Effect of Lysine at C-Terminus of the Dmt-Tic Opioid Pharmacophore

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Substitution of Gly with side-chain-protected or unprotected Lys in lead compounds containing the opioid pharmacophore Dmt-Tic [H-Dmt-Tic-Gly-NH-CH_2-Ph, μ agonist/δ antagonist; H-Dmt-Tic-Gly-NH-Ph, μ agonist/δ agonist; and H-Dmt-Tic-NH-CH_2-Bid, δ agonist (Bid = 1*H*-benzimidazole-2-yl)] yielded a new series of compounds endowed with distinct pharmacological activities. Compounds (**1**−**10**) included high δ- (K_i^{δ} = 0.068−0.64 nM) and μ-opioid affinities (K_i^{μ} = 0.13−5.50 nM), with a bioactivity that ranged from μ-opioid agonism {**10**, H-Dmt-Tic-NH-CH[$(CH_2)_4$-NH_2]-Bid (IC_{50} GPI = 39.7 nM)} to a selective μ-opioid antagonist [**3**, H-Dmt-Tic-Lys-NH-CH_2-Ph (pA_2^{μ} = 7.96)] and a selective δ-opioid antagonist [**5**, H-Dmt-Tic-Lys(Ac)-NH-Ph (pA_2^{δ} = 12.0)]. The presence of a Lys linker provides new lead compounds in the formation of opioid peptidomimetics containing the Dmt-Tic pharmacophore with distinct agonist and/or antagonist properties.

Introduction

The prototype δ-opioid receptor antagonist, H-Dmt-Tic-OH,[a,1−3] which evolved from H-Tyr-Tic-OH,[4] as a simplified form of TIP(P),[5] represents the minimal sequence that selectively interacts with δ-opioid receptors as a potent δ-opioid antagonist. This dipeptide underwent extensive modifications by rationale drug design principles and structure−activity studies,[6,7] which included *N*-terminal modification with alkyl substitutions;[3,8] replacement of Tic by heteroaliphatic or heteroaromatic nuclei,[9] or D-Phe;[10] alteration of the C-terminus of Tic by substituents containing hydrophobic groups;[3] and addition of a third aromatic center with or without inserting interposing linkers.[11−13] Many of the analogues had unique properties, including enhanced δ-opioid antagonism,[3,6−13] conversion from a δ-opioid antagonist to a δ-opioid agonist and vice versa,[11,14] mixed μ agonism/δ antagonism,[3,8,11] or development of irreversible fluorescent δ-opioid antagonists.[13] From these and other studies, it was concluded that small, discrete, and subtle modifications can drastically change the pharmacological profile in molecules related to the Dmt-Tic pharmacophore.[15−17]

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[a] Abbreviations: Ac, acetyl; Bid, 1*H*-benzimidazole-2-yl; Boc, *tert*-butyloxycarbonyl; DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]enkephalin; DELC, deltorphin II (H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2); DMF, *N*,*N*-dimethylformamide; DMSO-d_6, hexadeuteriodimethyl sulfoxide; Dmt, 2′,6′-dimethyl-L-tyrosine; GPI, guinea-pig ileum; HOBt, 1-hydroxybenzotriazole; HPLC, high performance liquid chromatography; MALDI-TOF, matrix assisted laser desorption ionization time-of-flight; MVD, mouse vas deferens; NMM, 4-methylmorpholine; pA_2, negative log of the molar concentration required to double the agonist concentration to achieve the original response; TFA, trifluoroacetic acid; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; TIP(P), H-Tyr-Tic-Phe-(Phe)-OH; TLC, thin-layer chromatography; WSC, 1-ethyl-3-[3′-dimethyl)aminopropyl]-carbodiimide hydrochloride; Z, benzyloxycarbonyl.

Rationale

Our recent efforts focused attention on the presence of side-chain-protected Lys in the Dmt-Tic pharmacophore. The substitution of the third amino acid in H-Dmt-Tic-Xaa peptides with H-Lys(Ac)-OH, gave the most potent δ antagonist of the series [H-Dmt-Tic-Lys(Ac)-OH; pA_2^{δ} = 10.07].[18] Substitution of the C-terminal Phe with H-Lys(Z)-OH in H-Dmt-Tic-Phe-Phe-OH [DIPP-OH; pA_2 (MVD) = 9.71] resulted in an increase of about 50-fold in δ-opioid antagonist activity [H-Dmt-Tic-Phe-Lys(Z)-OH; pA_2 = 11.43].[19] Starting from these considerations, we introduced the side-chain-protected or unprotected Lys into other peptides containing the Dmt-Tic pharmacophore: H-Dmt-Tic-Gly-NH-Ph (μ agonist/δ agonist), H-Dmt-Tic-Gly-NH-CH_2-Ph (μ agonist/δ antagonist), and H-Dmt-Tic-NH-CH_2-Bid (δ agonist) were selected as reference compounds. Compounds exhibiting pharmacological properties of μ agonism/δ agonism could be interesting clinical analgesics, which could have a low dependence for chronic use for the amelioration of pain.[20] Opioid ligands with a mixed μ agonist/δ antagonist activity profile may have diminished propensity to induce tolerance and, therefore, may have therapeutic advantages over μ agonist analgesics for long-term treatment of pain.[21,22] δ opioid receptor agonists, such as H-Dmt-Tic-NH-CH_2-Bid and H-Dmt-Tic-NH-CH(CH_2-COOH)-Bid, are attractive as potential analgesics, because δ opioid agonists exhibit strong antinociceptive activity with relatively few side effects.[23] Furthermore, δ opioid receptor agonists produce antidepressant-like and anxiolytic-like effects and regulate BDNF mRNA expression in rodents,[24,25] such that the regulation of BDNF mRNA expression could be useful in the treatment of multiple sclerosis and related diseases.[26] Moreover, δ-opioid receptor activation protects cortical neurons, producing hibernation and neuroprotection.[27−29] Activation of δ and κ opioid receptors affords cardioprotection.[30]

