# Evolution of the Bifunctional Lead $\mu$ Agonist/$\delta$ Antagonist Containing the 2′,6′-Dimethyl-L-tyrosine−1,2,3,4-Tetrahydroisoquinoline-3-carboxylic Acid (Dmt−Tic) Opioid Pharmacophore

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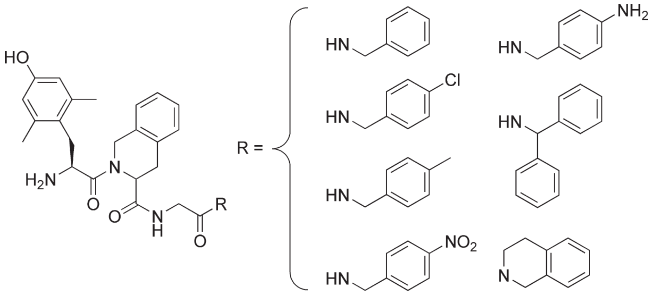
## Abstract




Based on a renewed importance recently attributed to bi- or multifunctional opioids, we report the synthesis and pharmacological evaluation of some analogues derived from our lead $\mu$ agonist/$\delta$ antagonist, H-Dmt-Tic-Gly-NH-Bzl (Dmt = 2′,6′-dimethyl-L-tyrosine, Tic = 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, Bzl = benzyl). Our previous studies focused on the importance of the C-teminal benzyl function in the induction of such bifunctional activity. The introduction of some substituents in the para position of the phenyl ring (−Cl, $-CH_3$, partially $-NO_2$, inactive $-NH_2$) was found to give a more potent $\mu$ agonist/antagonist effect associated with a relatively unmodified $\delta$ antagonist activity ($pA_2 = 8.28-9.02$). Increasing the steric hindrance of the benzyl group (using diphenylmethyl and tetrahydroisoquinoline functionalities) substantially maintained the $\mu$ agonist and $\delta$ antagonist activities of the lead compound. Finally and quite unexpectedly D-Tic[2], considered as a wrong opioid message now, inserted into the reference compound in lieu of L-Tic provided a $\mu$ agonist/$\delta$ agonist better than our reference ligand (H-Dmt-Tic-Gly-NH-Ph; Ph = phenyl) and was endowed with the same pharmacological profile.

**Keywords:** Bifunctional opioids, Dmt−Tic pharmacophore, opioid peptides, opioid receptors, angiogenesis, tolerance

Recently, bifunctional opioids characterized by a $\mu$ agonist/$\delta$ antagonist pharmacological profile are gaining renewed importance as potential drugs for the treatment of pain on the basis of their low propensity to induce tolerance and physical dependence (*1*−*3*). Furthermore, a project involving the use of our lead $\mu$ agonist/$\delta$ antagonist H-Dmt-Tic-Gly-NH-Bzl (*4*), (coded UFP-505) in the treatment of pain resulting from cancer was funded by the University of Leicester (UK) [https://swww2.le.ac.uk/ebulletin/news/press-releases/2000-2009/2008/12/nparticle.2008-12-05.9756874495].

In the 1990s, a series of articles demonstrated the potential utility of bifunctional ligands in pain relief with lower central or peripheral side effects (*5*−*14*). Although the majority of opioids act directly on the central nervous system to relieve pain, their activities on peripheral tissues are responsible for many of the secondary complications associated with the management of pain. A recently recognized peripheral effect of opioids and their receptors is the promotion of angiogenesis-dependent tumor growth (*15*). Opioids at physiologically relevant concentrations promote angiogenesis *in vitro*, as well as in breast cancer and in wound healing in rodents (*16*−*18*). Although naloxone and naltrexone can inhibit tumor growth in rodents (*16*, *19*, *20*), nonselective opioid antagonists cannot be used to counteract the unwanted effects of opioids without also compromising analgesia in a clinical setting. Therefore, it is important to identify agents that maintain the analgesic effect of opioids while inhibiting their angiogenic effect. This goal could be reached, for example, through the coadministration of morphine and the $\delta$ selective antagonist naltrindole, instead of the nonselective antagonists naloxone

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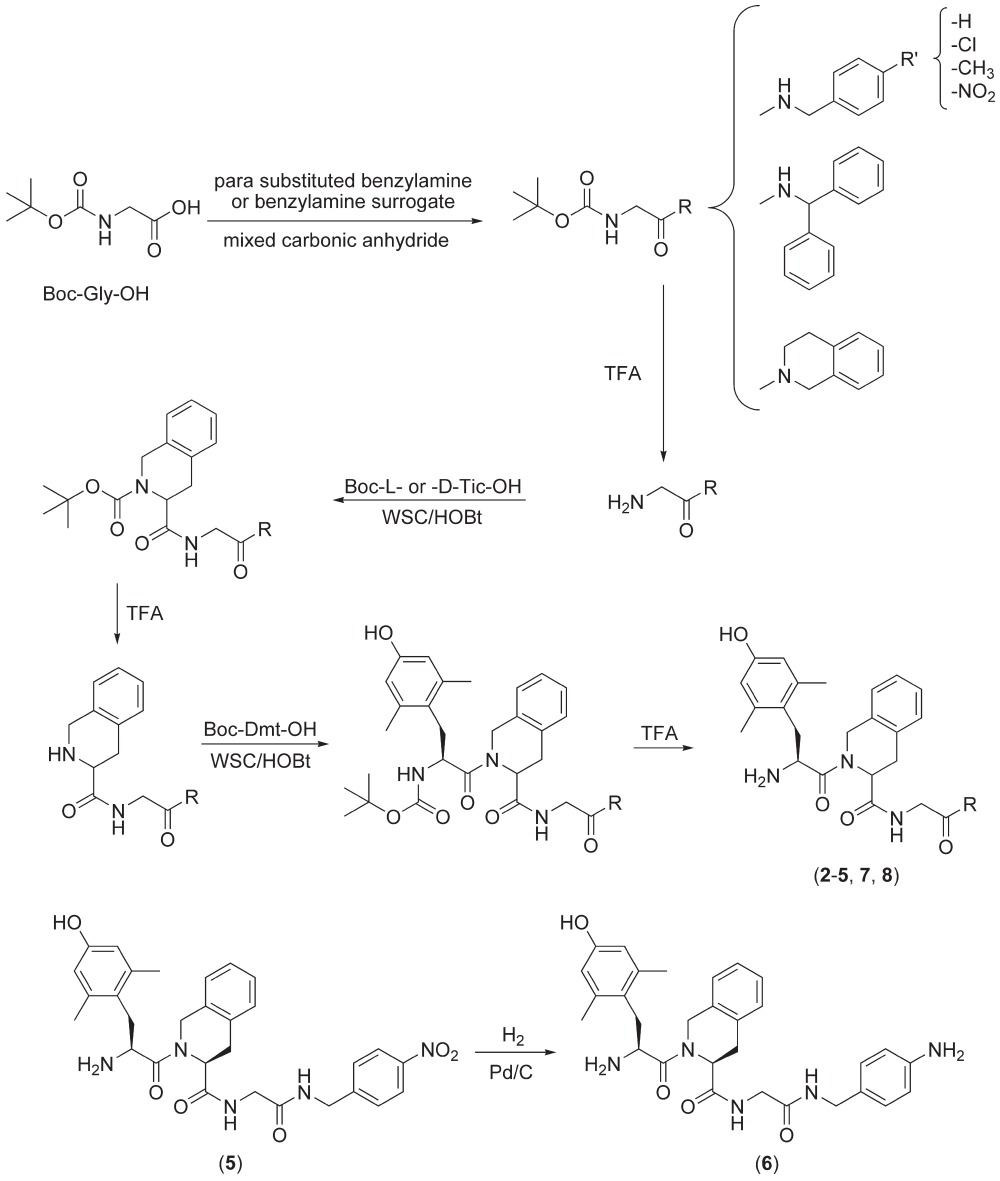
**Scheme 1.** General Synthetic Method for Compounds **2**−**8**




