylate [Boc-Tic-NH-CH_2-Bid(N^1-CH_2-CH=CH_2)].** This compound was obtained by alkylation of Boc-Tic-NH-CH_2-Bid with K_2CO_3 and allyl bromide as reported for Boc-Tic-NH-CH_2-Bid(N^1-CH_3): yield 0.20 g (91%); R_f (B) 0.85;

HPLC K' 6.40; mp 140-141 °C; $[\alpha]^{20}_D$ -10.7; MH^+ 448; ^1H NMR (DMSO-d_6) δ 1.30-1.40 (m, 9H), 2.92-3.17 (m, 2H), 4.17-4.27 (m, 2H), 4.40-4.46 (m, 4H), 4.92 (m, 1H), 5.15-5.17 (m, 2H), 5.83 (m, 1H), 6.96-7.70 (m, 8H).

***Tert*-butyl-3-((1-(cyclopropylmethyl)-1*H*-benzo[*d*]imidazol-2-yl)methylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate [Boc-Tic-NH-CH_2-Bid(N^1-CH_2-c(C_3H_5))]**. This compound was obtained by alkylation of Boc-Tic-NH-CH_2-Bid with K_2CO_3 and cyclopropylmethyl bromide as reported for Boc-Tic-NH-CH_2-Bid(N^1-CH_3): yield 0.16 g (84%); R_f (B) 0.84; HPLC K' 6.11; mp 142-144 °C; $[\alpha]^{20}_D$ -10.1; MH^+ 462 ^1H NMR (DMSO-d_6) δ 0.06- 0.31 (m, 5H), 1.30-1.40 (m, 9H), 2.92-3.17 (m, 2H), 3.69 (m, 2H), 4.17-4.27 (m, 2H), 4.46 (m, 2H), 4.92 (m, 1H), 6.96-7.70 (m, 8H).

2TFA·H-Tic-NH-CH_2-Bid(N^1-CH_3). Boc-Tic-NH-CH_2-Bid(N^1-CH_3) (0.18 g, 0.43 mmol) was treated with TFA (1 mL) for 0.5 h at room temperature. Et_2O/Pe (1:1, v/v) were added to the solution until the product precipitated: yield 0.22 g (93%); R_f (A) 0.49; HPLC K' 3.01; mp 175-177 °C; $[\alpha]^{20}_D$ -15.9; MH^+ 321.

2TFA·H-Tic-NH-CH_2-Bid(N^1-CH_2-C_6H_5). Boc-Tic-NH-CH_2-Bid(N^1-CH_2-C_6H_5) was treated with TFA as reported for 2TFA·H-Tic-NH-CH_2-Bid(N^1-CH_3): yield 0.16 g (91%); R_f (A) 0.53; HPLC K' 5.27; mp 176-178 °C; $[\alpha]^{20}_D$ -14.8; MH^+ 397.

2TFA·H-Tic-NH-CH_2-Bid(N^1-CH_2-CH=CH_2). Boc-Tic-NH-CH_2-Bid(N^1-CH_2-CH=CH_2) was treated with TFA as reported for 2TFA·H-Tic-NH-CH_2-Bid(N^1-CH_3): yield 0.18 g (92%); R_f (A) 0.51; HPLC K' 4.27; mp 168-170 °C; $[\alpha]^{20}_D$ -12.6; MH^+ 347.

2TFA·H-Tic-NH-CH_2-Bid(N^1-CH_2-c(C_3H_5)). Boc-Tic-NH-CH_2-Bid(N^1-CH_2-c(C_3H_5))

was treated with TFA as reported for 2TFA·H-Tic-NH-CH_2-Bid(N^1-CH_3): yield 0.19 g (92%); R_f (A) 0.35; HPLC K' 2.91; mp 168-170 °C; $[\alpha]^{20}_D$ -12.6; MH^+ 361.

Boc-Dmt-Tic-NH-CH_2-Bid(N^1-CH_3). To a solution of Boc-Dmt-OH (0.05 g, 0.16 mmol) and 2TFA·H-Tic-NH-CH_2-Bid(N^1-CH_3) (0.09 g, 0.16 mmol) in DMF (10 mL) at 0 °C, NMM (0.03 mL, 0.32 mmol), HOBt (0.03 g, 0.18 mmol) and WSC (0.04 g, 0.18 mmol) were added. The reaction mixture was stirred for 3 h at 0 °C and 24 h at room temperature. After DMF was evaporated, the residue was dissolved in EtOAc and washed with $NaHCO_3$ (5% in H_2O) and brine. The organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was precipitated from Et_2O/Pe (1:9, v/v): yield 0.09 g (88%); R_f (B) 0.87; HPLC K' 6.21; mp 157-159 °C; $[\alpha]^{20}_D$ -19.2; MH^+ 613; ^1H NMR (DMSO-d_6) δ 1.30-1.40 (m, 9H), 2.35 (s, 6H), 2.92-3.17 (m, 4H), 3.63 (s, 3H), 4.41-4.51 (m, 4H), 4.92 (m, 2H), 6.29 (s, 2H), 6.96-7.70 (m, 8H).