Chemistry

Peptides (**1**−**6**) and pseudopeptides (**7**−**10**) were prepared stepwise by solution peptide synthetic methods, as outlined in Schemes 1 and 2, respectively. Boc-Lys(Z)-OH or Boc-Lys-

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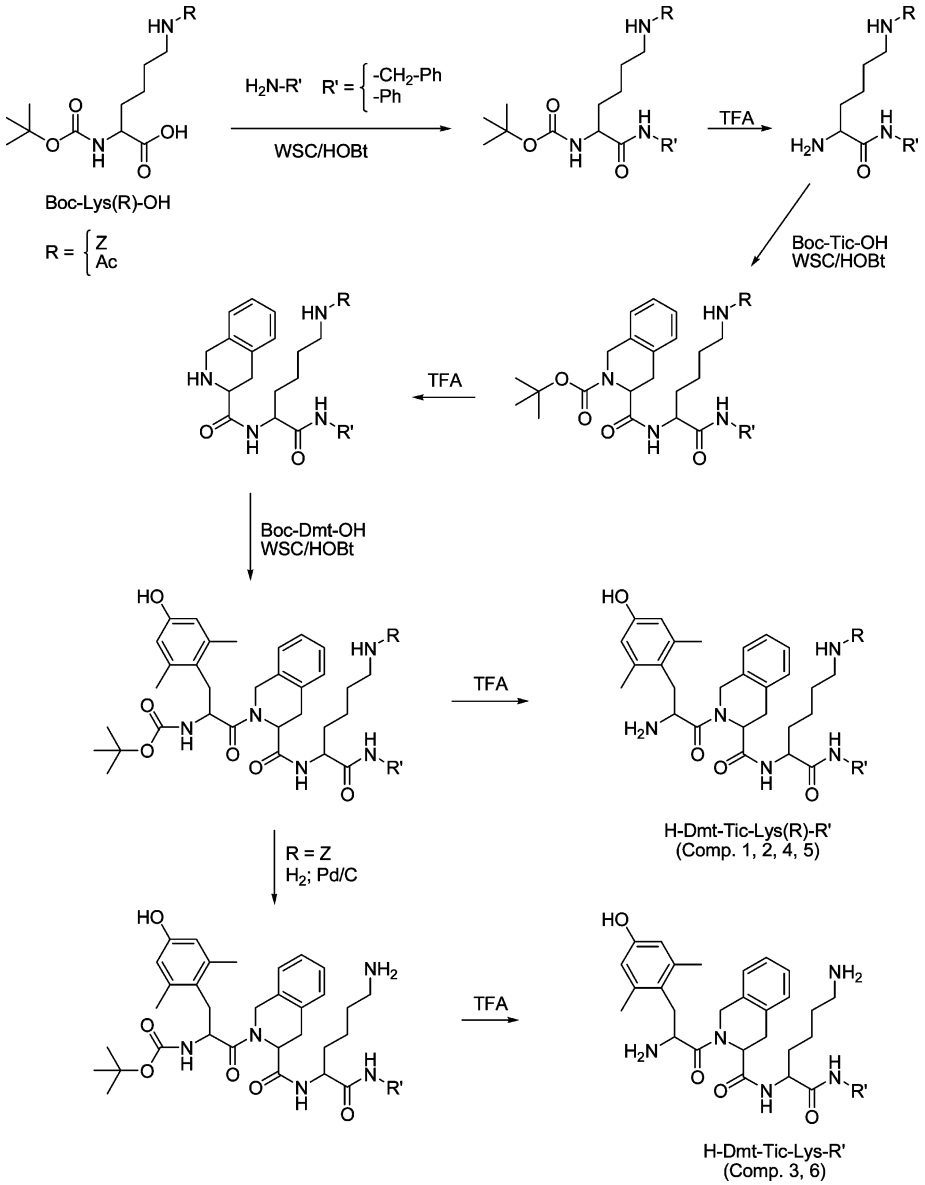
Scheme 1. Synthesis of Compounds **1**−**6**

