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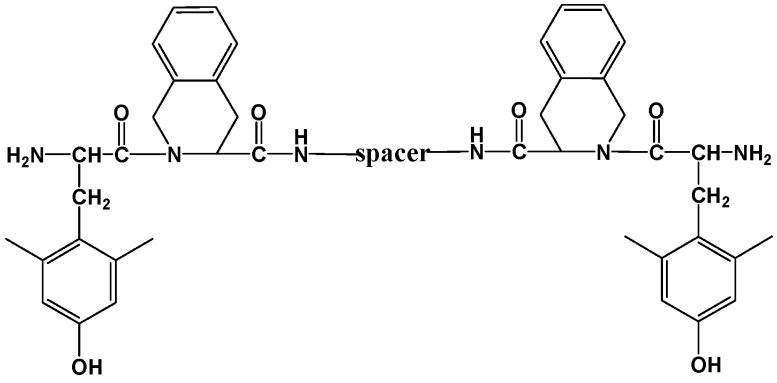

spacer = alkylene and pyrazinone

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Potent Dmt-Tic Pharmacophoric δ- and μ-Opioid Receptor Antagonists

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A series of dimeric Dmt-Tic (2′,6′-dimethyl-L-tyrosyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) analogues (**8**−**14**, **18**−**22**) were covalently linked through diaminoalkane and symmetric or asymmetric 3,6-diaminoalkyl-2(1*H*)-pyrazinone moieties. All the compounds exhibited high affinity for both δ-opioid receptors [$K_i(\delta) = 0.06-1.53$ nM] and μ-opioid receptors [$K_i(\mu) = 1.37-5.72$ nM], resulting in moderate δ-receptor selectivity [$K_i(\mu)/K_i(\delta) = 3-46$]. Regardless of the type of linker between the Dmt-Tic pharmacophores, δ-opioid-mediated antagonism was extraordinarily high in all analogues ($pA_2 = 10.42-11.28$), while in vitro agonism (MVD and GPI bioassays) was essentially absent (ca. 3 to >10 μM). While an unmodified *N*-terminus (**9**, **13**, **18**) revealed weak μ-opioid antagonism ($pA_2 = 6.78-6.99$), *N*,*N*′-dimethylation (**21**, **22**), which negatively impacts on μ-opioid-associated agonism (Balboni et al., *Bioorg. Med. Chem.* **2003**, *11*, 5435−5441), markedly enhanced μ-opioid antagonism ($pA_2 = 8.34$ and 7.71 for **21** and **22**, respectively) without affecting δ-opioid activity. These data are the first evidence that a single dimeric opioid ligand containing the Dmt-Tic pharmacophore exhibits highly potent δ- and μ-opioid antagonist activities.

Introduction[1]

The prototype δ-antagonist opioid H-Dmt-Tic-OH, developed from H-Tyr-Tic derivatives,[2] had high affinity [$K_i(\delta) = 0.022$ nM] and extraordinary selectivity relative to the μ-opioid receptor [$K_i(\mu)/K_i(\delta) = 150\,800$][3] and exhibited in vivo antagonistic activity against deltorphin II following systemic administration.[4] This pharmacophore underwent extensive modifications and structure−activity studies,[5] which included *N*-terminal modification with alkyl substitutions;[6] replacement of Tic by heteroaliphatic, heteroaromatic nuclei,[7] or D-Phe;[8] alteration of the C-terminus of Tic by substituents containing a hydrophobic groups;[6b] and addition of a third aromatic center with or without inserting interposing linkers.[9] Many of the analogues had unique properties, including enhanced δ-opioid antagonism,[5−9] conversion from a δ-antagonist to a δ-agonist,[9a] mixed μ-agonism/δ-antagonism,[6,9a] or development into an irreversible fluorescent δ-antagonist.[9c]

In addition, bivalent ligands containing two identical Dmt pharmacophoric moieties linked via alkyl or alkyl-pyrazinone spacers enhanced the potency and selectivity of the compounds to μ-opioid receptors relative to their monomeric lead compounds,[10,11] which would be attractive in drug design.[12] It is known that tail-to-tail condensation of pharmacophores significantly improved opioid receptor affinity and altered biological activity, which were speculatively claimed to result from the interaction with two spatially located receptors.[13,14] Several successful applications of this approach included, for example, dimeric dermorphin analogues, which increased affinities to both μ- and δ-opioid receptors and enhanced biological activity in vivo;[15] biphalin [(Tyr-D-Ala-Gly-Phe-NH-)$_2$], a dimeric enkephalin analogue that became antinociceptive after icv and systemic administration and induced less physical dependence than morphine;[14,16] condensation of [Dmt[1]]DALDA (selective μ-agonist) with TICP[ψ] (δ-antagonist) producing a chimeric analogue with a μ-agonist/δ-antagonist profile;[17] and the opiate norbinaltrophimine (norBNI), a selective κ-receptor antagonist, arising through dimerization of naltrexone derivatives.[18] Moreover, the formation of dimeric Dmt-derivatives developed from the observation that this single amino acid had an inherent and specific μ-opioid receptor affinity and bioactivity.[19] Dmt residues were linked through either diaminoalkanes[10] or 3,6-diaminoalkyl-2(*1H*)-pyrazinones,[11] and the resultant compounds exhibited remarkably high μ-opioid receptor affinities coupled with a potent bioactivities that included passage through the blood−brain barrier following systemic or oral administration.[11,20]

In this paper, we expand upon previously studies through the synthesis and analysis of the biological properties of a unique series of dimeric Dmt-Tic pharmacophores linked either through diaminoalkanes of

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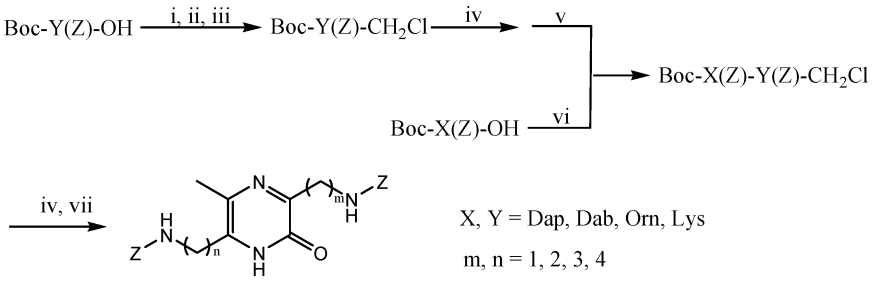
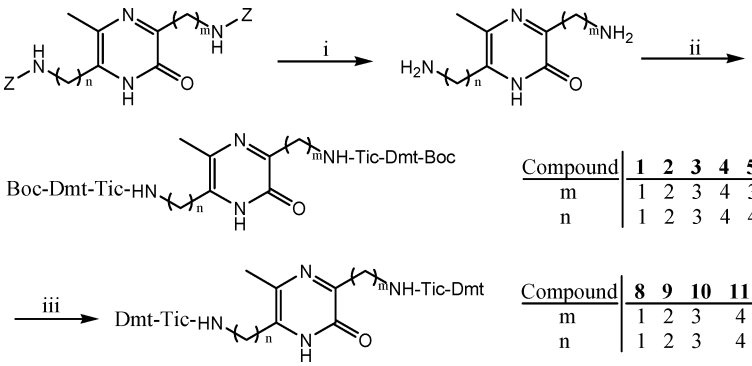
Scheme 1[a]



[a] Reagent and conditions: (i) IBCF, Et_3N, THF, −15 °C/10 min; (ii) CH_2N_2/Et_2O, rt/12 h; (iii) HCl/dioxane/15 min; (iv) HCl/dioxane; (v) Et_3N, DMF; (vi) IBCF, Et_3N, THF; (vii) MeOH reflux conditions for 1 h.

Scheme 2[a]



Compound	1	2	3	4	5	6	7
m	1	2	3	4	3	4	2
n	1	2	3	4	4	3	4

Compound	8	9	10	11	12	13	14
m	1	2	3	4	3	4	2
n	1	2	3	4	4	3	4

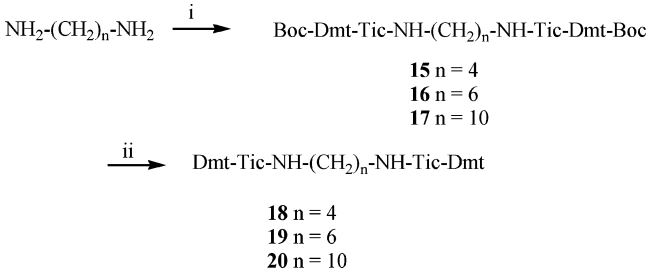
[a] (i) 25% HBr/AcOH; (ii) Boc-Dmt-Tic-OH, DIPEA, PyBop, DMF; (iii) TFA/anisole.

variable length or by symmetric or asymmetric 3,6-diaminoalkyl-2(1*H*)-pyrazinone derivatives. All the compounds had extraordinarily high δ-antagonism, greater than previously described in the literature,[5] coupled with high δ-opioid receptor affinities, which are common for Dmt-Tic-containing compounds[5] but now included concomitantly elevated μ-opioid receptor affinities, which were similarly noted with other Dmt derivatives.[10,11] However, while μ-agonism remained essentially minimal to undetectable (ca. 3 to >10 μM), we report herein the occurrence of a single opioid ligand containing potent dual δ- and μ-antagonist bioactivities.

Chemistry

Optically pure 2′,6′-dimethyl-L-tyrosine (Dmt) was prepared according to the method of Dygos et al.[21] Boc-Dmt-Tic-OH[6b] and *N*,*N*-dimethyl-Dmt-Tic-OH[6a] were prepared as described. Fmoc-NH-$(CH_2)_6$-NH-Boc was prepared from Boc-NH-$(CH_2)_6$-NH_2.[22] 3,6-Disubstituted-2(1*H*)-pyrazinone derivatives were synthesized following the procedure developed in this laboratory (Scheme 1).[23] Briefly, Boc-X(Z)-OH was coupled with H-Y(Z)-CH_2Cl by a mixed anhydride method to produce Boc-X(Z)-Y(Z)-CH_2Cl [X, Y = Dap (2,3-diaminopropionic acid), Dab (2,4-diaminobutyric acid), Orn, Lys]. After removal of the Boc group by HCl in dioxane, the corresponding amine hydrochloride in MeOH was treated under reflux conditions for 1 h to produce the Z-protected pyrazinone derivative. As shown in Scheme 2, the Z-protecting group was then removed by HBr/HOAc to release the amine group, which was then coupled with Boc-Dmt-Tic-OH using PyBop reagent to produce Boc-protected 3,6-bis(Dmt-Tic-aminoalkyl)pyrazinone derivatives (**1**−**7**). The Boc group was removed by TFA/anisole to give the 3,6-bis(Dmt-Tic-aminoalkyl)pyrazinone derivatives (**8**−**14**).