Boc-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-C_6H_5). This compound was obtained by condensation of Boc-Dmt-OH with 2TFA·H-Tic-NH-CH_2-Bid(N^1-CH_2-C_6H_5) via WSC/HOBt as reported for Boc-Dmt-Tic-NH-CH_2-Bid(N^1-CH_3): yield 0.07 g (83%); R_f (B) 0.93; HPLC K' 7.88; mp 161-163 °C; $[\alpha]^{20}_D$ -17.6; MH^+ 689; ^1H NMR (DMSO-d_6) δ 1.30-1.40 (m, 9H), 2.35 (s, 6H), 2.92-3.17 (m, 4H), 4.41-4.51 (m, 4H), 4.92-4.99 (m, 4H), 6.29 (s, 2H), 6.96-7.70 (m, 13H).

Boc-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-CH=CH_2). This compound was obtained by condensation of Boc-Dmt-OH with 2TFA·H-Tic-NH-CH_2-Bid(N^1-CH_2-CH=CH_2) via WSC/HOBt as reported for Boc-Dmt-Tic-NH-CH_2-Bid(N^1-CH_3): yield 0.08 g (84%); R_f (B) 0.79; HPLC K' 7.40; mp 155-157 °C; $[\alpha]^{20}_D$ -15.2; MH^+ 639; ^1H NMR (DMSO-d_6) δ

1.30-1.40 (m, 9H), 2.35 (s, 6H), 2.92-3.17 (m, 4H), 4.40-4.51 (m, 6H), 4.92 (m, 2H), 5.15-5.17 (m, 2H), 5.83 (m, 1H), 6.29 (s, 2H), 6.96-7.70 (m, 8H).

Boc-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-c(C_3H_5)). This compound was obtained by condensation of Boc-Dmt-OH with 2TFA·H-Tic-NH-CH_2-Bid(N^1-CH_2-c(C_3H_5)) via WSC/HOBt as reported for Boc-Dmt-Tic-NH-CH_2-Bid(N^1-CH_3): yield 0.06 g (76%); R_f (B) 0.86; HPLC K' 7.64; mp 158-160 °C; $[\alpha]^{20}_D$ -13.8; MH^+ 653; ^1H NMR (DMSO-d_6) δ 0.06- 0.31 (m, 5H), 1.30-1.40 (m, 9H), 2.35 (s, 6H), 2.92-3.17 (m, 4H), 3.69 (m, 2H), 4.41-4.51 (m, 4H), 4.92 (m, 2H), 6.29 (s, 2H), 6.96-7.70 (m, 8H).

Boc-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-COOH). To a solution of Boc-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-COOEt) (ref. [10]) (0.15 g, 0.22 mmol) in EtOH (10 mL) at room temperature, 1 N NaOH (0.33 mL, 0.33 mmol) was added. The reaction mixture was stirred for 3 h at room temperature. After EtOH was evaporated, the residue was directly purified by preparative HPLC without any treatment: yield 0.13 g (90%); R_f(B) 0.61; HPLC K' 7.01; mp 166-168 °C; $[\alpha]^{20}_D$ -24.5; MH^+ 657; ^1H NMR (DMSO-d_6) δ 1.30-1.40 (m, 9H), 2.35 (s, 6H), 2.92-3.17 (m, 4H), 4.41-4.51 (m, 4H), 4.67 (s, 2H), 4.92 (m, 2H), 6.29 (s, 2H), 6.96-7.70 (m, 8H).

Boc-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-$CONH_2$). A solution of Boc-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-COOH) (0.13 g, 0.2 mmol) and NMM (0.02 mL, 0.2 mmol) in DMF (10 mL) was treated at -20 °C with isobutyl chloroformate (1.2 mL, 0.03 mmol). After 10 min at -20 °C, NH_3(gas) was bubbled for 30 min. The reaction mixture was allowed to stir while slowly warming to room temperature (1 h) and was stirred 1 h at -20 °C and 3 h at room temperature. After DMF was evaporated, the residue was dissolved in EtOAc

and washed with $NaHCO_3$ (5% in H_2O) and brine. The organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was precipitated from Et_2O/Pe (1:9, v/v): yield 0.11 g (87%); R_f (B) 0.69; HPLC K' 7.88; mp 160-162 °C; $[\alpha]^{20}_D$ -20.8; MH^+ 656; ^1H NMR (DMSO-d_6) δ 1.30-1.40 (m, 9H), 2.35 (s, 6H), 2.92-3.17 (m, 4H), 4.41-4.51 (m, 4H), 4.62 (s, 2H), 4.92 (m, 2H), 6.29 (s, 2H), 6.96-7.70 (m, 8H).