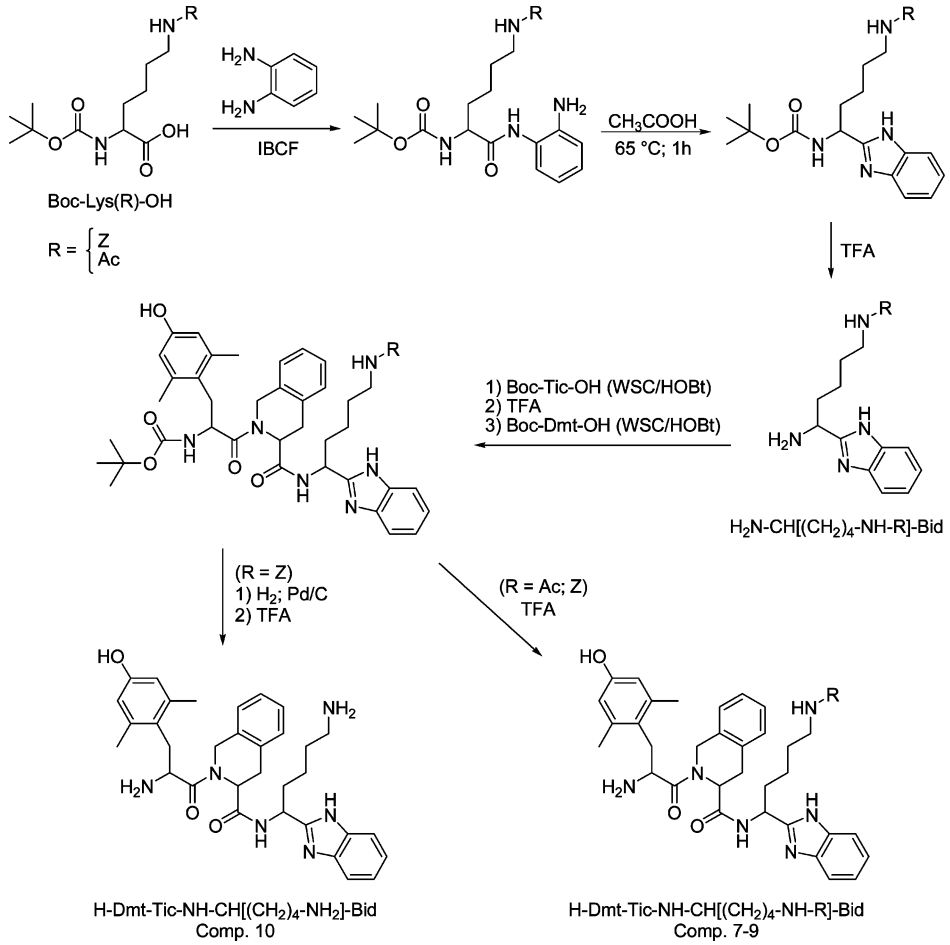


(Ac)-OH was condensed with benzylamine or aniline via WSC/HOBt. After N-terminal Boc deprotection with TFA, Lys side-chain-protected amides were condensed with Boc-Tic-OH via WSC/HOBt. N-terminal Boc-protected dipeptide amides were treated with TFA and condensed with Boc-Dmt-OH via WSC/HOBt. Final N-terminal Boc deprotection with TFA gave compounds (**1**, **2**, **4**, and **5**; Scheme 1). Catalytic hydrogenation (5% Pd/C) and TFA treatment of Boc-Dmt-Tic-Lys(Z)-amides gave the final products **3** and **6** (Scheme 1). Pseudopeptides (**7**−**10**), containing C-terminal 1*H*-benzimidazol-2-yl, were synthesized in a similar manner. Mixed carbonic anhydride coupling of Boc-Lys(Z)-OH or Boc-D-Lys(Z)-OH or Boc-Lys-(Ac)-OH with *o*-phenylendiamine gave the corresponding crude intermediate monoamides, which were converted without purification to the desired heteroaromatic derivatives by cyclization and dehydration in acetic acid, as outlined in Scheme 2. As detailed in Scheme 1, after N^{α} deprotection with TFA, each derivative was condensed with Boc-Tic-OH and then with Boc-Dmt-OH via WSC/HOBt. Final N-terminal Boc deprotection with TFA gave compounds (**7**−**9**; Scheme 2). Catalytic hydrogenation (5% Pd/C) and TFA treatment of Boc-Dmt-Tic-NH-CH[$(CH_2)_4$-NH-Z]-Bid gave the final product **10** (Scheme 2). Final compounds (**1**−**10**) were purified by preparative HPLC.