Scheme 3[a]



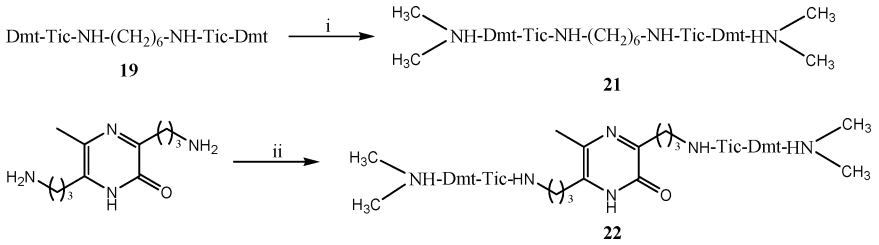
[a] (i) Boc-Dmt-Tic-OH, DIPEA, PyBop, DMF; (ii) TFA/anisole.

Symmetric pharmacophore compounds linked with diaminoalkanes (**18**−**20**) were synthesized as shown in Scheme 3. Boc-Dmt-Tic-OH was coupled with diaminoalkane to produce bis(Boc-Dmt-Tic-amino)alkanes (**15**−**17**), which were then treated with TFA/anisole to give bis(Dmt-Tic-amino)alkanes (**18**−**20**).

Compound **21** was prepared directly from **19** through reductive alkylation with formaldehyde and $NaBH_3CN$ in a H_2O and CH_3CN solution (Scheme 4). This method, however, failed in the preparation of compound **22** from compound **10**. Thus, after reductive alkylation, the unique 365-nm fluorescence of compound **10** was quenched, and the molecular weight of reaction product was 4 mass units greater than the desired one, which suggested that the two double bonds in the pyrazinone ring were reduced. Therefore, *N*,*N*-dimethyl-Dmt-Tic-OH was prepared through reductive alkylation of H-Dmt-Tic-OH as reported[6a] and coupled with 3,6-bis(3′-aminopropyl)pyrazinone by PyBop to give the desired compound (**22**).

The identification and purity of the final compounds were verified using MS, NMR, analytical HPLC, and elemental analysis. The elemental analysis data of the

Scheme 4[a]



[a] (i) 37% HCHO, $NaBH_3CN$, (ii) *N*,*N*-Dimethyl-Dmt-Tic-OH, DIPEA, PyBop, DMF.

Table 1. Rat Brain Membrane Receptor Binding Affinity of Dmt-Tic Dimers

compd	peptide	$K_i(\delta)$ (nM)[a]	n[c]	$K_i(\mu)$ (nM)[b]	n[c]	$K_i(\mu)/K_i(\delta)$
8	bis[(Dmt-Tic-NH)methyl]pyra[d]	0.163 ± 0.018	3	3.76 ± 0.30	4	23
9	bis[(Dmt-Tic-NH)ethyl]pyra	0.095 ± 0.001	3	2.83 ± 0.12	3	30
10	bis[(Dmt-Tic-NH)propyl]pyra	0.155 ± 0.016	3	3.08 ± 0.17	3	20
11	bis[(Dmt-Tic-NH)butyl]pyra	0.323 ± 0.007	3	1.74 ± 0.14	5	5
12	H-Dmt-Tic-propyl-pyra-butyl-Tic-Dmt-H	0.16 ± 0.03	3	1.56 ± 0.11	3	10
13	H-Dmt-Tic-butyl-pyra-propyl-Tic-Dmt-H	0.092 ± 0.010	3	2.28 ± 0.03	3	25
14	H-Dmt-Tic-ethyl-pyra-butyl-Tic-Dmt-H	0.107 ± 0.007	3	1.37 ± 0.13	3	13
18	bis[Dmt-Tic-NH]butyl	0.124 ± 0.016	3	5.72 ± 0.22	3	46
19	bis[Dmt-Tic-NH]hexyl	0.129 ± 0.030	3	1.79 ± 0.08	3	14
20	bis[Dmt-Tic-NH]decyl	1.53 ± 0.16	3	4.86 ± 0.41	4	3
21	bis[N,N-dimethyl-Dmt-Tic-NH]hexyl	0.06 ± 0.01	4	2.21 ± 0.08	6	37
22	3,6-bis[*N*,*N*-dimethyl-Dmt-Tic-NH-propyl]pyra	0.287 ± 0.015	3	1.68 ± 0.17	3	6

[a] Versus [^{3}H]delrorphin-II. [b] Versus [^{3}H]DAMGO. [c] The number of independent repetitions used different synaptosomal preparations. [d] pyra = pyrazinone.

final compounds are summarized in the Supporting Information. The compounds exhibiting greater than 98% purity were used for all biological assays.

Results and Discussion

Opioid Receptor Affinity. Affinities for μ- and δ-opioid receptors from rat brain membranes were computed by displacement of [^{3}H]DMAGO and [^{3}H]deltorphin-II, respectively, in equilibrium binding assays[6−9] (Table 1). Independent of the spacer used, most of the compounds exhibited high subnanomolar affinity to δ-opioid receptors [$K_i(\delta)$ = 0.06−0.323 nM], except compound **20** [$K_i(\delta)$ = 1.53 nM]; μ-opioid receptor affinities fell within the 1−5 nM range. Compared to the prototype δ-antagonist, H-Dmt-Tic-OH [$K_i(\delta)$ = 0.022 nM; $K_i(\mu)$ = 3320 nM],[3] μ-opioid receptor affinities increased by several orders of magnitude.

The application of opioid receptor affinities of the pyrazinone ring-containing compounds could not be used in order to assess the effect of the coupling of the alkyl linkers at either the 3 or 6 position of the pyrazinone ring in a comparable manner to the Dmt pharmacophoric compounds;[11] the data in Table 1 revealed that the affinities are quite similar. Furthermore, the selectivities of the compounds, which remain closely grouped (ranging from 5 to 25), demonstrated that the influence of Dmt in neutral or hydrophobic molecules shifted receptor discrimination toward μ-opioid receptors.[5,6,9]

Since the length of the pyrazinone ring is approximately equal to two carbon−carbon bonds, we assume that several pairs of compounds (**8** and **18**, **9** and **19**, **11** and **20**) would have a similar linker length between the Dmt-Tic pharmacophores. In two cases, however, a higher δ-opioid receptor affinity occurred with the pyrazinone ring-coupled compounds, suggesting that the pyrazinone ring imparts a distinctly consistent, albeit small, effect on the interaction within the receptor binding domain in compounds containing the Dmt-Tic pharmacophore. The decreased affinity of **8** compared with **18** might be due to several variables: steric effects imposed by the pyrazinone ring, the presence of methyl and keto groups, or the presence or position of nitrogen atoms. This latter possibility is suggested by the difference in the activity between the fourth position substituents of 5- and 6-quinoline in comparison to the naphthylene replacement of Phe^4-NH_2 in endormorphin-2.[24] The *N*,*N*′-dimethylated compounds **21** and **22** showed similar variation in δ- and μ-opioid affinities compared to the corresponding parent compounds **19** and **10**, respectively.

Functional Bioactivity. The functional biological activities were evaluated using isolated guinea pig ileum (GPI) for μ-opioid receptors and mouse vas deferens (MVD) for δ-opioid receptors (Table 2). Except **12**, **13**, and **20**, the other ligands listed in Table 2 showed no δ-opioid-receptor-mediated agonism; all the compounds were potent δ-antagonists, with pA_2 ranging from 10.42 to 11.28, which was greater than that of both naltrindole (pA_2 = 9.20) and H-Dmt-Tic-OH (pA_2 = 8.48) (Figure 1). The concentration−response curve of the δ-agonist deltorphin-II was markedly shifted to the right by the presence of Dmt-Tic dimeric compounds. Previous studies on the *N*,*N*′-alkylation of C-terminally modified Dmt-Tic pharmacophoric compounds, in which the nonalkylated derivatives exhibited increased μ-agonism, led to a marked loss of μ-associated biological activity.[9b] While it was reported that *N*-methylation increased the bioactivity in other compounds,[6a] **21** also had a 4.6-fold enhancement over its parent compound **19** (Table 2). In contrast to their μ-opioid receptor affinities, the compounds exhibited very weak to nonexistent agonism; especially, **21** and **22** exhibited absolutely pure and potent antagonism toward both δ- and μ-opioids (Table 2).

Table 2. Functional Bioactivity of Dmt-Tic Dimers

		MVD[a]		GPI[a]	
compd	peptide	agonist[b] IC_{50}, nM	antagoinst[c] pA_2 (nM)[e]	agonist[b] IC_{50}, nM	antagonist[d] pA_2 (nM)[e]
8	bis[(Dmt-Tic-NH)methyl]pyra[f]	>10000 (<1%)	11.22 (0.006)	>10000 (<15%)	ND
9	bis[(Dmt-Tic-NH)ethyl]pyra	>10000 (<1%)	10.73 (0.019)	>10000 (<27%)	6.78 (165)
10	bis[(Dmt-Tic-NH)propyl]pyra	>10000 (<1%)	10.56 (0.028)	7025 ± 2467	ND
11	bis[(Dmt-Tic-NH)butyl]pyra	>10000 (<1%)	11.06 (0.087)	>10000 (<34%)	ND
12	H-Dmt-Tic-propyl-pyra-butyl-Tic-Dmt-H	>10000 (<10%)	10.60 (0.025)	>10000 (<1%)	ND
13	H-Dmt-Tic-butyl-pyra-propyl-Tic-Dmt-H	>10000 (<22%)	10.47 (0.034)	>10000 (<9%)	6.95 (112)
14	H-Dmt-Tic-ethyl-pyra-butyl-Tic-Dmt-H	>10000 (<1%)	10.99 (0.010)	>10000 (<44%)	ND
18	bis[Dmt-Tic-NH]butyl	>10000 (<1%)	10.51 (0.031)	>10000 (<16%)	6.99 (102)
19	bis[Dmt-Tic-NH]hexyl	>10000 (<1%)	10.62 (0.024)	2715 ± 1359	ND
20	bis[Dmt-Tic-NH]decyl	>10000 (<28%)	10.97 (0.011)	5425 ± 1838	ND
21	1,6-bis(*N*,*N*-dimethyl-Dmt-Tic-NH)hexyl	>10000 (<1%)	11.28 (0.005)	>10000 (<1%)	8.34 (4)
22	3,6-bis(*N*,*N*-dimethyl-Dmt-Tic-NH-propyl)pyra	>10000 (<1%)	10.42 (0.04)	>10000 (<1%)	7.71 (19)
	H-Dmt-Tic-OH	none	8.48 (3.00)	ND	ND
	naltrindole[6a]		9.20 (0.60)		7.30 (50)

[a] The data are the means of over five independent repetitions used different isolated tissue preparations. [b] Values in parentheses indicate maximal inhibition of the tissue contraction at the concentration of 10 000 nM. [c] Versus deltorphin-II as the agonist. [d] Versus endomorphin-2. [e] The pA_2 values are expressed by antagonist concentrations within the parentheses at nM. [f] pyra = pyrazinone. [g] ND = not determined.

