Supporting Information

New Dmt Opioid Peptidomimetics Based on the Aba-Gly Scaffold: Development of Unique μ-Opioid Receptor Ligands

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Peptide Synthesis[1]

Boc-Aba-Gly-NH-CH_2-Ph. To a solution of Boc-Aba-Gly-OH[2] (0.1 g, 0.3 mmol) and benzylamine (0.03 mL, 0.3 mmol) in DMF (10 mL) at 0 °C, HOBt (0.05 g, 0.33 mmol), and WSC (0.06 g, 0.33 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 citric acid (10% in H_2O), $NaHCO_3$ (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 0.12 g (92%); *Rf*(B) 0.68; HPLC *K′* 8.59; mp 78-80 °C; $[\alpha]^{20}_D$ +4.2; MH^+ 425; ^{1}H-NMR (DMSO-d_6) δ 1.40 (m, 9H), 2.92-3.17 (m, 2H), 4.09-4.48 (m, 6H), 4.92 (m, 1H), 6.96-7.14 (m, 9H).

TFA·H-Aba-Gly-NH-CH_2-Ph. Boc-Aba-Gly-NH-CH_2-Ph (0.12 g, 0.28 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 0.12 g (96%); *Rf*(A) 0.42; HPLC *K′* 5.63; mp 93-95 °C; $[\alpha]^{20}_D$ +5.6; MH^+ 324.

Boc-Dmt-Aba-Gly-NH-CH_2-Ph. To a solution of Boc-Dmt-OH (0.05 g, 0.16 mmol) and TFA·H-Aba-Gly-NH-CH_2-Ph (0.07 g, 0.16 mmol) in DMF (10 mL) at 0 °C, NMM (0.02 mL, 0.16 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 citric acid (10% in H_2O), $NaHCO_3$ (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%); *Rf*(B) 0.67; HPLC *K′* 8.59; mp 105-107 °C; $[\alpha]^{20}_D$ -3.2; MH^+ 616; ^{1}H-NMR (DMSO-d_6) δ 1.40 (s, 9H), 2.35 (s, 6H), 2.92-3.17 (m, 4H), 4.09-4.48 (m, 6H), 4.92 (m, 2H), 6.29 (s, 2H), 6.96-7.14 (m, 9H).

TFA·H-Dmt-Aba-Gly-NH-CH_2-Ph (1). Boc-Dmt-Aba-Gly-NH-CH_2-Ph (0.09 g, 0.15 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 0.09 g (96%); *Rf*(A) 0.52; HPLC *K′* 6.79; mp 120-122 °C; $[\alpha]^{20}_D$ -4.8; MH^+ 516; ^{1}H-NMR (DMSO-d_6) δ 2.35 (s, 6H), 2.92-3.17 (m, 4H), 3.95-4.48 (m, 7H), 4.92 (m, 1H), 6.29 (s, 2H), 6.96-7.14 (m, 9H).

Boc-Aba-Gly-NH-Ph. This compound was obtained by condensation of Boc-Aba-Gly-OH[2] with aniline via WSC/HOBt as reported for Boc-Aba.Gly-NH-CH_2-Ph: yield 0.1 g (90%); *Rf*(B) 0.61; HPLC *K′* 8.35; mp 75-77 °C; $[\alpha]^{20}_D$ +4.9; MH^+ 410; ^{1}H-NMR (DMSO-d_6) δ 1.40 (m, 9H), 2.92-3.17 (m, 2H), 4.14-4.48 (m, 4H), 4.92 (m, 1H), 6.96-7.64 (m, 9H).

TFA·H-Aba-Gly-NH-Ph. Boc-Aba-Gly-NH-Ph was treated with TFA as reported for TFA·H-Aba-Gly-NH-CH_2-Ph: yield 0.09 g (97%); *Rf*(A) 0.37; HPLC *K′* 5.36; mp 98-100 °C; $[\alpha]^{20}_D$ +6.4; MH^+ 310.

