Synthesis of Opioidmimetics, 3-[H-Dmt-NH$(CH_2)_m$]-6-[H-Dmt-NH$(CH_2)_n$]-2(1*H*)-pyrazinones, and Studies on Structure-Activity Relationships

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Abstract: Opioidmimetics containing 3-[H-Dmt-NH-$(CH_2)_m$]-6-[H-Dmt-NH-$(CH_2)_n$]-2(1*H*)-pyrazinone symmetric (m = n, 1-4) (**1** - **4**) and asymmetric (m, n = 1 - 4) aliphatic chains (**5** - **16**) were synthesized using dipeptidyl chloromethylketone intermediates. They had high μ-affinity ($K_i\mu$ = 0.021 - 2.94 nM), δ-affinity ($K_i\delta$ = 1.06 - 152.6 nM), and μ selectivity ($K_i\delta/K_i\mu$ = 14 - 3,126). The opioidmimetics (**1** - **16**) exhibited μ agonism in proportion to their μ-receptor affinity. δ-Agonism was essentially lacking in the compounds except (**4**) and (**16**), and (**1**) and (**2**) indicated weak δ antagonism (pA_2 = 6.47 and 6.56, respectively). The data verify that a specific length of aliphatic linker is required between the Dmt pharmacophore and the pyrazinone ring to produce unique μ-opioid receptor ligands.

Key Words: Opioidmimetic, 2',6'-dimethyl-L-tyrosin (Dmt), pyrazinone platform, Dmt-dimerization, opioid receptor affinity, μ-agonism, δ-antagonism, structure-activity relationship.

INTRODUCTION

Of the endogenous opioid peptides [1-5], endomorphins [5,6] exhibit high μ-opioid receptor affinity and extraordinarily high selectivity over δ- and κ-opioid receptors [7], as such they would be potential candidates of analgesics for clinical and therapeutic use in the amelioration of pain associated with post-surgerical intervention, cancer, birth or other physical trauma. However, the oral bioavailability of bioactive peptides requires not only a means to overcome physiological and metabolic barriers, but also the ability to transit physical barriers, such as the epithelial membranes in the gastrointestinal tract [8] and at the blood-brain-barrier (BBB) [9, 10]. Detailed structure-activity relationship studies over the years revealed that despite the structural diversity among opioid peptides [1-5], an N-terminal Tyr residue is a common structural element except in nociceptin [11], which contains Phe in lieu of Tyr. According to the concept of the message and address domains [12,13], the Tyr residue acts within the message domain, which is involved in anchoring the opioid peptide in the receptor. While some unusual amino acids could replace Tyr in opioids [14], 2',6'-dimethyl-L-tyrosine (Dmt) dramatically enhanced receptor affinity to consistently alter receptor selectivity and functional bioactivity [14-16]. When Dmt replaced Tyr in opioids, such as enkephalin [17] and the endomorphins [15,16], it greatly increased both μ- and δ-opioid receptor affinities, which was initially demonstrated with the prototype opioid dipeptide pharmacophore, H-Dmt-Tic-OH (a δ-antagonist, Tic: 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid). H-Dmt-Tic-OH had high δ-opioid receptor affinity ($K_i\delta$ = 0.022 nM) and extraordinary δ-selectivity relative to the μ-opioid receptor ($K_i\mu/K_i\delta$ = 150,000) [18]. That [Dmt[1],Leu[5]]-enkephalin [17] resisted enzymatic degradation compared with [Leu[5]]-enkephalin and that the *in vivo* antinociceptive activity of [Dmt[1]]-endomorphin-2 was twice as active as the parent compound [19], indicated that stability and bioavailability of opioid substances would be potentially feasible for orally active drugs based on these molecular frameworks. Furthermore, presence of Dmt enhanced interaction with μ-opioid receptors than to the δ-type [15-17]. In fact, this was further demonstrated when H-Dmt-NH-CH_3 [20] specifically bound to the μ-opioid receptor ($K_i\mu$ = 7.45 nM) to a degree comparable to that of morphine; however, as a single amino acid residue Dmt was unable to trigger bioactivity to any appreciable extent on its own [21,22].

It is well known that dimerization of opioid peptides, such as dimeric enkephalin [22-24] and dermorphin [25], enhanced both receptor affinity and bioactivity. It is interesting that condensation of [Dmt[1]]DADLA (selective μ-agonist) with TICP[ψ] (δ-antagonist) produced a chimeric analogue with a mixed μ-agonist/δ-antagonist profile [26]. The opiates binaltrophimine (BNI) and norbinaltrophimine (norBNI), selective κ-receptor antagonists were produced through dimerization of naltrexone derivatives [27,28]. We previously demonstrated that pyrazinone ring-containing opioidmimetic substances, 3,6-bis[H-Dmt-NH-$(CH_2)_n$]-5-methyl-2(1*H*)-pyrazinones (**1-4**: n = 1-4) have antinociceptive properties [21]. This broad class of compounds bound to μ-opioid receptors with high affinity ($K_i\mu$ = 0.042-1.16 nM) and moderate μ-selectivity ($K_i\delta/K_i\mu$ = 14-307) and whose μ-agonism indicated that subtle differences in alkyl chain length covalently bound between positions 3 and 6 of pyrazinone ring and N-terminal Dmt residues profoundly affected their receptor affinity and bioactivity. One derivative exhibited considerably potent antinoceptive activity *in vivo* after (intracerebrov-

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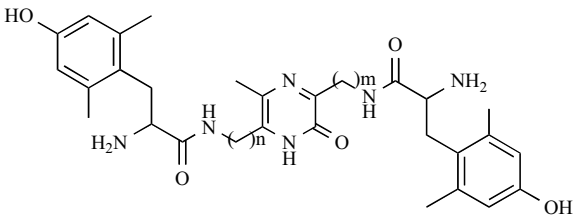
entricular, subcutaneous and oral) administration in mice, suggesting that pyrazinone derivatives might be more resistant to enzymatic degradation and pass through the epithelial membranes of the gastrointestinal tract and the blood-brain-barrier (BBB) [21]. Stability and permeability were also



(**1**) m = 1, n = 1 (**5**) m = 1, n = 2 (**9**) m = 2, n = 3 (**13**) m = 3, n = 4
(**2**) m = 2, n = 2 (**6**) m = 1, n = 3 (**10**) m = 2, n = 4 (**14**) m = 4, n = 1
(**3**) m = 3, n = 3 (**7**) m = 1, n = 4 (**11**) m = 3, n = 1 (**15**) m = 4, n = 2
(**4**) m = 4, n = 4 (**8**) m = 2, n = 1 (**12**) m = 3, n = 2 (**16**) m = 4, n = 3

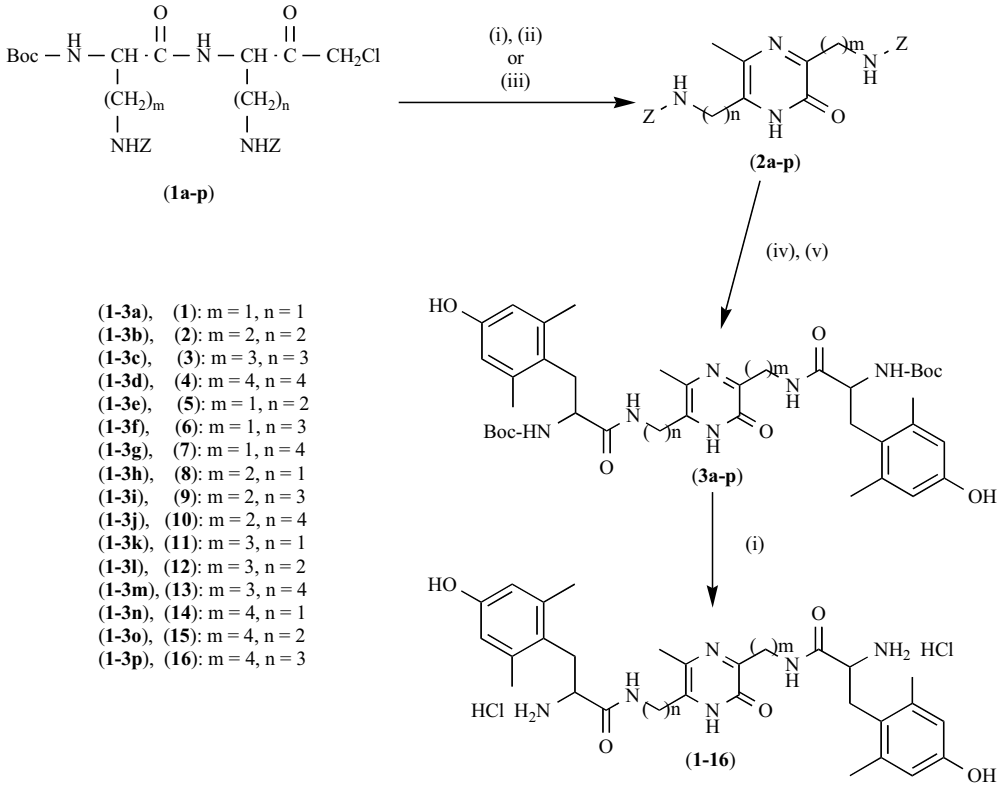
Fig. (1). Structure of Pyrazinone-Ring Containing Opioid Derivatives (**1-16**).

indicated by liquid chromatography-tandem mass spectrometry [29] and cell culture experiments [30]. Moreover, the asymmetric pyrazinone derivative, 3-(4'-H-Dmt-aminobutyl)-6-(3'-H-Dmt-aminopropyl)-5-methyl-2(1*H*)-pyrazinone (**16**) exhibited higher μ-opioid receptor affinity and more potent antinociceptive activity than the comparable symmetrical compound (**3**) [31,32], verifying that the length of the aliphatic spacers affected bioactivity. The subtle differences in chain length and hydrophobicity of the spacers can also impinge upon conformation, resulting in potentially new combination in topography to adopt to the structure of the receptor pocket to elicit a range of opioid activities.

In order to further investigate the effect of chain length of the spacer coupling the Dmt pharmacophore and the pyrazinone platform on receptor affinity and bioactivity, additional unsymmetrical aminoalkyl chains covalently bound at the 3 and 6 positions of pyrazinone were prepared using dipeptidyl chloromethylketone intermediates [33,34]. We discuss the synthesis of sixteen kinds of pyrazinone derivatives containing Dmt as the functional N-termini, 3-[H-Dmt-NH-$(CH_2)_m$]-6-[H-Dmt-NH-$(CH_2)_n$]-5-methyl-2(1*H*)-pyrazinones (Fig. **1**), examination of their opioid receptor affinities and *in vitro* bioactivity as a study on their structure-activity relationships.

CHEMISTRY

Optically pure 2',6'-dimethyl-L-tyrosine was prepared according to the method of Dygos *et al.* [35]. Dimeric Dmt derivatives were synthesized according to the Scheme **1**. Boc-X(Z)-OH was coupled with H-Y(Z)-CH_2Cl (Boc: *tert*-butyloxycaronyl, Z: benzyloxycarbonyl) by a mixed anhydride method to produce Boc-X(Z)-Y(Z)-CH_2Cl (1a-p) [X, Y



(**1a-p**)

(**1-3a**), (**1**): m = 1, n = 1
(**1-3b**), (**2**): m = 2, n = 2
(**1-3c**), (**3**): m = 3, n = 3
(**1-3d**), (**4**): m = 4, n = 4
(**1-3e**), (**5**): m = 1, n = 2
(**1-3f**), (**6**): m = 1, n = 3
(**1-3g**), (**7**): m = 1, n = 4
(**1-3h**), (**8**): m = 2, n = 1
(**1-3i**), (**9**): m = 2, n = 3
(**1-3j**), (**10**): m = 2, n = 4
(**1-3k**), (**11**): m = 3, n = 1
(**1-3l**), (**12**): m = 3, n = 2
(**1-3m**), (**13**): m = 3, n = 4
(**1-3n**), (**14**): m = 4, n = 1
(**1-3o**), (**15**): m = 4, n = 2
(**1-3p**), (**16**): m = 4, n = 3

Scheme (1). General Synthetic Method of Pyrazinone-Ring Containing Opioid Derivatives **(1-16)**.

(i) 7MHCl/dioxane (**1c, 1d, 1m** and **1p**); (ii) CH_3CN, at 60 °C (**1c, 1d, 1m** and **1p**); (iii) 3 M HCl : THF = 1 : 1, at 60 °C; (iv) 25% HBr/CH_3COOH; (v) Boc-Dmt-OH, PyBop, DIPEA.

= Dap (2,3-diaminopropionic acid), Dab (2,4-diaminobutyric acid), Orn, Lys]. After removal of the Boc group by HCl in dioxane, the resulting hydrochloride in MeOH (methanol) was heated at a reflux for 1 h to produce Z-protected pyrazinone derivatives (**2a-p**), in which the different and desired aminoalkyl chains bound at the 3 and 6 positions of pyrazinone ring. Next, Z-protection was removed by 25% HBr/AcOH to give the corresponding amine hydrobromide. In order to remove Z-protection, 25 % HBr/AcOH was recommended especially in the case of (**2a**, **2h**, **2k** and **2n** and **2b**, **2e**, **2l** and **2o**) (n = 1 and 2, respectively). Studies on the hydrogenation of Z-protected pyrazinone derivatives revealed that deamination occurred at the position 6 of compound (**2a**) due to the benzylic or allylic properties of the C-N bond at the position 6 [36, 37]. Because of the similarity in the C-N bond of (**2b**, **2e**, **2l** and **2o**), which also has homobenzylic or homoallylic properties, catalytic hydrogenation should be avoided and 25%HBr/AcOH was recommended. Boc-Dmt-OH was coupled with the resulting amine hydrobromide by PyBop (benzotriazol-1-yloxytrispyrrolidinophosphonium hexafluoro-phosphate) reagent to give Boc protected (**1-16**), namely (**3a-p**). Boc group of (3a-p) was removed by 7 M HCl/dioxane to give crude (**1-16**). All final products were purified by semipreparative reversed phase (RP) HPLC; each compound exhibited a single peak on analytical HPLC with unique retention time. Analysis by MALDI-TOF mass spectroscopy (MS), ^{1}H and ^{13}C-NMR and by elemental analysis revealed that the desired compounds were with greater than 98% purity.

RESULTS AND DISCUSSION

Opioid Receptor Affinity

Affinity for μ- and δ-opioid receptors from rat brain membranes were computed by displacement of [^{3}H]DAMGO and [^{3}H]deltorphin-II, respectively, using equilibrium binding assays (Table **1**). Of the symmetric 3,6-diaminoalkyl-2(1*H*)-pyrazinone derivatives (**1-4**) [21], compound (**3**; m = n : 3) exhibited the highest affinity for μ-opioid receptor, while (**1**; m = n : 1) was the weakest (Table **1**). In terms of the δ-opioid receptor, (**2**; m = n: 2) had the highest affinity and (**4**; m = n : 4) the lowest affinity (Table **1**) as previously reported [21]. In a new series of asymmetrical Dmt dimers (**5-16**), most compounds exhibited subnanomolar affinities for the μ-opioid receptor (K_iμ = 0.021-0.850 nM) except compound (**7**; m = 1, n = 4; K_iμ = 2.94 nM, K_iδ/K_iμ = 28). Compound (**16**; m = 4, n = 3) had 2-fold higher affinity (K_iμ = 0.021 nM; K_iδ/K_iμ = 1,519) than symmetrical (**3**) with an increase in μ-selectivity [31,32] and compound (**14**) exhibited the higtest μ-selectivity (K_iδ/K_iμ = 3,126). The δ-opioid affinities are considerably less than that recorded for the μ-opoid receptor as clearly detailed in Table **1** and documented in other publications [21,31,32].

In Vitro Functional Bioactivity

The functional bioactivities were evaluated using isolated guinea pig ileum (GPI) for μ-opioid receptors and mouse vas deferens (MVD) for δ-opioid receptors (Table **2**). As previously reported [21], compounds (**3** and **4**) are potent μ-

Table 1. Opioid Receptor Affinity of Compounds (1-16)

no.	K_iμ (nM)[c]	(*n*)[e]	K_iδ (nM)[d]	(*n*)[e]	K_iδ/K_iμ
(**1**) m = 1, n = 1[a]	1.16 ± 0.18	(3)	15.7 ± 2.10	(3)	14
(**2**) m = 2, n = 2[a]	0.115 ± 0.003	(3)	7.26 ± 1.20	(3)	63
(**3**) m = 3, n = 3[a]	0.042 ± 0.003	(3)	13.2 ± 1.70	(3)	307
(**4**) m = 4, n = 4[a]	0.114 ± 0.008	(3)	23.2 ± 2.5	(3)	204
(**5**) m = 1, n = 2	0.850 ± 0.060	(3)	15.5 ± 1.00	(3)	18
(**6**) m = 1, n = 3	0.640 ± 0.125	(3)	218.0 ± 20.00	(3)	338
(**7**) m = 1, n = 4	2.94 ± 0.32	(5)	81.7 ± 12.0	(4)	28
(**8**) m = 2, n = 1	0.315 ± 0.035	(3)	152.6 ± 25.00	(3)	484
(**9**) m = 2, n = 3	0.048 ± 0.001	(5)	23.1 ± 2.50	(4)	354
(**10**) m = 2, n = 4	0.065 ± 0.003	(3)	1.060 ± 0.006	(3)	16.3
(**11**) m = 3, n = 1	0.042 ± 0.005	(4)	158.8 ± 32.00	(5)	1,524
(**12**) m = 3, n = 2	0.080 ± 0.008	(5)	112.1 ± 10.00	(4)	369
(**13**) m = 3, n = 4[b]	0.051 ± 0.009	(5)	18.8 ± 2.90	(3)	1,325
(**14**) m = 4, n = 1	0.081 ± 0.005	(3)	123.2 ± 16.00	(4)	3,126
(**15**) m = 4, n = 2	0.090 ± 0.017	(3)	33.9 ± 6.50	(3)	21
(**16**) m = 4, n = 3[b]	0.021 ± 0.003	(3)	31.9 ± 2.90	(5)	1,519

[a]The experimental data of compounds (**1-4**) were cited from ref. [21]. [b]The experimental data of compounds (**13** and **16**) were cited from ref. [32]. [c]Versus [^{3}H]DAMGO. [d]Versus [^{3}H]Deltorphin II. [e]The number of independent repetitions (*n*) conducted for each analogue using 5-8 doses of each compound.