Results and Discussion

Receptor Affinity Analysis. Receptor binding and functional bioactivities are reported in Table 1. All new compounds (**1**−**10**) had subnanomolar affinity for δ-opioid receptors (K_i^{δ} = 0.068−0.64 nM). As expected, the lack of a free carboxylic function induces an increase in the μ-receptor affinity (K_i^{μ} = 0.13−5.50 nM).[18,31] In general, none of these compounds (**1**−**10**) are highly receptor selective; however, the benzyl amides (**1**−**3**) and phenyl amides (**4**−**6**) were slightly more selective for δ receptors, and **7**−**10**, containing Bid at the C-terminus, exhibited a modest selectivity for μ-opioid receptors. Selectivity (K_i^{μ}/K_i^{δ}) of pseudopeptides containing Bid at the C-terminus depends on the side-chain of the amino acid transformed in Bid. In fact, selectivity decreased from 122 in H-Dmt-Tic-NH-CH-(CH_2-COOH)-Bid (carboxylic function) to 14 in H-Dmt-Tic-NH-CH_2-Bid (no side chain) and further declined to 0.33 (K_i^{δ}/K_i^{μ} = 3.1) in **10** (H-Dmt-Tic-NH-CH[$(CH_2)_4$-NH_2]-Bid, with an amine function). The functionalization of the C-terminal carboxylic acid in H-Dmt-Tic-Lys(Ac)-OH with benzylamine

Scheme 2. Synthesis of Compounds **7**−**10**



(**2**), aniline (**5**), and Bid (**9**) caused a highly significant drop in selectivity by several orders of magnitude (276-, 4472-, and 31 057-fold, respectively).

Functional Bioactivity. Compounds (**1**−**10**) were tested in the electrically stimulated MVD and GPI assays for intrinsic functional bioactivity (Table 1). We and other investigators have previously discussed the discrepancy of the correlation between receptor binding affinities and functional bioactivity; unfortunately, we have neither definitive nor comprehensive answers for these observations.[18] Our data reveal that all of the analogues were inactive as δ opioid agonists in the MVD assay. Substitution of side-chain-protected or unprotected Lys in the δ agonists [H-Dmt-Tic-NH-CH_2-Bid and H-Dmt-Tic-NH-CH(CH_2-COOH)-Bid] and δ agonist/μ agonist (H-Dmt-Tic-Gly-NH-Ph) reference compounds caused a complete loss of δ agonist activity. The new compounds containing Bid at the C-terminus (**7**−**10**) show μ opioid agonism in the same order of magnitude as the endogenous μ agonist endomorphin-2.[32] The Lys side chain (unprotected or protected as an acetyl) in place of the Asp side chain is able to transform a selective δ agonist into selective μ agonists (**7**−**10**). Unexpectedly, and contrary to our preceding results,[31] the stereochemistry of Lys seems to be quite important; in fact, H-Dmt-Tic-NH-CH[$(CH_2)_4$-NH_2]-Bid is 6-fold less-active than the corresponding diastereoisomer containing D-Lys (**8**). Among the C-terminal phenyl amide compounds (**4**−**6**) having μ agonist activity in the μM range, only H-Dmt-Tic-Lys(Ac)-NH-Ph (**5**) is a very potent and selective δ antagonist (GPI, IC_{50} = 1248 nM; MVD, pA_2 = 12.0). Among C-terminal benzyl amide derivatives, **1** is almost inactive as an agonist or an antagonist; H-Dmt-Tic-Lys(Ac)-NH-CH_2-Ph is a nonselective δ and μ antagonist (MVD pA_2 = 10.4; GPI pA_2 = 8.16). Finally, **3** (H-Dmt-Tic-Lys-NH-CH_2-Ph) shows an interesting selective μ antagonist bioactivity (GPI, pA_2 = 7.96).

Conclusions

In light of the objectives of this study, we evaluated the possibility to improve the potency of some reference opioid compounds (δ agonists, μ agonist/δ agonist, and μ agonist/δ antagonist) through the substitution of Gly with a side-chain-unprotected or -protected (Z or Ac) Lys. Quite surprisingly, as seen in Table 1, while none of the new compounds confirmed our hypothesis (substitution of Gly with Lys could improve potency), considerably more interesting results were obtained. Starting from the prototype δ selective antagonist pharmacophore (Dmt-Tic) in our previous studies, we were able to transform this pharmacophore into selective δ agonists and vice versa, nonselective δ agonists/μ agonists and nonselective μ agonists/δ antagonists. The introduction of a C-terminal Lys residue further increased the versatility of this pharmacophore; in fact, we now obtained μ-selective agonists H-Dmt-Tic-NH-CH[$(CH_2)_4$-NH_2]-Bid (GPI; IC_{50} = 39.7 nM) and a μ-selective antagonist H-Dmt-Tic-Lys-NH-CH_2-Ph (GPI; pA_2 = 7.96) endowed with a potency comparable to the reference μ antagonists CTOP [D-Phe-c(Cys-Tyr-D-Trp-Orn-Thr-Pen)-Thr-NH_2] and CTAP [D-Phe-c(Cys-Tyr-D-Trp-Arg-Thr-Pen)-Thr-NH_2].[33] The μ-opioid antagonist H-Dmt-Tic-Lys-NH-CH_2-Ph could find wide use as a pharmacological tool in opioid research and may also have potential