or naltrexone. Considering that bifunctional ligands may have advantages compared with drug combinations, such as more predictable pharmacokinetic and pharmacodynamic relationship as a consequence of the administration of a single drug (*1*), we focused our attention on the lead $\mu$ agonist/$\delta$ antagonist H-Dmt-Tic-Gly-NH-Bzl (*4*) as a potential analgesic displaying a lower induction of tolerance, a lower physical dependence, and a lower angiogenetic propensity compared with morphine. In previous studies regarding the Dmt−Tic opioid pharmacophore, it was demonstrated that the importance of the C-terminal group in the induction of different pharmacological profiles were as follows: -NH-Ph, $\mu$ agonist/$\delta$ agonist (*4*); -NH-Bzl, $\mu$ agonist/$\delta$ antagonist (*4*); and -NH-$CH_2$-Bid (*4*), -NH-CH($CH_3$)-Bid (*21*), -NH-CH($CH_2$-COOH)-Bid (*21*), $\delta$ agonists.

On the basis of the new interest in opioid bifunctional $\mu$ agonist/$\delta$ antagonist ligands, it would be valuable to increase the number of compounds endowed with such a profile for more thorough pharmacological investigations in the future. As a result, H-Dmt-Tic-Gly-NH-Bzl (**1**) was selected as a starting point for the synthesis of some new analogues modified on the C-terminal benzyl group. A minireview by Schiller et al. (*22*) prompted us to evaluate the electronic and steric hindrance effects by inserting electron-donating or -withdrawing groups in the para position of the phenyl ring (**3**−**6**) and by the employment of benzyl surrogates (**7**, **8**). Finally, although the Tyr/Dmt-D-Tic pharmacophore is considered



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**Table 1.** $K_i$ Values of the Inhibition of $\mu$, $\delta$ and $\kappa$ Opioid Binding to CHO Membranes

| Cp | Structure | $K_i$ (nM)±SEM[a] | | | Selectivity | | |
|---|---|---|---|---|---|---|---|
| | | [$^3$H]DAMGO ($\mu$) | [$^3$H]Naltrindole ($\delta$) | [$^3$H]U69,593 ($\kappa$) | $K_i^{\mu}/K_i^{\delta}$ | $K_i^{\kappa}/K_i^{\delta}$ | $K_i^{\kappa}/K_i^{\mu}$ |
| 1 | H-Dmt-Tic-Gly–NH– | 2.2±0.22 | 0.26±0.015 | 39±0.78 | 8.46 | 150 | 17.7 |
| 2 | H-Dmt-D-Tic-Gly–NH– | 0.068±0016 | 3.2±0.51 | 11±0.53 | 47.1[b] | 3.44 | 162 |
| 3 | H-Dmt-Tic-Gly–NH– (Cl) | 2.9±0.11 | 0.26±0.051 | 140±3.9 | 11.1 | 538 | 48 |
| 4 | H-Dmt-Tic-Gly–NH– | 3.7±0.21 | 0.31±0.029 | 240±10 | 11.9 | 774 | 64.9 |
| 5 | H-Dmt-Tic-Gly–NH– ($NO_2$) | 4.5±0.074 | 0.39±0.022 | 180±9.7 | 11.5 | 462 | 40.0 |
| 6 | H-Dmt-Tic-Gly–NH– ($NH_2$) | 34±1.2 | 2.0±0.33 | 750±33 | 17 | 375 | 22.1 |
| 7 | H-Dmt-Tic-Gly–NH– | 1.71±0.12 | 0.94±0.075 | 82±8.7 | 1.82 | 87.2 | 48.0 |
| 8 | H-Dmt-Tic-Gly–N | 3.3±0.16 | 0.75±0.10 | 130±2.2 | 4.40 | 173 | 39.4 |

[a] See Methods section. The $K_d$ values for [$^3$H]DAMGO, [$^3$H]U69,593, and [$^3$H]naltrindole were 0.56, 0.34, and 0.10 nM, respectively. These values were used to calculate the $K_i$ values. [b] Selectivity = $K_i^{\delta}/K_i^{\mu}$.

to contain the wrong stereochemistry (*23*−*25*), our curiosity encouraged us to synthesize the diastereomer of the lead compound (**1**), which resulted in the formation of H-Dmt-D-Tic-Gly-NH-Bzl (**2**).

## Chemistry

All peptides (**2**−**8**) were prepared stepwise in solution using conventional synthetic methods as outlined in Scheme 1. Boc-Gly-OH was condensed with either benzylamine, a para-substituted derivative, or a benzyl surrogate (containing a diphenylmethyl or a tetrahydroisoquinoline functionality) via a mixed carbonic anhydride. Each Boc-protected intermediate was deprotected with TFA and condensed with Boc-Tic-OH or Boc-D-Tic-OH via WSC/HOBt. After Tic deprotection by TFA, the dipeptides were subsequently condensed with Boc-Dmt-OH via WSC/HOBt. Final removal of the N-terminal protecting groups using TFA gave the crude final compounds (**2**−**5**, **7**, and **8**). Compound **6** was obtained by catalytic hydrogenation of the *para*-nitrobenzyl group of compound **5**. All peptides were purified by reverse-phase preparative HPLC.