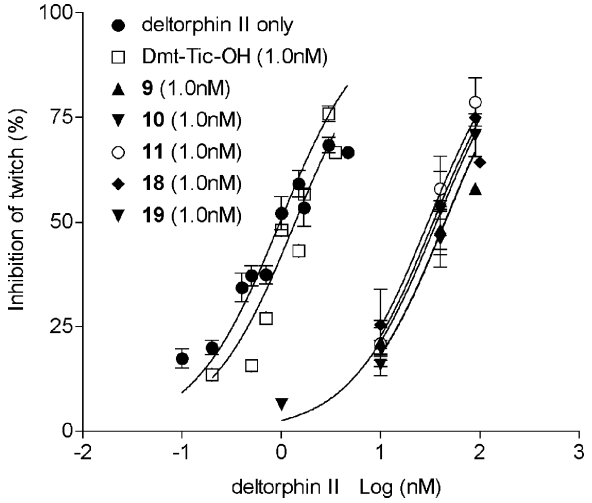


Figure 1. Antagonism by Dmt-Tic pharmacophoric dimeric compounds (**9**, **10**, **11**, **18**, **19**) in the MVD bioassay.

The μ-opioid receptor antagonist activities of **21** and **22** exceed that of some other known peptidic[25] and nonpeptidic[26] antagonists. Relative to the [D-Arg^2]-dermorphin(1−4) analogues, which had both weak μ-affinities and μ-antagonism (pA_2 = 5.3−6.1) and lacked interaction with δ-opioid receptors,[25b] the potency of **21** and **22** is greater by more than 2 orders of magnitude. Furthermore, compound **21** is 3-fold more potent than the cyclic somatostatin derivative CTOP (pA_2 = 7.9).[25a]

Comparison between most of the analogues (**9**, **10**, **12**, **13**, **18**, **19**) in the MVD assay failed to show any major difference, suggesting that δ-antagonism was due to the Dmt-Tic pharmacophore.[5] Portoghese et al.[13c] reported that the distance between the recognition sites of homodimeric μ−μ-opioid receptors with a TM5−TM6 interface is ∼27 Å. If this distance could be applicable to homodimeric δ−δ-opioid receptors based on receptor sequence homology (∼60% amino acid identity),[2,27] we assume that the spacer distances, from ∼8 Å (**8**) to ∼18 Å (**11** and **20**), would be incompatible with a ligand interacting between two binding sites located in different membrane-bound receptor molecules. However, regardless of the *N*,*N*′-dimethylation of compound **21**, which has identical δ-antagonism to compound **8**, despite the difference in the length of the distance between pharmacophores, other structural features, such as folding or the pyrazinone ring, must be taken into account, as discussed with the bis-Dmt-alkyl derivatives.[10]

On the basis of the κ-opioid receptor dimeric antagonist, Portoghese et al.[13] suggested that the κ-opioid receptor site comprises two key subsites: one recognizes the tyramine moiety in norBNI, while the second interacts with another element of the second pharmacophore. This description might be applicable to δ- and μ-opioid receptors, particularly the model of the δ-opioid receptor developed by computational chemistry[28] since both δ- and μ-opioid receptors recognize Dmt-Tic pharmacophore ligands with agonist and antagonist activities. Furthermore, two overlapping recognition sites were suggested for δ-opioid receptors.[28] Although the irreversible interaction of β-fulnaltrexamine to the μ-opioid receptor is through covalent binding to Lys^{233}, which joins TM-5 at the C-terminal region of extracellular loop 2,[29] this residue may not be involved with the effect observed with **21** on the basis of the observation that alkaloid opiates and peptide opioids apparently differentially affect opioid receptor internalization.[30]

While **10** and **19** exhibited very weak μ-agonism (Table 2), *N*,*N*′-dimethylation yielded **22** and **21**, forming substantial μ-antagonists. Moreover, these analogues retained potent δ antagonism, which is greater than other Dmt-Tic derivatives[6] or naltrindole[6a] (Table 2).

Theoretically there should be a correlation between the binding affinity and the antagonism in isolated tissues for an antagonist. However, some discrepancies were observed in our experiments on this point (Tables 1 and 2), as well as in many other published works,[3,6−8,25b] suggesting possible differences between the receptors in rat brain membranes and in GPI or MVD.[31]

Conclusion

These compounds represent unique opioid substances that express both δ- and μ-antagonism in the same molecule. Our data support the following conclusions:

(a) regardless of the spacer length or structural composition (alkyl or alkylpyrazinone), both types of compounds had high affinity for δ- and μ-opioid receptors; (b) all compounds exhibited extraordinarily high δ-antagonism, which correlated with the high affinity for δ-opioid receptors, and represent the most potent δ-antagonists described in the literature;[5] and (c) the extraordinary dual δ-and μ-antagonism of **21** and **22** qualify these compounds as potential pharmacological tools for potential application in the clinical and therapeutic treatment of drug addiction and alcohol dependency.[12] Moreover, considering that bis-Dmt analogues containing alkylpyrazinone are orally active and pass through the blood−brain barrier,[11,20] we would anticipate that **21** and **22** might show similar properties or may be more potent due to their increased hydrophobicity, which is a prerequisite for transit across membrane barriers.[32] Current studies are ongoing to determine the validity of these conclusions.

Experimental Section

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. TLC was performed on precoated plates of silica gel F254 (Merck, Darmstada, Germany). R_f values refer to the following solvent systems: (1) AcOEt:hexane = 3:1, (2) AcOEt:MeOH = 10:1, (3) $CHCl_3$:MeOH = 10:1, (4) AcOEt:hexane = 2:1, (5) *n*-BnOH:H_2O:AcOH = 4:1:5, (6) *n*-BnOH:H_2O:AcOH:pyridine = 4:1:1:2. Optical rotations were determined with a DIP-1000 automatic polarimeter (Japan Spectroscopic Co.). Analytical RP-HPLC and semipreparative RP-HPLC used a Waters Delta 600 with COSMOSIL C18 column (4.6 mm × 250 mm) and COSMOSIL C18 column (20 mm × 250 mm), respectively. The solvent for analytical HPLC was as follows: A, 0.05% TFA in water; B, 0.05% TFA in CH_3CN. The column was eluted at a flow rate of 1 mL/min with a linear gradient of 90% A to 10% A in 30 min; the retention time (t_R) is reported in minutes. Mass spectra were measured with a KRATOS MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight mass spectrometry). ^{1}H and ^{13}C NMR spectra were measured on a Bruker DPX-400 spectrometer at 25 °C. Chemical shift values are expressed as ppm downfield from tetramethylsilane.

General Procedure for Synthesis of Compound 1−7. 3,6-Bis(Z-aminoalkyl)-2(1*H*)-pyrazinone (0.27 mmol) was stirred in 25% HBr/AcOH (1.88 mL, 7.80 mmol) with an ice bath for 10 min and then room temperature for 3 h. The resulting amine was precipitated with ether and dried in vacuo. The precipitate was dissolved in DMF (10 mL) containing DIPEA (237 μL, 1.36 mmol), to which Boc-Dmt-Tic-OH (277 mg, 0.59 mmol) and PyBop (324 mg, 0.62 mmol) were added. The solution was first stirred in an ice bath for 10 min and then at room temperature for 4 h. After removal of the solvent in vacuo, the residue was extracted with AcOEt, which was washed with 10% citric acid (3 × 10 mL), 5% $NaHCO_3$ (3 × 10 mL), and saturated aqueous NaCl solution (3 × 15 mL) and dried over Na_2SO_4. After filtration, the solvent was evaporated in vacuo, the crude compound was purified by silica gel chromatography, and the compound was precipitated with ether. Elemental analyses of **1**−**7** are summarized in the Supporting Information.

3,6-Bis(Boc-Dmt-Tic-NH-methyl)-2(1*H*)-pyrazinone (1). Starting from 3,6-bis(Z-NH-CH_2)-2(1*H*)-pyrazinone (70 mg, 0.16 mmol), the crude compound was purified by silica gel chromatography (2.8 cm × 33 cm; AcOEt:MeOH = 13:1): yield 144 mg (84%); mp 165−167 °C; R_{f3} = 0.57; $[\alpha]^{25}_D$ +2.49° (*c* = 0.45, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.59 (br, 1H), 8.99 (d, 1H, *J* = 9.11 Hz), 8.05 (s, 1H), 7.59 (s, 1H), 7.30−7.00 (m, 8H), 6.85−5.85 (m, 3.6H), 5.80 (d, 1H, *J* = 5.21 Hz), 5.48 (d, 0.9H, *J* = 8.79 Hz), 5.36−5.20 (m 1H), 5.20−4.96 (m, 3.9H), 4.86−4.52 (m, 3.2H), 4.48−4.33 (m, 1.9H), 4.19 (d, 1H, *J* = 17.86 Hz), 3.97−3.72 (m, 1.9H), 3.60 (d, 0.9H, *J* = 15.55 Hz), 3.28−2.86 (m, 5.2H), 2.71 (d, 1H, *J* = 11.56 Hz), 2.63−1.85 (m, 16H), 1.50−0.50 (m, 18H); ^{13}C NMR ($CDCl_3$) δ 173.04, 172.96, 171.45, 169.91, 156.88, 156.74, 155.83, 155.04, 154.59, 151.72, 138.68, 133.21, 132.72, 130.91, 130.73, 129.82 (16q), 129.51, 128.55, 128.46, 126.97, 126.62, 126.54, 126.41, 126.30, 126.11 (9t), 123.60, 122.98 (2q), 116.58, 115.75 (2t), 81.24, 80.11 (2q), 55.77, 51.20, 50.60, 48.83 (4t), 46.34, 46.29, 45.11, 44.04, 40.70, 37.09, 34.27, 31.68, 29.48 (9s), 28.38 (t), 28.15 (s), 27.49 (t), 26.46, 26.38 (2s), 20.48, 19.90, 18.40 (3p).