Table 1. Receptor Binding and Functional Bioactivity

		receptor affinity[a]		selectivity	functional bioactivity			
comp	structure	$K_i\delta$ (nM)	$K_i\mu$ (nM)	$K_i\mu/K_i\delta$	MVD IC_{50} (nM)[b]	GPI IC_{50} (nM)[b]	MVD pA_2[c]	GPI pA_2[c]
	H-Dmt-Tic-Gly-OH	1.38 ± 0.09 (7)	529.0 ± 50 (6)	383			8.85	
	H-Dmt-Tic-Gly-NH-CH_2-Ph[d]	0.031	0.16	5.3		2.69	9.25	
	H-Dmt-Tic-Gly-NH-Ph[d]	0.042	0.16	3.6	3.02	2.57		
	H-Dmt-Tic-NH-CH_2-Bid[d]	0.035	0.50	14	0.13	26.92		
	H-Dmt-Tic-NH-CH(CH_2-COOH)-Bid[e]	0.443	53.9	122	0.12	1724		
	H-Dmt-Tic-Lys(Ac)-OH[f]	0.047	1051	22 361			10.07	
	H-Dmt-Tic-Phe-Lys(Z)-OH[g]	0.019	2.75	145			11.43	
	endomorphin-2					13.7		
	deltorphin-II				0.371			
1	H-Dmt-Tic-Lys(Z)-NH-CH_2-Ph	0.31 ± 0.02 (3)	4.41 ± 0.52 (5)	14	>10 000	>10 000	6.21	5.82
2	H-Dmt-Tic-Lys(Ac)-NH-CH_2-Ph	0.068 ± 0.009 (3)	5.50 ± 0.18 (3)	81	>10 000	>10 000	10.4	8.16
3	H-Dmt-Tic-Lys-NH-CH_2-Ph	0.50 ± 0.07 (4)	4.05 ± 0.54 (5)	8	>10 000	>10 000	6.01	7.96
4	H-Dmt-Tic-Lys(Z)-NH-Ph	0.57 ± 0.06 (3)	1.47 ± 0.20 (4)	3	>10 000	438 ± 65	5.77	
5	H-Dmt-Tic-Lys(Ac)-NH-Ph	0.13 ± 0.003 (4)	0.63 ± 0.065 (3)	5	>10 000	1248 ± 194	12.0	
6	H-Dmt-Tic-Lys-NH-Ph	0.42 ± 0.02 (4)	0.93 ± 0.05 (4)	2	>10 000	1254 ± 205	5.38	
7	H-Dmt-Tic-NH-CH[(CH_2)$_4$NH-Z]-Bid	0.64 ± 0.02 (3)	0.37 ± 0.040 (3)	1.7[h]	9049 ± 1128	375 ± 33	<5	
8	H-Dmt-Tic-NH-(*D*)-CH[(CH_2)$_4$NH-Z]-Bid	0.40 ± 0.08 (4)	0.15 ± 0.018 (3)	2.7[h]	>10 000	62.5 ± 13.5	<5	
9	H-Dmt-Tic-NH-CH[(CH_2)$_4$NH-Ac]-Bid	0.18 ± 0.03 (4)	0.13 ± 0.02 (4)	1.4[h]	>10 000	53.9 ± 7.3	n.a.[i]	
10	H-Dmt-Tic-NH-CH[(CH_2)$_4$$NH_2$]-Bid	0.49 ± 0.04 (3)	0.16 ± 0.015 (3)	3.1[h]	>10 000	39.7 ± 6.9	n.a.[i]	

[a] The K_i values (nM) were determined according to Chang and Prusoff.[39] The mean ± SE with *n* repetitions in parentheses is based on independent duplicate binding assays with five to eight peptide doses using several different synaptosomal preparations. [b] Agonist activity was expressed as IC_{50} obtained from dose−response curves. These values represent the mean ± SE for at least five to six fresh tissue samples. Deltorphin II and endomorphin-2 were the internal standards for MVD (δ-opioid receptor bioactivity) and GPI (μ-opioid receptor bioactivity) tissue preparation, respectively. [c] The pA_2 values of opioid antagonists against the agonists (deltorphin II and endomorphin-2) were determined by the method of Kosterlitz and Watt.[40] [d] Data taken from Balboni et al.[11] [e] Data taken from Balboni et al.[31] [f] Data taken from Balboni et al.[18] [g] Data taken from Balboni et al.[19] [h] μ Selectivity K_i^{δ}/K_i^{μ}. [i] n.a. = no antagonism.

as a therapeutic agent as in the regulation of food intake[34] and in the treatment of alcoholism.[35]