Table 2. ***In Vitro*** **Bioactivity for μ- and δ- Receptor Functions of Compounds (1-16).**

no.	Guinea Pig Ileum (GPI) assay[c](μ)	Mouse Vas Deferens (MVD) assay[c](δ)	
	IC_{50} (nM)[d]	IC_{50} (nM)[d]	δ-antagonism (pA_2[e])
(**1**) m = 1, n = 1[a]	1,695 ± 365.0	>10,000	+[h] (6.47)
(**2**) m = 2, n = 2[a]	12.9 ± 2.40	>10,000	+[h] (6.56)
(**3**) m = 3, n = 3[a]	1.33 ± 0.20	>10,000	n.d.
(**4**) m = 4, n = 4[a]	1.90 ± 0.67	41.5 ± 10.4	n.d.
(**5**) m = 1, n = 2	142 ± 27.0	>10,000	+[h]
(**6**) m = 1, n = 3	792 ± 108	>10,000	n.d.
(**7**) m = 1, n = 4	809 ± 128	>10,000	+[h]
(**8**) m = 2, n = 1	88.9 ± 8.90	>10,000	n.d.
(**9**) m = 2, n = 3	4.91 ± 0.83	>10,000	+[h]
(**10**) m = 2, n = 4	5.38 ± 1.76	>10,000	+[h]
(**11**) m = 3, n = 1	8.12 ± 2.43	>10,000	n.d.
(**12**) m = 3, n = 2	12.1 ± 0.70	>10,000	n.d.
(**13**) m = 3, n = 4[b]	4.93 ± 1.73	>10,000[f]	n.d.
(**14**) m = 4, n = 1	8.71 ± 4.50	>10,000	n.d.
(**15**) m = 4, n = 2	51.4 ± 5.10	>10,000	+[h]
(**16**) m = 4, n = 3[b]	1.79 ± 0.57	25.8 ± 4.30[g]	n.d.

[a]The experimental data of compounds (**1-4**) were cited from ref. [21]. [b]The experimental data of compounds (**13** and **16**) were cited from ref. [32]. [c]The data are the means of over five independent repetitions used different isolated tissue preparations. [d]IC_{50}: concentration required for 50% inhibition of the electrically induced contration in muscle derived from a dose-response curve. [e]Negative log of the molar concentration of an antagonist that is necessary to double the concentration of agonist (deltorphin II) concentration to achieve the original response. [f]Partial agonist (max inhibition 44% at the concentration of 10,000 nM). [g]Partial agonist (max inhibition 60% at the concentration of 10,000 nM). n.d. = not determined. [h]Antagonism (+) denotes the IC_{50} value of the δ-agonist (deltorphin II: 0.86 nM) required to increase by twice or more in the presence of the compound (1,000 nM) in the MVD assay.

agonists (IC_{50} = 1.33 and 1.90 nM, respectivery). It can be theorized that one Dmt residue interacts within the message-binding domain, while another might lie in the address-binding domain in the receptor, however, the binding mode between each ligand and regions in the receptor differs due to the subtle difference in the lengths of aminoalkyl chains bound at the 3 and 6 positions of the pyrazinone ring. Similarly, compounds (**1-4**) bind to δ-opioid receptors with modest K_iδ values (7.26-23.2 nM) but each compound exhibited different bioactivity (agonism and antagonism) as shown in Table **2**.

In this new dimeric opioidmimetic series, Table **2** reveals that the compounds are divisible into three types: (i) relatively good μ agonists (IC_{50} = 1.79-8.12 nM) (**9, 10, 13, 14** and **16**), (ii) those with moderate to weak μ agonism (IC_{50} = 8.79-88.9 nM) (**8**, **11, 12** and **15**), and (iii) the remainings are very weak and essentially inactive μ-agonists (IC_{50} = 142-809 nM) (**5**, **6,** and **7**). Except compounds (**13**) and (**16**), which exhibited partial δ-agonism (maximal inhibition: 44 and 60%, respectively), compounds (**5-15**) did not exhibit significant δ-agonism (Table **2**). Although (**10**) had nanomolar δ-opioid receptor affinity (K_iδ = 1.06 nM), it was totally devoid of δ-agonism. Some compounds (**5**, **7**, **9**, **10** and **15**) having higher affinity for δ-receptor exhibited weak antagonism as compounds **1** and **2** demonstrated against the selective δ-receptor agonist deltorphin II.

CONCLUSION

A series of Dmt dimers linked with various unsymmetrical aminoalkyl chains bound at the 3 and 6 positions of pyrazinone ring was successfully synthesized using synthetic procedures developed in our laboratory [33,34]. All the compounds in Fig. (**1**) exhibited μ-opioid receptor affinity and μ-agonism with low or no δ-agonism. These data indicate that length of the aliphatic spacers between the Dmt pharmacophore and the pyrazinone ring platform affected both receptor affinity and bioactivity. Of compounds (**1-16**), compounds (**3**: m = 3, n = 3) and (**16**: m = 4, n = 3) exhibited the highest μ-opioid receptor affinity (K_iμ = 0.042 and 0.021 nM, respectively) with high selectivity over δ-receptor (K_iδ/K_iμ = 307 and 1,519, respectively) and showed potent μ-agonistic activity (IC_{50}: 1.33 and 1.79 nM, respectively) in GPI assay. Accumulation of these data provides us with important information to theoretically design additional opioid agonists that might be valuable in therapeutic applications.

EXPERIMENTAL SECTION

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. TLC was

performed on precoated plates of silica gel F254 (Merck, Darmstadt, Germany). R_f values refer to the following solvent systems: (1) AcOEt (ethyl acetate) : Hexane = 1 : 1, (2) $CHCl_3$: MeOH : AcOH (acetic acid) = 90 : 8 : 2 and (3) *n*-BuOH (*n*-butanol) : 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 semi-preparative RP-HPLC used are Waters Delta 600 with COSMOSIL C18 column and YMC Pack R&D ODS C18 column (4.6 mm × 250 mm) and COSMOSIL C18 column or YMC Pack R&D ODS 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 90% A to 50% A in 40 min; the retention time is reported as t_R (min). Mass spectra were measured with a KRATOS MALDI-TOF MS (matrix-assisted laser desorption ionization time-of-flight mass spectrometry). ^{1}H and ^{13}C NMR spectra were measured on a Bruker DPX-400 or DPX-500 (for **1m**, **1p**, **2m** and **2p**) spectrometer at 20 °C. Purified compounds (20 mg) were dissolved in 1.0 mL of $CDCl_3$ (chloroform-*d*: 99.8% isotopic purity), pyridine-d_5 (99.9% isotopic purity) or DMSO (dimethyl sulfoxide-d_6: 99.9% isotopic purity). ^{1}H pattern was described using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Chemical shift values are expressed as ppm downfield from tetramethylsilane, used as an internal standard (δ-value), and *J* values are given in hertz (Hz). ^{13}C signals were assigned with the aid of Distortionless Enhancement by Polarization Transfer (DEPT) and two-dimentional experiments, and multiplicities are indicated by p (primary), s (secondary), t (tertiary) or q (quaternary). The experimental data of compounds (**1a-3a, 1**, **1b-3b**, **2, 1c-3c, 3, 1d-3d** and **4**) were described in reference [21].

General Procedure for Synthesis of Boc-X(Z)-Y(Z)-CH_2Cl (1e-p, X: Dap, Dab, Orn, Lys, Y: Dap, Dab, Orn, Lys)

A mixed anhydride [prepared from Boc-X(Z)-OH (1.2 mmol), IBCF (isobutyl chloroformate: 0.15 mL, 1.2 mmol) and NMM (*N*-methylmorpholine: 0.17 mL, 1.2 mmol) in the usual manner] in THF (tetrahydrofuran: 30 mL) was added to a solution of HCl·H-Y(Z)-CH_2Cl [prepared from Boc-Y(Z)-CH_2Cl (1.0 mmol) and 7 M HCl/dioxane (1.7 mL, 12 mmol) in the usual manner] in DMF (*N*, *N*-dimethylformamide: 30 mL) containing NMM (0.21 mL, 1.5 mmol) at 0 °C. The reaction mixture was stirred at the same temperature for 1 h and at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt (ethyl acetate). The extract was washed with 10% aqueous citric acid, 5% aqueous Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to give crystals, which were collected by filtration and recrystallized from ether and EtOH (ethanol) and purified by silicagel chromatography using AcOEt : Hexane = 1 : 1 as eluent.

Boc-Dap(Z)-Dab(Z)-CH_2Cl (1e)

Yield 1.53g (54.1%), mp 162-164 °C, $[\alpha]_D^{25}$ -15.0° (*c* = 1.0, MeOH), R_{f1} = 0.48, Anal. Calcd for $C_{29}H_{37}ClN_4O_8$: C, 57.6; H, 6.16; N, 9.26. Found: C, 57.5; H, 6.12; N, 9.17. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 7.47-7.25 [m, 10H, 2 × phenyl of Z (benzyloxycarbonyl)], 7.03 (br, 1H, α-NH of Dab), 5.86 (d, *J* = 6.1 Hz, 1H, α-NH of Dap), 5.56 (br, 1H, β-NH of Dap), 5.16 and 5.09 (2s, 4H, 2 × CH_2 of Z), 4.76-4.63 (m, 1H, α-CH of Dap), 4.25 (br, 1H, α-CH of Dab), 4.25 and 3.56 (each br, each 1H, CH_2Cl), 3.78-3.19 (m, 2H, γ-CH_2 of Dap), 3.63 (br, 1H, γ-NH of Dab), 3.56 (br, 2H, β-CH_2 of Dap), 2.20-1.68 (m, 2H, β-CH_2 of Dab), 1.42 (s, 9H, *tert*-butyl), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 169.4 (q), 150.7 (q), 136.2 and 136.1 (q, 2 × phenyl of Z), 135.0 (q), 128.8-128.1 (t, 2 × phenyl of Z), 118.8 (q), 81.0 (q, *tert*-butyl), 68.7 and 67.3 (s, 2 × CH_2 of Z), 55.5 (t, α-CH of Dap), 51.6 (t, α-CH of Dab), 45.7 (s, γ-CH_2 of Dab), 45.5 (s, CH_2Cl), 42.8 (s, β-CH_2 of Dap), 28.3 (p, *tert*-butyl), 28.2 (s, β-CH_2 of Dab).

Boc-Dap(Z)-Orn(Z)-CH_2Cl (1f)

Yield 944 mg (62.2%), mp 204-205 °C, $[\alpha]_D^{23}$ -32.3° (*c* = 1.0, DMF), R_{f1} = 0.63, Anal. Calcd for $C_{30}H_{39}ClN_4O_8$: C, 58.2; H, 6.35; N, 9.05. Found: C, 58.1; H, 6.24; N, 9.12. ^{1}H-NMR (400.1 MHz, DMSO-d_6) δ: 8.39 (d, *J* = 7.6 Hz, 1H, α-NH of Orn), 7.38-7.19 (m, 12H, 2 × phenyl of Z, β-NH of Dap, δ-NH of Orn,), 6.87 (d, *J* = 6.3 Hz, 1H, α-NH of Dap), 5.02 and 5.00 (2s, 4H, 2 × CH_2 of Z), 4.57 and 4.50 (AB-q, *J* = 14.2 Hz, 2H, CH_2Cl), 4.32 (br, 1H, α-CH of Orn), 4.02 (q, *J* = 6.5 Hz, 1H, α-CH of Dap), 3.38-3.22 (m, 2H, β-CH_2 of Dap), 3.03-2.89 (m, 2H, δ-CH_2 of Orn), 1.83-1.69 and 1.58-1.47 (each m, 4H, β, γ-CH_2 of Orn), 1.38 (s, 9H, *tert*-butyl), ^{13}C-NMR (100.6 MHz, DMSO-d_6) δ: 191.3 (q, *C*OCH_2Cl), 177.5 (q), 161.5 (q), 147.0 (q), 146.7 (q), 127.9 and 127.6 (q, 2 × phenyl of Z), 119.0-118.3 (t, 2 × phenyl of Z), 69.2 (q, *tert*-butyl), 56.1 and 55.8 (s, 2 × CH_2 of Z), 46.9 (t, α-CH of Orn), 45.5 (t, α-CH of Dap), 38.5 (s, CH_2Cl), 32.5 (s, β-CH_2 of Dap), 30.4 (s, δ-CH_2 of Orn), 18.7 (p, *tert*-butyl), 17.2 (s, γ-CH_2 of Orn), 16.1 (s, β-CH_2 of Orn).

Boc-Dap(Z)-Lys(Z)-CH_2Cl (1g)

Yield 1.76g (71.8%), mp 175-178 °C, $[\alpha]_D^{20}$ -32.7° (*c* = 0.34, DMSO), R_{f1} = 0.59, Anal. Calcd for $C_{31}H_{41}ClN_4O_8 \cdot 0.5H_2O$: C, 57.8; H, 6.59; N, 8.73. Found: C, 58.0; H, 6.29; N, 8.83. ^{1}H-NMR (400.1 MHz, DMSO-d_6) δ: 8.39 (d, *J* = 6.9 Hz, 1H, α-NH of Lys), 7.34 (s, 10H, 2 × phenyl of Z), 7.25 (br, 1H, β-NH of Dap), 7.19 (br, 1H, ε-NH of Lys), 6.88 (br, 1H, α-NH of Dap), 5.02 and 5.00 (2s, 4H, 2 × CH_2 of Z), 4.59 and 4.52 (AB-q, *J* = 16.3 Hz, 2H, CH_2Cl), 4.30 (br, 1H, α-CH of Lys), 4.10-3.98 (m, 1H, α-CH of Dap), 3.32 (br, 2H, β-CH_2 of Dap), 3.02-2.87 (m, 2H, ε-CH_2 of Lys), 1.73 (br, 2H, β-CH_2 of Lys), 1.53 (br, 2H, γ-CH_2 of Lys), 1.38 (s, 9H, *tert*-butyl), 1.36-1.17 (m, 2H, δ-CH_2 of Lys), ^{13}C-NMR (100.6 MHz, DMSO-d_6) δ: 200.7 (q, *C*OCH_2Cl), 177.6 (q), 172.7 (q), 170.7 (q), 156.2 (q), 137.2 (q), 136.9 (q), 128.2-127.5 (t, 2 × phenyl of Z), 78.4 (q, *tert*-butyl), 65.4 and 65.0 (s, 2 × CH_2 of Z), 56.5 (t, α-CH of Lys), 54.8 (t, α-CH of Dap), 47.8 (s, CH_2Cl), 41.8 (s, ε-CH_2 of Lys), 29.0 (s, β-CH_2 of Dap), 28.9 (s, γ-CH_2 of Lys), 28.6 (p, *tert*-butyl), 24.7 (s, δ-CH_2 of Lys), 22.2 (s, β-CH_2 of Lys).

Boc-Dab(Z)-Dap(Z)-CH_2Cl (1h)

Yield 0.30g (41.9%), mp 133-134 °C, $[\alpha]_D^{25}$ -3.36° (*c* = 1.0, CH_3OH), R_{f1} = 0.48, Anal. Calcd for $C_{29}H_{37}ClN_4O_8$: C,

57.6; H, 6.10; N, 9.26. Found: C, 57.3; H, 5.92; N, 9.29. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 7.85 (br, 1H, α-NH of Dap), 7.41-7.25 (m, 10H, 2 × phenyl of Z), 5.75 (br, 1H, β-NH of Dap), 5.45 (br, 2H, α, γ-NH of Dab), 5.08 (s, 4H, 2 × CH_2 of Z), 4.84 (br, 1H, α-CH of Dap), 4.42 and 4.30 (AB-q, J = 15.7 Hz, each 1H, CH_2Cl), 4.14 (br, 1H, α-CH of Dab), 3.78 and 3.61 (each br, each 1H, β-CH_2 of Dap), 3.49 and 3.13 (each br, each 1H, γ-CH_2 of Dab), 1.90 and 1.80 (each br, each 1H, β-CH_2 of Dab), 1.41 (s, 9H, *tert*-butyl), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 199.4 (q, *C*OCH_2Cl), 157.2 (q), 156.9 (q), 136.24 and 136.17 (q, 2 × phenyl of Z), 128.6-128.0 (t, 2 × phenyl of Z), 80.2 (q, *tert*-butyl), 67.1 (s, 2 × CH_2 of Z), 57.2 (t, α-CH of Dap), 52.1 (t, α-CH of Dab), 46.5 (s, CH_2Cl), 41.1 (s, β-CH_2 of Dap), 37.4 (s, γ-CH_2 of Dab), 33.2 (s, β-CH_2 of Dab), 28.3 (p, *tert*-butyl).

Boc-Dab(Z)-Orn(Z)-CH_2Cl (1i)

Yield 254.6 mg (53.5%), mp 135-137 °C, $[\alpha]_D^{23}$ +1.50° (*c* = 1.0, CH_3OH), R_{f1} = 0.70, Anal. Calcd for $C_{31}H_{41}ClN_4O_8$: C, 58.8; H, 6.53; N, 8.85, Found: C, 58.7; H, 6.50; N, 9.07. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 7.89 (br, 1H, α-NH of Dab), 7.32 (s, 10H, 2 × phenyl of Z), 7.29 (br, 1H, α-NH of Orn), 5.58-5.40 (m, 1H, δ-NH of Orn), 5.19 (br, 1H, γ-NH of Dab), 5.07 (s, 4H, 2 × CH_2 of Z), 4.68 (br, 1H, α-CH of Dab), 4.24 (s, 2H, CH_2Cl), 4.21 (br, 1H, α-CH of Orn), 3.50 and 3.19 (each br, each 1H, δ-CH_2 of Orn, 2H, γ-CH_2 of Dab), 2.19-1.70 (m, 4H, β, γ-CH_2 of Orn), 1.70-1.47 (m, 2H, β-CH_2 of Dab), 1.42 (s, 9H, *tert*-butyl), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 172.3 (q), 157.7 (q), 157.1 (q), 155.9 (q), 136.8 (q), 128.8-128.1 (t, 2 × phenyl of Z), 80.4 (q, *tert*-butyl), 67.3 and 67.0 (s, 2 × CH_2 of Z), 56.6 (t, α-CH of Dab), 51.7 (t, α-CH of Orn), 47.0 (s, CH_2Cl), 40.4 (s, γ-CH_2 of Dab), 37.7 (s, δ-CH_2 of Orn), 34.3 (s, γ-CH_2 of Orn), 28.6 (p, *tert*-butyl), 27.6 (s, β-CH_2 of Orn), 26.4 (s, β-CH_2 of Dab).