Boc-Dmt-Aba-Gly-NH-Ph. This compound was obtained by condensation of Boc-Dmt-OH with TFA·H-Aba-Gly-NH-Ph via WSC/HOBt as reported for Boc-Dmt-Aba-Gly-NH-CH_2-Ph: yield 0.08 g (89%); *Rf*(B) 0.59; HPLC *K′* 8.21; mp 111-113 °C; $[\alpha]^{20}_D$ -4.8; MH^+ 602; ^{1}H-NMR (DMSO-d_6) δ 1.40 (s, 9H), 2.35 (s, 6H), 2.92-3.17 (m, 4H), 4.14-4.48 (m, 4H), 4.92 (m, 2H), 6.29 (s, 2H), 6.96-7.64 (m, 9H).

TFA·H-Dmt-Aba-Gly-NH-Ph (2). Boc-Dmt-Aba-Gly-NH-Ph was treated with TFA as reported for TFA·H-Dmt-Aba-Gly-NH-CH_2-Ph: yield 0.07 g (95%); *Rf*(A) 0.46; HPLC *K′* 6.33; mp 126-128 °C; $[\alpha]^{20}_D$ -5.3; MH^+ 502; ^{1}H-NMR (DMSO-d_6) δ 2.35 (s, 6H), 2.92-3.17 (m, 4H), 3.95-4.48 (m, 5H), 4.92 (m, 1H), 6.29 (s, 2H), 6.96-7.64 (m, 9H).

tert-butyl 2-((1H-benzo[*d*]imidazol-2-yl)methyl)-2,3,4,5-tetrahydro-3-oxo-1H-benzo[*c*]-azepin-4-ylcarbamate [Boc-Aba-Gly*-Bid]. A solution of Boc-Aba-Gly-OH[2] (0.1 g, 0.3 mmol)

and NMM (0.03 mL, 0.3 mmol) in DMF (10 mL) was treated at -20 °C with IBCF, (0.04 mL, 0.3 mmol). After 10 min at -20 °C, *o*-phenylendiamine (0.03 g, 0.3 mmol) was added. The reaction mixture was allowed to stir while slowly warming to room temperature (1 h) and was then stirred for 3 h. The solvent was evaporated, and the residue was partitioned between EtOAc and H_2O. The EtOAc layer was washed with 5% $NaHCO_3$ and brine and dried over Na_2SO_4. The solution was filtered, the solvent was evaporated, and the residual solid was dissolved in glacial AcOH (10 mL). The solution was heated at 65 °C for 1 h. After the solvent was evaporated, the residue was crystallized from Et_2O/Pe (1:9, v/v): yield 0.1 g (82%); *Rf*(B) 0.51; HPLC *K′* 7.23; mp 85-87 °C; $[\alpha]^{20}_D$ +6.4; MH^+ 407; ^{1}H-NMR (DMSO-d_6) δ 1.40 (m, 9H), 2.92-3.17 (m, 2H), 4.45-4.48 (m, 4H), 4.92 (m, 1H), 6.96-7.70 (m, 8H).

2TFA·H-Aba-Gly*-Bid. Boc-Aba-Gly*-Bid was treated with TFA as reported for TFA·H-Aba-Gly-NH-CH_2-Ph: yield 0.09 g (96%); *Rf*(A) 0.28; HPLC *K′* 4.40; mp 102-104 °C; $[\alpha]^{20}_D$ +6.9; MH^+ 307.

Boc-Dmt-Aba-Gly*-Bid. To a solution of Boc-Dmt-OH (0.12 g, 0.4 mmol) and 2TFA·H-Aba-Gly*-Bid (0.21 g, 0.4 mmol) in DMF (10 mL) at 0 °C, NMM (0.09 mL, 0.8 mmol), HOBt (0.07 g, 0.44 mmol), and WSC (0.08 g, 0.44 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 0.21 g (88%); *Rf*(B) 0.46; HPLC *K′* 6.98; mp 115-117 °C; $[\alpha]^{20}_D$ +4.9; MH^+ 599; ^{1}H-NMR (DMSO-d_6) δ 1.40 (m, 9H), 2.35 (s, 6H), 2.92-3.17 (m, 4H), 4.45-4.48 (m, 4H), 4.92 (m, 2H), 6.29 (s, 2H), 6.96-7.70 (m, 8H).