## Results and Discussion

### Receptor Affinity Analysis

The tripeptides (**1**−**8**) were evaluated for their affinity and selectivity for $\mu$, $\delta$, and $\kappa$ opioid receptors using Chinese hamster ovary (CHO) cell membranes stably expressing the opioid receptors. The data are summarized in Table 1. All compounds (**1**−**8**) had nanomolar or subnanomolar affinity for both $\mu$ ($K_i$ = 0.068−4.5 nM) and $\delta$ ($K_i$ = 0.26−3.2 nM) receptors. Only the *para*-aminobenzyl analogue (**6**) resulted in a lower $\mu$ affinity ($K_i$ = 34 nM). As expected for peptides containing the Dmt−Tic pharmacophore, $\kappa$ affinity was low ($K_i$ = 11−750 nM). However, the reference compound (**1**) and its diastereomer (**2**) containing the C-terminal unmodified benzyl amide yielded higher $\kappa$ affinity (39 and 11 nM, respectively). Only diastereomer **2**, containing D-Tic, appears to be weakly $\mu$ selective



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**Table 2.** $EC_{50}$ and $E_{max}$ Values for the Stimulation of [$^{35}$S]GTP$\gamma$S Binding and $IC_{50}$ and $I_{max}$ Values for the Inhibition of Agonist-Stimulated [$^{35}$S]GTP$\gamma$S Binding to the Human $\mu$ and $\delta$ Opioid Receptors[a]

| | agonism | | | | antagonism | | | |
|---|---|---|---|---|---|---|---|---|
| | $\mu$ | | $\delta$ | | $\mu$ | | $\delta$ | |
| compd | $EC_{50}$ (nM) | $E_{max}$ (%) | $EC_{50}$ (nM) | $E_{max}$ (%) | $IC_{50}$ (nM) | $I_{max}$ (%) | $IC_{50}$ (nM) | $I_{max}$ (%) |
| **1** | 19 ± 1.9 | 84 ± 10 | *c* | 6.8 ± 1.8 | *b* | *b* | 1.3 ± 0.031 | 95 ± 1.1 |
| **2** | 0.90 ± 0.032 | 92 ± 4.0 | 2.8 ± 0.60 | 44 ± 6.1 | *b* | *b* | *b* | *b* |
| **3** | 33 ± 2.9 | 52 ± 5.5 | *c* | 4.5 ± 0.85 | 460 ± 93 | 43 ± 3.3 | 0.95 ± 0.058 | 93 ± 2.2 |
| **4** | 63 ± 3.5 | 34 ± 6.4 | *c* | 0.4 ± 1.43 | 820 ± 150 | 65 ± 1.1 at 25 $\mu$M[e] | 1.0 ± 0.28 | 86 ± 5.0 |
| **5** | 61 ± 15 | 16 ± 2.5 | *c* | 3.1 ± 0.43 | 810 ± 27.0 | 74 ± 6.9 | 1.6 ± 0.76 | 79 ± 3.8 |
| **6** | *d* | *d* | *c* | 13 ± 1.0 | *d* | *d* | 5.2 ± 0.13 | 78 ± 2.7 |
| **7** | 4.8 ± 0.16 | 45 ± 1.2 | *c* | −1.9 ± 0.17 | *b* | *b* | 8.3 ± 0.26 | 100 ± 7.4 |
| **8** | 22 ± 6.8 | 43 ± 6.6 | *c* | −1.4 ± 0.14 | *b* | *b* | 2.3 ± 0.27 | 99 ± 3.3 |

[a] See Methods section. Data are the mean $E_{max}$ and $EC_{50}$ values (SEM from at least three separate experiments, performed in triplicate). For calculation of the $E_{max}$ values, the basal [$^{35}$S]GTP$\gamma$S binding was set at 0%. [b] No inhibition. [c] Not active. [d] Not tested. [e] Compound **4** at a concentration of 25 $\mu$M inhibited 65% ± 1.1% of DAMGO-stimulated [$^{35}$S]GTP$\gamma$S binding. An $IC_{50}$ value was not reported because higher concentrations could not be used without having the DMSO vehicle interfere with the assay. A concentration of 200 nM DAMGO, which gave 96% ± 3.1% stimulation, was used to measure inhibition of DAMGO-stimulated [$^{35}$S]GTP$\gamma$S binding. A concentration of 100 nM U50,488, which gave 64% ± 1.9% stimulation, was used to measure inhibition of U50,488-stimulated [$^{35}$S]GTP$\gamma$S binding, and 10 nM SNC 80, which gave 66% ± 4.3% stimulation, was used to measure inhibition of [$^{35}$S]GTP$\gamma$S binding, mediated by the $\delta$ opioid receptor.

($K_i^{\delta}/K_i^{\mu}$ = 47.1) whereas all other compounds (**1**, **3**−**8**) are substantially less selective ($K_i^{\mu}/K_i^{\delta}$ = 1.82−17).

## Functional Bioactivity

Table 2 indicates the agonist and antagonist properties of the new compounds (**1**−**8**) in stimulating [$^{35}$S]-GTP$\gamma$S binding mediated by the $\mu$ and $\delta$ opioid receptors; [$^{35}$S]-GTP$\gamma$S binding mediated by $\kappa$ receptors is not reported due to their low binding affinity data (Table 1). This new data confirmed the $\mu$ agonist ($EC_{50}$ = 19 nM)/$\delta$ antagonist ($IC_{50}$ = 1.3 nM) activity of the reference compound **1** in comparison with the old data obtained using functional pharmacological assays (GPI, $EC_{50}$ = 2.69 nM; MVD, $K_e$ = 0.56 nM) (*4*). Compound **3**, bearing a chlorine atom at the para position of the benzyl amide, exhibited an agonist effect on $\mu$ receptors ($EC_{50}$ = 33 nM) comparable to other $\mu$ agonists containing the Dmt-Tic pharmacophore (*26*), a low but potentially interesting $\mu$ antagonist effect ($IC_{50}$ = 460 nM), and a potent $\delta$ antagonist effect ($IC_{50}$ = 0.95 nM). Compounds **4** and **5**, containing methyl and nitro groups, respectively, in the para position, had a similar but less interesting behavior, especially toward $\mu$ receptors. Compound **6** exhibited $\delta$ antagonist activity; however the functional bioactivity on $\mu$ receptors was not evaluated due to its poor affinity. Compounds **7** and **8** containing C-terminal benzyl amide surrogates revealed a $\mu$ agonism/$\delta$ antagonism behavior very similar to the reference compound **1**. Finally, H-Dmt-D-Tic-Gly-NH-Bzl **2**, which incorporated D-Tic, unexpectedly displayed a potent $\mu$ agonist ($EC_{50}$ = 0.9 nM)/$\delta$ agonist ($EC_{50}$ = 2.8 nM) opioid activity, superimposable to $\mu$ agonist/$\delta$ agonist lead compound H-Dmt-Tic-Gly-Ph (GPI, $EC_{50}$ = 2.57 nM; MVD, $EC_{50}$ = 3.02 nM) (*4*).