Boc-Dab(Z)-Lys(Z)-CH_2Cl (1j)

Yield 461.5 mg (27.5%), mp 106-109 °C, $[\alpha]_D^{25}$ -2.33° (*c* = 0.1, MeOH), R_{f1} = 0.54, Anal. Calcd for $C_{32}H_{43}ClN_4O_8 \cdot 0.5H_2O$: C, 58.6; H, 6.76; N, 8.54. Found: C, 58.7; H, 6.67, N, 8.47. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 7.69 (br, 1H, γ-NH of Dab), 7.32-7.29 (m, 10H, 2 × phenyl of Z), 5.42 (br, 2H, α, ε-NH of Lys), 5.08 (s, 5H, 2 × CH_2 of Z, α-NH of Dab), 4.67 (br, 1H, α-CH of Dab), 4.24 (s, 3H, CH_2Cl, α-CH of Lys), 3.62-3.47 and 3.26-3.04 (each m, each 1H, ε-CH_2 of Lys), 3.26-3.04 (m, 2H, δ-CH_2 of Lys), 1.95-1.49 (m, 6H, γ-CH_2 of Lys, β, γ-CH_2 of Dab), 1.42 (s, 11H, *tert*-butyl, β-CH_2 of Lys), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 201.0 (q, *C*OCH_2Cl), 172.1 (q), 157.4 (q), 156.7 (q), 155.7 (q), 136.6 (q), 136.4 (q), 128.5-127.9 (t, 2 × phenyl of Z), 80.2 (q, *tert*-butyl), 67.0 and 66.8 (s, 2 × CH_2 of Z), 56.4 (t, α-CH of Dab), 51.5 (t, α-CH of Lys), 46.7 (s, CH_2Cl), 40.1 (s, δ-CH_2 of Lys), 37.4 (s, ε-CH_2 of Lys), 34.0 (s, γ-CH_2 of Lys), 29.3 (s, β, γ-CH_2 of Dab), 28.3 (p, *tert*-butyl), 22.4 (s, β-CH_2 of Lys).

Boc-Orn(Z)-Dap(Z)-CH_2Cl (1k)

Yield 1.40 g (88.4%), mp 99-104 °C, $[\alpha]_D^{20}$ -33.6° (*c* = 1.27, DMF), R_{f1} = 0.42, Anal. Calcd for $C_{30}H_{39}ClN_4O_8$: C, 58.2; H, 6.35; N, 9.05. Found: C, 58.1; H, 6.35; N, 9.07. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 7.31 (s, 11H, 2 × phenyl of Z, α-NH of Dap), 5.63 (br, 1H, β-NH of Dap), 5.07 (br, 6H, α-NH of Orn, δ-NH of Orn, 2 × CH_2 of Z), 4.87 (br, 1H, α-CH of Dap), 4.42 and 4.28 (each br, each 1H, CH_2Cl), 4.17 (br, 1H, α-CH of Orn), 3.75 and 3.60 (each br, each 1H, β-CH_2 of Dap), 3.32 and 3.14 (each br, each 1H, δ-CH_2 of Orn), 1.76 and 1.57 (each br, each 1H, β-CH_2 of Orn), 1.57 (br, 2H, γ-CH_2 of Orn), 1.43 (s, 9H, *tert*-butyl), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 190.6 (q, *C*OCH_2Cl), 172.6 (q), 157.2 (q), 136.4 (q), 136.2 (q), 128.5-128.0 (t, 2 × phenyl of Z), 80.3 (q, *tert*-butyl), 67.1 and 66.9 (s, 2 × CH_2 of Z), 57.0 (t, α-CH of Dap, Orn), 46.4 (s, CH_2Cl), 40.1 (s, δ-CH_2 of Orn), 29.8 (s, β-CH_2 of Dap), 28.3 (p, *tert*-butyl), 26.2 (s, β, γ-CH_2 of Orn).

Boc-Orn(Z)-Dab(Z)-CH_2Cl (1l)

Yield 2.1g (83.8%), mp 65-66 °C, $[\alpha]_D^{22}$ -35.4° (*c* = 1.0, DMF), R_{f1} = 0.24, Anal. Calcd for $C_{31}H_{41}ClN_4O_8$: C, 58.8; H, 6.53; N, 8.85. Found: C, 58.6; H, 6.43; N, 8.72. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 7.34 and 7.30 (2s, 10H, 2 × phenyl of Z), 6.80 (br, 1H, δ-NH of Orn), 5.28 (br, 1H, γ-NH of Dab), 5.22-5.00 (m, 6H, α-NH of Dab, Orn, 2 × CH_2 of Z), 4.73 (br, 2H, δ-CH_2 of Orn), 4.20 (br, 2H, γ-CH_2 of Dab), 3.72 and 3.29 (each br, each 1H, CH_2Cl), 3.60 (br, 1H, α-CH of Dab), 3.29 (br, 2H, β-CH_2 of Orn), 3.16 (br, 1H, α-CH of Orn), 2.20 and 1.82 (each br, each 1H, γ-CH_2 of Orn), 1.58 (br, 2H, β-CH_2 of Dab), 1.42 (s, 9H, *tert*-butyl), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 233.7 (q, *C*OCH_2Cl), 172.4 (q), 172.3 (q), 156.9 (q), 156.8 (q), 156.3 (q), 136.5 and 136.4 (q, 2 × phenyl of Z), 128.7-127.7 (t, 2 × phenyl of Z), 80.0 (q, *tert*-butyl), 66.7 (s, 2 × CH_2 of Z), 54.6 (s, δ-CH_2 of Orn), 45.2 (s, γ-CH_2 of Dab), 44.8 (t, α-CH of Orn, Dab, s, CH_2Cl), 40.1 (s, β-CH_2 of Orn), 28.3 (p, *tert*-butyl), 26.2 (s, β-CH_2 of Dab), 21.1 (s, γ-CH_2 of Orn).

Boc-Orn(Z)-Lys(Z)-CH_2Cl (1m)

Yield 2.60g (27.2%), mp 150-151 °C, $[\alpha]_D^{22}$ -49.2° (*c* = 0.1, CH_3OH), R_{f1} = 0.58, Anal. Calcd for $C_{33}H_{45}ClN_4O_8$: C, 60.0; H, 6.86; N, 8.47. Found: C, 59.9; H, 6.84; N, 8.52. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 7.36-7.28 (m, 10H, 2 × phenyl of Z), 7.20 (br, 1H, α-NH of Lys), 5.21 (d, J = 7.3 Hz, 1H, α-NH of Orn), 5.11-5.04 (m, 6H, 2 × CH_2 of Z, δ-NH of Orn, ε-NH of Lys), 4.72-4.65 (m, 1H, α-CH of Lys), 4.29 (br, 1H, α-CH of Orn), 4.22 (s, 2H, CH_2Cl), 3.35 and 3.19 (each br, each 1H, ε-CH_2 of Lys), 3.14 (quintet, J = 6.6 Hz, 2H, δ-CH_2 of Orn), 1.87-1.50 (m, 4H, β-CH_2 of Lys, β-CH_2 of Orn), 1.65-1.44 (m, 6H, γ-CH_2 of Orn, γ, δ-CH_2 of Lys), 1.42 (s, 9H, *tert*-butyl), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 200.9 (q, *C*OCH_2Cl), 172.8, 157.1, 156.7 and 155.9 (q, 3 × OCONH, CONH), 136.6 and 136.5 (q, 2 × phenyl of Z), 128.6-128.0 (t, 2 × phenyl of Z), 80.1 (q, *tert*-butyl), 66.79 and 66.75 (s, 2 × CH_2 of Z), 56.1 (t, α-CH of Lys), 53.1 (t, α-CH of Orn), 46.6 (s, CH_2Cl), 40.2 (s, δ-CH_2 of Orn), 39.7 (s, ε-CH_2 of Lys), 30.2 (s, β-CH_2 of Lys), 29.8 (s, β-CH_2 of Orn), 29.3 (s, γ-CH_2 of Lys), 28.3 (p, *tert*-butyl), 26.2 (s, δ-CH_2 of Lys), 22.4 (s, γ-CH_2 of Orn).

Boc-Lys(Z)-Dap(Z)-CH_2Cl (1n)

Yield 194.1 mg (38.0%), mp 93-98 °C, $[\alpha]_D^{25}$ -33.5° (*c* = 1.0, DMF), R_{f1} = 0.53, Anal. Calcd for $C_{31}H_{41}ClN_4O_8$: C, 58.8; H, 6.53; N, 8.85. Found: C, 59.0; H, 6.52; N, 8.93. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 7.33 and 7.31 (2s, 11H, 2 × phenyl of Z, α-NH of Dap), 5.71 (br, 1H, β-NH of Dap), 5.20 (br, 1H, α-NH of Lys), 5.08 and 5.06 (2s, 4H, 2 × CH_2 of Z), 4.96 (br, 1H, ε-NH of Lys), 4.86 (br, 1H, α-CH of Dap), 4.42 and 4.29 (AB-q, *J* = 15.0 Hz, each 1H, CH_2Cl), 4.02 (br, 1H, α-CH of Lys), 3.77 and 3.66 (each br, each 1H, β-CH_2 of Dap), 3.17 (q, *J* = 12.6 Hz, 2H, ε-CH_2 of Lys), 1.81 and 1.68 (each br, 2H, β-CH_2 of Lys), 1.61 (br, 2H, δ-CH_2 of Lys), 1.41 (s, 11H, *tert*-butyl, γ-CH_2 of Lys), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 157.4 (q), 156.8 (q), 136.6 (q), 128.6-128.1 (t, 2 × phenyl of Z), 121.3 (q), 91.8 (q), 84.7 (q, *tert*-butyl), 67.2 and 66.8 (s, 2 × CH_2 of Z), 57.3 (t, α-CH of Dap), 54.9 (t, α-CH of Lys), 46.4 (s, CH_2Cl), 41.6 (s, β-CH_2 of Dap), 40.6 (s, ε-CH_2 of Lys), 29.5 (s, δ-CH_2 of Lys), 28.3 (p, *tert*-butyl, β-CH_2 of Lys), 22.5 (s, γ-CH_2 of Lys).

Boc-Lys(Z)-Dab(Z)-CH_2Cl (1o)

Yield 1.66 g (65.9%), mp 60-66 °C, $[\alpha]_D^{23}$ -6.14° (*c* = 1.0, DMSO), R_{f1} = 0.19, Anal. Calcd for $C_{32}H_{43}ClN_4O_8$: C, 59.4; H, 6.70; N, 8.66. Found: C, 59.1; H, 6.72; N, 8.51. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 7.35 and 7.34 (2s, 10H, 2 × phenyl of Z), 6.62 (br, 1H, α-NH of Dab), 5.27 (br, 1H, α-NH of Lys), 5.16 and 5.09 (2s, 4H, 2 × CH_2 of Z), 4.93 (m, 1H, γ-NH of Dab), 4.72 (q, *J* = 9.2 Hz, 1H, α-CH of Dab), 4.23 (d, *J* = 11.6 Hz, 1H, ε-NH of Lys), 4.05 (br, 1H, α-CH of Lys), 3.82-3.70 and 3.35-3.23 (each m, each 1H, CH_2Cl), 3.73 (t, *J* = 9.0 Hz, 2H, γ-CH_2 of Dab), 3.65-3.54 and 3.23-3.10 (each m, each 1H, ε-CH_2 of Lys), 3.19 (br, 2H, δ-CH_2 of Lys), 2.26-2.12 and 1.60-1.52 (each m, each 1H, β-CH_2 of Lys), 1.94-1.78 and 1.73-1.60 (each m, each 1H, β-CH_2 of Dab), 1.51 (br, 2H, γ-CH_2 of Lys), 1.42 (s, 9H, *tert*-butyl), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 176.5 (q), 172.2 (q), 156.7 (q), 155.7 (q), 137.5 and 136.0 (q, 2 × phenyl of Z), 128.6-127.9 (t, 2 × phenyl of Z), 88.7 (q, *tert*-butyl), 66.8 and 66.7 (s, 2 × CH_2 of Z), 54.7 (t, α-CH of Lys), 54.6 (s, CH_2Cl), 52.0 (t, α-CH of Dab), 45.2 (s, γ-CH_2 of Dab), 44.6 (s, ε-CH_2 of Lys), 40.3 (s, δ-CH_2 of Lys), 29.5 (s, γ-CH_2 of Lys), 28.3 (p, *tert*-butyl), 22.5 (s, β-CH_2 of Dab), 12.1 (s, β-CH_2 of Lys).

Boc-Lys(Z)-Orn(Z)-CH_2Cl (1p)

Yield 0.55 g (83.3%), mp 108-110 °C, $[\alpha]_D^{23}$ -7.67° (*c* = 0.1, CH_3OH), R_{f1} = 0.48, Anal. Calcd for $C_{33}H_{45}ClN_4O_8$: C, 60.0; H, 6.89; N, 8.47. Found: C, 59.9; H, 6.74; N, 8.42. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 7.35-7.25 (m, 11H, 2 × phenyl of Z, α-NH of Lys), 5.37 (br, 1H, α-NH of Orn), 5.28 (br, 1H, δ-NH of Orn), 5.11 (t, *J* = 5.3 Hz, 1H, ε-NH of Lys), 5.04 (s, 4H, 2 × CH_2 of Z), 4.70 (br, 1H, α-CH of Lys), 4.240 and 4.196 (AB-q, *J* = 16.1 Hz, each 1H, CH_2Cl), 4.09 (br, 1H, α-CH of Orn), 3.17 (br, 4H, ε-CH_2 of Lys, δ-CH_2 of Orn), 1.89-1.27 (m, 10H, β, γ, δ-CH_2 of Lys, β, γ-CH_2 of Orn,), 1.41 (s, 9H, *tert*-butyl), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 200.9 (q, *C*OCH_2Cl), 172.9, 156.86, 156.72 and 155.9 (q, 3 × OCONH, CONH), 136.7 and 136.6 (q, 2 × phenyl of Z), 128.5-128.1 (t, 2 × phenyl of Z), 80.3 (q, *tert*-butyl), 66.8 and 66.7 (s, 2 × CH_2 of Z), 56.0 (t, α-CH of Lys), 54.4 (t, α-CH of Orn), 46.6 (s, CH_2Cl), 40.3 (s, ε-CH_2 of Lys), 40.2 (s, δ-CH_2 of Orn), 31.6 (s, β-CH_2 of Lys), 29.4 (s, δ-CH_2 of Lys), 28.3 (p, *tert*-butyl), 27.7 (s, γ-CH_2 of Lys), 26.2 (s, γ-CH_2 of Orn), 22.5 (s, β-CH_2 of Orn).

General Procedure for Synthesis of 3,6-Bis(benzyloxycarbonylaminoalkyl)-5-methyl-2(1*H*)-pyrazinone (2e-p)

[Method 1] A solution of HCl·H-X(Z)-Y(Z)-CH_2Cl (X: Orn, Lys; Y: Orn, Lys) [prepared from Boc-X(Z)-Y(Z)-CH_2Cl (**2c**, **2d**, **2m** and **2p**; 1.0 mmol) and 7 M HCl/dioxane (1.43 mL, 10.0 mmol) in the usual manner] in CH_3CN or MeOH (300 mL) was refluxed for 3 h. After removal of the solvent, the residue was extracted with $CHCl_3$ and the extract was washed with a little amount of water, dried over Na_2SO_4 and evaporated. Ether was added to the residue to give crystals, which were collected by filtration and recrystallized from EtOH and ether and purified by silicagel chromatography using AcOEt : MeOH = 10 : 1 as eluent.

[Method 2] Boc-X(Z)-Y(Z)-CH_2Cl (X: Dap, Dab, Orn, Lys; Y: Dap, Dab, Orn, Lys) except for (**2c**, **2d**, **2m** and **2p**) was dissolved in THF(compound concentration: 0.05 mmol/mL), to which 3M HCl was droped in a stepwise manner during 30 min at 60 °C. The solution (3 M HCl : THF = 1 : 1) was stirred for 3 h at the same temperature. After removal of the solvent, the residue was extracted with $CHCl_3$ and the extract was washed with a little amount of water, dried over Na_2SO_4 and evaporated. Ether was added to the residue to give crystals, which were collected by filtration and recrystallized from EtOH and ether and purified by silicagel chromatography using AcOEt : MeOH = 10 : 1 as eluent.

6-(2'-Benzyloxycarbonylaminoethyl)-3-(benzyloxycarbonylaminomethyl)-5-methyl-2(1*H*)-pyrazinone (2e)

Yield 213.6 mg (52.2%), mp 144-146 °C, R_{f2} = 0.63, Anal. Calcd for $C_{24}H_{26}N_4O_5 \cdot 0.25H_2O$: C, 63.4; H, 5.87; N, 12.3. Found: C, 63.2; H, 5.79; N, 12.1. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 13.4 (br, 1H, 1-NH), 7.36-7.24 (m, 10H, 3,6-phenyl of Z), 5.93 (br, 1H, 3-CH_2-N*H*), 5.74 (br, 1H, 6-CH_2-CH_2-N*H*), 5.14 and 4.96 (2s, 4H, CH_2 of 3,6-Z), 4.44 (br, 2H, 3-C*H*$_2$-NH), 3.45 (br, 2H, 6-CH_2-C*H*$_2$-NH), 2.75 (br, 2H, 6-C*H*$_2$-CH_2-NH), 2.28 (s, 3H, 5-CH_3), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 156.52 (q), 156.48 (q), 156.3 (q), 136.6 (q), 136.3 (q), 133.2 (q), 130.9 (q, 3,6-phenyl of Z), 128.5-127.7 (t, 3,6-phenyl of Z), 66.9 and 66.6 (s, CH_2 of 3,6-Z), 42.1 (s, 3-CH_2-NH), 39.5 (s, 6-CH_2-*C*H_2-NH), 30.4 (s, 6-*C*H_2-CH_2-NH), 18.3 (p, 5-CH_3).