2TFA·H-Dmt-Aba-Gly*-Bid (3). Boc-Dmt-Aba-Gly*-Bid was treated with TFA as reported for

TFA·H-Dmt-Aba-Gly-NH-CH_2-Ph: yield 0.15 g (90%); *Rf*(B) 0.46; HPLC *K′* 4.95; mp 134-136 °C; $[\alpha]^{20}_D$ +5.5; MH^+ 499; ^{1}H-NMR (DMSO-d_6) δ 2.35 (s, 6H), 2.92-3.17 (m, 4H), 3.95 (m, 1H), 4.45-4.48 (m, 4H), 4.92 (m, 1H), 6.29 (s, 2H), 6.96-7.70 (m, 8H).

References

(1) Abbreviations. In addition to the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol.* Chem. **1985**, *260*, 14-42), this paper uses the following additional symbols and abbreviations: H-Aba-Gly-OH, 2-(4-amino-4,5-dihydro-3-oxo-1*H*-ben- zo[*c*]azepin-2(3H)-yl)acetic acid; Aba-Gly*-Bid, 2-((1*H*-benzo[*d*]imidazol-2-yl)methyl)-4-amino-1,2,4,5-tetrahydrobenzo[*c*]azepin-3-one; Ac, acetyl; Asp*-Bid, -NH-CH(CH_2-COOH)-1*H*-benzimidazole-2-yl; Boc, *tert*-butyl- oxycarbonyl; DMF, *N,N*-dimethylformamide; DMSO-d_6, hexa- deuteriodimethyl sulfoxide; Dmt, 2′,6′-dimethyl-L-tyrosine; Gly*-Bid, -NH-CH_2-1*H*-benzimidazole-2-yl; 1-hydroxybenzotriazole; TFA, trifluoroacetic acid; WSC, 1-ethyl-3-[3′-dimethyl)aminopropyl]-carbodiimide HCl; Z, benzyloxycarbonyl; NMM, 4-methylmorpholine; MALDI-TOF, matrix assisted laser desorption ionization time-of-flight; *Rf*, relative mobility in thin-layer chromatography solvents.

(2) Tourwé, D.; Verschueren, K.; Frycia, A.; Davis, P.; Porreca, F.; Hruby, V.J.; Toth, G.; Jaspers, H.; Verheyden, P.; Van Binst, G. Conformational restriction of Tyr and Phe side chains in opioid peptides: information about preferred and bioactive side-chain topology. *Biopolymers* **1995** *38*, 1–12.