3,6-Bis(Boc-Dmt-Tic-NH-ethyl)-2(1*H*)-pyrazinone (2). Starting from 3,6-bis[Z-NH-$(CH_2)_2$]-2(1*H*)-pyrazinone (125 mg, 0.27 mmol), the crude compound was purified by silica gel chromatography (2.6 cm × 34 cm; AcOEt:MeOH = 20:1). Yield 186 mg (63%); mp 177−178 °C; R_{f2} = 0.56; $[\alpha]^{25}_D$ −21.2° (*c* = 0.38, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (br, 1H), 7.80−7.40 (m, 1H), 7.20−6.73 (m, 8H), 6.73−6.28 (m, 4H), 5.72−5.38 (m, 1.7H), 5.38−4.85 (m, 3.3H), 4.85−4.15 (m, 3H), 4.00−3.60 (m, 2.3H), 3.60−2.36 (m, 11H), 2.36−1.65 (m, 21H), 1.65−1.15 (m, 19.6H); ^{13}C NMR ($CDCl_3$) δ 174.01, 173.96, 173.54, 173.17, 170.54, 170.16, 170.00, 169.89, 156.65, 156.50, 156.28, 155.98, 155.80, 155.50, 155.13, 155.03, 139.16, 138.82, 133.81, 133.72, 133.28, 132.62, 131.28, 131.23 (24q), 128.52, 128.44, 128.01, 127.96, 127.89, 127.53, 127.09, 127.05, 126.86, 126.08, 125.83 (11t), 124.45, 124.05, 123.92, 123.22 (4q), 116.04, 115.99, 115.66 (3t), 80.77, 80.17, 80.08, 80.02 (4q), 55.77, 54.60, 50.37, 49.32 (4t), 45.70, 44.04, 37.73, 37.66, 36.72, 36.58, 33.31, 30.75, 30.21 (9s), 28.39, 28.37, 20.43, 20.28, 19.96, 18.00 (6p).

3,6-Bis(Boc-Dmt-Tic-NH-propyl)-2(1*H*)-pyrazinone (3). Starting from 3,6-bis[Z-NH-$(CH_2)_3$]-2(1*H*)-pyrazinone (132 mg, 0.27 mmol), the crude compound was purified by silica gel chromatography (2.6 cm × 34 cm; AcOEt:MeOH = 20:1): yield 157 mg (52%); mp 165−166 °C; R_{f2} = 0.56; $[\alpha]^{25}_D$ −9.37° (*c* = 0.36, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (br, 1H), 8.00−7.48 (m, 1H), 7.20−6.70 (m, 8H), 6.70−6.35 (m, 4H), 5.75−5.34 (m, 2H), 5.34−4.80 (m, 3.4H), 4.80−4.30 (m, 3.0H), 4.05−3.70 (m, 1.6H), 3.50−2.85 (m, 10.5H), 2.85−1.63 (m, 20.6H), 1.63−1.05 (m, 22.3H); ^{13}C NMR ($CDCl_3$) δ 173.91, 173.70, 173.19, 171.02, 170.85, 169.72, 169.52, 156.45, 156.23, 155.93, 155.71, 155.69, 155.54, 155.21, 155.10, 138.96, 138.81, 138.68, 133.58, 133.54, 133.46, 132,87, 131.63, 131.44, 131.12, 131.07 (26q), 128.47, 127.68, 127.55, 127.34, 126.95, 126.74, 126.70, 126.66, 126.21, 125.87 (10t), 124.03, 123.83, 123.03 (3q), 115.85, 115.79, 115.60, 115.34 (4t), 80.68, 80.63, 80.18, 80.04 (4q), 55.96, 54.16, 50.79, 49.42 (4t), 45.59, 44.65, 38.95, 38.02, 33.47, 31.95, 31.36, 31.16 (8s), 28.37, 28.33 (2p), 26.84, 25.70 (2s), 20.43, 20.38, 19.97, 17.84 (4p).

3,6-Bis(Boc-Dmt-Tic-NH-butyl)-2(1*H*)-pyrazinone (4). Starting from 3,6-bis[Z-NH-$(CH_2)_4$]-2(1*H*)-pyrazinone (130 mg, 0.25 mmol), the crude compound was purified with silica gel chromatography (2.8 cm × 36 cm; $CHCl_3$:MeOH = 20:1): yield 226 mg (79%); mp 162−164 °C; R_{f3} = 0.62; $[\alpha]^{25}_D$ −4.1°(*c* = 0.38, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62−7.40 (m, 0.9H), 7.20−6.69 (m, 8.3H), 6.69−6.35 (m, 4H), 5.65−5.40 (m, 1.8H), 5.24−4.83 (m, 3.4H), 4.83−4.30 (m, 3.6H), 4.05−3.70 (m, 1.9H), 3.50−2.75 (m, 10.8H), 2.75−1.72 (m, 21.9H), 1.65−0.60 (m, 26.3H); ^{13}C NMR ($CDCl_3$) δ 174.00, 173.93, 173.35, 173.23, 170.81, 170.44, 170.00, 169.50, 156.90, 156.55, 156.04, 155.73, 155.36, 15.17, 138.99, 138.87, 138.73, 138.58, 133.76, 133.68, 131.25, 131.10, 131.55, 131.27 (24q), 128.38, 128.11, 127.73, 127.48, 127.06, 126.81, 126.68, 126.61, 126.40, 126.06, 125.87, 125.59 (12t), 124.21, 123.90, 123.07, 122.97 (4q), 115.99, 115.81, 115.67, 115.54 (4t), 80.51, 80.39, 80.14, 80.04 (4q), 55.91, 54.46, 50.77, 49.48 (4t), 45.76, 44.52, 39.35, 38.98, 38.13, 37.70, 33.43, 33.30, 32.18, 31.96, 31.43, 30.33, 28.94, 28.53 (14s), 28.36, 28.34 (2p), 24.46, 23.72 (2s), 20.38, 19.97, 19.92, 18.00, 17.84 (5p).

3-(Boc-Dmt-Tic-NH-propyl)-6-(Boc-Dmt-Tic-NH-butyl)-2(1*H*)-pyrazinone (5). Starting from 3-[Z-NH-$(CH_2)_3$]-6-[Z-NH-$(CH_2)_4$]-2(1*H*)-pyrazinone (127 mg, 0.25 mmol), the crude compound was purified with silica gel chromatography (2.8 cm × 36 cm; $CHCl_3$:MeOH = 20:1): yield 203 mg (73%); mp 164−166 °C; R_{f3} = 0.56; $[\alpha]^{25}_D$ −8.12° (*c* = 0.36, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75−7.43 (m, 1H), 7.25−6.72 (m, 8H), 6.72−6.40 (m, 4H), 5.68−5.41 (m, 1.8H), 5.31−4.97 (m,

3.1H), 4.85−4.33 (m, 3.5H), 4.02−3.73 (m, 1.8H), 3.64−2.88 (m, 10.6H), 2.88−2.05 (m, 20.9H), 2.05−1.53 (m, 1.7H), 1.53−0.52 (m, 23.7H); ^{13}C NMR ($CDCl_3$) δ 173.63, 173.36, 171.15, 170.52, 169.71, 169.44, 156.76, 156.39, 156.06, 155.84, 155.55, 155.32, 155.18, 155.10, 138.99, 138.89, 138.80, 138.55, 133.80, 133.54, 133.50, 133.27, 131.66, 131.44, 131.06, 130.92 (26q), 128.54, 128.30, 127.99, 127.50, 127.07, 126.71, 126.64, 126.17, 125.91, 125.62 (10t), 124.24, 123.99, 123.55, 122.96 (4q), 116.14, 116.05, 115.78, 115.53 (4t), 8.057, 80.45, 80.12, 80.04 (4q), 55.96, 53.80, 50.84, 49.36 (4t), 45.61, 44.68, 38.83, 38.00, 37.21, 33.46, 32.12, 31.52, 30.85, 30.49, 28.96, 28.79 (12s), 28.36, 28.35 (2p), 28.05, 27.04, 24.07, 23.84 (4s), 20.42, 20.39, 19.99, 19.92, 17.84 (5p).

3-(Boc-Dmt-Tic-NH-butyl)-6-(Boc-Dmt-Tic-NH-propyl)-2(1*H*)-pyrazinone (6). Starting from 3-[Z-NH-$(CH_2)_4$]-6-[Z-NH-$(CH_2)_3$]-2(1*H*)-pyrazinone (136 mg, 0.27 mmol), the crude compound was purified with silica gel chromatography (2.6 cm × 34 cm; AcOEt:MeOH = 20:1): yield 145 mg (47%); mp 164−165 °C; $R_{f2} = 0.57$; $[\alpha]^{25}_D$ −4.0° ($c = 0.33$, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (br, 1H), 7.95−7.32 (m, 1H), 7.18−6.73 (m, 8H), 6.70−6.40 (m, 4H), 5.64−5.33 (m, 1.8H), 5.33−4.85 (m, 3.1H), 4.85−4.25 (m, 3.2H), 4.00−3.70 (m, 1.6H), 3.45−2.72 (m, 10.5H), 2.70−1.77 (m, 21.4H), 1.60−0.80 (m, 25H); ^{13}C NMR ($CDCl_3$) δ 173.92, 173.28, 171.13, 170.96, 170.06, 169.91, 156.55, 156.33, 156.23, 155.90, 155.82, 177.71, 155.49, 155.26, 155.15, 138.99, 138.75, 138.64, 133.69, 133.66, 133.48, 133.02, 131.63, 131.28 (24q), 128.41, 128.35, 128.23, 127.71, 127.40, 126.94, 126.82, 126.38, 126.23, 125.90 (10t), 124.09, 122.94 (2q), 115.86, 115.70 (2t), 80.70, 80.39, 80.19, 80.05 (4q), 55.93, 54.54, 54.13, 50.85, 50.43, 49.50 (6t), 45.75, 44.66, 44.10, 38.96, 38.20, 37.93, 33.50, 32.00, 31.45, 30.57, 30.12, 28.70 (12s), 28.37, 28.32 (2p), 26.41, 25.55, 23.72, 23.58 (4s), 20.43, 20.35, 20.17, 19.97, 17.90 (5p).