## Conclusions

In this SAR study, our attention was focused on the elaboration of the C-terminal benzyl amide of our lead $\mu$ agonist/$\delta$ antagonist H-Dmt-Tic-Gly-NH-Bzl (*4*). This amide functionality, considered responsible for the pharmacological profile in peptides containing the Dmt-Tic pharmacohore, was modified by the addition of electron-withdrawing or -donating groups in the para position or by increasing its steric hindrance. The pharmacological characterization of these analogues gave some interesting results: (1) affinities for the $\mu$, $\delta$, and $\kappa$ opioid receptors from CHO cell membranes and agonist/antagonist properties determined by stimulating [$^{35}$S]-GTP$\gamma$S binding confirmed the previous data reported for the reference compound **1** using brain $P_2$ synaptosomal membranes for affinity data and GPI and MVD tissues for agonism/antagonism (*4*); (2) the introduction of substituents in the para position of the phenyl ring and the increase of the steric hindrance further decreased the low $\kappa$ affinity of the unmodified benzyl amide; (3) compound **3**, containing the *para*-chlorobenzyl amide functionality, displayed the same $\mu$ agonist/$\delta$ antagonist profile of compound **1** and showed that a low $\mu$ antagonism could be useful in the trafficking of these receptors, thus avoiding their internalization without decreasing their analgesic effect (*27*−*29*); (4) compound **2** containing D-Tic[2] can be considered a derivative of the almost forgotten (Tyr)/Dmt-D-Tic pharmacophore (*23*−*25*). Initially, (Tyr)/Dmt-Tic and (Tyr)/Dmt-D-Tic were considered equally important opioid pharmacophores; in fact H-Tyr-Tic-$NH_2$(*24*) and H-Tyr-D-Tic-$NH_2$ (*24*) are commercially available as selective $\delta$ antagonist and nonselective



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$\mu$ agonist, respectively. Regarding the (Tyr)/Dmt-D-Tic pharmacophore, to the best of our knowledge only 12 analogues, which were not completely pharmacologically characterized were published from 1992 to 1995 (*23*−*25*) potentially due to being considered to contain the wrong stereochemistry. Unexpectedly, the new analogue H-Dmt-D-Tic-Gly-NH-Bzl (**2**) was endowed with interesting bifunctional $\mu/\delta$ agonist activity comparable to H-Dmt-Tic-Gly-NH-Ph(*4*) and confirmed the nonselective $\mu$ agonist activity evidenced years ago (*24*). On the basis of the new trend in opioid medicinal chemistry regarding the synthesis of bi- or multifunctional ligands, (*1*−*3*) the last two compounds mentioned above require more detailed pharmacological *in vivo* studies. In fact, Negus et al. stated that "delta opioid agonists can selectively enhance the antinociceptive effects of mu opioid agonists without enhancing some other, potentially undesirable mu agonist effects" (*30*). In summary, it appears that an increase in the steric hindrance of the benzyl amide function does not influence the activity; a substituent in the para position can induce further $\mu$ antagonism, and D-Tic$^2$, at least in this case, can reverse a $\delta$ antagonism into $\delta$ agonism without afffecting the $\mu$ activity.

# Methods

## Chemistry

**General Methods.** Crude peptides and pseudopeptides were purified by preparative reversed-phase HPLC [Waters Delta Prep 4000 system with Waters Prep LC 40 mm Assembly column C18 (30 cm × 4 cm, 15 $\mu$m particle)] and eluted at a flow rate of 20 mL/min with mobile phase solvent A (10% acetonitrile + 0.1% TFA in $H_2O$, v/v), and a linear gradient from 10% to 60% 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 × 4.6 mm, 5 $\mu$m 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 5% 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. $^1$H NMR ($\delta$) spectra were measured, when not specified, in DMSO-$d_6$ solution using a Bruker AC-200 spectrometer, and peak positions are given in parts per million downfield from tetramethylsilane as internal standard. The purity of tested compounds was determined by combustion elemental analyses conducted by the Microanalytical Laboratory of the Chemistry Department, University of Ferrara, with a Yanagimoto MT-5 CHN recorder elemental analyzer. All tested compounds possess a purity of at least 95% of the theoretical values.

**Boc-D-Tic-Gly-NH-Bzl.** To a solution of Boc-D-Tic-OH (0.2 g, 0.72 mmol) and TFA·H-Gly-NH-Bzl (*31*) (0.2 g, 0.72 mmol) in DMF (10 mL) at 0 °C, NMM (0.08 mL, 0.72 mmol), HOBt (0.12 g, 0.79 mmol), and WSC (0.15 g, 0.79 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 0.26 g (85%); $R_f$(B) 0.72; HPLC $K'$ 11.02; mp 123−125 °C; $[\alpha]_D^{20}$ +35.2; $m/z$ 425 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.39−1.41 (d, 9H), 2.92−3.17 (m, 2H), 4.09−4.27 (m, 4H), 4.46−4.92 (m, 3H), 6.96−7.14 (m, 9H).

**TFA·H-D-Tic-Gly-NH-Bzl.** Boc-D-Tic-Gly-NH-Bzl (0.21 g, 0.5 mmol) was treated with TFA (1.5 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 0.21 g (94%); $R_f$(A) 0.38; HPLC $K'$ 6.14; mp 151−153 °C; $[\alpha]_D^{20}$ +30.8; $m/z$ 324 $(M + H)^+$.

**Boc-Dmt-D-Tic-Gly-NH-Bzl.** To a solution of Boc-Dmt-OH (0.05 g, 0.16 mmol) and TFA·H-D-Tic-Gly-NH-Bzl (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 0.08 g (83%); $R_f$(B) 0.64; HPLC $K'$ 9.82; mp 138−140 °C; $[\alpha]_D^{20}$ +20.2; $m/z$ 616 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.39−1.41 (d, 9H), 2.35 (s, 6H), 2.92−3.17 (m, 4H), 4.09−4.51 (m, 6H), 4.90−4.94 (m, 2H), 6.29 (s, 2H), 6.96−7.14 (m, 9H).