3-(Benzyloxycarbonylaminomethyl)-6-(3'-benzyloxycarbonylaminopropyl)-5-methyl-2(1*H*)-pyrazinone (2f)

Yield 117.9 mg (52.9%), mp 148-149 °C, R_{f2} = 0.42, Anal. Calcd for $C_{25}H_{28}N_4O_5$: C, 64.6; H, 6.08; N, 12.1. Found: C, 64.4; H, 5.88; N, 12.0. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 13.5 (br, 1H, 1-NH), 7.36-7.27 (m, 10H, 3,6-phenyl of Z), 6.12 (br, 1H, 6-CH_2-CH_2-CH_2-N*H*), 5.88 (br, 1H, 3-CH_2-N*H*), 5.12 and 5.10 (2s, 4H, CH_2 of 3,6-Z), 4.44 (d, *J* = 4.5 Hz, 2H, 3-C*H*$_2$-NH), 3.21 (q, *J* = 12.4 Hz, 2H, 6-CH_2-CH_2-C*H*$_2$-NH), 2.60 (t, *J* = 7.0 Hz, 2H, 6-C*H*$_2$-CH_2-CH_2-NH), 2.28 (s, 3H, 5-CH_3), 1.83 (quintet, *J* = 6.8 Hz, 2H, 6-CH_2-

C*H*$_2$-CH$_2$-NH), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 156.8 (q), 156.7 (q), 156.3 (q), 136.7 (q), 136.6 (q), 135.4 (q), 130.8 (q, 3,6-phenyl of Z), 128.5-128.0 (t, 3,6-phenyl of Z), 66.8 and 66.7 (s, CH_2 of 3,6-Z), 42.1 (s, 3-CH_2-NH), 39.3 (s, 6-CH_2-CH_2-*C*H_2-NH), 28.5 (s, 6-CH_2-*C*H_2-CH_2-NH), 26.8 (s, 6-*C*H_2-CH_2-CH_2-NH), 18.3 (p, 5-CH_3).

6-(4'-Benzyloxycarbonylaminobutyl)-3-(benzyloxycarbonylaminomethyl)-5-methyl-2(1*H*)-pyrazinone (2g)

Yield 81.0 mg (55.0%), mp 184-185 °C, R_{f2} = 0.55, Anal. Calcd for $C_{26}H_{30}N_4O_5 \cdot 0.3H_2O$: C, 64.5; H, 6.37; N, 11.6. Found: C, 64.6; H, 6.34; N, 11.6. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 13.2 (br, 1H, 1-NH), 7.37-7.26 (m, 10H, 3,6-phenyl of Z), 5.97 (t, *J* = 9.0 Hz, 1H, 3-CH_2-N*H*), 5.52 (br, 1H, 6-CH_2-CH_2-CH_2-CH_2-N*H*), 5.13 and 5.05 (2s, each 2H, CH_2 of 3,6-Z), 4.46 (d, *J* = 4.4 Hz, 2H, 3-C*H*$_2$-NH), 3.25 (q, *J* = 11.8 Hz, 2H, 6-CH_2-CH_2-CH_2-C*H*$_2$-NH), 2.56 (t, *J* = 7.2 Hz, 2H, 6-C*H*$_2$-CH_2-CH_2-CH_2-NH), 2.27 (s, 3H, 5-CH_3), 1.74-1.52 (m, 4H, 6-CH_2-C*H*$_2$-C*H*$_2$-CH_2-NH), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 156.74 (q), 156.65 (q), 156.4 (q), 136.64 (q), 136.58 (q), 135.8 (q), 129.5 (q, 3,6-phenyl of Z), 128.5-128.0 (t, 3,6-phenyl of Z), 66.9 and 66.6 (s, CH_2 of 3,6-Z), 41.9 (s, 3-*C*H_2-NH), 39.9 (s, 6-CH_2-CH_2-CH_2-*C*H_2-NH), 29.3 (s, 6-*C*H_2-CH_2-CH_2-CH_2-NH), 29.2 (s, 6-CH_2-*C*H_2-CH_2-CH_2-NH), 25.4 (s, 6-CH_2-CH_2-*C*H_2-CH_2-NH), 18.3 (p, 5-CH_3).

3-(2'-Benzyloxycarbonylaminoethyl)-6-(benzyloxycarbonylaminomethyl)-5-methyl-2(1*H*)-pyrazinone (2h)

Yield 50.0 mg (67.2%), mp 161-164 °C, R_{f2} = 0.43, Anal. Calcd for $C_{34}H_{26}N_4O_5 \cdot 0.6H_2O$: C, 62.5; H, 5.90; N, 12.2. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 12.8 (br, 1H, 1-NH), 7.31 and 7.29 (2s, 10H, 3,6-phenyl of Z), 6.39 (br, 1H, 6-CH_2-N*H*), 5.56 (br, 1H, 3-CH_2-CH_2-N*H*), 5.07 and 5.04 (2s, 4H, CH_2 of 3,6-Z), 4.21 (br, 2H, 6-C*H*$_2$-NH), 3.54 (br, 2H, 3-CH_2-C*H*$_2$-NH), 2.90 (br, 2H, 3-C*H*$_2$-CH_2-NH), 2.36 (s, 3H, 5-CH_3), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 157.2 (q), 156.7 (q), 156.3 (q), 155.0 (q), 136.6 (q), 136.1 (q), 131.2 and 130.2 (q, 3,6-phenyl of Z), 128.5-127.7 (t, 3,6-phenyl of Z), 67.3 and 66.6 (s, CH_2 of 3,6-Z), 39.2 (s, 6-CH_2-NH), 38.3 (s, 3-CH_2-*C*H_2-NH), 32.9 (s, 3-*C*H_2-CH_2-NH), 18.4 (p, 5-CH_3).

3-(2'-Benzyloxycarbonylaminoethyl)-6-(3'-benzyloxycarbonylaminopropyl)-5-methyl-2(1*H*)-pyrazinone (2i)

Yield 433 mg (51.4%), mp 163-168 °C, R_{f2} = 0.78, Anal. Calcd for $C_{26}H_{30}N_4O_5 \cdot 0.4H_2O$: C, 64.5; H, 6.31; N, 11.5. Found: C, 64.5; H, 6.18; N, 11.5. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 13.6 (br, 1H, 1-NH), 7.32-7.27 (m, 10H, 3,6-phenyl of Z), 6.26 (br, 1H, 6-CH_2-CH_2-CH_2-N*H*), 5.55 (br, 1H, 3-CH_2-CH_2-N*H*), 5.09 and 5.03 (2s, each 2H, CH_2 of 3,6-Z), 3.46 (br, 2H, 3-CH_2-C*H*$_2$-NH), 3.21 (br, 2H, 6-CH_2-CH_2-C*H*$_2$-NH), 2.90 (t, *J* = 11.6 Hz, 2H, 3-C*H*$_2$-CH_2-NH), 2.54 (t, *J* = 13.6 Hz, 2H, 6-C*H*$_2$-CH_2-CH_2-NH), 2.25 (s, 3H, 5-CH_3), 1.81 (br, 2H, 6-CH_2-C*H*$_2$-CH_2-NH), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 157.8 (q), 156.8 (q), 156.4 (q), 153.5 (q), 136.7 and 136.6 (q, 3,6-phenyl of Z), 128.5-127.9 (t, 3,6-phenyl of Z), 66.8 and 66.6 (s, CH_2 of 3,6-Z), 39.4 (s, 6-CH_2-CH_2-*C*H_2-NH), 38.7 (s, 3-CH_2-*C*H_2-NH), 32.7 (s, 3-*C*H_2-CH_2-NH), 28.4 (s, 6-CH_2-*C*H_2-CH_2-NH), 26.7 (s, 6-*C*H_2-CH_2-CH_2-NH), 18.5 (p, 5-CH_3).

6-(4'-Benzyloxycarbonylaminobutyl)-3-(2'-benzyloxycarbonylaminoethyl)-5-methyl-2(1*H*)-pyrazinone (2j)

Yield 325.9 mg (56.5%), mp 159-160 °C, R_{f2} = 0.53, Anal. Calcd for $C_{27}H_{32}N_4O_5$: C, 65.8; H, 6.55; N, 11.4. Found: C, 65.6; H, 6.51; N, 11.3. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 13.2 (br, 1H, 1-NH), 7.30 and 7.28 (2s, 10H, 3,6-phenyl of Z), 5.65 (br, 1H, 3-CH_2-CH_2-N*H*), 5.54 (br, 1H, 6-CH_2-CH_2-CH_2-CH_2-N*H*), 5.05 and 5.04 (2s, 4H, CH_2 of 3,6-Z), 3.56 (br, 2H, 3-CH_2-C*H*$_2$-NH), 3.25 (br, 2H, 6-CH_2-CH_2-CH_2-C*H*$_2$-NH), 2.95 (br, 2H, 3-C*H*$_2$-CH_2-NH), 2.50 (br, 2H, 6-C*H*$_2$-CH_2-CH_2-CH_2-NH), 2.25 (s, 3H, 5-CH_3), 1.61 (br, 4H, 6-CH_2-C*H*$_2$-C*H*$_2$-CH_2-NH), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 156.7 (q), 156.4 (q), 153.2 (q), 136.7 (q), 136.6 (q), 135.2 (q), 129.8 (q, 3,6-phenyl of Z), 128.5-127.9 (t, 3,6-phenyl of Z), 66.6 and 66.5 (s, CH_2 of 3,6-Z), 40.0 (s, 6-CH_2-CH_2-CH_2-*C*H_2-NH), 38.7 (s, 3-CH_2-*C*H_2-NH), 32.7 (s, 3-*C*H_2-CH_2-NH), 29.3 (s, 6-*C*H_2-CH_2-CH_2-CH_2-NH), 29.1 (s, 6-CH_2-CH_2-*C*H_2-CH_2-NH), 25.4 (s, 6-CH_2-*C*H_2-CH_2-CH_2-NH), 18.5 (p, 5-CH_3).

3-(3'-Benzyloxycarbonylaminopropyl)-6-(benzyloxycarbonylaminomethyl)-5-methyl-2(1*H*)-pyrazinone (2k)

Yield 54.9 mg (43.0%), mp 140-144 °C, R_{f2} = 0.51, Anal. Calcd for $C_{25}H_{28}N_4O_5$: C, 64.6; H, 6.08; N, 12.1. Found: C, 64.4; H, 6.12; N, 11.8. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 12.9 (br, 1H, 1-NH), 7.33 and 7.29 (2s, 10H, 3,6-phenyl of Z), 6.31 (br, 1H, 6-CH_2-N*H*), 5.42 (br, 1H, 3-CH_2-CH_2-CH_2-N*H*), 5.07 (s, 4H, CH_2 of 3,6-Z), 4.17 (br, 2H, 6-C*H*$_2$-NH), 3.15 (br, 2H, 3-CH_2-CH_2-C*H*$_2$-NH), 2.75 (br, 2H, 3-C*H*$_2$-CH_2-CH_2-NH), 2.36 (s, 3H, 5-CH_3), 1.83 (br, 2H, 3-CH_2-C*H*$_2$-CH_2-NH), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 157.1 (q), 156.6 (q), 156.4 (q), 154.4 (q), 136.7 (q), 136.1 (q), 130.80 and 130.76 (q, 3,6-phenyl of Z), 128.5-128.0 (t, 3,6-phenyl of Z), 67.3 and 66.6 (s, CH_2 of 3,6-Z), 40.4 (s, 3-CH_2-CH_2-*C*H_2-NH), 39.1 (s, 6-CH_2-NH), 29.6 (s, 3-*C*H_2-CH_2-CH_2-NH), 27.0 (s, 3-CH_2-*C*H_2-CH_2-NH), 18.3 (p, 5-CH_3).

6-(2'-Benzyloxycarbonylaminoethyl)-3-(3'-benzyloxycarbonylaminopropyl)-5-methyl-2(1*H*)-pyrazinone (2l)

Yield 62.0 mg (48.1%), mp 148-150 °C, R_{f2} = 0.57, Anal. Calcd for $C_{26}H_{30}N_4O_5 \cdot 0.3H_2O$: C, 64.7; H, 6.37; N, 11.6. Found: C, 64.5; H, 6.26; N, 11.6. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 13.5 (br, 1H, 1-NH), 7.42-7.15 (m, 10H, 3,6-phenyl of Z), 5.82 (br, 1H, 6-CH_2-CH_2-N*H*), 5.52 (br, 1H, 3-CH_2-CH_2-CH_2-N*H*), 5.04 and 4.97 (2s, 4H, CH_2 of 3,6-Z), 3.39 (br, 2H, 6-CH_2-C*H*$_2$-NH), 3.19 (br, 2H, 3-CH_2-CH_2-C*H*$_2$-NH), 2.77 (br, 2H, 3-C*H*$_2$-CH_2-CH_2-NH), 2.66 (br, 2H, 6-C*H*$_2$-CH_2-NH), 2.26 (s, 3H, 5-CH_3), 1.86 (br, 2H, 3-CH_2-C*H*$_2$-CH_2-NH), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 157.6 (q), 156.5 (q), 155.5 (q), 136.7 (q), 136.4 (q), 132.0 and 131.2 (q, 3,6-phenyl of Z), 128.5-127.7 (t, 3,6-phenyl of Z), 66.7 and 66.6 (s, CH_2 of 3,6-Z), 40.3 (s, 3-CH_2-CH_2-*C*H_2-NH), 39.6 (s, 6-CH_2-*C*H_2-NH), 30.4 (s, 6-*C*H_2-CH_2-NH), 29.6 (s, 3-*C*H_2-CH_2-CH_2-NH), 27.2 (s, 3-CH_2-*C*H_2-CH_2-NH), 18.4 (p, 5-CH_3).

6-(4'-Benzyloxycarbonylaminobutyl)-3-(3'-benzyloxycarbonylaminopropyl)-5-methyl-2(1*H*)-pyrazinone (2m)

Yield 1.15 g (75.3%), mp 171-172 °C, R_{f2} = 0.49, Anal. Calcd for $C_{28}H_{34}N_4O_5$: C, 66.4; H, 6.58; N, 11.1. Found: C,

66.3; H, 6.66; N, 11.0. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 13.3 (br, 1H, 1-NH), 7.33-7.24 (m, 10H, 3,6-phenyl of Z), 5.56 (t, *J* = 5.6 Hz, 1H, 3-CH_2-CH_2-CH_2-CH_2-N*H*), 5.53 (t, *J* = 5.6 Hz, 1H, 6-CH_2-CH_2-CH_2-N*H*), 5.06 and 5.05 (2s, 4H, 3,6-CH_2 of Z), 3.22 (br, 4H, 3-CH_2-CH_2-C*H*$_2$-NH, 6-CH_2-CH_2-CH_2-C*H*$_2$-NH), 2.80 (t, *J* = 7.3 Hz, 2H, 3-C*H*$_2$-CH_2-CH_2-NH), 2.48 (t, *J* = 7.4 Hz, 2H, 6-C*H*$_2$-CH_2-CH_2-CH_2-NH), 2.25 (s, 3H, 5-CH_3), 1.88 (quintet, *J* = 6.9 Hz, 2H, 3-CH_2-C*H*$_2$-CH_2-NH), 1.63 (quintet, *J* = 7.0 Hz, 2H, 6-CH_2-C*H*$_2$-CH_2-CH_2-NH), 1.56 (quintet, *J* = 6.7 Hz, 2H, 6-CH_2-CH_2-C*H*$_2$-CH_2-NH), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 157.8 and 155.1 (q, 2, 3), 156.7 and 156.5 (q, CO of 3,6-Z), 136.8 and 136.7 (q, 3,6-phenyl of Z), 134.8 and 129.8 (q, 5, 6), 128.5-128.0 (t, 3,6-phenyl of Z), 66.58 and 66.52 (s, 3,6-CH_2 of Z), 40.6 (s, 3-CH_2-CH_2-*C*H$_2$-NH), 40.0 (s, 6-CH_2-CH_2-CH_2-*C*H$_2$-NH), 29.7 (s, 3-*C*H$_2$-CH_2-CH_2-NH), 29.3 (s, 6-*C*H$_2$-CH_2-CH_2-CH_2-NH), 29.1 (s, 6-CH_2-CH_2-*C*H$_2$-CH_2-NH), 27.2 (s, 3-CH_2-*C*H$_2$-CH_2-NH), 25.5 (s, 6-CH_2-*C*H$_2$-CH_2-CH_2-NH), 18.4 (p, 5-CH_3).

3-(4'-Benzyloxycarbonylaminobutyl)-6-(benzyloxycarbonylaminomethyl)-5-methyl-2(1*H*)-pyrazinone (2n)

Yield 41.3 mg (45.4%), mp 139-141 °C, R_{f2} = 0.60, Anal. Calcd for $C_{26}H_{30}N_4O_5 \cdot 0.3H_2O$: C, 64.5; H, 6.37; N, 11.6. Found: C, 64.7; H, 6.26; N, 11.6. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 13.0 (br, 1H, 1-NH), 7.33 and 7.28 (2s, 10H, 3,6-phenyl of Z), 6.34 (t, *J* = 5.4 Hz, 1H, 6-CH_2-N*H*), 5.07 (s, 5H, CH_2 of 3,6-Z, 3-CH_2-CH_2-CH_2-CH_2-N*H*), 4.25 (d, *J* = 6.0 Hz, 2H, 6-C*H*$_2$-NH), 3.17 (q, *J* = 6.2 Hz, 2H, 3-CH_2-CH_2-CH_2-C*H*$_2$-NH), 2.73 (t, *J* = 7.2 Hz, 2H, 3-C*H*$_2$-CH_2-CH_2-CH_2-NH), 2.38 (s, 3H, 5-CH_3), 1.69 (quintet, *J* = 7.5 Hz, 2H, 3-CH_2-CH_2-C*H*$_2$-CH_2-NH), 1.51 (quintet, *J* = 7.0 Hz, 2H, 3-CH_2-C*H*$_2$-CH_2-CH_2-NH), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 157.2 (q), 157.0 (q), 156.5 (q), 156.4 (q), 136.7 (q), 136.1 (q), 130.6 and 130.2 (q, 3,6-phenyl of Z), 128.5-127.7 (t, 3,6-phenyl of Z), 67.2 and 66.5 (s, CH_2 of 3,6-Z), 40.7 (s, 3-CH_2-CH_2-CH_2-*C*H$_2$-NH), 39.1 (s, 6-CH_2-NH), 32.2 (s, 3-*C*H$_2$-CH_2-CH_2-CH_2-NH), 29.2 (s, 3-CH_2-*C*H$_2$-CH_2-CH_2-NH), 23.8 (s, 3-CH_2-CH_2-*C*H$_2$-CH_2-NH), 18.3 (p, 5-CH_3).