3-(Boc-Dmt-Tic-NH-ethyl)-6-(Boc-Dmt-Tic-NH-butyl)-2(1*H*)-pyrazinone (7). Starting from 3-[Z-NH-$(CH_2)_2$]-6-[Z-NH-$(CH_2)_4$]-2(1*H*)-pyrazinone (130 mg, 0.26 mmol), the crude compound was purified with silica gel chromatography (3.2 cm × 30 cm; $CHCl_3$:MeOH = 20:1): yield 185 mg (62%); mp 172−174 °C; $R_{f3} = 0.57$; $[\alpha]^{25}_D$ −6.0° ($c = 0.36$, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92−7.36 (m, 0.5H), 713−6.40 (m, 12H), 5.66−5.40 (m, 1.7H), 5.26−4.97 (m, 3.3H), 4.77−4.20 (m, 3.3H), 3.94−3.60 (m, 2.5H), 3.50−2.58 (m, 11.4H), 2.50−1.78 (m, 19.8H), 1.51−0.67 (m, 22.5H); ^{13}C NMR ($CDCl_3$) δ 173.95, 173.29, 171.16, 170.33, 170.06, 169.93, 157.07, 156.41, 155.93, 155.84, 155.70, 155.52, 155.20, 155.13, 152.13, 151.88, 138.98, 138.85, 138.76, 138.57, 133.79, 133.67, 133.64, 133.14, 131.61, 131.23, 131.17, 131.14 (28q), 128.43, 127.99, 127.81, 127.42, 127.07, 126.99, 126.95, 126.72, 126.65, 126.09, 125.88, 125.63 (12q), 124.30, 124.00, 122.98 (3q), 116.22, 116.07, 115.61, 115.50 (4t), 80.43, 80.25, 80.11, 80.05 (4q), 55.92, 54.59, 50.68, 49.36 (4t), 45.74, 44.52, 37.91, 37.36, 36.84, 33.33, 32.27, 31.70, 31.36, 30.41, 28.92 (11s), 28.37, 28.34 (2p), 28.17, 24.19 (2s), 20.37, 20.30, 20.03, 19.91, 17.97, 17.79 (6p).

General Procedure for Synthesis of Compound 8−14. 3,6-Bis(Boc-Dmt-Tic-NH-alkyl)-2(1*H*)-pyrazinones (compounds **1**−**7**, 0.12 mmol) were treated with TFA (0.8 mL, 10 mmol) and anisole (40 μL) for 1 h at room temperature. The reaction solution was diluted with hexane, the solid was collected by filtration, dried over KOH pellets, and purified by semi-preparative RP-HPLC. The purified peptide was lyophilized from water containing 1 mol/L HCl (250 μL) three times to give an amorphous powder.

3,6-Bis(Dmt-Tic-NH-methyl)-2(1*H*)-pyrazinone·2HCl (8): yield 95 mg (83%); mp 224−226 °C (dec); $R_{f5} = 0.15$; $R_{f6} = 0.73$; $t_R = 14.44$ min; $[\alpha]^{25}_D$ +27.02° ($c = 0.35$, H_2O); *m/z* 870 (MH^+); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.22 (br, 2H), 8.78 (s, 5H), 8.60−8.22 (m, 3.4H), 7.22−6.70 (m, 8.6H), 6.55−6.20 (m, 4H), 5.05−4.90 (m, 1H), 4.68−4.44 (m, 1.5H), 4.44−3.86 (m, 8.5H), 3.23−2.88 (m, 5H), 2.88−2.67 (m, 1.5H), 2.32−1.83 (m, 15H), 1.57−1.36 (m, 1.5H); ^{13}C NMR (DMSO-d_6) δ 169.04, 168.98, 168.40, 168.05, 156.07, 155.87, 154.13, 138.48, 138.44, 138.41, 138.30, 132.00, 131.88, 131.45, 131.24 (15q), 127.87, 127.74, 126.44, 126.29, 126.09, 125.99, 125.86, 125.60 (8t, aryl C of Tic), 121.34, 121.25, 121.04, 120.99 (4q), 115.07 (t, aryl C of Dmt), 54.96, 54.89, 52.55, 51.62 (4t, α-C of Tic), 47.81, 47.69, 48.82, 48.43 (4t, α-C of Dmt), 43.24, 42.70 (2s, 1-C of Tic), 39.81, 39.40 (2s, $NHCH_2$), 31.06, 30.38 (2s, β-C of Dmt), 28.99, 28.37 (2s, β-C of Tic), 20.02, 19.98, 19.51 (3p, CH_3 of Dmt), 17.94 (p, 5-CH_3 of pyrazinone).

3,6-Bis(Dmt-Tic-NH-ethyl)-2(1*H*)-pyrazinone·2HCl (9): yield 97 mg (77%); mp 226−228 °C; $R_{f5} = 0.16$; $R_{f6} = 0.74$; $t_R = 14.46$ min; $[\alpha]^{25}_D$ +10.04° ($c = 0.48$, H_2O); *m/z* 898 (MH^+); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.95−8.37 (m, 6H), 8.27 (br, 0.8H), 8.08 (br, 0.8H), 7.86 (br, 0.2H), 7.55 (br, 0.2H), 7.20−6.73 (m, 8H), 6.54−6.22 (m, 4H), 5.08−4.95 (m, 0.4H), 4.86−4.72 (m, 1.6H), 4.60−4.10 (m, 4H), 3.93−3.80 (m, 1.6H), 3.55−3.45 (m, 0.4H), 3.34−2.85 (m, 9.3H), 2.85−2.35 (m, 5H), 2.35−1.80 (m, 14.7H), 1.54−1.36 (m, 1.5H); ^{13}C NMR (DMSO-d_6) δ 169.09, 169.06, 168.30, 168.13, 156.04, 138.47, 138.39, 132.06, 131.88, 131.40, 131.35 (11q), 127.76, 126.39, 126.05, 125.99, 125.92, 125.43 (6t, aryl C of Tic), 121.39, 121.06, 120.98 (3q), 115.06 (t, aryl C of Dmt), 55.09, 54.99, 52.20 (3t, α-C of Tic), 48.80, 47.83 (2t, α-C of Dmt), 43.21, 43.07 (2s, 1-C of Tic), 37.66, 37.37 (2s, $NHCH_2CH_2$), 31.09 (s, $NHCH_2CH_2$), 31.00, 30.45 (2s, β-C of Dmt), 29.15, 28.97 (2s, β-C of Tic), 20.02, 19.58, 19.54, 19.49 (4p, CH_3 of Dmt), 17.18 (p, 5-CH_3 of pyrazinone).

3,6-Bis(Dmt-Tic-NH-propyl)-2(1*H*)-pyrazinone·2HCl (10): yield 81 mg (74%); mp 222−223 °C; $R_{f5} = 0.16$; $R_{f6} = 0.75$; $t_R = 14.89$ min; $[\alpha]^{25}_D$ +22.07° ($c = 0.42$, H_2O); *m/z* 926 (MH^+); ^{1}H NMR (DMSO-d_6) δ 8.95−8.36 (m, 6H), 8.24 (br, 0.8H), 8.11 (br, 0.8H), 7.49 (br, 0.4H), 7.18−6.77 (m, 8H), 6.54−6.25 (m, 4H), 5.05−4.95 (m, 0.4H), 4.82−4.66 (m, 1.6H), 4.57−4.13 (m, 4H), 3.92−4.76 (m, 1.6H), 3.55 (dd, 0.4H, $J = 4.20$, 15.16 Hz), 3.25−2.65 (m, 10.9H), 2.58−1.85 (m, 18.7H), 1.67−1.27 (m, 5.5H); ^{13}C NMR (DMSO-d_6) δ 169.10, 169.01, 168.09, 167.97, 156.08, 156.04, 155.98, 155.57, 138.46, 138.38, 132.14, 132.10, 131.63, 131.48 (14q), 127.75, 127.67, 126.43, 126.08, 126.04, 125.99, 125.4 (7t, aryl C of Tic), 121.39, 121.03, 121.02 (3q), 115.10 (t, aryl C of Dmt), 55.03, 54.99, 52.70, 52.50 (4t, α-C of Tic), 48.70, 48.01, 47.93 (3t, α-C of Dmt), 44.62, 43.41, 43.26 (3s, 1-C of Tic), 38.47, 38.28, 37.80 (3s, $NHCH_2(CH_2)_2$), 31.06, 30.36 (2s, β-C of Dmt), 29.83, 29.46, 29.35 (3s, β-C of Tic), 28.43 (s, $NH(CH_2)_2CH_2$), 27.60 (s, $NHCH_2CH_2CH_2$), 26.74 (s, $NH(CH_2)_2CH_2$), 26.12 (s, $NHCH_2CH_2CH_2$), 20.02, 19.51, 19.49 (3p, CH_3 of Dmt), 17.26 (p, 5-CH_3 of pyrazinone).