**TFA·H-Dmt-D-Tic-Gly-NH-Bzl (2).** Boc-Dmt-D-Tic-Gly-NH-Bzl (0.05 g, 0.08 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 0.05 g (97%); $R_f$(A) 0.41; HPLC $K'$ 5.21; mp 147−149 °C; $[\alpha]_D^{20}$ +21.5; $m/z$ 516 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.35 (s, 6H), 2.92−3.17 (m, 4H), 3.93−3.97 (m, 1H), 4.09−4.51 (m, 6H), 4.90−4.94 (m, 1H), 6.29 (s, 2H), 6.96−7.14 (m, 9H). Anal. $C_{32}H_{35}F_3N_4O_6$: C; H; N.

**Boc-Gly-NH-Bzl(pCl).** A solution of Boc-Gly-OH (0.5 g, 2.86 mmol) and NMM (0.31 mL, 2.86 mmol) in DMF (10 mL) was treated at −20 °C with isobutyl chloroformate (IBCF, 0.37 mL, 2.86 mmol). After 10 min at −20 °C, 4-chlorobenzylamine (0.35 mL, 2.86 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



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partitioned between EtOAc and $H_2O$. The AcOEt layer was 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 0.76 g (89%); $R_f$(B) 0.42; HPLC $K'$ 4.25; mp 110−112 °C; $m/z$ 300 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.40 (s, 9H), 3.83−3.87 (d, 2H), 4.46 (s, 2H), 7.00−7.15 (m, 4H).

**TFA·H-Gly-NH-Bzl(pCl).** Boc-Gly-NH-Bzl(pCl) (0.71 g, 2.37 mmol) was treated with TFA (2.5 mL) for 0.5 h at room temperature. $Et_2O$/Pe (1:1, v/v) was added to the solution until the product precipitated: yield 0.69 g (93%); $R_f$(A) 0.39; HPLC $K'$ 3.67; mp 126−128 °C; $m/z$ 200 $(M + H)^+$.

**Boc-Tic-Gly-NH-Bzl(pCl).** This intermediate was obtained by condensation of Boc-Tic-OH with TFA·H-Gly-NH-Bzl-(pCl) via WSC/HOBt as reported for Boc-D-Tic-Gly-NH-Bzl: yield 0.52 g (88%); $R_f$(B) 0.81; HPLC $K'$ 11.15; mp 131−133 °C; $[\alpha]_D^{20}$ −30.2; $m/z$ 459 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.38−1.42 (d, 9H), 2.92−3.17 (m, 2H), 4.09−4.27 (m, 4H), 4.46−4.92 (m, 3H), 6.96−7.15 (m, 8H).

**TFA·H-Tic-Gly-NH-Bzl(pCl).** Boc-Tic-Gly-NH-Bzl(pCl) was treated with TFA as reported for TFA·H-D-Tic-Gly-NH-Bzl: yield 0.3 g (90%); $R_f$(A) 0.43; HPLC $K'$ 6.51; mp 156−158 °C; $[\alpha]_D^{20}$ −28.6; $m/z$ 359 $(M + H)^+$.

**Boc-Dmt-Tic-Gly-NH-Bzl(pCl).** This intermediate was obtained by condensation of Boc-Dmt-OH with TFA·H-Tic-Gly-NH-Bzl(pCl) via WSC/HOBt as reported for Boc-Dmt-D-Tic-Gly-NH-Bzl: yield 0.12 g (85%); $R_f$(B) 0.70; HPLC $K'$ 9.91; mp 144−146 °C; $[\alpha]_D^{20}$ −20.5; $m/z$ 650 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.38−1.42 (d, 9H), 2.35 (s, 6H), 2.92−3.17 (m, 4H), 4.09−4.51 (m, 6H), 4.90−4.94 (m, 2H), 6.29 (s, 2H), 6.96−7.15 (m, 8H).

**TFA·H-Dmt-Tic-Gly-NH-Bzl(pCl) (3).** Boc-Dmt-Tic-Gly-NH-Bzl(pCl) was treated with TFA as reported for TFA·H-Dmt-D-Tic-Gly-NH-Bzl: yield 0.07 g (96%); $R_f$(A) 0.46; HPLC $K'$ 5.36; mp 155−157 °C; $[\alpha]_D^{20}$ −18.6; $m/z$ 550 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.35 (s, 6H), 2.92−3.17 (m, 4H), 3.95−4.09 (m, 3H), 4.41−4.51 (m, 4H), 4.91−4.93 (m, 1H), 6.29 (s, 2H), 6.96−7.15 (m, 8H). Anal. $C_{32}H_{34}ClF_3N_4O_6$: C; H; N.

**Boc-Gly-NH-Bzl(pMe).** This intermediate was obtained by condensation of Boc-Gly-OH with 4-methylbenzylamine via mixed carbonic anhydride as reported for Boc-Gly-NH-Bzl(pCl): yield 0.72 g (86%); $R_f$(B) 0.45; HPLC $K'$ 4.36; mp 119−121 °C; $m/z$ 279 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.40 (s, 9H), 2.35 (s, 3H), 3.83−3.87 (d, 2H), 4.46 (s, 2H), 6.94−7.13 (m, 4H).

**TFA·H-Gly-NH-Bzl(pMe).** Boc-Gly-NH-Bzl(pMe) was treated with TFA as reported for TFA·H-Gly-NH-Bzl(pCl): yield 0.57 g (91%); $R_f$(A) 0.43; HPLC $K'$ 3.74; mp 133−135 °C; $m/z$ 179 $(M + H)^+$.

**Boc-Tic-Gly-NH-Bzl(pMe).** This intermediate was obtained by condensation of Boc-Tic-OH with TFA·H-Gly-NH-Bzl(pMe) via WSC/HOBt as reported for Boc-D-Tic-Gly-NH-Bzl: yield 0.46 g (87%); $R_f$(B) 0.84; HPLC $K'$ 11.23; mp 133−135 °C; $[\alpha]_D^{20}$ −28.4; $m/z$ 439 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.38−1.42 (d, 9H), 2.35 (s, 3H), 2.92−3.17 (m, 2H), 4.09−4.27 (m, 4H), 4.46−4.92 (m, 3H), 6.94−7.02 (m, 8H).