3-(4'-Benzyloxycarbonylaminobutyl)-6-(2'-benzyloxycarbonylaminoethyl)-5-methyl-2(1*H*)-pyrazinone (2o)

Yield 24.4 mg (64.0%), mp 161-163 °C, R_{f2} = 0.49, Anal. Calcd for $C_{27}H_{32}N_4O_5 \cdot 0.5H_2O$: C, 64.7; H, 6.63; N, 11.2. Found: C, 64.7; H, 6.51; N, 11.2. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 13.3 (br, 1H, 1-NH), 7.31-7.27 (m, 10H, 3,6-phenyl of Z), 5.75 (br, 1H, 6-CH_2-CH_2-N*H*), 5.10 (br, 1H, 3-CH_2-CH_2-CH_2-CH_2-N*H*), 5.05 and 4.98 (2s, 4H, CH_2 of 3,6-Z), 3.40 (br, 2H, 6-CH_2-C*H*$_2$-NH), 3.20 (br, 2H, 3-CH_2-CH_2-CH_2-C*H*$_2$-NH), 2.72 (br, 4H, 3-C*H*$_2$-CH_2-CH_2-CH_2-NH, 6-C*H*$_2$-CH_2-NH), 2.26 (s, 3H, 5-CH_3), 1.74 (br, 2H, 3-CH_2-C*H*$_2$-CH_2-CH_2-NH), 1.50 (br, 2H, 3-CH_2-CH_2-C*H*$_2$-CH_2-NH), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 157.5 (q), 156.5 (q), 156.1 (q), 136.7 (q), 136.4 (q), 131.8 (q), 130.9 (q, 3,6-phenyl of Z), 128.5-127.8 (t, 3,6-phenyl of Z), 66.6 and 66.5 (s, CH_2 of 3,6-Z), 40.7 (s, 3-CH_2-CH_2-CH_2-*C*H$_2$-NH), 39.6 (s, 6-CH_2-*C*H$_2$-NH), 32.0 (s, 3-*C*H$_2$-CH_2-CH_2-CH_2-NH), 30.3 (s, 6-*C*H$_2$-CH_2-NH), 29.2 (s, 3-CH_2-CH_2-*C*H$_2$-CH_2-NH), 23.9 (s, 3-CH_2-*C*H$_2$-CH_2-CH_2-NH), 18.4 (p, 5-CH_3).

3-(4'-Benzyloxycarbonylaminobutyl)-6-(3'-benzyloxycarbonylaminopropyl)-5-methyl-2(1*H*)-pyrazinone (2p)

Yield 0.25 g (60.5%), mp 144-146 °C, R_{f2} = 0.52, Anal. Calcd for $C_{28}H_{34}N_4O_5$: C, 66.4; H, 6.58; N, 11.1. Found: C, 66.1; H, 6.69; N, 11.0. ^{1}H-NMR (400.1 MHz, $CDCl_3$) δ: 13.4 (br, 1H, 1-NH), 7.35-7.25 (m, 10H, 3,6-phenyl of Z), 6.23 (br, 1H, 6-CH_2-CH_2-CH_2-N*H*), 5.09 and 5.07 (2s, 4H, CH_2 of 3,6-Z), 5.02 (br, 1H, 3-CH_2-CH_2-CH_2-CH_2-N*H*), 3.23 (q, *J* = 5.5 Hz, 2H, 6-CH_2-CH_2-C*H*$_2$-NH), 3.14 (q, *J* = 6.1 Hz, 2H, 3-CH_2-CH_2-CH_2-C*H*$_2$-NH), 2.74 (t, *J* = 7.2 Hz, 2H, 3-C*H*$_2$-CH_2-CH_2-CH_2-NH), 2.58 (t, *J* = 7.0 Hz, 2H, 6-C*H*$_2$-CH_2-CH_2-NH), 2.26 (s, 3H, 5-CH_3), 1.83 (quintet, *J* = 6.0 Hz, 2H, 6-CH_2-C*H*$_2$-CH_2-NH), 1.63 (quintet, *J* = 7.2 Hz, 2H, 3-CH_2-C*H*$_2$-CH_2-CH_2-NH), 1.44 (quintet, *J* = 7.0 Hz, 2H, 3-CH_2-CH_2-C*H*$_2$-CH_2-NH), ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 157.6 and 155.7 (q, 2, 3), 156.9 and 156.5 (q, CO of 3,6-Z), 136.8 and 136.7 (q, 3,6-phenyl of Z), 134.1 (q, 6), 130.3 (q, 5), 128.5-128.0 (t, 3,6-phenyl of Z), 66.7 and 66.5 (s, CH_2 of 3,6-Z), 40.8 (s, 3-CH_2-CH_2-CH_2-*C*H$_2$-NH), 39.4 (s, 6-CH_2-CH_2-*C*H$_2$-NH), 31.9 (s, 3-*C*H$_2$-CH_2-CH_2-CH_2-NH), 29.2 (s, 3-CH_2-CH_2-*C*H$_2$-CH_2-NH), 28.5 (s, 6-CH_2-*C*H$_2$-CH_2-NH), 26.6 (s, 6-*C*H$_2$-CH_2-CH_2-NH), 23.8 (s, 3-CH_2-*C*H$_2$-CH_2-CH_2-NH), 18.4 (p, 5-CH_3).

General Procedure for Synthesis of 3,6-Bis(*N*$^{\alpha}$-Boc-Dmt-aminoalkyl)-5-methyl-2(1*H*)-pyrazinone (3e-p)

3,6-Bis(aminoalkyl)-5-methyl-2(1H)-pyrazinone·2HBr (1.5 mmol) [prepared from 3,6-bis(benzyloxycarbonylaminoalkyl)-5-methyl-2(1*H*)-pyrazinone (2.0 mmol) and 25% HBr/AcOH (2.5 mL, 40.0 mmol) by the usual method] in DMF (10 mL) containing DIPEA (diisopropylethylamine: 0.7 mL, 4.0 mmol), was added to a solution of Boc-Dmt-OH (0.9 g, 3.0 mmol) and DIPEA (0.5 mL, 3.0 mmol) and Py-Bop (benzotriazol-1-yloxytrispyrrolidinophosphonium hexafluorophosphate: 1.8 g, 3.5 mmol), in DMF (30 mL). The reaction mixture was then stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt, and the extract washed with 10% aqueous citric acid, 5% aqueous Na_2CO_3 and water, dried over Na_2SO_4 then evaporated down. Ether was added to the residue to give crystals, which were collected by filtration, and purified by silicagel chromatography using 3% MeOH/AcOEt as eluent.

6-[2'-*N*$^{\alpha}$-Boc-Dmt-aminoethyl]-3-[*N*$^{\alpha}$-Boc-Dmt-aminomethyl]-5-methyl-2(1*H*)-pyrazinone (3e)

Yield 220.2 mg (51.8%), mp 177-179 °C, $[\alpha]_D^{25}$ +1.47° (*c* = 0.1, MeOH), R_{f2} = 0.23, Anal. Calcd. for $C_{40}H_{56}N_6O_9 \cdot H_2O$: C, 61.4; H, 7.47; N, 10.7. Found: C, 61.3; H, 7.20; N, 10.5. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 13.5 (br, 1H, 1-NH), 11.0 and 10.9 (each br, each 1H, 2 × OH of Dmt), 9.04 (t, *J* = 8.0 Hz, 1H, 6-CH_2-CH_2-N*H*), 8.51 (br, 1H, 3-CH_2-N*H*), 8.02 (d, *J* = 7.2 Hz, 1H, 3 or 6-α-NH of Dmt), 8.05 (d, *J* = 6.5 Hz, 1H, 3 or 6-α-NH of Dmt), 6.86 and 6.85 (2s, 4H, 3,6-phenyl of Dmt), 5.10-4.70 (m, 4H, 3,6-α-CH of Dmt, 3-C*H*$_2$-NH), 3.80-3.13 (m, 6H, 6-CH_2-C*H*$_2$-NH, 3,6-β-CH_2 of Dmt), 3.97-3.66 (m, 2H, 6-C*H*$_2$-CH_2-NH), 2.44 and 2.38 (2s, 12H, 4 × CH_3 of Dmt), 2.31 (s, 3H, 5-CH_3), 1.48 and 1.47 (2s, 18H, 3,6-*tert*-butyl), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 172.8 (q), 172.4 (q), 171.9 (q), 156.7 (q), 156.0 (q), 155.9 (q),

138.9 and 138.8 (q, 3,6-phenyl of Dmt), 125.5 (q), 125.3 (q), 115.8 (t, 3,6-phenyl of Dmt), 78.44 and 78.40 (q, *tert*-butyl), 55.5 and 55.2 (t, 3,6-α-CH of Dmt), 41.0 (s, 3-CH_2-NH), 38.2 (s, 6-CH_2-*C*H_2-NH), 33.4 (s, 3,6-β-CH_2 of Dmt), 31.5 (s, 6-*C*H_2-CH_2-NH), 28.3 (p, 3,6-*tert*-butyl), 20.3 (p, 4 × CH_3 of Dmt), 18.7 (p, 5-CH_3).

3-[N^{α}-Boc-Dmt-aminomethyl]-6-[3'-N^{α}-Boc-Dmt-aminopropyl]-5-methyl-2(1*H*)-pyrazinone (3f)

Yield 102.7 mg (52.9%), mp 155-157 °C, $[\alpha]_D^{24}$ +1.36° (*c* = 0.1, MeOH), R_{f2} = 0.29, Anal. Calcd for $C_{41}H_{58}N_6O_9 \cdot 1.5H_2O$: C, 61.1; H, 7.63; N, 10.4. Found: C, 61.4; H, 7.51; N, 10.1. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 8.88 (br, 1H, 6-CH_2-CH_2-CH_2-N*H*), 8.47 (br, 1H, 3-CH_2-N*H*), 8.06 (d, *J* = 6.0 Hz, 1H, 3 or 6-α-NH of Dmt), 7.97 (d, *J* = 6.0 Hz, 1H, 3 or 6-α-NH of Dmt), 6.83 (s, 4H, 3,6-phenyl of Dmt), 4.91-4.71 (m, 4H, 3,6-α-CH of Dmt, 3-C*H*$_2$-NH), 3.56-3.11 (m, 6H, 6-CH_2-CH_2-C*H*$_2$-NH, 3,6-β-CH_2 of Dmt), 2.50 (br, 2H, 6-C*H*$_2$-CH_2-CH_2-NH), 2.40 and 2.34 (2s, 12H, 4 × CH_3 of Dmt), 2.21 (s, 3H, 5-CH_3), 1.82 (br, 2H, 6-CH_2-C*H*$_2$-CH_2-NH), 1.44 (s, 18H, 3,6-*tert*-butyl), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 173.2 (q), 172.6 (q), 157.0 (q), 156.9 (q), 156.21 (q), 156.15 (q), 139.1 and 139.0 (q, 3,6-phenyl of Dmt), 125.8 (q), 125.5 (q), 116.04 and 115.99 (t, 3,6-phenyl of Dmt), 78.7 and 78.6 (q, 3,6-*tert*-butyl), 55.7 and 55.6 (t, 3,6-α-CH of Dmt), 41.3 (s, 3-CH_2-NH), 39.2 (s, 6-CH_2-CH_2-*C*H_2-NH), 33.4 (s, 3,6-β-CH_2 of Dmt), 28.8 (s, 6-*C*H_2-CH_2-CH_2-NH), 28.7 (s, 6-CH_2-*C*H_2-CH_2-NH), 28.5 (p, 3,6-*tert*-butyl), 20.6 and 20.5 (p, 4 × CH_3 of Dmt), 18.7 (p, 5-CH_3).

6-[4'-N^{α}-Boc-Dmt-aminobutyl]-3-[N^{α}-Boc-Dmt-aminomethyl]-5-methyl-2(1*H*)-pyrazinone (3g)

Yield 50.5 mg (44.8%), mp 160-165 °C, $[\alpha]_D^{26}$ -0.80° (*c* = 0.1, MeOH), R_{f2} = 0.15, Anal. Calcd. for $C_{42}H_{60}N_6O_9 \cdot 1.6H_2O$: C, 61.6; H, 7.45; N, 10.1. Found: C, 61.6; H, 7.45; N, 9.83. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 8.65 (br, 1H, 6-CH_2-CH_2-CH_2-CH_2-N*H*), 8.48 (br, 1H, 3-CH_2-N*H*), 7.89 (br, 2H, 3,6-α-NH of Dmt), 6.83 (s, 4H, 3,6-phenyl of Dmt), 5.10-4.70 (m, 4H, 3,6-α-CH of Dmt, 3-C*H*$_2$-NH), 3.52-3.05 (m, 6H, 3,6-β-CH_2 of Dmt, 6-CH_2-CH_2-CH_2-C*H*$_2$-NH), 2.48 (t, *J* = 8.0 Hz, 2H, 6-C*H*$_2$-CH_2-CH_2-CH_2-NH), 2.41 and 2.35 (2s, 12H, 4 × CH_3 of Dmt), 2.21 (s, 3H, 5-CH_3), 1.56 (br, 2H, 6-CH_2-C*H*$_2$-CH_2-CH_2-NH), 1.45 (s, 18H, 3,6-*tert*-butyl), 1.40 (br, 2H, 6-CH_2-CH_2-C*H*$_2$-CH_2-NH), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 173.0 (q), 172.9 (q), 159.3 (q), 157.0 (q), 156.1 (q), 156.0 (q), 139.1 and 139.0 (q, 3,6-phenyl of Dmt), 125.9 (q), 125.8 (q), 116.0 (t, 3,6-phenyl of Dmt), 78.9 (q, 3,6-*tert*-butyl), 55.6 (t, 3,6-α-CH of Dmt), 41.5 (s, 3-CH_2-NH), 39.2 (s, 6-CH_2-CH_2-CH_2-*C*H_2-NH), 33.8 (s, 3,6-β-CH_2 of Dmt), 31.1 (s, 6-*C*H_2-CH_2-CH_2-CH_2-NH), 29.4 (s, 6-CH_2-CH_2-*C*H_2-CH_2-NH), 28.5 (p, 3,6-*tert*-butyl), 26.1 (s, 6-CH_2-*C*H_2-CH_2-CH_2-NH), 20.6 (p, 4 × CH_3 of Dmt), 18.8 (p, 5-CH_3).

3-[2'-N^{α}-Boc-Dmt-aminoethyl]-6-[N^{α}-Boc-Dmt-aminomethyl]-5-methyl-2(1*H*)-pyrazinone (3h)

Yield 41.3 mg (27.0%), mp 159-163 °C, $[\alpha]_D^{25}$ -1.04° (*c* = 0.1, MeOH), R_{f2} = 0.70, Anal. Calcd for $C_{40}H_{56}N_6O_9 \cdot H_2O$: C, 61.4; H, 7.47; N, 10.7. Found: C, 61.6; H, 7.50; N, 10.2. ^{1}H-NMR (400.1 MHz, DMSO-d_6) δ: 11.7 (br, 1H, 1-NH), 9.90 (br, 2H, 2 × OH of Dmt), 8.10 (br, 1H, 6-CH_2-N*H*), 7.77 (br, 1H, 3-CH_2-CH_2-N*H*), 6.89 (d, *J* = 3.2 Hz, 1H, 3 or 6-α-NH of Dmt), 6.69 (d, *J* = 7.7 Hz, 1H, 3 or 6-α-NH of Dmt), 6.35 (s, 4H, 3,6-phenyl of Dmt), 4.13-3.95 (m, 2H, 3,6-α-CH of Dmt), 3.28 (br, 2H, 6-C*H*$_2$-NH), 3.17 (br, 2H, 3-CH_2-C*H*$_2$-NH), 2.98-2.57 (m, 4H, 3,6-β-CH_2 of Dmt), 2.17 and 2.09 (2s, 14H, 3-C*H*$_2$-CH_2-NH, 4 × CH_3 of Dmt), 2.14 (s, 3H, 5-CH_3), 1.30 (s, 18H, 3,6-*tert*-butyl), ^{13}C-NMR (100.6 MHz, DMSO-d_6) δ: 172.6 (q), 172.1 (q), 156.8 (q), 139.1 and 139.0 (q, 3,6-phenyl of Dmt), 121.2 (q), 117.6 (t, 3,6-phenyl of Dmt), 79.0 (q, 3,6-*tert*-butyl), 54.6 (t, 3,6-α-CH of Dmt), 46.9 (s, 6-CH_2-NH), 46.4 (s, 3-CH_2-*C*H_2-NH), 40.0 (s, 3,6-β-CH_2 of Dmt), 30.5 (s, 3-*C*H_2-CH_2-NH), 28.4 (p, 3,6-*tert*-butyl), 20.2 (p, 4 × CH_3 of Dmt), 18.8 (p, 5-CH_3).

3-[2'-N^{α}-Boc-Dmt-aminoethyl]-6-[3'-N^{α}-Boc-Dmt-aminopropyl]-5-methyl-2(1*H*)-pyrazinone (3i)

Yield 42.9 mg (51.8%), mp 150-153 °C, $[\alpha]_D^{24}$ +2.00° (*c* = 0.1, MeOH), R_{f2} = 0.21, Anal. Calcd for $C_{42}H_{60}N_6O_9 \cdot H_2O$: C, 62.2; H, 7.71; N, 10.4. Found: C, 62.3; H, 7.47; N, 10.1. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 13.3 (br, 1H, 1-NH), 11.0 (br, 2H, 2 × OH of Dmt), 8.70 (br, 2H, 3-CH_2-CH_2-N*H*, 6-CH_2-CH_2-CH_2-N*H*), 7.95 (d, 1H, *J* = 8.6 Hz, 3 or 6-α-NH of Dmt), 7.82 (d, 1H, *J* = 8.5 Hz, 3 or 6-α-NH of Dmt), 6.88 and 6.86 (2s, 4H, 3,6-phenyl of Dmt), 4.90-4.75 (m, 2H, 3,6-α-CH of Dmt), 4.10-3.86 (m, 2H, 3-CH_2-C*H*$_2$-NH), 3.52-3.40 and 3.30-3.11 (each m, each 2H, 3,6-β-CH_2 of Dmt), 3.40-3.30 (m, 2H, 6-CH_2-CH_2-C*H*$_2$-NH), 3.30-3.11 (m, 2H, 3-C*H*$_2$-CH_2-NH-), 2.52 (t, *J* = 7.2 Hz, 2H, 6-C*H*$_2$-CH_2-CH_2-NH), 2.43 and 2.38 (2s, 12H, 4 × CH_3 of Dmt), 2.24 (s, 3H, 5-CH_3), 1.61 (br, 2H, 6-CH_2-C*H*$_2$-CH_2-NH), 1.46 (s, 18H, 3,6-*tert*-butyl), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 172.8 (q), 172.4 (q), 157.0 (q), 156.91 (q), 156.88 (q), 156.1 (q), 139.2 and 139.0 (q, 3,6-phenyl of Dmt), 126.5 (q), 125.7 (q), 116.0 and 115.5 (t, 3,6-phenyl of Dmt), 78.7 and 78.6 (q, 3,6-*tert*-butyl), 55.6 (t, 3,6-α-CH of Dmt), 39.3 (s, 6-CH_2-CH_2-*C*H_2-NH), 37.5 (s, 3-CH_2-*C*H_2-NH), 33.8 (s, 3,6-β-CH_2 of Dmt), 33.5 (s, 3-*C*H_2-CH_2-NH), 32.9 (s, 6-*C*H_2-CH_2-CH_2-NH), 28.5 (p, 3,6-*tert*-butyl), 26.2 (s, 6-CH_2-*C*H_2-CH_2-NH), 20.6 (p, 4 × CH_3 of Dmt), 18.8 (p, 5-CH_3).