Experimental Section

Peptide Synthesis. Boc-Lys(Z)-NH-CH_2-Ph.[42] To a solution of Boc-Lys(Z)-OH (0.23 g, 0.62 mmol) and benzylamine (0.07 mL, 0.62 mmol) in DMF (10 mL) at 0 °C, HOBt (0.10 g, 0.68 mmol) and WSC (0.13 g, 0.68 mmol) were added. The reaction mixture was stirred for 3 h at 0 °C and 24 h at room temperature. After DMF was evaporated, the residue was dissolved in EtOAc and washed with citric acid (10% in H_2O), $NaHCO_3$ (5% in H_2O), and brine. The organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was precipitated from Et_2O/Pe (1:9, v/v): yield 0.26 g (89%); R_f (B) 0.94; HPLC K' 5.55; mp 93−95 °C; $[\alpha]^{20}_D$ −12.1; *m*/*z* 471 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.79 (m, 15H), 2.96 (t, 2H), 4.46−4.53 (m, 3H), 5.34 (s, 2H), 7.06−7.19 (m, 10H).

TFA·H-Lys(Z)-NH-CH_2-Ph. Boc-Lys(Z)-NH-CH_2-Ph (0.20 g, 0.43 mmol) was treated with TFA (1 mL) for 0.5 h at room temperature. Et_2O/Pe (1:1, v/v) were added to the solution until the product precipitated: yield 0.16 g (98%); R_f (A) 0.77; HPLC K' 3.55; mp 112−114 °C; $[\alpha]^{20}_D$ −13.4; *m*/*z* 371 $(M + H)^+$.

Boc-Tic-Lys(Z)-NH-CH_2-Ph. To a solution of Boc-Tic-OH (0.13 g, 0.46 mmol) and TFA·H-Lys(Z)-NH-CH_2-Ph (0.22 g, 0.46 mmol) in DMF (10 mL) at 0 °C, NMM (0.05 mL, 0.46 mmol), HOBt (0.07 g, 0.51 mmol), and WSC (0.09 g, 0.51 mmol) were added. The reaction mixture was stirred for 3 h at 0 °C and 24 h at room temperature. After DMF was evaporated, the residue was dissolved in EtOAc and washed with citric acid (10% in H_2O), $NaHCO_3$ (5% in H_2O), and brine. The organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was precipitated from Et_2O/Pe (1:9, v/v): yield 0.23 g (80%); R_f (B) 0.82; HPLC K' 5.61; mp 105−107 °C; $[\alpha]^{20}_D$ −18.2; *m*/*z* 630 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.79 (m, 15H), 2.92−3.17 (m, 4H), 4.17−4.53 (m, 5H), 4.92−5.34 (m, 3H), 6.96−7.19 (m, 14H).

TFA·H-Tic-Lys(Z)-NH-CH_2-Ph. Boc-Tic-Lys(Z)-NH-CH_2-Ph (0.17 g, 0.27 mmol) was treated with TFA (1 mL) for 0.5 h at room temperature. Et_2O/Pe (1:1, v/v) were added to the solution until the product precipitated: yield 0.16 g (96%); R_f (A) 0.49; HPLC K' 4.28; mp 118−120 °C; $[\alpha]^{20}_D$ −20.3; *m*/*z* 530 $(M + H)^+$.

Boc-Dmt-Tic-Lys(Z)-NH-CH_2-Ph. To a solution of Boc-Dmt-OH (0.10 g, 0.32 mmol) and TFA·H-Tic-Lys(Z)-NH-CH_2-Ph (0.21 g, 0.32 mmol) in DMF (10 mL) at 0 °C, NMM (0.03 mL, 0.32 mmol), HOBt (0.05 g, 0.35 mmol), and WSC (0.07 g, 0.35 mmol) were added. The reaction mixture was stirred for 3 h at 0 °C and 24 h at room temperature. After DMF was evaporated, the residue was dissolved in EtOAc and washed with citric acid (10% in H_2O), $NaHCO_3$ (5% in H_2O), and brine. The organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was precipitated from Et_2O/Pe (1:9, v/v): yield 0.25 g (95%); R_f (B) 0.78; HPLC K' 5.41; mp 132−134 °C; $[\alpha]^{20}_D$ −17.1; *m*/*z* 821 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.79 (m, 15H), 2.35 (s, 6H), 2.92−3.17 (m, 6H), 4.41−4.53 (m, 5H), 4.92−5.34 (m, 4H), 6.29 (s, 2H), 6.96−7.19 (m, 14H).

TFA·H-Dmt-Tic-Lys(Z)-NH-CH_2-Ph (1). Boc-Dmt-Tic-Lys(Z)-NH-CH_2-Ph (0.19 g, 0.23 mmol) was treated with TFA (1 mL) for 0.5 h at room temperature. Et_2O/Pe (1:1, v/v) was added to the solution until the product precipitated: yield 0.16 g (96%); R_f (A) 0.45; HPLC K' 4.81; mp 128−130 °C; $[\alpha]^{20}_D$ −15.1; *m*/*z* 721 $(M + H)^+$;^{1}H NMR (DMSO-d_6) δ 1.29−1.79 (m, 6H), 2.35 (s, 6H), 2.92−3.17 (m, 6H), 3.95−4.53 (m, 4H), 4.92−5.34 (m, 3H), 6.29 (s, 2H), 6.96−7.19 (m, 14H).