**TFA·H-Tic-Gly-NH-Bzl(pMe).** Boc-Tic-Gly-NH-Bzl(pMe) was treated with TFA as reported for TFA·H-D-Tic-Gly-NH-Bzl: yield 0.27 g (93%); $R_f$(A) 0.47; HPLC $K'$ 6.62; mp 163−165 °C; $[\alpha]_D^{20}$ −26.8; $m/z$ 338 $(M + H)^+$.

**Boc-Dmt-Tic-Gly-NH-Bzl(pMe).** This intermediate was obtained by condensation of Boc-Dmt-OH with TFA·H-Tic-Gly-NH-Bzl(pMe) via WSC/HOBt as reported for Boc-Dmt-D-Tic-Gly-NH-Bzl: yield 0.11 g (87%); $R_f$(B) 0.72; HPLC $K'$ 9.95; mp 149−151 °C; $[\alpha]_D^{20}$ −18.3; $m/z$ 630 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.38−1.42 (d, 9H), 2.34 (s, 6H), 2.38 (s, 3H), 2.92−3.17 (m, 4H), 4.09−4.51 (m, 6H), 4.90−4.94 (m, 2H), 6.29 (s, 2H), 6.94−7.02 (m, 8H).

**TFA·H-Dmt-Tic-Gly-NH-Bzl(pMe) (4).** Boc-Dmt-Tic-Gly-NH-Bzl(pMe) was treated with TFA as reported for TFA·H-Dmt-D-Tic-Gly-NH-Bzl: yield 0.05 g (93%); $R_f$(A) 0.50; HPLC $K'$ 5.42; mp 160−162 °C; $[\alpha]_D^{20}$ −17.5; $m/z$ 530 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.33 (s, 6H), 2.37 (s, 3H), 2.92−3.17 (m, 4H), 3.95−4.09 (m, 3H), 4.41−4.51 (m, 4H), 4.91−4.93 (m, 1H), 6.29 (s, 2H), 6.94−7.02 (m, 8H). Anal. $C_{33}H_{37}F_3N_4O_6$: C; H; N.

**Boc-Gly-NH-Bzl(pNO$_2$) (*32*).** This intermediate was obtained by condensation of Boc-Gly-OH with 4-nitrolbenzylamine via mixed carbonic anhydride as reported for Boc-Gly-NH-Bzl-(pCl): yield 0.96 g (88%); $R_f$(B) 0.35; HPLC $K'$ 4.08; mp 117−119 °C; $m/z$ 310 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.40 (s, 9H), 3.83−3.87 (d, 2H), 4.46 (s, 2H), 7.32−8.07 (m, 4H).

**TFA·H-Gly-NH-Bzl(pNO$_2$).** Boc-Gly-NH-Bzl(pNO$_2$) was treated with TFA as reported for TFA·H-Gly-NH-Bzl(pCl): yield 0.85 g (94%); $R_f$(A) 0.33; HPLC $K'$ 3.48; mp 134−136 °C; $m/z$ 210 $(M + H)^+$.

**Boc-Tic-Gly-NH-Bzl(pNO$_2$).** This intermediate was obtained by condensation of Boc-Tic-OH with TFA·H-Gly-NH-Bzl-(pNO$_2$) via WSC/HOBt as reported for Boc-D-Tic-Gly-NH-Bzl: yield 0.73 g (87%); $R_f$(B) 0.75; HPLC $K'$ 9.97; mp 136−138 °C; $[\alpha]_D^{20}$ −24.6; $m/z$ 469 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.38−1.42 (d, 9H), 2.92−3.17 (m, 2H), 4.09−4.27 (m, 4H), 4.46−4.92 (m, 3H), 6.96−8.07 (m, 8H).

**TFA·H-Tic-Gly-NH-Bzl(pNO$_2$).** Boc-Tic-Gly-NH-Bzl-(pNO$_2$) was treated with TFA as reported for TFA·H-D-Tic-Gly-NH-Bzl: yield 0.61 g (93%); $R_f$(A) 0.39; HPLC $K'$ 6.36; mp 161−163 °C; $[\alpha]_D^{20}$ −23.4; $m/z$ 369 $(M + H)^+$.

**Boc-Dmt-Tic-Gly-NH-Bzl(pNO$_2$).** This intermediate was obtained by condensation of Boc-Dmt-OH with TFA·H-Tic-Gly-NH-Bzl(pNO$_2$) via WSC/HOBt as reported for Boc-Dmt-D-Tic-Gly-NH-Bzl: yield 0.34 g (85%); $R_f$(B) 0.62; HPLC $K'$ 8.85; mp 153−155 °C; $[\alpha]_D^{20}$ −16.6; $m/z$ 661 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.38−1.42 (d, 9H), 2.35 (s, 6H), 2.92−3.17 (m, 4H), 4.09−4.51 (m, 6H), 4.90−4.94 (m, 2H), 6.29 (s, 2H), 6.96−8.07 (m, 8H).

**TFA·H-Dmt-Tic-Gly-NH-Bzl(pNO$_2$) (5).** Boc-Dmt-Tic-Gly-NH-Bzl(pNO$_2$) was treated with TFA as reported for TFA·H-Dmt-D-Tic-Gly-NH-Bzl: yield 0.25 g (95%); $R_f$(A) 0.38; HPLC $K'$ 5.03; mp 161−163 °C; $[\alpha]_D^{20}$ −13.3; $m/z$ 561 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.35 (s, 6H), 2.92−3.17 (m, 4H), 3.95−4.09 (m, 3H), 4.41−4.51 (m, 4H), 4.91−4.93 (m, 1H), 6.29 (s, 2H), 6.96−8.07 (m, 8H). Anal. $C_{32}H_{34}F_3N_5O_8$: C; H; N.



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**2TFA·H-Dmt-Tic-Gly-NH-Bzl(pNH$_2$) (6).** To a solution of TFA·H-Dmt-Tic-Gly-NH-Bzl(pNO$_2$) (0.12 g, 0.18 mmol) in methanol (30 mL) was added Pd/C (10%, 0.07 g), and $H_2$ was bubbled for 1 h at room temperature. After filtration, the solution was evaporated to dryness. The residue was crystallized from $Et_2O$/Pe (1:1, v/v): yield 0.12 g (90%); $R_f$(A) 0.34; HPLC $K'$ 4.82; mp 169−171 °C; $[\alpha]_D^{20}$ −15.1; $m/z$ 531 (M + H)$^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.35 (s, 6H), 2.92−3.17 (m, 4H), 3.95−4.09 (m, 3H), 4.41−4.51 (m, 4H), 4.91−4.93 (m, 1H), 6.29 (s, 2H), 6.34−7.02 (m, 8H). Anal. $C_{34}H_{37}F_6N_5O_8$: C; H; N.