3,6-Bis(Dmt-Tic-NH-butyl)-2(1*H*)-pyrazinone·2HCl (11): yield 98 mg (85%); mp 219−220 °C; $R_{f5} = 0.14$; $R_{f6} = 0.74$; $t_R = 14.74$ min; $[\alpha]^{25}_D$ +22.54° ($c = 0.42$, H_2O); *m/z* 954 (MH^+); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.98−8.40 (m, 6H), 8.14 (s, 0.8H), 8.04 (s, 0.9H), 7.23−6.75 (m, 8.3H), 6.52−6.25 (m, 4H), 5.02 (t, 0.4H, $J = 4.56$ Hz), 4.75−4.64 (m, 1.6H), 4.75−4.64 (m, 0.8H), 4.57−4.40 (m, 0.8H), 4.38−4.24 (m, 1.6H), 3.85−3.75 (m, 1.6H), 3.54 (d, 0.4H, $J = 15.01$ Hz), 3.26−2.85 (m, 9.2H), 2.78 (d, 1.6H, $J = 15.97$ Hz), 2.62−2.45 (m, 1.2H), 2.43−1.95 (m, 17H), 1.67−0.97 (m, 10H); ^{13}C NMR (DMSO-d_6) δ 169.10, 169.07, 168.98, 168.94, 167.85, 167.83, 156.09, 156.06, 155.98, 155.66, 138.43, 138.36, 138.43, 132.56, 132.37, 132.21, 132.15, 131.66, 131.53 (19q), 127.68, 126.66, 126.40, 126.35, 126.17, 126.08, 126.03, 125.98, 125.95, 125.45 (10t, aryl C of Tic), 121.36, 121.03 (2q), 115.11 (t, aryl C of Dmt), 55.02, 52.70 (2t, α-C of Tic), 48.62, 48.07 (2t, α-C of Dmt), 44.71, 43.57, 43.52 (3s, 1-C of Tic), 38.41, 38.06 (2s, $NHCH_2CH_2$), 31.00, 30.76 (2s, β-C of Dmt), 29.66, 29.40 (2s, β-C of Tic), 28.77 (s, $NH(CH_2)_3CH_2$), 28.55, 28.30 (2s, $NHCH_2CH_2$), 24.95, 23.55 (2s, $NH(CH_2)_2CH_2$), 19.98, 19.50, 19.47 (3p, CH_3 of Dmt), 17.06 (p, 5-CH_3 of pyrazinone).

3-(Dmt-Tic-NH-propyl)-6-(Dmt-Tic-NH-butyl)-2(1*H*)-pyrazinone·2HCl (12): yield 105 mg (91%); mp 222−224 °C; $R_{f5} = 0.17$; $R_{f6} = 0.74$; $t_R = 15.01$ min; $[\alpha]^{25}_D$ +24.05° ($c = 0.36$, H_2O); *m/z* 940 (MH^+); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93−8.35 (m, 6H), 8.18−7.97 (m, 1.6H), 7.49 (br, 0.2H), 7.19−6.75 (m, 8.2H), 6.52−6.25 (m, 4H), 4.96−4.87 (m, 0.4H), 4.72 (t, 1.6H, $J = 16.70$ Hz), 4.58−4.10 (m, 4H), 3.87−3.73 (m, 1.6H), 3.55 (dd, 0.4H, $J = 7.73$, 15.32 Hz), 3.38−2.84 (m, 9.2H), 2.79 (d, 1.7H, $J = 13.90$ Hz), 2.60−1.88 (m, 18.6H), 1.58−1.47 (m, 3.5H), 1.37−0.94 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 169.11, 169.08, 169.04, 168.97, 167.98, 167.83, 156.09, 156.04, 155.94,

155.59, 138.43, 138.36, 132.55, 132.49, 132.47, 132.28, 132.20, 132.11, 131.63, 131.53 (20q), 127.68, 126.43, 126.35, 126.04, 125.98, 125.95, 125.44 (7t, aryl C of Tic), 121.39, 121.35, 121.03, 121.02 (4q), 115.11 (t, aryl C of Dmt), 55.03, 55.00, 52.68, 52.53 (4t, α-H of Tic), 48.72, 48.61, 48.02 (3t, α-H of Dmt), 44.72, 44.63, 43.52, 43.41 (4s, 1-C of Tic), 38.51, 38.22, 38.05, 37.67 (4s, $NHCH_2$), 31.04, 30.98, 30.40 (3s, β-C of Dmt), 29.55, 29.44 (2s, β-C of Tic), 28.74 (s, 6-$CH_2(CH_2)_3NH$), 28.55 (s, 3-$CH_2(CH_2)_2NH$), 28.41, 28.30 (2s, 6-$(CH_2)_2CH_2CH_2NH$), 26.18 (s, 3-$CH_2CH_2CH_2NH$), 24.97 (s, 6-$CH_2CH_2(CH_2)_2NH$), 20.03, 19.98, 19.50, 19.49 (4p, CH_3 of Dmt), 17.04, 17.31 (2p, 5-CH_3 of pyrazinone).

3-(Dmt-Tic-NH-butyl)-6-(Dmt-Tic-NH-propyl)-2(1*H*)-pyrazinone·2HCl (13): yield 78 mg (88%); mp 224−226 °C; $R_{f5} = 0.15$; $R_{f6} = 0.73$; $t_R = 14.78$ min; $[\alpha]^{25}_D$ +28.10° ($c = 0.41$, H_2O); *m/z* 940 (MH^+); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02−8.37 (m, 6H), 8.25 (br, 0.8H), 8.03 (br, 0.8H), 7.56−6.74 (m, 8.5H), 6.64−6.14 (m, 4H), 5.05−4.95 (m, 0.4H), 4.85−4.60 (m, 1.6H), 4.60−4.17 (m, 4H), 3.97−3.72 (m, 1.6H), 3.60−3.50 (m, 0.4H), 3.31−2.67 (m, 10.5H), 2.40−1.80 (m, 17.3H), 1.72−1.08 (m, 9.2H); ^{13}C NMR (DMSO-d_6) δ 169.29, 169.22, 169.10, 168.93, 168.09, 167.84, 165.08, 156.03, 155.97, 155.65, 138.45, 138.43, 138.39, 138.35, 132.15, 131.65, 131.48 (17q), 127.76, 127.65, 126.43, 126.39, 126.08, 126.02, 125.44 (7t, aryl C of Tic), 121.37, 121.03 (2q), 115.10 (t, aryl C of Dmt), 55.03, 52.70, 52.61 (3t, α-C of Tic), 48.71, 48.62, 48.07, 47.92 (4t, α-C of Dmt), 44.70, 43.58, 43.35 (3s, 1-C of Tic), 38.38, 38.27, 37.78 (3s, $NHCH_2$), 31.03 (s, β-C of Dmt), 30.68 (s, 3-$CH_2(CH_2)_3NH$), 30.34 (s, β-C of Dmt), 29.66, 29.34 (2s, β-C of Tic), 28.75, 28.53 (2s, 3-$(CH_2)_2CH_2CH_2NH$), 27.78, 27.59 (2s, 6-$CH_2CH_2CH_2NH$), 26.83 (s, 6-$CH_2(CH_2)_2NH$), 23.52 (s, 3-$CH_2CH_2(CH_2)_2NH$), 20.02, 19.98, 19.51, 19.47 (4p, CH_3 of Dmt), 17.00 (p, 5-CH_3 of pyrazinone).

3-(Dmt-Tic-NH-ethyl)-6-(Dmt-Tic-NH-butyl)-2(1*H*)-pyrazinone·2HCl (14): yield 106 mg (98%); mp 225−227 °C; $R_{f5} = 0.16$; $R_{f6} = 0.74$; $t_R = 14.59$ min; $[\alpha]^{25}_D$ +17.66° ($c = 0.40$, H_2O); *m/z* 926 (MH^+); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.94−8.36 (m, 6H), 8.28−7.93 (m, 1.7H), 7.17−6.74 (m, 8.3H), 6.53−6.18 (m, 4H), 5.06−4.96 (m, 0.4H), 4.87−4.63 (m, 1.6H), 4.56−4.10 (m, 4H), 3.90−3.75 (m, 1.6H), 3.58−3.47 (m, 0.4), 3.33−2.54 (m, 13.2H), 2.47−1.93 (m, 15.8H), 1.63−1.00 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 169.11, 169.09, 169.05, 168.99, 168.20, 167.86, 156.12, 156.07, 156.05, 155.99, 138.48, 138.36, 132.16, 132.02, 131.55, 131.32 (16q), 127.78, 127.68, 126.70, 126.39, 126.07, 125.99, 125.94, 125.45 (8t, aryl C of Tic), 121.41, 121.36, 121.09, 121.02 (4q), 115.11, 115.05 (2t, aryl C of Dmt), 55.09, 55.02, 52.69, 52.51 (4t, α-C of Dmt), 48.75, 48.64, 48.02, 47.83 (4t, α-C of Tic), 44.71, 44.54, 43.52, 43.05 (4s, 1-C of Tic), 38.11, 37.72 (2s, 6-$(CH_2)_3CH_2NH_2$), 37.56, 36.05 (2s, 3-CH_2CH_2-NH), 31.61 (s, 3-$CH_2CH_2NH_2$), 30.99, 30.33 (2s, β-C of Dmt), 29.56, 28.92 (2s, β-C of Tic), 28.55 (s, 6-$CH_2(CH_2)_3NH$), 28.25 (s, 6-$(CH_2)_2CH_2CH_2NH$), 25.07 (s, 6-$CH_2CH_2(CH_2)_2NH$), 20.02, 19.98, 19.57, 19.50 (4p, CH_3 of Dmt), 17.65, 16.89 (2p, 5-CH_3 of pyrazinone).

General Procedure for Synthesis of 15−17. To a solution of diaminoalkane (0.26 mmol) in DMF (10 mL) were added Boc-Dmt-Tic-OH (0.53 mmol), DIPEA (112 μL, 0.64 mmol), and PyBop (290 mg, 0.56 mmol). The reaction mixture was stirred at 0 °C for 10 min and then room temperature for 4 h. After removal of solvent, the residue was diluted with AcOEt. The organic phase was washed with ice cold 10% citric acid (3 × 10 mL), 5% Na_2CO_3 (3 × 10 mL), and saturated aqueous NaCl (3 × 10 mL); dried over Na_2SO_4; and evaporated to dryness. The residue was purified by flash chromatography. The compound was precipitated with hexane, filtered, and dried in vacuo. Elemental analyses of **15−17** are summarized in the Supporting Information.