Boc-Lys(Ac)-NH-CH_2-Ph. This compound was obtained by condensation of Boc-Lys(Ac)-OH with benzylamine via WSC/HOBt, as reported for Boc-Lys(Z)-NH-CH_2-Ph: yield 0.32 g (82%); R_f (B) 0.88; HPLC K' 3.76; mp 101−103 °C; $[\alpha]^{20}_D$ −13.0; *m*/*z* 379 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.79 (m, 15H), 2.02 (s, 3H), 3.20−4.53 (m, 5H), 7.06−7.14 (m, 5H).

TFA·H-Lys(Ac)-NH-CH_2-Ph. Boc-Lys(Ac)-NH-CH_2-Ph was treated with TFA, as reported for TFA·H-Lys(Z)-NH-CH_2-Ph: yield

0.19 g (98%); R_f (A) 0.74; HPLC K' 2.14; mp 120−122 °C; $[\alpha]^{20}_D$ −14.3; *m/z* 279 $(M + H)^+$.

Boc-Tic-Lys(Ac)-NH-CH_2-Ph. This compound was obtained by condensation of Boc-Tic-OH with TFA·H-Lys(Ac)-NH-CH_2-Ph via WSC/HOBt, as reported for Boc-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.46 g (83%); R_f (B) 0.76; HPLC K' 5.02; mp 111−113 °C; $[\alpha]^{20}_D$ −19.1; *m/z* 537 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.79 (m, 15H), 2.02 (s, 3H), 3.05−3.20 (m, 4H), 4.22−4.92 (m, 7H), 4.92−5.34 (m, 3H), 6.96−7.14 (m, 9H).

TFA·H-Tic-Lys(Ac)-NH-CH_2-Ph. Boc-Tic-Lys(Ac)-NH-CH_2-Ph was treated with TFA, as reported for TFA·H-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.32 g (98%); R_f (A) 0.46; HPLC K' 3.02; mp 124−126 °C; $[\alpha]^{20}_D$ −21.2; *m/z* 437 $(M + H)^+$.

Boc-Dmt-Tic-Lys(Ac)-NH-CH_2-Ph. This compound was obtained by condensation of Boc-Dmt-OH with TFA·H-Tic-Lys(Ac)-NH-CH_2-Ph via WSC/HOBt. as reported for Boc-Dmt-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.15 g (85%); R_f (B) 0.72; HPLC K' 4.94; mp 138−140 °C; $[\alpha]^{20}_D$ −18.0; *m/z* 729 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.79 (m, 15H), 2.02 (s, 3H), 2.35 (s, 6H), 3.05−3.20 (m, 6H), 4.46−4.53 (m, 5H), 4.92 (m, 3H), 6.29 (s, 2H), 6.96−7.14 (m, 9H).

TFA·H-Dmt-Tic-Lys(Ac)-NH-CH_2-Ph (2). Boc-Dmt-Tic-Lys-(Ac)-NH-CH_2-Ph was treated with TFA, as reported for TFA·H-Dmt-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.07 g (95%); R_f (A) 0.42; HPLC K' 3.62; mp 134−136 °C; $[\alpha]^{20}_D$ −16.0; *m/z* 629 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.79 (m, 6H), 2.02 (s, 3H), 2.35 (s, 6H), 3.05−3.20 (m, 6H), 3.95−4.53 (m, 6H), 4.92 (m, 1H), 6.29 (s, 2H), 6.96−7.14 (m, 9H).

Boc-Dmt-Tic-Lys-NH-CH_2-Ph. To a solution of Boc-Dmt-Tic-Lys(Z)-NH-CH_2-Ph (0.10 g, 0.12 mmol) in methanol (30 mL) was added Pd/C (10%, 0.07 g), and H_2 was bubbled for 1 h at room temperature. After filtration, the solution was evaporated to dryness. The residue was crystallized from Et_2O/Pe (1:9, v/v): yield 0.08 g (96%); R_f (B) 0.65; HPLC K' 4.99; mp 141−143 °C; $[\alpha]^{20}_D$ −18.3; *m/z* 687 $(M + H)^+$.

2TFA·H-Dmt-Tic-Lys-NH-CH_2-Ph (3). Boc-Dmt-Tic-Lys-NH-CH_2-Ph was treated with TFA, as reported for TFA·H-Dmt-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.07 g (95%); R_f (A) 0.39; HPLC K' 3.32; mp 147−149 °C; $[\alpha]^{20}_D$ −16.2; *m/z* 587 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.79 (m, 6H), 2.35 (s, 6H), 2.65−3.17 (m, 6H), 3.95−4.53 (m, 6H), 4.92 (m, 1H), 6.29 (s, 2H), 6.96−7.14 (m, 9H).