Legend to Table

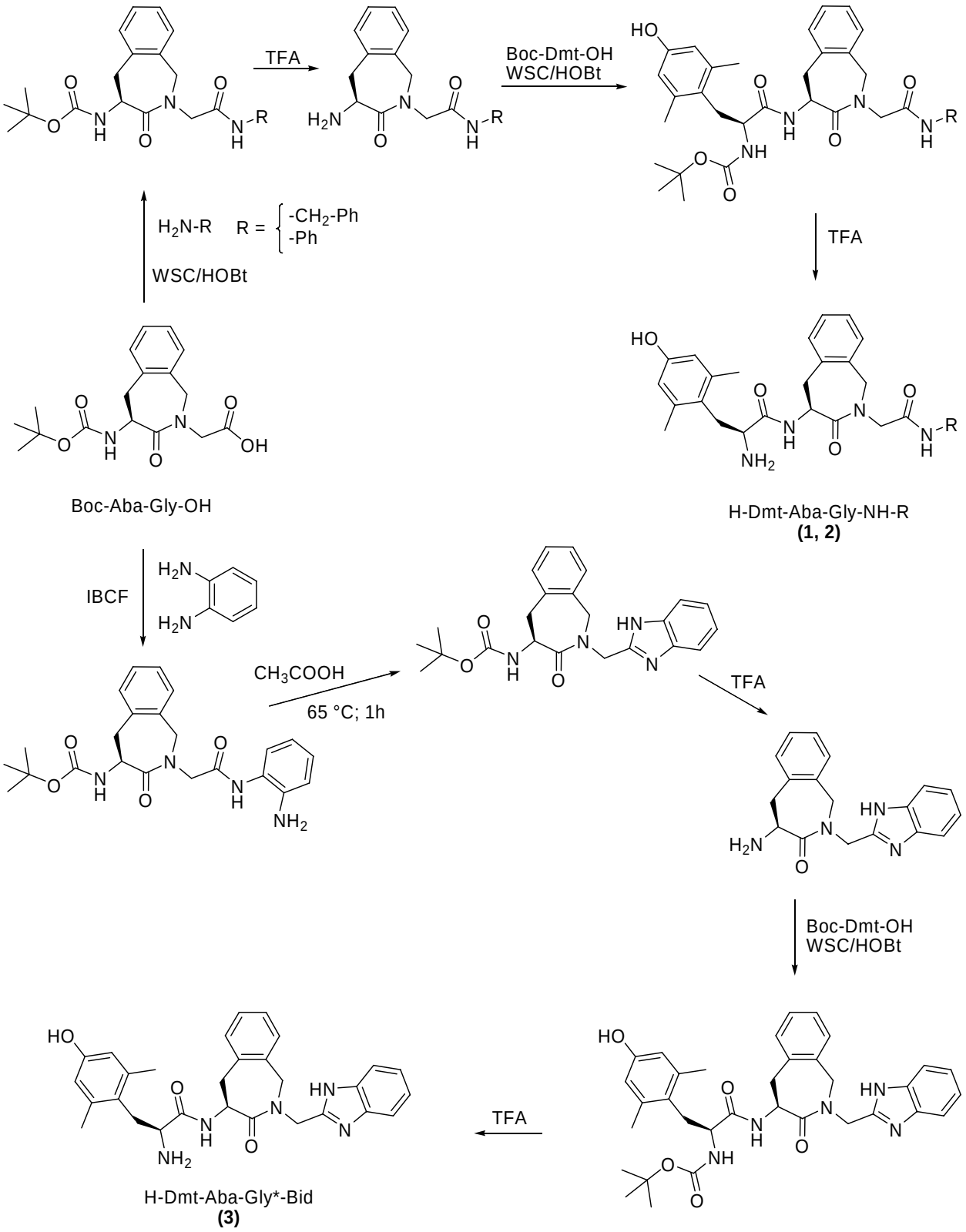
Table. Elemental analysis of compounds **1-3**. [a]Only the analysis of the new compounds, detailed in the **Experimental Section**, are included.

Legend to Scheme

Scheme. Synthesis of compounds **1-3**.

Table. Elemental analysis of compounds **1**-**3**.

no.[a]	Formula	MH^+, *m/z*		C	H	N
1	$C_{32}H_{35}F_3N_4O_6$	516	Calc	61.14	5.61	8.91
			Found	60.98	5.54	8.84
2	$C_{31}H_{33}F_3N_4O_6$	502	Calc	60.58	5.41	9.12
			Found	60.87	5.57	8.94
3	$C_{33}H_{33}F_6N_5O_7$	499	Calc	54.62	4.58	9.65
			Found	54.88	4.72	9.77


TFA
Boc-Dmt-OH
WSC/HOBt
TFA
H2N-R R = -CH2-Ph -Ph
WSC/HOBt
Boc-Aba-Gly-OH
H-Dmt-Aba-Gly-NH-R
(1, 2)
IBCF
CH3COOH
65 °C; 1h
TFA
Boc-Dmt-OH
WSC/HOBt
TFA
H-Dmt-Aba-Gly*-Bid
(3)

Scheme 1.