2TFA·H-Dmt-Tic-NH-CH_2-Bid(N^1-CH_3) (1). Boc-Dmt-Tic-NH-CH_2-Bid(N^1-CH_3) (0.09 g, 0.15 mmol) was treated with TFA (1 mL) for 30 min. at room temperature. Et_2O/Pe (1:1, v/v) were added to the solution until the product precipitated: yield 0.1 g (95%); R_f (A) 0.45; HPLC K' 4.32; mp 158-160 °C; $[\alpha]^{20}_D$ -22.4; MH^+ 513; ^1H NMR (DMSO-d_6) δ 2.35 (s, 6H), 2.92-3.17 (m, 4H), 3.63 (s, 3H), 3.95 (m, 1H); 4.41-4.51 (m, 4H), 4.92 (m, 1H), 6.29 (s, 2H), 6.96-7.70 (m, 8H).

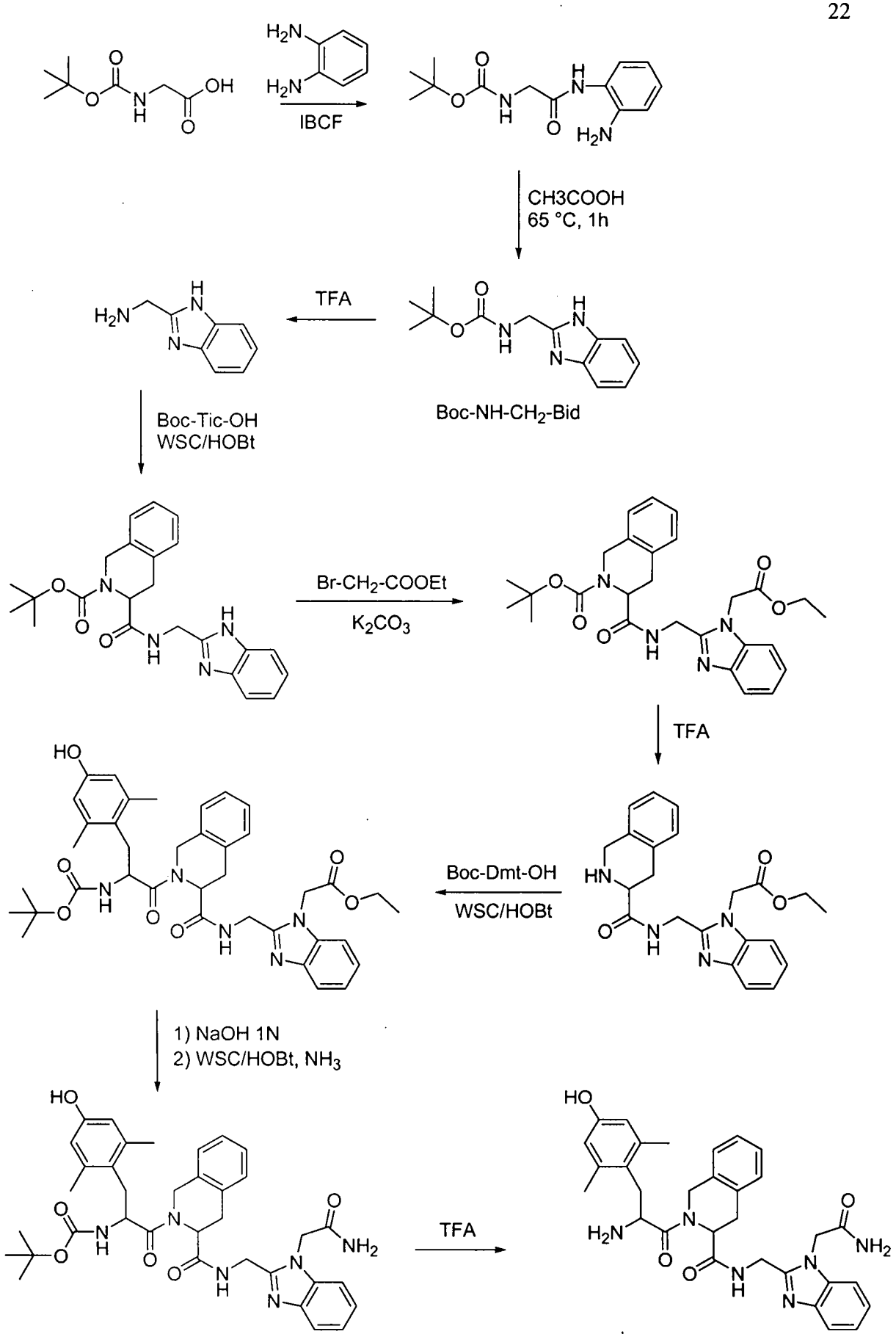
2TFA·H-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-C_6H_5) (2). Boc-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-C_6H_5) was treated with TFA as reported for 2TFA·H-Dmt-Tic-NH-CH_2-Bid(N^1-CH_3): yield 0.04 g (93%); R_f (A) 0.58; HPLC K' 6.99; mp 161-163 °C; $[\alpha]^{20}_D$ -16.7; MH^+ 589; ^1H NMR (DMSO-d_6) δ 2.35 (s, 6H), 2.92-3.17 (m, 4H), 3.95 (m, 1H), 4.41-4.51 (m, 4H), 4.92-4.99 (m, 3H), 6.29 (s, 2H), 6.96-7.70 (m, 13H).