***tert*-Butyl (Benzhydrylcarbamoyl)methylcarbamate [Boc-Gly-NH-Benzhydryl].** This intermediate was obtained by condensation of Boc-Gly-OH with benzhydrylamine via mixed carbonic anhydride as reported for Boc-Gly-NH-Bzl(pCl): yield 0.68 g (85%); $R_f$(B) 0.58; HPLC $K'$ 5.51; mp 127−129 °C; $m/z$ 341 (M + H)$^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.40 (s, 9H), 3.83−3.87 (d, 2H), 6.16 (s, 1H), 7.06−7.14 (m, 10H).

**TFA·H-Gly-NH-Benzhydryl.** Boc-Gly-NH-benzhydryl was treated with TFA as reported for TFA·H-Gly-NH-Bzl(pCl): yield 0.62 g (94%); $R_f$(A) 0.45; HPLC $K'$ 4.21; mp 135−137 °C; $m/z$ 241 (M + H)$^+$.

**Boc-Tic-Gly-NH-Benzhydryl.** This intermediate was obtained by condensation of Boc-Tic-OH with TFA·H-Gly-NH-Benzhydryl via WSC/HOBt as reported for Boc-D-Tic-Gly-NH-Bzl: yield 0.5 g (86%); $R_f$(B) 0.85; HPLC $K'$ 11.24; mp 136−138 °C; $[\alpha]_D^{20}$ −20.6; $m/z$ 501 (M + H)$^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.38−1.42 (d, 9H), 2.92−3.17 (m, 2H), 4.09−4.27 (m, 4H), 4.90−4.94 (m, 1H), 6.16 (s, 1H), 7.06−7.14 (m, 10H).

**TFA·H-Tic-Gly-NH-Benzhydryl.** Boc-Tic-Gly-NH-Benzhydryl was treated with TFA as reported for TFA·H-D-Tic-Gly-NH-Bzl: yield 0.35 g (92%); $R_f$(A) 0.52; HPLC $K'$ 7.03; mp 161−163 °C; $[\alpha]_D^{20}$ −21.2; $m/z$ 400 (M + H)$^+$.

**Boc-Dmt-Tic-Gly-NH-Benzhydryl.** This intermediate was obtained by condensation of Boc-Dmt-OH with TFA·H-Tic-Gly-NH-Benzhydryl via WSC/HOBt as reported for Boc-Dmt-D-Tic-Gly-NH-Bzl: yield 0.15 g (83%); $R_f$(B) 0.78; HPLC $K'$ 10.05; mp 149−151 °C; $[\alpha]_D^{20}$ −16.3; $m/z$ 691 (M + H)$^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.38−1.42 (d, 9H), 2.35 (s, 6H), 2.92−3.17 (m, 4H), 4.09−4.51 (m, 4H), 4.90−4.94 (m, 2H), 6.16 (s, 1H), 6.29 (s, 2H), 6.96−7.14 (m, 14H).

**TFA·H-Dmt-Tic-Gly-NH-Benzhydryl (7).** Boc-Dmt-Tic-Gly-NH-Benzhydryl was treated with TFA as reported for TFA·H-Dmt-D-Tic-Gly-NH-Bzl: yield 0.08 g (94%); $R_f$(A) 0.53; HPLC $K'$ 5.51; mp 161−163 °C; $[\alpha]_D^{20}$ −15.5; $m/z$ 592 (M + H)$^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.35 (s, 6H), 2.92−3.17 (m, 4H), 3.95−4.09 (m, 3H), 4.41−4.51 (m, 2H), 4.91−4.93 (m, 1H), 6.16 (s, 1H), 6.29 (s, 2H), 6.96−7.14 (m, 4H). Anal. $C_{38}H_{39}F_3N_4O_6$: C; H; N.

***tert*-Butyl 2-(3,4-Dihydroisoquinolin-2(1*H*)-yl)-2-oxoethylcarbamate [Boc-Gly-1,2,3,4-tetrahydroisoquinoline] (*33*).** This intermediate was obtained by condensation of Boc-Gly-OH with 1,2,3,4-tetrahydroisoquinoline via mixed carbonic anhydride as reported for Boc-Gly-NH-Bzl(pCl): yield 0.63 g (83%); $R_f$(B) 0.51; HPLC $K'$ 5.24; mp 125−127 °C; $m/z$ 291 (M + H)$^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.40 (s, 9H), 2.79−2.83 (m, 2H), 3.53−3.85 (m, 4H), 4.44−4.48 (m, 2H), 6.96−7.02 (m, 4H).

**TFA·H-Gly-1,2,3,4-tetrahydroisoquinoline.** Boc-Gly-1,2,3,4-tetrahydroisoquinoline was treated with TFA as reported for TFA·H-Gly-NH-Bzl(pCl): yield 0.54 g (93%); $R_f$(A) 0.42; HPLC $K'$ 4.14; mp 130−132 °C; $m/z$ 191 (M + H)$^+$.

**Boc-Tic-Gly-1,2,3,4-tetrahydroisoquinoline.** This intermediate was obtained by condensation of Boc-Tic-OH with TFA·H-Gly-1,2,3,4-tetrahydroisoquinoline via WSC/HOBt as reported for Boc-D-Tic-Gly-NH-Bzl: yield 0.46 g (88%); $R_f$(B) 0.82; HPLC $K'$ 11.00; mp 132−134 °C; $[\alpha]_D^{20}$ −18.6; $m/z$ 451 (M + H)$^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.38−1.42 (d, 9H), 2.81−3.17 (m, 4H), 3.51−3.55 (m, 2H), 4.09−4.27 (m, 4H), 4.44−4.48 (m, 2H), 4.90−4.94 (m, 1H), 6.96−7.02 (m, 8H).

**TFA·H-Tic-Gly-1,2,3,4-tetrahydroisoquinoline.** Boc-Tic-Gly-1,2,3,4-tetrahydroisoquinoline was treated with TFA as reported for TFA·H-D-Tic-Gly-NH-Bzl: yield 0.31 g (92%); $R_f$(A) 0.46; HPLC $K'$ 6.91; mp 153−155 °C; $[\alpha]_D^{20}$ −19.3; $m/z$ 350 (M + H)$^+$.