6-[4'-N^{α}-Boc-Dmt-aminobutyl]-3-[2'-N^{α}-Boc-Dmt-aminoethyl]-5-methyl-2(1*H*)-pyrazinone (3j)

Yield 121.2 mg (74.1%), mp 150-154 °C, $[\alpha]_D^{25}$ +3.29° (*c* = 1.0, MeOH), R_{f2} = 0.34, Anal. Calcd for $C_{43}H_{62}N_6O_9 \cdot 1.5H_2O$: C, 61.9; H, 7.86; N, 10.1. Found: C, 61.6; H, 7.56; N, 10.0. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 8.90 (br, 1H, 6-CH_2-CH_2-CH_2-CH_2-N*H*), 8.67 (br, 1H, 3-CH_2-CH_2-N*H*), 8.07 (d, *J* = 8.0 Hz, 1H, 3 or 6-α-NH of Dmt), 7.81 (d, *J* = 8.0 Hz, 1H, 3 or 6-α-NH of Dmt), 6.87 (s, 4H, 3,6-phenyl of Dmt), 4.85 (br, 2H, 3,6-α-CH of Dmt), 4.01 and 3.89 (each br, each 1H, 3-CH_2-C*H*$_2$-NH), 3.49 and 3.41 (each br, each 4H, 3,6-β-CH_2 of Dmt, 6-CH_2-CH_2-C*H*$_2$-C*H*$_2$-NH), 2.56 (br, 2H, 6-C*H*$_2$-CH_2-CH_2-CH_2-NH), 2.44 and 2.39 (2s, 12H, 4 × CH_3 of Dmt), 2.25 (s, 3H, 5-CH_3), 1.88 (br, 2H, 6-CH_2-C*H*$_2$-CH_2-CH_2-NH), 1.47 (s, 18H, 3,6-*tert*-butyl), ^{13}C-NMR (100.6

MHz, pyridine-d_5) δ: 173.2 (q), 172.3 (q), 157.0 (q), 156.9 (q), 156.2 (q), 156.1 (q), 139.1 and 139.0 (q, 3,6-phenyl of Dmt), 125.63 (q), 125.57 (q), 116.12 and 116.06 (t, 3,6-phenyl of Dmt), 78.7 and 78.6 (q, 3,6-*tert*-butyl), 55.7 and 55.6 (t, 3,6-α-CH of Dmt), 39.2 (s, 6-CH_2-CH_2-CH_2-*C*H_2-NH), 37.5 (s, 3-CH_2-*C*H_2-NH), 33.8 and 33.5 (s, 3,6-β-CH_2 of Dmt), 32.8 (s, 6-CH_2-CH_2-*C*H_2-CH_2-NH), 28.9 (s, 6-*C*H_2-CH_2-CH_2-CH_2-NH), 28.5 (p, 3,6-*tert*-butyl), 20.6 (p, 4 × CH_3 of Dmt), 18.8 (p, 5-CH_3).

6-[*N*$^{\alpha}$-Boc-Dmt-aminomethyl]-3-[3'-*N*$^{\alpha}$-Boc-Dmt-aminopropyl]-5-methyl-2(1*H*)-pyrazinone (3k)

Yield 63.2 mg (36.6%), mp 157-160 °C, $[\alpha]_D^{21}$ +21.2° (*c* = 1.0, MeOH), R_{f2} = 0.32, Anal. Calcd for $C_{41}H_{58}N_6O_9 \cdot 0.9H_2O$: C, 61.9; H, 7.58; N, 10.6. Found: C, 61.7; H, 7.25; N, 10.3. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 13.2 (br, 1H, 1-NH), 11.0 and 10.9 (each br, each 1H, 2 × OH of Dmt), 9.27 (br, 1H, 6-CH_2-N*H*), 8.72 (br, 1H, 3-CH_2-CH_2-CH_2-N*H*), 8.11 and 7.83 (each br, each 1H, 3,6-α-NH of Dmt), 6.87 and 6.83 (2s, 4H, 3,6-phenyl of Dmt), 4.92 (br, 4H, 3,6-β-CH_2 of Dmt), 4.89-4.75(m, 2H, 3-CH_2-CH_2-C*H*$_2$-NH), 4.47 (br, 4H, 6-C*H*$_2$-NH, 3-CH_2-CH_2-C*H*$_2$-NH), 3.46 and 3.19 (each br, each 1H, 3,6-α-CH of Dmt), 3.00-2.82 (m, 2H, 3-C*H*$_2$-CH_2-CH_2-NH), 2.41 and 2.30 (2s, 15H, 4 × CH_3 of Dmt, 5-CH_3), 2.06 (br, 2H, 3-CH_2-C*H*$_2$-CH_2-NH), 1.45 (s, 18H, 3,6-*tert*-butyl), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 172.6 (q), 172.4 (q), 157.0 (q), 156.9 (q), 156.5 (q), 139.1 and 139.0 (q, 3,6-phenyl of Dmt), 136.0 (q), 116.1 (t, 3,6-phenyl of Dmt), 78.7 and 78.6 (q, 3,6-*tert*-butyl), 55.6 (s, 3-CH_2-CH_2-*C*H_2-NH), 55.5 (s, 3,6-β-CH_2 of Dmt), 40.8 (s, 6-CH_2-NH), 39.7 (t, 3,6-α-CH of Dmt), 30.3 (s, 3-*C*H_2-CH_2-CH_2-NH), 28.5 (p, 3,6-*tert*-butyl), 27.1 (s, 3-CH_2-*C*H_2-CH_2-NH), 20.6 and 20.4 (p, 4 × CH_3 of Dmt), 15.5 (p, 5-CH_3).

6-[2'-*N*$^{\alpha}$-Boc-Dmt-aminethyl]-3-[3'-*N*$^{\alpha}$-Boc-Dmt-aminopropyl]-5-methyl-2(1*H*)-pyrazinone (3l)

Yield 90.9 mg (54.1%), mp 170-172 °C, $[\alpha]_D^{25}$ +4.10° (*c* = 0.1, MeOH), R_{f2} = 0.30, Anal. Calcd for $C_{42}H_{60}N_6O_9 \cdot 1.1H_2O$: C, 62.1; H, 7.71; N, 10.3. Found: C, 62.2; H, 7.80; N, 9.94. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 9.17 (br, 1H, 6-CH_2-CH_2-N*H*), 8.84 (br, 1H, 3-CH_2-CH_2-CH_2-N*H*), 7.87 (d, *J* = 8.4 Hz, 1H, 3 or 6-α-NH of Dmt), 7.73 (d, *J* = 8.6 Hz, 1H, 3 or 6-α-NH of Dmt), 6.91 (s, 4H, 3,6-phenyl of Dmt), 4.90-4.77 (m, 2H, 3,6-α-CH of Dmt), 3.68-3.45 (m, 4H, 6-CH_2-C*H*$_2$-NH, 3-CH_2-CH_2-C*H*$_2$-NH), 3.52-3.38 and 3.24-3.12 (each m, each 2H, 3,6-β-CH_2 of Dmt), 2.92 (br, 2H, 3-C*H*$_2$-CH_2-CH_2-NH), 2.92 and 2.78 (each br, each 1H, 6-C*H*$_2$-CH_2-NH), 2.43 and 2.38 (2s, 12H, 4 × CH_3 of Dmt), 2.31 (s, 3H, 5-CH_3), 2.08 (br, 2H, 3-CH_2-C*H*$_2$-CH_2-NH), 1.47 and 1.45 (2s, 18H, 3,6-*tert*-butyl), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 175.0 (q), 174.5 (q), 174.0 (q), 160.6 (q), 156.2 (q), 155.4 (q), 138.3 (q, 3,6-phenyl of Dmt), 115.29 and 115.25 (t, 3,6-phenyl of Dmt), 78.2 (q, 3,6-*tert*-butyl), 54.7 (t, 3,6-α-CH of Dmt), 38.9 (s, 3-CH_2-CH_2-*C*H_2-NH), 37.9 (s, 6-CH_2-*C*H_2-NH), 32.8 (s, 3,6-β-CH_2 of Dmt), 32.6 (s, 6-*C*H_2-CH_2-NH), 30.6 (s, 3-*C*H_2-CH_2-CH_2-NH), 29.5 (p, 3,6-*tert*-butyl), 27.7 (s, 3-CH_2-*C*H_2-CH_2-NH), 19.8 and 19.7 (p, 4 × CH_3 of Dmt), 18.0 (p, 5-CH_3).

6-[4'-*N*$^{\alpha}$-Boc-Dmt-aminobutyl]-3-[3'-*N*$^{\alpha}$-Boc-Dmt-aminopropyl]-5-methyl-2(1*H*)-pyrazinone (3m)

Yield 214.3 mg (88.0%), mp 133-135 °C, $[\alpha]_D^{21}$ +23.7° (*c* = 0.1, MeOH), R_{f2} = 0.39, Anal. Calcd for $C_{44}H_{64}N_6O_9 \cdot H_2O$: C, 63.0; H, 7.93; N, 10.0. Found: C, 62.9; H, 7.59; N, 10.3. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 8.81 (br, 1H, 6-CH_2-CH_2-CH_2-CH_2-N*H*), 8.67 (br, 1H, 3-CH_2-CH_2-CH_2-N*H*), 8.01 and 7.88 (each br, each 1H, 3,6-α-NH of Dmt), 7.00-6.71 (m, 4H, 3,6-phenyl of Dmt), 5.18 and 4.76 (each br, each 1H, 3,6-α-CH of Dmt), 3.62-3.37 (m, 4H, 3,6-β-CH_2 of Dmt), 3.20-3.19 (m, 4H, 6-CH_2-CH_2-C*H*$_2$-C*H*$_2$-NH), 3.19 (br, 2H, 3-CH_2-CH_2-C*H*$_2$-NH), 2.46 (s, 3H, 5-CH_3), 2.43-2.26 (m, 12H, 4 × CH_3 of Dmt), 2.14 (t, *J* = 8.0 Hz, 2H, 6-C*H*$_2$-CH_2-CH_2-CH_2-NH), 1.67 (t, *J* = 6.6 Hz, 2H, 3-C*H*$_2$-CH_2-CH_2-NH), 1.51-1.47 (m, 2H, 6-CH_2-C*H*$_2$-CH_2-CH_2-NH), 1.46 (s, 18H, 3,6-*tert*-butyl), 1.45 (br, 2H, 3-CH_2-C*H*$_2$-CH_2-NH), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 172.5 (q), 172.4 (q), 156.9 (q), 139.12 (q), 139.09 and 139.08 (q, 3,6-phenyl of Dmt), 121.4 (q), 116.3 and 116.2 (t, 3,6-phenyl of Dmt), 79.1 and 78.6 (q, 3,6-*tert*-butyl), 46.7 and 46.6 (s, 3,6-β-CH_2 of Dmt), 38.9 (s, 6-CH_2-CH_2-CH_2-*C*H_2-NH), 33.2 (s, 6-CH_2-CH_2-*C*H_2-CH_2-NH), 31.4 (s, 3-CH_2-CH_2-*C*H_2-NH), 30.3 (s, 6-CH_2-*C*H_2-CH_2-CH_2-NH), 29.1 (s, 3-CH_2-*C*H_2-CH_2-NH), 28.5 (p, 3,6-*tert*-butyl), 26.7 (s, 6-*C*H_2-CH_2-CH_2-CH_2-NH), 26.6 (s, 3-*C*H_2-CH_2-CH_2-NH), 20.6 (p, 4 × CH_3 of Dmt), 15.5 (p, 5-CH_3).

3-[4'-*N*$^{\alpha}$-Boc-Dmt-aminobutyl]-6-[*N*$^{\alpha}$-Boc-Dmt-aminomethyl]-5-methyl-2(1*H*)-pyrazinone (3n)

Yield 166.4 mg (72.8%), mp 149-153 °C, $[\alpha]_D^{25}$ +36.2° (*c* = 0.1, MeOH), R_{f2} = 0.41, Anal. Calcd for $C_{42}H_{60}N_6O_9 \cdot H_2O$: C, 62.2; H, 7.45; N, 10.1. Found: C, 62.2; H, 7.71; N, 10.4. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 13.2 (br, 1H, 1-NH), 11.1 and 11.0 (each br, each 1H, 2 × OH of Dmt), 9.30 (br, 1H, 6-CH_2-N*H*), 8.65 (br, 1H, 3-CH_2-CH_2-CH_2-CH_2-N*H*), 8.14 (d, *J* = 7.9 Hz, 1H, 3 or 6-α-NH of Dmt), 7.89 (d, *J* = 8.1 Hz, 1H, 3 or 6-α-NH of Dmt), 6.88 and 6.85 (2s, 4H, 3,6-phenyl of Dmt), 4.89-4.78 (m, 2H, 3,6-α-CH of Dmt), 4.64-4.55 and 4.48-4.40 (each m, each 1H, 6-C*H*$_2$-NH), 3.55-3.57 (m, 2H, 3-CH_2-CH_2-CH_2-C*H*$_2$-NH), 3.38-3.26 and 3.24-3.13 (each m, each 2H, 3,6-β-CH_2 of Dmt), 2.90 (t, *J* = 7.2 Hz, 2H, 3-C*H*$_2$-CH_2-CH_2-CH_2-NH), 2.41 and 2.32 (2s, 12H, 4 × CH_3 of Dmt), 2.36 (s, 3H, 5-CH_3), 1.81 (quintet, *J* = 8.0 Hz, 2H, 3-CH_2-C*H*$_2$-CH_2-CH_2-NH), 1.60 (br, 2H, 3-CH_2-CH_2-C*H*$_2$-CH_2-NH), 1.46 (s, 18H, 3,6-*tert*-butyl), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 172.2 (q), 156.9 (q), 155.8 (q), 139.0 (q, 3,6-phenyl of Dmt), 116.0 (t, 3,6-phenyl of Dmt), 55.2 (t, 3,6-α-CH of Dmt), 40.8 (s, 6-CH_2-NH), 39.4 (s, 3-CH_2-CH_2-CH_2-*C*H_2-NH), 33.6 and 33.2 (s, 3,6-β-CH_2 of Dmt), 32.7 (s, 3-*C*H_2-CH_2-CH_2-CH_2-NH), 29.6 (s, 3-CH_2-CH_2-*C*H_2-CH_2-NH), 28.4 (p, 3,6-*tert*-butyl), 24.9 (s, 3-CH_2-*C*H_2-CH_2-CH_2-NH), 20.3 (p, 4 × CH_3 of Dmt), 18.8 (p, 5-CH_3).

3-[4'-*N*$^{\alpha}$-Boc-Dmt-aminobutyl]-6-[2'-*N*$^{\alpha}$-Boc-Dmt-aminoethyl]-5-methyl-2(1*H*)-pyrazinone (3o)

Yield 275.2 mg (84.0%), mp 145-148 °C, $[\alpha]_D^{25}$ +3.69° (*c* = 0.1, MeOH), R_{f2} = 0.15, Anal. Calcd for $C_{43}H_{62}N_6O_9 \cdot H_2O$:

C, 62.6; H, 7.82; N, 10.2. Found: C, 62.6; H, 7.71; N, 9.92. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 13.6 (br, 1H, 1-NH), 11.0 (br, 2H, 2 × OH of Dmt), 9.10 (br, 1H, 6-CH_2-CH_2-N*H*), 8.68 (br, 1H, 3-CH_2-CH_2-CH_2-CH_2-N*H*), 8.03 (d, *J* = 8.5 Hz, 1H, 3 or 6-α-NH of Dmt), 7.88 (d, *J* = 8.5 Hz, 1H, 3 or 6-α-NH of Dmt), 6.88 (s, 4H, 3,6-phenyl of Dmt), 4.84 (br, 2H, 3,6-α-CH of Dmt), 3.68 (br, 2H, 6-CH_2-C*H*$_2$-NH), 3.60-3.27 (m, 6H, 3,6-β-CH_2 of Dmt, 3-CH_2-CH_2-CH_2-C*H*$_2$-NH), 3.00-2.70 (m, 4H, 3-C*H*$_2$-CH_2-CH_2-CH_2-NH, 6-C*H*$_2$-CH_2-NH), 2.41 and 2.39 (2s, 12H, 4 × CH_3 of Dmt), 2.37 (s, 3H, 5-CH_3), 1.84 (br, 2H, 3-CH_2-C*H*$_2$-CH_2-CH_2-NH), 1.63 (br, 2H, 3-CH_2-CH_2-C*H*$_2$-CH_2-NH), 1.48 and 1.47 (2s, 18H, 3,6-*tert*-butyl), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 173.0 (q), 172.5 (q), 156.98 (q), 156.94 (q), 156.90 (q), 156.1 (q), 139.1 (q), 125.7 and 125.6 (q, 3,6-phenyl of Dmt), 116.1 (t, 3,6-phenyl of Dmt), 78.7 and 78.6 (q, 3,6-*tert*-butyl), 55.5 (t, 3,6-α-CH of Dmt), 38.7 (s, 3-CH_2-CH_2-CH_2-*C*H_2-NH), 38.6 (s, 6-CH_2-*C*H_2-NH), 32.5 (s, 3-*C*H_2-CH_2-CH_2-CH_2-NH), 32.1 (s, 6-*C*H_2-CH_2-NH), 29.7 (s, 3-CH_2-CH_2-*C*H_2-CH_2-NH), 28.5 (p, 3,6-*tert*-butyl), 24.9 (s, 3-CH_2-*C*H_2-CH_2-CH_2-NH), 20.6 (p, 4 × CH_3 of Dmt), 19.1 (p, 5-CH_3).