2TFA·H-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-CH=CH_2) (3). Boc-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-CH=CH_2) was treated with TFA as reported for 2TFA·H-Dmt-Tic-NH-CH_2-Bid(N^1-CH_3): yield 0.05 g (91%); R_f (A) 0.56; HPLC K' 5.38; mp 166-168 °C; $[\alpha]^{20}_D$ -17.6; MH^+ 539; ^1H NMR (DMSO-d_6) δ 2.35 (s, 6H), 2.92-3.17 (m, 4H), 3.95 (m, 1H), 4.40-4.51 (m, 6H), 4.92 (m, 1H), 5.15-5.17 (m, 2H), 5.83 (m, 1H), 6.29 (s, 2H), 6.96-7.70 (m, 8H).

2TFA·H-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-c(C_3H_5)) (4). Boc-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-c(C_3H_5)) was treated with TFA as reported for 2TFA·H-Dmt-Tic-NH-CH_2-Bid(N^1-CH_3): yield 0.04 g (89%); R_f (A) 0.54; HPLC K' 5.89; mp 164-166 °C; $[\alpha]^{20}_D$ -14.9; MH^+ 553; ^1H NMR (DMSO-d_6) δ 0.06- 0.31 (m, 5H), 2.35 (s, 6H), 2.92-3.17 (m, 4H), 3.69 (m, 2H), 3.95 (m, 1H) 4.41-4.51 (m, 4H), 4.92 (m, 1H), 6.29 (s, 2H), 6.96-7.70 (m, 8H).

2TFA·H-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-COOEt) (5). Boc-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-COOEt))[10] was treated with TFA as reported for 2TFA·H-Dmt-Tic-NH-CH_2-Bid(N^1-CH_3): yield 0.05 g (93%); R_f (A) 0.81; HPLC K' 6.40; mp 162-164 °C; $[\alpha]^{20}_D$ -34.7; MH^+ 585; ^1H NMR (DMSO-d_6) δ 1.30 (t, 3H), 2.35 (s, 6H), 2.92-3.17 (m, 4H), 3.95 (m, 1H), 4.12 (q, 2H), 4.41-4.69 (m, 6H), 4.92 (m, 1H), 6.29 (s, 2H), 6.96-7.70 (m, 8H).

2TFA·H-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-$CONH_2$) (6). Boc-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-$CONH_2$) was treated with TFA as reported for 2TFA·H-Dmt-Tic-NH-CH_2-Bid(N^1-CH_3): yield 0.04 g (90%); R_f (A) 0.77; HPLC K' 5.51; mp 167-169 °C; $[\alpha]^{20}_D$ -30.2; MH^+ 556; ^1H NMR (DMSO-d_6) δ 2.35 (s, 6H), 2.92-3.17 (m, 4H), 3.95 (m, 1H), 4.41-4.62 (m, 6H), 4.92 (m, 1H), 6.29 (s, 2H), 6.96-7.70 (m, 8H).



Scheme. Synthesis of compound **6** (H-Dmt-Tic-NH-CH_2-Bid(N^1-CH_2-$CONH_2$)**.**

Table. Physicochemical properties and elemental analysis of compounds **1-6**.

Comp.	Formula	MH^+, *m/z*		C	H	N
1	$C_{34}H_{35}F_6N_5O_7$	513	Calc	55.21	4.77	9.47
			Found	55.05	4.70	9.30
2	$C_{40}H_{39}F_6N_5O_7$	589	Calc	58.89	4.82	8.59
			Found	59.18	4.98	8.27
3	$C_{36}H_{37}F_6N_5O_7$	539	Calc	56.47	4.87	9.15
			Found	56.73	5.01	9.27
4	$C_{37}H_{39}F_6N_5O_7$	553	Calc	56.99	5.04	8.98
			Found	56.86	4.86	8.84
5	$C_{37}H_{39}F_6N_5O_9$	585	Calc	54.75	4.84	8.36
			Found	54.59	4.77	8.19
6	$C_{35}H_{36}F_6N_6O_8$	556	Calc	53.71	4.64	10.74
			Found	54.00	4.80	10.42