**Boc-Dmt-Tic-Gly-1,2,3,4-tetrahydroisoquinoline.** This intermediate was obtained by condensation of Boc-Dmt-OH with TFA·H-Tic-Gly-1,2,3,4-tetrahydroisoquinoline via WSC/HOBt as reported for Boc-Dmt-D-Tic-Gly-NH-Bzl: yield 0.12 g (82%); $R_f$(B) 0.74; HPLC $K'$ 9.86; mp 140−142 °C; $[\alpha]_D^{20}$ −17.8; $m/z$ 642 (M + H)$^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.38−1.42 (d, 9H), 2.35 (s, 6H), 2.81−3.17 (m, 6H), 3.53 (m, 2H), 4.09−4.51 (m, 6H), 4.90−4.94 (m, 2H), 6.29 (s, 2H), 6.96−7.02 (m, 8H).

**TFA·H-Dmt-Tic-Gly-1,2,3,4-tetrahydroisoquinoline (8).** Boc-Dmt-Tic-Gly-1,2,3,4-tetrahydroisoquinoline was treated with TFA as reported for TFA·H-Dmt-D-Tic-Gly-NH-Bzl: yield 0.06 g (92%); $R_f$(A) 0.49; HPLC $K'$ 5.26; mp 157−159 °C; $[\alpha]_D^{20}$ −16.9; $m/z$ 542 (M + H)$^+$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.35 (s, 6H), 2.81−3.17 (m, 6H), 3.53 (m, 2H), 3.95−4.51 (m, 7H), 4.90−4.94 (m, 1H), 6.29 (s, 2H), 6.96−7.02 (m, 8H). Anal. $C_{34}H_{37}F_3N_4O_6$: C; H; N.

## Pharmacology

**Radiolabeled Ligand Binding Assays.** Binding assays used to screen compounds are similar to those previously reported (*34*). Membrane protein from CHO cells that stably expressed one type of the human opioid receptor were incubated with 12 different concentrations of the compound in the presence of either 1 nM [$^3$H]U69,593 (*35*) ($\kappa$), 0.25 nM [$^3$H]DAMGO (*36*) ($\mu$), or 0.2 nM [$^3$H]naltrindole (*37*) ($\delta$) in a final volume of 1 mL of 50 mM Tris-HCl, pH 7.5, at 25 °C. Incubation times of 60 min were used for [$^3$H]U69,593 and [$^3$H]DAMGO. Because of a slower association of [$^3$H]naltrindole with the receptor, a 3 h incubation was used with this radioligand. Samples incubated with [$^3$H]naltrindole also contained 10 mM $MgCl_2$ and 0.5 mM phenylmethylsulfonyl fluoride. Nonspecific binding was measured by inclusion of 10 $\mu$M naloxone. The binding was terminated by filtering the samples through Schleicher & Schuell no. 32 glass fiber filters using a Brandel 48-well cell harvester. The filters were subsequently washed three times with 3 mL of cold 50 mM Tris-HCl, pH 7.5, and were counted in 2 mL of ScintiSafe 30% scintillation fluid. For [$^3$H]naltrindole and [$^3$H]U69,593 binding, the filters were soaked in 0.1% polyethylenimine for at least 60 min before use. $IC_{50}$ values were calculated by least-squares fit to a logarithm-probit analysis. $K_i$ values of unlabeled compounds were calculated



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from the equation $K_i = (IC_{50})/(1 + S)$ where $S$ = (concentration of radioligand)/($K_d$ of radioligand) (*38*). Data are the mean ± SEM from at least three experiments performed in triplicate.

**[$^{35}$S]GTP$\gamma$S Binding Assays.** In a final volume of 0.5 mL, 12 different concentrations of each test compound were incubated with 10 $\mu$g ($\delta$) or 7.5 $\mu$g ($\mu$) of CHO cell membranes that stably expressed either the human $\delta$ or $\mu$ opioid receptor. The assay buffer consisted of 50 mM Tris-HCl, pH 7.4, 3 mM $MgCl_2$, 0.2 mM EGTA, 3 $\mu$M GDP, and 100 mM NaCl. The final concentration of [$^{35}$S]GTP$\gamma$S was 0.080 nM. Nonspecific binding was measured by inclusion of 10 $\mu$M GTP$\gamma$S. Binding was initiated by the addition of the membranes. After an incubation of 60 min at 30 °C, the samples were filtered through Schleicher & Schuell no. 32 glass fiber filters. The filters were washed three times with cold 50 mM Tris-HCl, pH 7.5, and were counted in 2 mL of Ecoscint scintillation fluid. Data are the mean $E_{max}$ and $EC_{50}$ values ± SEM from at least three separate experiments, performed in triplicate. For calculation of the $E_{max}$ values, the basal [$^{35}$S]GTP$\gamma$S binding was set at 0%. To determine antagonist activity of a compound at the $\mu$ opioid receptors, CHO membranes expressing the $\mu$ opioid receptor were incubated with 12 different concentrations of the compound in the presence of 200 nM of the $\mu$ agonist DAMGO. To determine whether a compound was an antagonist at $\delta$ receptors, CHO membranes expressing the $\delta$ receptor were incubated with 12 different concentrations of the test compound in the presence of 10 nM of the $\delta$-selective agonist SNC 80.

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**Author Contributions**

G.B., S.S., and J.L.N. designed research; C.T. and X.P. synthesized the compounds; B.I.K., J.M.B., and L.H.L. generated and analyzed the pharmacological data; G.B. and J.L.N. wrote the paper.

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## Abbreviations

In addition to those of the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* 1985, *260*, 14−42), this paper uses the following additional symbols and abbreviations: AcOEt, ethyl acetate; Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; DAMGO, [D-Ala$^2$,*N*-Me-Phe$^4$,Gly-ol$^5$]enkephalin; DMF, *N*,*N*-dimethylformamide; DMSO-$d_6$, hexadeuteriodimethyl sulfoxide; Dmt, 2′,6′-dimethyl-L-tyrosine; $Et_2O$, diethyl ether; GPI, guinea pig ileum; HOBt, 1-hydroxybenzotriazole; HPLC, high-performance liquid chromatography; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; MVD, mouse vas deferens; NMM, 4-methylmorpholine; p$A_2$, negative log of the molar concentration required to double the agonist concentration to achieve the original response; Pe, petroleum ether; Ph, phenyl; TFA, trifluoroacetic acid; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; TLC, thin-layer chromatography; WSC, 1-ethyl-3-[(3′-dimethyl)aminopropyl]-carbodiimide hydrochloride.

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