3-[4'-*N*$^{\alpha}$-Boc-Dmt-aminobutyl]-6-[3'-*N*$^{\alpha}$-Boc-Dmt-aminopropyl]-5-methyl-2(1*H*)-pyrazinone (3p)

Yield 273.4 mg (56.2%), mp 144-145 °C, $[\alpha]_D^{23}$ +58.7° (*c* = 0.1, MeOH), R_{f2} = 0.78, Anal. Calcd for $C_{44}H_{64}N_6O_9$: C, 63.0; H, 7.93; N, 10.0. Found: C, 62.8; H, 8.04; N, 9.45. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 10.9 (br, 2H, 2 × OH of Dmt), 8.83 (t, *J* = 4.0 Hz, 1H, 6-CH_2-CH_2-CH_2-N*H*), 8.59 (t, *J* = 6.0 Hz, 1H, 3-CH_2-CH_2-CH_2-CH_2-N*H*), 8.01 (d, *J* = 8.5 Hz, 1H, 3 or 6-α-NH of Dmt), 7.77 (d, *J* = 8.5 Hz, 1H, 3 or 6-α-NH of Dmt), 6.84 (s, 2H, 3 or 6-phenyl of Dmt), 6.82 (s, 2H, 3 or 6-phenyl of Dmt), 4.86-4.75 (m, 2H, 3,6-α-CH of Dmt), 3.56-3.38 (m, 2H, 3-CH_2-CH_2-CH_2-C*H*$_2$-NH), 3.56-3.12 (m, 4H, 3,6-β-CH_2 of Dmt), 3.38-3.22 (m, 2H, 6-CH_2-CH_2-C*H*$_2$-NH), 2.92 (t, *J* = 7.5 Hz, 2H, 3-C*H*$_2$-CH_2-CH_2-CH_2-NH), 2.54 (t, *J* = 7.6 Hz, 2H, 6-C*H*$_2$-CH_2-CH_2-NH), 2.38 and 2.35 (2s, 12H, 4 × CH_3 of Dmt), 2.28 (s, 3H, 5-CH_3), 1.91-1.75 (m, 4H, 3-CH_2-C*H*$_2$-CH_2-CH_2-NH, 6-CH_2-C*H*$_2$-CH_2-NH), 1.67-1.55 (m, 2H, 3-CH_2-CH_2-C*H*$_2$-CH_2-NH), 1.45 (s, 18H, 3,6-*tert*-butyl), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 167.0 (q), 157.5 (q), 157.0 (q), 156.9 (q), 156.2 (q), 139.1 and 139.0 (q, 3.6-phenyl of Dmt), 121.5 (q), 116.1-116.0 (t, 3,6-phenyl of Dmt), 78.5 (q, 3,6-*tert*-buyl), 55.6 (t, 3,6-α-CH of Dmt), 39.7 (s, 3-CH_2-CH_2-CH_2-*C*H_2-NH), 39.3 (s, 6-CH_2-CH_2-*C*H_2-NH), 33.5 (s, 3,6-β-CH_2 of Dmt), 29.6 (s, 3-CH_2-CH_2-*C*H_2-CH_2-NH), 28.3 (p, 3,6-*tert*-butyl), 29.0 and 24.0 (s, 3-CH_2-*C*H_2-CH_2-CH_2-NH, 6-CH_2-*C*H_2-CH_2-NH), 20.6 (p, 4 × CH_3 of Dmt), 18.9 (p, 5-CH_3).

General Procedure for Synthesis of 3,6-Bis[H-Dmt-aminoalkyl]-5-methyl-2(1*H*)-pyrazinone·2HCl (5-16)

A solution of 3,6-bis[*N*$^{\alpha}$-Boc-Dmt-aminoalkyl]-5-methyl-2(1*H*)-pyrazinone (0.12 mmol) in 7 M HCl/dioxane (0.85 mL, 6.0 mmol) was stored at 0 °C for 5 min and at room temperature for 3 h. After reaction, ether was added to the solution to form a white precipitate, which was collected by filtration. The crude product was purified by semi-preparative RP-HPLC under the following conditions: column, COSMOSIL C18 (20 mm x 250 mm); solvents, A : B (90 : 10) to A : B (50 : 50) in 40 min; flow rate, 10 mL/min. Analytical HPLC conditions: column, COSMOSIL C18 or YMC Pack R&D ODS C18 (4.6 mm × 250 mm); solvents, A : B (90 : 10) to A : B (50 : 50) in 40 min, flow late 1.0 mL/min; detection, 220 nm. The retention times (min), t_{R1} and t_{R2} are by YMC Pack R&D ODS C18 column and COSMOSIL C18 column, respectively.

6-[2'-(H-Dmt)-aminoethyl]-3-[(H-Dmt)-aminomethyl]-5-methyl-2(1*H*)-pyrazinone·3HCl (5)

Yield 59.0 mg (88.1%), $[\alpha]_D^{23}$ -3.64° (*c* = 0.1, H_2O), R_{f3} = 0.64, t_{R1} = 13.2, t_{R2} = 11.0 (min), *m/z* 565.89 $(M+1)^+$, Calcd. 565.68, Anal. Calcd for $C_{30}H_{40}N_6O_5$·3HCl·4H_2O: C, 48.3; H, 6.82; N, 11.3. Found. C, 48.4; H, 7.09; N, 11.9. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 9.59 (br, 1H, 6-CH_2-CH_2-N*H*), 8.90 (br, 1H, 3-CH_2-N*H*), 6.82 (s, 4H, 3,6-phenyl of Dmt), 4.84 (br, 2H, 3,6-α-CH of Dmt), 4.72 (br, 2H, 3-C*H*$_2$-NH), 3.89 (br, 2H, 6-CH_2-C*H*$_2$-NH), 3.80-3.41 (m, 4H, 3,6-β-CH_2 of Dmt), 2.98 and 2.80 (each br, each 1H, 6-C*H*$_2$-CH_2-NH), 2.39 (s, 12H, 4 × CH_3 of Dmt), 2.19 (s, 3H, 5-CH_3), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 175.7 (q), 174.1 (q), 169.8 (q), 168.7 (q), 157.0 (q), 156.7 (q), 139.5 and 139.3 (q, 3,6-phenyl of Dmt), 116.3 and 116.2 (t, 3,6-phenyl of Dmt), 55.2 (t, 3,6-α-CH of Dmt), 34.7 (s, 3,6-β-CH_2 of Dmt), 32.3 (s, 3-CH_2-NH), 27.9 (s, 6-*C*H_2-CH_2-NH), 23.1 (s, 6-CH_2-*C*H_2-NH), 20.6 (p, 4 × CH_3 of Dmt), 18.1 (p, 5-CH_3).

3-[(H-Dmt)-aminomethyl]-6-[3'-(H-Dmt)-aminopropyl]-5-methyl-2(1*H*)-pyrazinone·2HCl (6)

Yield 27.0 mg (58.9%), $[\alpha]_D^{24}$ +3.81° (*c* = 0.1, H_2O), R_{f3} = 0.48, t_{R1} = 14.6, t_{R2} = 14.4 (min), *m/z* 579.82 $(M+1)^+$, Calcd. 579.72, Anal. Calcd for $C_{31}H_{42}N_6O_5$·2HCl·2.5H_2O: C, 53.4; H, 7.09; N, 12.1. Found. C, 53.5; H, 7.18; N, 12.4. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 9.58 (br, 1H, 6-CH_2-CH_2-CH_2-N*H*), 6.89 and 6.82 (2s, 5H, 3,6-phenyl of Dmt, 3-CH_2-N*H*), 4.98-4.70 (m, 4H, 3,6-α-CH of Dmt, 3-C*H*$_2$-NH), 3.86-3.83 and 3.78-3.53 (each m, 4H, 3,6-β-CH_2 of Dmt), 3.78-3.53 and 3.22-3.09 (each br, each 1H, 6-CH_2-CH_2-C*H*$_2$-NH), 2.80 and 2.70 (each br, each 1H, 6-C*H*$_2$-CH_2-CH_2-NH), 2.42 and 2.36 (2s, 12H, 4 × CH_3 of Dmt), 2.16 (s, 3H, 5-CH_3), 1.98 and 1.80 (each br, each 1H, 6-CH_2-C*H*$_2$-CH_2-NH), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 173.4 (q), 170.2 (q), 170.1 (q), 157.5 (q), 157.4 (q), 156.7 (q), 156.9 (q), 139.5 and 139.3 (q, 3,6-phenyl of Dmt), 116.3 and 116.2 (t, 3,6-phenyl of Dmt), 54.1 (t, 3,6-α-CH of Dmt), 41.9 (s, 3-CH_2-NH), 39.0 (s, 6-CH_2-CH_2-*C*H_2-NH), 31.0 (s, 3,6-β-CH_2 of Dmt), 28.1 (s, 6-CH_2-*C*H_2-CH_2-NH), 23.7 (s, 6-*C*H_2-CH_2-CH_2-NH), 20.63 and 20.56 (p, 4 × CH_3 of Dmt), 18.4 (p, 5-CH_3).

6-[4'-(H-Dmt)-aminobutyl]-3-[(H-Dmt)-aminomethyl]-5-methyl-2(1*H*)-pyrazinone·2HCl (7)

Yield 30.3 mg (46.8%), $[\alpha]_D^{25}$ +1.29° (*c* = 0.1, H_2O), R_{f3} = 0.48, t_{R1} = 22.2 t_{R2} = 18.1 (min), *m/z* 593.8 $(M+1)^+$, Calcd. 593.7, Anal. Calcd for $C_{32}H_{44}N_6O_5$·2HCl·3H_2O: C, 53.4; H, 7.28; N, 11.7. Found: C, 53.2; H, 7.28; N, 12.0. ^{1}H-NMR

(400.1 MHz, pyridine-d_5) δ: 9.38 (br, 1H, 6-CH_2-CH_2-CH_2-CH_2-N*H*), 8.85 (br, 1H, 3-CH_2-N*H*), 6.89 and 6.84 (2s, 4H, 3,6-phenyl of Dmt), 4.93-4.73 (m, 4H, 3,6-α-CH of Dmt, 3-C*H*$_2$-NH), 3.95-3.55 (m, 4H, 3,6-β-CH_2 of Dmt), 3.55-3.12 (m, 2H, 6-CH_2-CH_2-CH_2-C*H*$_2$-NH), 2.64-2.46 (m, 2H, 6-C*H*$_2$-CH_2-CH_2-CH_2-NH), 2.41 and 2.38 (2s, 12H, 4 × CH_3 of Dmt), 2.20 (s, 3H, 5-CH_3), 1.68 (br, 2H, 6-CH_2-C*H*$_2$-CH_2-CH_2-NH), 1.46 (br, 2H, 6-CH_2-CH_2-C*H*$_2$-CH_2-NH), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 172.4 (q), 170.1 (q), 169.9 (q), 157.4 (q), 156.1 (q), 152.1 (q), 139.5 and 139.3 (q, 3,6-phenyl of Dmt), 116.3 and 116.1 (t, 3,6-phenyl of Dmt), 54.0 and 53.8 (t, 3,6-α-CH of Dmt), 41.6 (s, 3-CH_2-NH), 39.2 (s, 6-CH_2-CH_2-CH_2-*C*H_2-NH-), 31.9 (s, 3,6-β-CH_2 of Dmt), 29.5 (s, 6-*C*H_2-CH_2-CH_2-CH_2-NH), 28.9 (s, 6-CH_2-CH_2-*C*H_2-CH_2-NH), 26.1 (s, 6-CH_2-*C*H_2-CH_2-CH_2-NH), 20.7 and 20.6 (p, 4 × CH_3 of Dmt), 18.5 (p, 5-CH_3).

3-[2'-(H-Dmt)-aminoethyl]-6-[(H-Dmt)-aminomethyl]-5-methyl-2(1*H*)-pyrazinone·3HCl (8)

Yield 23.5 mg (94.0%), $[\alpha]_D^{25}$ +9.44° (*c* = 0.1, H_2O), R_{f3} = 0.45, t_{R1} = 12.5, t_{R2} = 11.0 (min), *m/z* 566.01 $(M+1)^+$, Calcd. 565.68, Anal. Calcd for $C_{30}H_{40}N_6O_5 \cdot 3HCl \cdot 1.7H_2O$: C, 50.0; H, 6.18; N, 12.2. Found: C, 49.9; H, 6.11; N, 11.9. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 9.83 (br, 1H, 6-CH_2-N*H*), 9.01 (br, 1H, 3-CH_2-CH_2-N*H*), 6.85 and 6.80 (2s, 4H, 3,6-phenyl of Dmt), 4.86-4.56 (m, 2H, 3,6-α-CH of Dmt), 4.76 and 4.30 (each br, each 1H, 6-C*H*$_2$-NH), 3.96-3.79 (m, 2H, 3-CH_2-C*H*$_2$-NH), 3.79-3.50 (m, 4H, 3,6-β-CH_2 of Dmt), 3.16-2.99 (m, 2H, 3-C*H*$_2$-CH_2-NH), 2.41 and 2.36 (2s, 12H, 4 × CH_3 of Dmt), 2.26 (s, 3H, 5-CH_3), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 172.4 (q), 170.1 (q), 169.9 (q), 165.1 (q), 157.4 (q), 153.1 (q), 139.4 and 139.3 (q, 3,6-phenyl of Dmt), 116.3 and 116.2 (t, 3,6-phenyl of Dmt), 54.0 (t, 3,6-α-CH of Dmt), 40.0 (s, 6-CH_2-NH), 37.8 (s, 3-CH_2-*C*H_2-NH), 32.3 and 31.5 (s, 3,6-β-CH_2 of Dmt), 29.5 (s, 3-*C*H_2-CH_2-NH), 20.7 and 20.6 (p, 4 × CH_3 of Dmt), 18.9 (p, 5-CH_3).

3-[2'-(H-Dmt)-aminoethyl]-6-[3'-(H-Dmt)-aminopropyl]-5-methyl-2(1*H*)-pyrazinone·2HCl (9)

Yield 10.0 mg (44.6%), $[\alpha]_D^{25}$ +1.28° (*c* = 0.1, H_2O), R_{f3} = 0.28, t_{R1} = 16.2, t_{R2} = 14.6 (min), *m/z* 594.11 $(M+1)^+$, Calcd. 593.73, Anal. Calcd for $C_{32}H_{44}N_6O_5 \cdot 2HCl \cdot 5.5H_2O$: C, 50.3; H, 7.51; N, 11.0. Found: C, 50.0; H, 7.62; N, 11.0. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 9.41 (br, 1H, 6-CH_2-CH_2-CH_2-N*H*), 9.15 (br, 1H, 3-CH_2-CH_2-N*H*), 6.89 and 6.86 (2s, 4H, 3,6-phenyl of Dmt), 4.80 (br, 1H, 3 or 6-α-CH of Dmt), 4.72 (br, 1H, 3 or 6-α-CH of Dmt), 4.09-3.71 (m, 8H, 3-C*H*$_2$-C*H*$_2$-NH, 3,6-β-CH_2 of Dmt), 3.61 and 3.11 (each br, 2H, 6-CH_2-CH_2-C*H*$_2$-NH), 3.25-3.05 (m, 2H, 6-C*H*$_2$-CH_2-CH_2-NH), 2.45 and 2.43 (2s, 12H, 4 × CH_3 of Dmt), 2.17 (s, 3H, 5-CH_3), 1.88-1.57 (m, 2H, 6-CH_2-C*H*$_2$-CH_2-NH), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 178.9 (q), 178.2 (q), 171.8 (q), 169.6 (q), 157.1 (q), 156.8 (q), 139.2 and 139.1 (q, 3,6-phenyl of Dmt), 116.0 (t, 3,6-phenyl of Dmt), 53.8 (t, 3,6-α-CH of Dmt), 41.0, (s, 6-*C*H_2-CH_2-CH_2-NH), 39.2 (s, 6-CH_2-CH_2-*C*H_2-NH), 35.1 (s, 3-*C*H_2-CH_2-NH), 31.5 (s, 3,6-β-CH_2 of Dmt), 29.6 (s, 3-CH_2-*C*H_2-NH), 20.5 (p, 4 × CH_3 of Dmt), 18.3 (p, 5-CH_3), 16.0 (s, 6-CH_2-*C*H_2-CH_2-NH).

6-[4'-(H-Dmt)-aminobutyl]-3-[2'-(H-Dmt)-aminoethyl]-5-methyl-2(1*H*)-pyrazinone·2HCl (10)

Yield 5.1 mg (17.0%), $[\alpha]_D^{25}$ +2.52° (*c* = 0.1, H_2O), R_{f3} = 0.34, t_{R1} = 18.7, t_{R2} = 17.2 (min), *m/z* 607.57 $(M+1)^+$, Calcd. 606.76, Anal. Calcd for $C_{33}H_{46}N_6O_5 \cdot 2HCl \cdot 5H_2O$: C, 49.6; H, 6.90; N, 9.92. Found: C, 49.0; H, 6.60; N, 10.4. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 9.55 (br, 1H, 3-CH_2-CH_2-N*H*), 9.14 (br, 1H, 6-CH_2-CH_2-CH_2-CH_2-N*H*), 6.87 and 6.83 (2s, 4H, 3,6-phenyl of Dmt), 4.85 (br, 1H, 3 or 6-α-CH of Dmt), 4.71 (br, 1H, 3 or 6-α-CH of Dmt), 3.72-3.66 (m, 8H, 6-CH_2-CH_2-CH_2-C*H*$_2$-NH, 3,6-β-CH_2 of Dmt, 3-C*H*$_2$-CH_2-NH), 3.17 and 3.02 (each br, 4H, 3-CH_2-C*H*$_2$-NH, 6-C*H*$_2$-CH_2-CH_2-CH_2-NH), 2.80 and 2.64 (each br, each 1H, 6-CH_2-C*H*$_2$-CH_2-CH_2-NH), 2.40 (s, 12H, 4 × CH_3 of Dmt), 2.08 (s, 3H, 5-CH_3), 1.90 and 1.72 (each br, each 1H, 6-CH_2-CH_2-C*H*$_2$-CH_2-NH), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 177.7 (q), 175.4, (q), 171.7 (q), 170.0 (q), 169.8 (q), 157.2 (q), 139.3 and 139.2 (q, 3,6-phenyl of Dmt), 116.1 and 116.0 (t, 3,6-phenyl of Dmt), 68.0 (s, 6-*C*H_2-CH_2-CH_2-CH_2-NH), 54.01 and 53.99 (t, 3,6-α-CH of Dmt), 39.6 (s, 3,6-β-CH_2 of Dmt), 37.6 (s, 6-CH_2-CH_2-CH_2-*C*H_2-NH), 32.4 (s, 3-CH_2-*C*H_2-NH), 31.6 (s, 3-*C*H_2-CH_2-NH), 28.2 (s, 6-CH_2-CH_2-*C*H_2-CH_2-NH), 20.6 (p, 4 × CH_3 of Dmt), 18.4 (p, 5-CH_3).