1,4-Bis(Boc-Dmt-Tic-amino)butane (15). Starting from 1,4-diaminobutane (23 mg, 0.26 mmol), the crude compound was purified by silica gel chromatography (3.2 cm × 33 cm; AcOEt:hexane = 4:1): yield 145 mg (57%); mp 154−155 °C; $R_{f1} = 0.27$; $[\alpha]^{25}_D$ −8.08° ($c = 0.27$, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40−7.18 (m, 1H), 7.18−6.70 (m, 8.4H), 6.64−6.40 (m, 4H), 5.60−5.30 (m, 2H), 5.30−4.83 (m, 3.5H), 4.83−4.55 (m, 2H), 4.55−4.27 (m, 1.7H), 4.03−3.85 (m, 0.9H), 3.83−3.66 (m, 1H), 3.40−2.65 (m, 10.6H), 2.65−1.95 (m, 13.7H), 1.95−1.72 (m, 0.7H), 1.60−1.27 (m, 18.3H), 1.10−0.50 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 173.92, 173.90, 173.88, 173.12, 170.63, 170.28, 170.03, 155.98, 155.69, 155.62, 155.36, 155.09, 138.82, 133.64, 133.44, 132.80, 131.54, 131.41, 131.20 (19q), 128.38, 128.30, 128.17, 127.75, 127.61, 127.24, 127.14, 126.91, 126.80, 126.68, 126.15, 125.77 (12t), 123.96, 123.78, 123.40 (3q), 115.66, 115.57, 115.51, 115.47 (4t), 80.71, 80.14, 80.07 (3q), 55.87, 54.46, 54.27, 50.34, 49.63, 49.38 (6t), 45.65, 44.05, 39.26, 38.80, 38.57, 33.51, 33.37, 32.44, 31.59, 31.42, 31.33, 30.03 (12s), 28.37, 28.33, 28.30 (3p), 26.27, 25.76, 25.44, 22.65 (4s), 2.83, 20.34, 19.96 (3p).

1,6-Bis(Boc-Dmt-Tic-amino)hexane (16). Starting from 1,6-diaminohexane (30 mg, 0.26 mmol), the crude compound was purified by silica gel chromatography (3.2 cm × 37 cm; AcOEt:hexane = 3:1): yield 170 mg (65%); mp 159−160 °C; $R_{f1} = 0.48$; $[\alpha]^{25}_D$ +0.03° ($c = 0.30$, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.30−8.15 (br, 1.5H), 7.90−7.58 (m, 1.2H), 7.44−6.75 (m, 10.4H), 6.52−6.10 (m, 4H), 4.90−4.35 (m, 4.7H), 4.30−3.95 (m, 3H), 3.20−2.65 (m, 11.3H), 2.35−1.83 (m, 12.8H), 1.80−0.67 (m, 26.2H); ^{13}C NMR (DMSO-d_6) δ 172.00, 171.20, 169.82, 168.24, 168.21, 155.54, 155.39, 155.04, 154.65, 138.34, 138.01, 137.91, 133.68, 133.57, 132.70, 131.79 (16q), 127.76, 127.60, 126.83, 126.25, 126.16, 125.96, 125.87, 125.84, 125.31 (9t), 124.44, 122.94 (2q), 115.12, 114.89, 114.65 (3t), 78.60, 78.56, 77.88 (3q), 54.85, 53.28, 50.18, 49.47, 48.21 (5t), 44.71, 42.86, 38.69, 38.20, 31.62, 31.18, 30.85, 29.18, 28.93, 28.51, 28.43 (11s), 28.04, 27.94, 27.62, 27.45 (4p), 25.75, 25.66, 25.60 (3s), 20.16, 19.46, 19.41 (3p).

1,10-Bis(Boc-Dmt-Tic-amino)decane (17). Starting from 1,10-diaminodecane (54 mg, 0.31 mmol), the crude compound was purified by silica gel chromatography (3.2 cm × 35 cm; AcOEt:hexane = 3:1): yield 208 mg (62%); mp 145−147 °C; $R_{f1} = 0.54$; $[\alpha]^{25}_D$ −2.14° ($c = 0.44$, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.98 (br, 2H), 7.85−7.62 (m, 1.5H), 7.30−6.75 (m, 10.3H), 6.48−6.30 (m, 4H), 5.04−3.94 (m, 7.5H), 3.71−3.50 (m, 0.5H), 3.15−2.70 (m, 11.5H), 2.30−2.00 (m, 12.5H), 1.73−1.58 (m, 1.3H), 1.43−0.80 (m, 22.7H); ^{13}C NMR (DMSO-d_6) δ 172.01, 169.83, 169.28, 168.21, 167.93, 156.03, 155.53, 155.40, 155.03, 154.64, 138.33, 138.00, 132.71, 131.79 (14q), 127.77, 126.79, 126.22, 125.94, 125.77, 125.30 (6t), 124.41, 122.92 (2q), 115.06, 114.88, 114.64 (3t), 78.54, 77.87 (2q), 54.82, 53.27, 50.16, 49.47 (4t), 44.65, 42.83, 38.40, 38.27, 31.58, 31.19, 30.87, 29.11, 28.99, 28.80, 28.73, 28.54, 28.48 (13s), 28.04, 27.94 (2p), 26.01,25.96 (2s), 20.15, 19.44, 19.40 (3p).

General Procedue for Synthesis of Compound 18−20. Bis(Boc-Dmt-Tic-amino)alkane (compounds **15−17**, 0.17 mmol) was treated with TFA (1.0 mL, 13 mmol) and anisole (50 μL) for 1 h at room temperature. The reaction solution was diluted with hexane and the solid was collected by filtration, dried over KOH pellets, and purified by semipreparative RP-HPLC. The purified peptide was lyophilized from water containing 1 mol/L HCl (250 μL) for three times to give an amorphous powder.

1,4-Bis(Dmt-Tic-amino)butane·2HCl (18): yield 137 mg (95%); mp 215−217 °C; $R_{f5} = 0.19$; $R_{f6} = 0.74$; $t_R = 14.50$ min; $[\alpha]^{25}_D$ +23.55° ($c = 0.41$, H_2O); *m/z* 790 (MH^+); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.27 (br, 2H), 8.95−8.30 (m, 6H), 8.04−7.73 (m, 1.4H), 7.22−6.72 (m, 8.6H), 6.52−6.25 (m, 4H), 5.02−4.92 (m, 0.4H), 4.75−4.58 (m, 1.6H), 4.55−4.13 (m, 4H), 3.81−3.66 (m, 1.6H), 3.57−3.48 (m, 0.4H), 3.20−2.65 (m, 10.5H), 2.34−1.87 (m, 12H), 1.65−1.53 (m, 1.5H), 1.07−0.60 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 169.18, 169.09, 169.07, 168.92, 167.81, 167.78, 156.05, 155.97, 138.42, 138.34, 132.57, 132.39, 132.24, 132.20, 131.65 (15q), 127.66, 126.73, 126.46, 126.12, 125.97, 125.41 (6t, aryl C of Tic), 121.36, 120.98 (2q), 115.09 (t, aryl C of Dmt), 54.99, 52.65 (2t, α-C of Tic), 48.67, 48.06 (2t, α-C of Dmt), 44.66, 43.58 (2s, 1-C of Tic), 38.18, 37.81 (2s, $NHCH_2$-CH_2), 31.27 (s, β-C of Tic), 30.97, 30.34 (2s, β-C of Dmt), 29.65 (s, β-C of Tic), 25.85, 25.78 (2s, $NHCH_2CH_2$), 19.98, 19.57 (2p, CH_3 of Dmt).

1,6-Bis(Dmt-Tic-amino)hexane·2HCl (19): yield 100 mg (94%); mp 215−217 °C; $R_{f5} = 0.22$; $R_{f6} = 0.74$; $t_R = 15.01$ min;

$[\alpha]^{25}_D$ +32.3° (c = 0.41, H_2O); *m/z* 818 (MH^+); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.28 (s, 2H), 8.90−8.30 (m, 6H), 8.03−7.88 (m, 1.5H), 7.18−6.75 (m, 8.5H), 6.55−6.28 (m, 4H), 5.05−4.98 (m, 0.4H), 4.74−4.60 (m, 1.6H), 4.58−4.41 (m, 0.9H), 4.38−4.25 (m, 1.6H), 4.22−4.05 (m, 1.5H), 3.82−3.70 (m, 1.6H), 3.58−3.50 (m, 0.4H), 3.20−2.70 (m, 10.5H), 2.40−1.90 (m, 12H), 1.67−1.56 (m, 1.5H), 1.15−0.62 (m, 8H); ^{13}C NMR (DMSO-d_6) δ 169.32, 169.11, 168.84, 167.68, 156.08, 156.01, 1388.42, 138.35, 132.50, 132.21, 132.15, 132.10, 131.55 (13q), 127.74, 127.64, 126.75, 126.47, 126.23, 126.19, 125.98, 125.45 (8t, aryl C of Tic), 121.33, 121.00 (2q), 115.10 (t, aryl C of Dmt), 55.01, 52.59 (2t, α-C of Tic), 48.58, 48.05 (2t, α-C of Dmt), 44.67, 43.63 (2s, 1-C of Tic), 38.49, 38.13 (2s, NH*C*H_2CH$_2$), 31.24 (s, β-C of Tic), 31.00, 30.36 (2s, β-C of Dmt), 29.62 (s, β-C of Tic), 28.85, 28.65 (2s, NHCH$_2$*C*H$_2$), 25.61, 25.53 (2s, NH(CH$_2$)$_2$*C*H$_2$), 19.96, 19.47 (2p, *C*H$_3$ of Dmt).

1,10-Bis(Dmt-Tic-amino)decane·2HCl (20): yield 108 mg (81.6%); mp 205−207 °C; R_{f5} = 0.32; R_{f6} = 0.75; t_R = 17.22 min; $[\alpha]^{25}_D$ +5.36° (c = 0.55, H_2O); *m/z* 874 (MH^+); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (br, 2H), 8.90−8.30 (m, 6H), 8.00 (t, 1.6H, J = 5.74 Hz), 7.25−6.75 (m, 8.4H), 6.52−6.25 (m, 4H), 5.05−4.98 (m, 0.4H), 4.72−4.64 (m, 1.6H), 4.56−4.42 (m, 0.8H), 4.36−4.28 (m, 1.6H), 4.22−4.07 (m, 1.6H), 3.56−3.52 (m, 0.4H), 3.20−2.70 (m, 10.5H), 2.30−1.88 (m, 12H), 1.60 (dd, 1.5H, J = 5.2, 15.6 Hz), 1.25−0.78 (m, 16H); ^{13}C NMR (DMSO-d_6) δ 169.38, 169.12, 168.85, 167.69, 156.09, 156.04, 138.41, 138.34, 132.56, 132.22, 132.17, 131.59 (12q), 127.74, 127.66, 126.69, 126.42, 126.20, 126.12, 125.98, 125.48 (8t, aryl C of Tic), 121.34, 121.02 (2q), 115.11 (t, aryl C of Dmt), 55.02, 52.61 (2t, α-C of Tic), 48.56, 48.05 (2t, α-C of Dmt), 44.71, 43.60 (2s, 1-C of Tic), 38.56, 38.20 (2s, NH*C*H_2CH$_2$), 31.22 (s, β-C of Tic), 31.01, 30.36 (2s, β-C of Dmt), 29.63 (s, β-C of Tic), 28.96, 28.79, 28.76, 28.67, 28.61, 28.58 (6s, NHCH$_2$(*C*H$_2$)$_3$CH$_2$), 25.90 (s, NH(CH$_2$)$_4$*C*H$_2$), 19.96, 19.48 (2p, *C*H$_3$ of Dmt).