3-[3'-(H-Dmt)-aminopropyl]-6-[(H-Dmt)-aminomethyl]-5-methyl-2(1*H*)-pyrazinone·2HCl (11)

Yield 21.3 mg (63.6%), $[\alpha]_D^{23}$ +1.30° (*c* = 0.1, H_2O), R_{f3} = 0.48, t_{R1} = 17.3, t_{R2} = 16.7 (min), *m/z* 579.3 $(M+1)^+$, Calcd. 579.7, Anal. Calcd for $C_{31}H_{42}N_6O_5 \cdot 2HCl \cdot 3H_2O$: C, 52.8; H, 7.14; N, 11.9. Found: C, 52.7; H, 7.16; N, 12.2. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 9.82 (br, 1H, 6-CH_2-N*H*), 9.20 (br, 1H, 3-CH_2-CH_2-CH_2-N*H*), 6.86 and 6.83 (2s, 4H, 3,6-phenyl of Dmt), 4.75 (br, 1H, 3 or 6-α-CH of Dmt), 4.62 (br, 2H, 3 or 6-α-CH of Dmt), 4.62 and 4.42 (each br, each 1H, 6-C*H*$_2$-NH), 3.75-3.37 (m, 6H, 3,6-β-CH_2 of Dmt, 3-CH_2-CH_2-C*H*$_2$-NH), 2.85 (br, 2H, 3-C*H*$_2$-CH_2-CH_2-NH), 2.38 and 2.30 (2s, 12H, 4 × CH_3 of Dmt), 2.28 (s, 3H, 5-CH_3), 2.02 (br, 2H, 3-CH_2-C*H*$_2$-CH_2-NH), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 170.2 (q), 170.0 (q), 157.9 (q), 157.5 (q), 157.4 (q), 156.2 (q), 139.5 and 139.4 (q, 3,6-phenyl of Dmt), 116.3 and 116.2 (t, 3,6-phenyl of Dmt), 54.1 and 54.0 (t, 3,6-α-CH of Dmt), 39.9 (s, 3,6-β-CH_2 of Dmt), 35.1 (s, 6-CH_2-NH), 32.1 (s, 3-CH_2-CH_2-*C*H_2-NH), 30.1 (s, 3-*C*H_2-CH_2-CH_2-NH), 26.7 (s, 3-CH_2-*C*H_2-CH_2-NH), 20.6 (p, 4 × CH_3 of Dmt), 19.0 (p, 5-CH_3).

6-[2'-(H-Dmt)-aminoethyl]-3-[3'-(H-Dmt)-aminopropyl]-5-methyl-2(1*H*)-pyrazinone·2HCl (12)

Yield 23.1 mg (91.7%), $[\alpha]_D^{22}$ +105.5° (*c* = 0.1, H_2O), R_{f3} = 0.55, t_{R1} = 17.4, t_{R2} = 13.2 (min), *m/z* 593.81 $(M+1)^+$, Calcd. 593.73, Anal. Calcd for $C_{32}H_{44}N_6O_5 \cdot 2HCl \cdot 3.4H_2O$: C, 52.9; H, 7.32; N, 11.6. Found. C, 52.9; H, 7.32; N, 11.9. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 9.68 (br, 1H, 6-CH_2-CH_2-N*H*), 9.27 (br, 1H, 3-CH_2-CH_2-CH_2-N*H*), 6.89 and 6.87 (2s, 4H, 3,6-phenyl of Dmt), 4.88-4.72 (m, 2H, 3,6-α-CH of Dmt), 3.97-3.76 (m, 4H, 3,6-β-CH_2 of Dmt), 3.77-3.48 (m, 2H, 6-CH_2-C*H*$_2$-NH), 3.55-3.32 (m, 2H, 6-C*H*$_2$-CH_2-NH),

3.41 and 2.93 (each br, each 1H, 3-CH_2-CH_2-CH_2-NH), 2.82 (t, J = 6.9 Hz, 2H, 3-CH_2-CH_2-CH_2-NH), 2.443 and 2.437 (2s, 12H, 4 × CH_3 of Dmt), 2.19 (s, 3H, 5-CH_3), 2.10-1.90 (m, 2H, 3-CH_2-CH_2-CH_2-NH), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 170.1 (q), 169.9 (q), 157.4 (q), 156.6 (q), 139.5 and 139.4 (q, 3,6-phenyl of Dmt), 116.3 and 116.2 (t, 3,6-phenyl of Dmt), 54.1 and 54.0 (t, 3,6-α-CH of Dmt), 39.8 (s, 6-*C*H_2-CH_2-NH), 38.62 (s, 3,6-β-CH_2 of Dmt), 38.60 (s, 6-CH_2-*C*H_2-NH), 31.8 (s, 3-CH_2-CH_2-*C*H_2-NH), 30.0 (s, 3-*C*H_2-CH_2-CH_2-NH), 26.4 (s, 3-CH_2-*C*H_2-CH_2-NH), 20.7 (p, 4 × CH_3 of Dmt), 18.7 (p, 5-CH_3).

6-[4'-(H-Dmt)-aminobutyl]-3-[3'-(H-Dmt)-aminopropyl]-5-methyl-2(1*H*)-pyrazinone·2HCl (13)

Yield 9.0 mg (59.5%), $[\alpha]_D^{22}$ +32.4° (c = 0.1, MeOH), R_{f3} = 0.64, t_{R1} = 19.4, t_{R2} = 17.4 (min), *m/z* 622.05 $(M+1)^+$, Calcd. 621.78, Anal. Calcd for $C_{34}H_{48}N_6O_5{\cdot}2HCl{\cdot}5.5H_2O$: C, 47.2; H, 6.42; N, 9.71. Found. C, 47.0; H, 6.23; N, 9.99. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 8.56 (br, 1H, 6-CH_2-CH_2-CH_2-CH_2-N*H*), 8.10 (br, 1H, 3-CH_2-CH_2-CH_2-N*H*), 6.40 (s, 4H, 3,6-phenyl of Dmt), 4.60 and 4.17 (each br, each 1H, 3,6-α-CH of Dmt), 3.69 (br, 2H, 6-CH_2-CH_2-CH_2-CH_2-NH), 3.01-2.89 (m, 8H, 6-CH_2-CH_2-CH_2-CH_2-NH, 3-CH_2-CH_2-CH_2-NH, 3,6-β-CH_2 of Dmt), 2.52 (br, 2H, 6-CH_2-CH_2-CH_2-CH_2-NH), 2.38 (t, J = 7.1 Hz, 2H, 3-CH_2-CH_2-CH_2-NH), 2.19-2.17 (m, 15H, 5-CH_3, 4×CH_3 of Dmt), 1.61-1.56 (m, 2H, 6-CH_2-CH_2-CH_2-CH_2-NH), 1.32 (quintet, J = 7.3 Hz, 2H, 3-CH_2-CH_2-CH_2-NH), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 191.0 (q), 168.0 (q), 167.8 (q), 155.5 (q), 138.2 (q), 122.6 (q), 114.9 and 114.8 (t, 3,6-phenyl of Dmt), 51.7 (s, 6-CH_2-CH_2-CH_2-*C*H_2-NH), 38.5 (s, 6-CH_2-CH_2-*C*H_2-CH_2-NH), 38.2 (s, 3-CH_2-CH_2-*C*H_2-NH), 30.5 (s, 3-CH_2-*C*H_2-CH_2-NH), 30.4 (s, 6-*C*H_2-CH_2-CH_2-CH_2-NH), 30.4 (s, 3-*C*H_2-CH_2-CH_2-NH), 27.7 (s, 6-CH_2-*C*H_2-CH_2-CH_2-NH), 19.8 (p, 4 × CH_3 of Dmt), 17.6 (p, 5-CH_3).

3-[4'-(H-Dmt)-aminobutyl]-6-[(H-Dmt)-aminomethyl]-5-methyl-2(1*H*)-pyrazinone·2HCl (14)

Yield 115.5 mg (98.7%), $[\alpha]_D^{25}$ +92.1° (c = 0.1, H_2O), R_{f3} = 0.40, t_{R1} = 16.8, t_{R2} = 18.8 (min), *m/z* 593.9 $(M+1)^+$, Calcd. 593.7, Anal. Calcd for $C_{31}H_{42}N_6O_5{\cdot}2HCl{\cdot}2H_2O$: C, 55.6; H, 7.66; N, 11.4. Found: C, 55.7; H, 7.97; N, 11.5. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 9.87 (br, 1H, 6-CH_2-N*H*), 9.11 (br, 1H, 3-CH_2-CH_2-CH_2-CH_2-N*H*), 6.81 and 6.80 (2s, 4H, 3,6-phenyl of Dmt), 4.74 (br, 1H, 3 or 6-α-CH of Dmt), 4.63 (br, 1H, 3 or 6-α-CH of Dmt), 4.63 and 4.35 (each 1H, 2H, 6-CH_2-NH), 3.72-3.55 (m, 4H, 3,6-β-CH_2 of Dmt), 3.35-3.28 (m, 2H, 3-CH_2-CH_2-CH_2-CH_2-NH), 2.77 (br, 2H, 3-CH_2-CH_2-CH_2-CH_2-NH), 2.34 and 2.28 (2s, 15H, 4 × CH_3 of Dmt, 5-CH_3), 1.87 (br, 2H, 3-CH_2-CH_2-CH_2-CH_2-NH), 1.49 (br, 2H, 3-CH_2-CH_2-CH_2-CH_2-NH), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 179.0 (q), 170.7 (q), 169.9 (q), 157.5 (q), 157.4 (q), 156.5 (q), 139.4 and 139.3 (q, 3,6-phenyl of Dmt), 116.4 and 116.3 (t, 3,6-phenyl of Dmt), 54.0 and 53.9 (t, 3,6-α-CH of Dmt), 39.78 (s, 6-CH_2-NH), 39.75 (s, 3-CH_2-CH_2-CH_2-*C*H_2-NH), 32.4 (s, 3-*C*H_2-CH_2-CH_2-CH_2-NH), 31.9 (s, 3,6-β-CH_2 of Dmt), 28.9 (s, 3-CH_2-CH_2-*C*H_2-CH_2-NH), 24.7 (s, 3-CH_2-*C*H_2-CH_2-CH_2-NH), 20.6 and 20.5 (p, 4 × CH_3 of Dmt), 19.2 (p, 5-CH_3).

3-[4'-(H-Dmt)-aminobutyl]-6-[2'-(H-Dmt)-aminoethyl]-5-methyl-2(1*H*)-pyrazinone·2HCl (15)

Yield 5.1 mg (70.5%), $[\alpha]_D^{25}$ +11.9° (c = 0.1, H_2O), R_{f3} = 0.44, t_{R1} = 17.7, t_{R2} = 15.8 (min), *m/z* 607.06 $(M+1)^+$, Calcd. 606.76, Anal. Calcd for $C_{33}H_{46}N_6O_5{\cdot}2HCl{\cdot}4H_2O$: C, 57.6; H, 8.21; N, 12.2. Found: C, 57.7; H, 8.54; N, 12.0. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 9.70 (br, 1H, 6-CH_2-CH_2-N*H*), 9.18 (br, 1H, 3-CH_2-CH_2-CH_2-CH_2-N*H*), 6.89 and 6.88 (2s, 4H, 3,6-phenyl of Dmt), 4.83 (br, 1H, 3 or 6-α-CH of Dmt), 4.76 (br, 1H, 3 or 6-α-CH of Dmt), 3.96-3.50 (m, 6H, 6-CH_2-CH_2-NH, 3,6-β-CH_2 of Dmt), 3.64 (t, J = 12.0 Hz, 2H, 6-CH_2-CH_2-NH), 3.50-3.38 (m, 2H, 3-CH_2-CH_2-CH_2-CH_2-NH), 3.05-2.70 (m, 2H, 3-CH_2-CH_2-CH_2-CH_2-NH), 2.43 (s, 12H, 4 × CH_3 of Dmt), 2.24 (s, 3H, 5-CH_3), 1.72 (br, 2H, 3-CH_2-CH_2-CH_2-CH_2-NH), 1.55 (br, 2H, 3-CH_2-CH_2-CH_2-CH_2-NH), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 170.5 (q), 170.1 (q), 157.8 (q), 157.0 (q), 156.5 (q), 139.8 (q), 116.63 and 116.56 (t, 3,6-phenyl of Dmt), 54.4 (t, 3,6-α-CH of Dmt), 40.1, (s, 3-CH_2-CH_2-CH_2-*C*H_2-NH), 39.0 (s, 6-CH_2-*C*H_2-NH), 32.8 (s, 6-*C*H_2-CH_2-NH), 32.0 (s, 3-*C*H_2-CH_2-CH_2-CH_2-NH), 29.4 (s, 3-CH_2-CH_2-*C*H_2-CH_2-NH), 24.9 (s, 3-CH_2-*C*H_2-CH_2-CH_2-NH), 21.09 and 21.06 (p, 4 × CH_3 of Dmt), 19.1 (p, 5-CH_3).

3-[4'-(H-Dmt)-aminobutyl]-6-[3'-(H-Dmt)-aminopropyl]-5-methyl-2(1*H*)-pyrazinone·2HCl (16)

Yield 7.0 mg (23.2%), $[\alpha]_D^{25}$ +76.3° (c = 0.1, H_2O), R_{f3} = 0.48, t_{R1} = 18.3, t_{R2} = 20.5 (min), *m/z* 621.88 $(M+1)^+$, Calcd. 621.78, Anal. Calcd for $C_{34}H_{48}N_6O_5{\cdot}2HCl{\cdot}6H_2O$: C, 50.9; H, 7.49; N, 10.5. Found: C, 50.7; H, 7.23; N, 10.1. ^{1}H-NMR (400.1 MHz, pyridine-d_5) δ: 9.62 (t, J = 5.5 Hz, 1H, 6-CH_2-CH_2-CH_2-N*H*), 9.11 (t, J = 5.5 Hz, 1H, 3-CH_2-CH_2-CH_2-CH_2-N*H*), 6.88 and 6.87 (2s, 4H, 3,6-phenyl of Dmt), 4.88-4.81 (m, 1H, 3 or 6-α-CH of Dmt), 4.72-4.64 (m, 1H, 3 or 6-α-CH of Dmt), 3.83-3.74 (m, 2H, 6-CH_2-CH_2-CH_2-NH), 3.68-3.60 (m, 2H, 3-CH_2-CH_2-CH_2-CH_2-NH), 3.59-3.32 (m, 4H, 3,6-β-CH_2 of Dmt), 2.85-2.82 (m, 2H, 3-CH_2-CH_2-CH_2-CH_2-NH), 2.85-2.65 (m, 2H, 6-CH_2-CH_2-CH_2-NH), 2.42 and 2.41 (2s, 12H, 4 × CH_3 of Dmt), 2.20 (s, 3H, 5-CH_3), 1.77-1.75 (m, 2H, 6-CH_2-CH_2-CH_2-NH), 1.57-1.51 (m, 2H, 3-CH_2-CH_2-CH_2-CH_2-NH), ^{13}C-NMR (100.6 MHz, pyridine-d_5) δ: 170.2 (q), 170.0 (q), 157.5 (q), 157.4 (q), 139.5 and 139.4 (q, 3,6-phenyl of Dmt), 116.3 and 116.2 (t, 3,6-phenyl of Dmt), 54.1and 54.0 (t, 3,6-α-CH of Dmt), 39.6 (s, 3,6-β-CH_2 of Dmt), 39.1 (s, 3-CH_2-CH_2-CH_2-*C*H_2-NH), 32.5 (s, 3-CH_2-CH_2-*C*H_2-CH_2-NH), 31.9 (s, 6-CH_2-CH_2-*C*H_2-NH), 29.0 (s, 3-*C*H_2-CH_2-CH_2-CH_2-NH), 28.8 (s, 6-*C*H_2-CH_2-CH_2-NH), 24.6 (s, 6-CH_2-*C*H_2-CH_2-NH), 24.4 (s, 3-CH_2-*C*H_2-CH_2-CH_2-NH), 20.7 (p, 4 × CH_3 of Dmt), 18.5 (p, 5-CH_3).

Competitive Opioid Receptor Binding Assays

Opioid receptor binding affinities were determined under equilibrium conditions (2.5h at 22°C) in a competition assay using rat brain P_2 synaptosomes membranes [38-43].

The synaptosomes were preincubated to remove endogenous opioids, extensively washed in ice cold buffer containing protease inhibitor, resuspended in buffered 20% glycerol, and stored at -80 ^{o}C [25]. δ- and μ-Opioid receptors were radiolabeled with [^{3}H]deltorphin II (45 Ci/mmol, PerkinElmer, Boston, MA) and [^{3}H]DAMGO (50 Ci/mmol, Amersham Biosciences, Arlington, IL), respectively. Excess unlabeled peptide (2 μM) established nonspecific binding background. Radiolabeled membranes were rapidly filtered on Whatman GF/C glass fiber filters presoaked in 0.1% polyethylenimine to enhance the signal-to-noise ratio, washed with ice-cold BSA buffer, and dried at 75 ^{o}C for 60 min, and radioactivity was determined using CytoScint (INC, CostaMesa, CA). The analogues were analyzed in duplicate assays using 5-8 dosages and 3-5 independent repetitions (*n* values noted in parentheses in Table **1**) with different synaptsomal preparations to ensure statistical significance and listed as mean ± S.E. (Prism 3.03). The affinity constants (K_i) were calculated according to Cheng and Prusoff [44].

Functional Bioassay in Isolated Preparations

The myenteric plexus longitudinal muscle preparations (2-3 cm segments) were surgically removed from the small intestine of guinea pigs (GPI) and used to measure μ-opioid receptor agonism [45]. A single mouse vas deferens (MVD), containing primarily δ-opioid receptors, was used to determine agonism or antagonism for δ-opioid receptor activity [45]. 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 concentration-response curves in comparison to endomorphin-2 with GPI and deltorpin II for MVD. IC_{50} values represent the mean ± S.E. of five to seven separate tissue samples. δ-Antagonism (+) denotes that the IC_{50} value (0.86 nM) of deltorphin II increased by twice or more in the presence of the compound (1,000 nM). pA_2 value is the negative logarithm of the concentration of an antagonist that is necessary to double the concentration of agonist (deltorphin II) concentration to achieve the original response.

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