1,6-Bis(*N*,*N*-Dimethyl-Dmt-Tic-NH)hexane·2HCl (21). To a stirred solution of 1,6-bis(Dmt-Tic-amino)hexane·2TFA (**19**, 105 mg, 0.1 mmol) in 37% aqueous formaldehyde (149 μL, 2 mmol) in H_2O (4.5 mL) and acetonitrile (4.5 mL) was added sodium cyanoborohydride (38 mg, 0.6 mmol). Glacial acetic acid (20 μL) was added over 10 min and stirring was continued for 15 min. The solution was adjusted to pH 5 with TFA, evaporated, and lyophilized, and the residue was purified by RP-HPLC: yield 67 mg (71%); mp 195−197 °C; R_{f5} = 0.10; t_R = 15.0 min; $[\alpha]^{25}_D$ −12.73° (c = 0.44, H_2O); *m/z* 874 (MH^+); ^{1}H NMR (MeOD-d_3) δ 7.18−6.85 (m, 8H), 6.60−6.30 (m, 4H), 5.12−5.70 (m, 0.4H), 4.83−4.78 (m, 0.4H), 4.74−4.67 (m, 0.4H), 4.63−4.48 (m, 3.3H), 4.30 (dd, 1.6H, J = 4.0, 12.04 Hz), 3.89−3.83 (m, 1.6H), 3.73−3.65 (m, 0.4H), 3.50−3.20 (m, 4H), 3.20−3.74 (m, 18H), 2.72−2.62 (m, 0.4H), 2.58−2.00 (m, 12H), 1.97−1.83 (m, 1.6H), 1.13−0.80 (m, 2H), 0.80−0.58 (m, 2H); ^{13}C NMR (MeOD-d_3) δ 171.45, 170.65, 170.02, 169.74, 158.14, 157.96, 140.52, 140.44, 134.25, 132.96, 132.86, 132.68 (12q), 129.20, 128.93, 128.36, 128.30, 128.24, 128.20, 127.46, 127.00 (8t, aryl C of Tic), 121.69, 121.65 (2q), 116.89, 116.79 (2t, aryl C of Dmt), 65.44, 65.00 (2t, α-C of Dmt), 57.39, 56.25 (2t, α-C of Tic), 46.80, 45.97 (2s, 1-C of Tic), 44.37, 43.52, 41.57 (3p, (*C*H$_3$)$_2$N), 40.52, 40.10 (2s, NH*C*H_2CH$_2$), 32.75, 31.78 (2s, β-C of Tic), 30.30, 30.12, 30.06, 29.43 (4s, β-C of Dmt and NHCH$_2$*C*H$_2$), 27.07, 26.85 (2s, NH(CH$_2$)$_2$*C*H$_2$), 20.70, 20.03 (2p, *C*H$_3$ of Dmt).

3,6-Bis(*N*,*N*-dimethyl-Dmt-Tic-NH-propyl)-2(1*H*)-pyrazinone·2HCl (22). 3,6-Bis[Z-NH-(CH$_2$)$_3$]-2(1*H*)-pyrazinone (58 mg, 0.12 mmol) was stirred in 25% HBr/AcOH (420 μL, 1.76 mmol) with an ice bath for 10 min and then room temperature for 3 h. The resulting amine was precipitated with ether and dried in vacuo. The precipitate was dissolved in DMF (10 mL) containing DIPEA (147 μL, 0.85 mmol), to which *N*,*N*-dimethyl-Dmt-Tic-OH·TFA (120 mg, 0.24 mmol) and PyBop (128 mg, 0.25 mmol) were added. The solution was first stirred in an ice bath for 10 min and then room temperature for 4 h. After removal of the solvent in vacuo, the residue was diluted with AcOEt (60 mL), and the precipitate was collected by filtration, dried, and purified by RP-HPLC: yield 58 mg (46%); mp 214−216 °C; R_{f5} = 0.09; R_{f6} = 0.59; t_R = 14.7 min; $[\alpha]^{25}_D$ +0.92° (c = 0.38, H_2O); *m/z* 982 (MH^+); ^{1}H NMR (DMSO-d_6) δ 10.85−10.04 (m, 1.7H), 8.30−8.00 (m, 1.7H), 7.25−6.75 (m, 8.4H), 6.65−6.08 (m, 4H), 4.82−4.38 (m, 5.8H), 4.18−3.96 (m, 1.8H), 3.50−2.66 (m, 23.5H), 2.66−1.65 (m, 18H), 1.65−1.20 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 169.20, 168.21, 168.14, 168.12, 168.00, 167.90, 156.11, 155.99, 138.54, 138.34 (10q), 131.59, 131.55, 131.27, 131.22, 127.63, 126.41, 126.27, 126.11 (8t, aryl C of Tic), 120.27, 120.20 (2q), 115.12, 114.90 (2t, aryl C of Dmt), 61.80, 61.72 (2t, α-C of Tic), 55.36, 54.41 (2t, α-C of Dmt), 45.00, 44.07, 43.95 (3s, NH*C*H_2,), 41.78, 41.55, 40.41, 40.20 (4p, N(*C*H$_3$)$_2$), 38.46, 38.27, 37.81 (3s, NH*C*H$_2$), 29.84, 29.71 (2s, β-C of Tic), 28.39, 28.27 (2s, β-C of Dmt), 27.43 (s, NHCH$_2$*C*H$_2$CH$_2$), 26.58, 26.10 (2s, NH(CH$_2$)$_2$*C*H$_2$), 25.86 (s, NHCH$_2$*C*H$_2$CH$_2$), 20.01, 19.68 (2p, *C*H$_3$ of Dmt), 17.25, 16.82 (2p, 5-*C*H$_3$ of pyrazinone).

Opioid Receptor Binding Assays. Opioid receptor affinities were determined under equilibrium conditions [2.5 h at room temperature (23 °C)] in a competition assay using brain P_2 synaptosomal membranes prepared from Sprague−Dawley rats.[33] Synaptosomes were preincubated to remove endogenous opioids, washed in excess ice-cold buffer containing protease inhibitor, and stored in a glycerol-containing buffer with protease inhibitor at −80 °C as described previously.[33] The δ- and μ-opioid receptors were radiolabeled with [^{3}H]deltorphin-II and [^{3}H]DAMGO, respectively,[10,11,33] and excess unlabeled peptide (2 μM) established the level of nonspecific binding. [^{3}H]Deltorphin-II was substituted for [^{3}H]DPDPE, used in previous publications,[10,11] due to the instability of [^{3}H]DPDPE preparations; comparison of the affinities in our laboratory and others in the literature demonstrated analogous K_i values for identical opioids, despite inherent variations in rat brain synaptosomal preparations. After incubation, the radiolabeled membranes were rapidly filtered on Whatman GF/C glass fiber filters presoaked in 0.1% polyethylenimine in order to optimize the signal-to-noise ratio, washed with ice-cold BSA buffer, and dried at 75−80 °C. Radioactivity was determined using EcoLume (ICN, Costa Mesa, CA). All compounds are analyzed in duplicate using 5−8 peptide dosages and several synaptosomal preparations in independent repetitions (n values noted in Table 1) to ensure statistical significance. The affinity constants (K_i) were calculated according to Cheng and Prusoff[34] using K_d values for [^{3}H]deltorphin-II and [^{3}H]DAMGO published in the literature using this standard receptor assay system.

Biological Activity in Isolated Tissue Preparation. The myenteric plexus longitudinal muscle preparations (2−3 cm segments) from the small intestine of male Hartley strain guinea pigs (GPI) measured μ-opioid receptor agonism, and a single mouse vas deferens (MVD) was used to determine δ-opioid receptor agonism as described previously.[35] The isolated tissues were suspended in organ baths containing balanced salt solutions in a physiological buffer, pH 7.5. Agonists were tested for the inhibition of electrically evoked contraction and expressed as IC_{50} (nM) obtained from the dose−response curves. The IC_{50} values represent the mean ± SE of five or six separate assays. δ-Antagonist potencies in the MVD assay were determined against the δ-agonist deltorphin-II; μ-antagonism in the GPI assay used the μ-agonist endomorphin-2 and both are expressed as pA_2 determined using the Schild Plot.[36] The Schild slopes of all analogues were 0.86−1.

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Supporting Information Available: Elemental analysis of all the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(1) In additional to the IUPAC−IUB Commission on Biochemical Nomenclature (*J. Bio. Chem.*, **1985**, *260*, 14−42), this paper uses the following symbols and abbreviations: Ac_2O, acetic anhydride; AcOEt, ethyl acetate; Boc, *tert*-butyloxycarbonyl; BSA, bovine serum albumin; DALDE, [D-Ala,^{2}D-Leu^5]enkephalin; DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]enkephalin; deltorphin II, Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2; DIPEA, diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; Dmt, 2′,6′-dimethyl-L-tyrosine; Fmoc, 9-fluorenylmethoxycarbonyl; GPI, guinea pig ileum; IBCF, isobutyl chloroformate; IC_{50}, concentration required for 50% inhibition of the electrically induced contraction in muscle derived from a dose−response curve; MVD, mouse vas deferens; NMR, nuclear magnetic resonance; PyBOP, benzoltriazol-1-yloxytrispyrrolidinophosphonium hexafluorphosphate; RP-HPLC, reverse-phase high performance liquid chromatography; TFA, trifluoroacetic acid; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxyl acid; TLC, thin-layer chromatography; Z, benzyloxycarbonyl.

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