Boc-Lys(Z)-NH-Ph. This compound was obtained by condensation of Boc-Lys(Z)-OH with aniline via WSC/HOBt, as reported for Boc-Lys(Z)-NH-CH_2-Ph: yield 0.23 g (82%); R_f (B) 0.89; HPLC K' 5.15; mp 90−92 °C; $[\alpha]^{20}_D$ −15.2; *m/z* 456 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.89 (m, 15H), 2.96 (t, 2H), 4.53−5.34 (m, 3H), 7.00−7.64 (m, 10H).

TFA·H-Lys(Z)-NH-Ph. Boc-Lys(Z)-NH-Ph was treated with TFA, as reported for TFA·H-Lys(Z)-NH-CH_2-Ph: yield 0.13 g (97%); R_f (A) 0.65; HPLC K' 3.63; mp 110−112 °C; $[\alpha]^{20}_D$ −15.9; *m/z* 356 $(M + H)^+$.

Boc-Tic-Lys(Z)-NH-Ph. This compound was obtained by condensation of Boc-Tic-OH with TFA·H-Lys(Z)-NH-Ph via WSC/HOBt, as reported for Boc-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.26 g (88%); R_f (B) 0.77; HPLC K' 5.47; mp 97−99 °C; $[\alpha]^{20}_D$ −19.5; *m/z* 616 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.79 (m, 15H), 2.92−3.17 (m, 4H), 4.17−4.53 (m, 2H), 4.92−5.34 (m, 3H), 6.96−7.19 (m, 14H).

TFA·H-Tic-Lys(Z)-NH-Ph. Boc-Tic-Lys(Z)-NH-Ph was treated with TFA, as reported for TFA·H-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.17 g (98%); R_f (A) 0.44; HPLC K' 4.37; mp 114−116 °C; $[\alpha]^{20}_D$ −20.4; *m/z* 516 $(M + H)^+$.

Boc-Dmt-Tic-Lys(Z)-NH-Ph. This compound was obtained by condensation of Boc-Dmt-OH with TFA·H-Tic-Lys(Z)-NH-Ph via WSC/HOBt, as reported for Boc-Dmt-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.22 g (84%); R_f (B) 0.73; HPLC K' 5.44; mp 127−129 °C; $[\alpha]^{20}_D$ −16.4; *m/z* 807 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.89 (m, 15H), 2.35 (s, 6H), 2.92−3.17 (m, 6H), 4.41−4.53 (m, 3H), 4.92−5.34 (m, 4H), 6.29 (s, 2H), 6.96−7.64 (m, 14H).

TFA·H-Dmt-Tic-Lys(Z)-NH-Ph (4). Boc-Dmt-Tic-Lys(Z)-NH-Ph was treated with TFA, as reported for TFA·H-Dmt-Tic-Lys-(Z)-NH-CH_2-Ph: yield 0.13 g (93%); R_f (A) 0.38; HPLC K' 4.70; mp 124−126 °C; $[\alpha]^{20}_D$ −14.4; *m/z* 707 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.89 (m, 6H), 2.35 (s, 6H), 2.96−3.05 (m, 4H), 3.95−4.53 (m, 4H), 4.92−5.34 (m, 3H), 6.29 (s, 2H), 6.96−7.64 (m, 14H).

Boc-Lys(Ac)-NH-Ph. This compound was obtained by condensation of Boc-Lys(Ac)-OH with aniline via WSC/HOBt, as reported for Boc-Lys(Z)-NH-CH_2-Ph: yield 0.35 g (92%); R_f (B) 0.83; HPLC K' 3.85; mp 96−98 °C; $[\alpha]^{20}_D$ −16.1; *m/z* 365 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.89 (m, 15H), 2.02 (s, 3H), 3.20−4.53 (m, 3H), 7.00−7.64 (m, 5H).

TFA·H-Lys(Ac)-NH-Ph. Boc-Lys(Ac)-NH-Ph was treated with TFA, as reported for TFA·H-Lys(Z)-NH-CH_2-Ph: yield 0.21 g (98%); R_f (A) 0.62; HPLC K' 2.31; mp 116−118 °C; $[\alpha]^{20}_D$ −16.8; *m/z* 265 $(M + H)^+$.

Boc-Tic-Lys(Ac)-NH-Ph. This compound was obtained by condensation of Boc-Tic-OH with TFA·H-Lys(Ac)-NH-Ph via WSC/HOBt, as reported for Boc-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.45 g (83%); R_f (B) 0.71; HPLC K' 4.86; mp 103−105 °C; $[\alpha]^{20}_D$ −20.4; *m/z* 523 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.89 (m, 15H), 2.02 (s, 3H), 3.05−3.20 (m, 4H), 4.22−4.92 (m, 5H), 6.96−7.64 (m, 9H).

TFA·H-Tic-Lys(Ac)-NH-Ph. Boc-Tic-Lys(Ac)-NH-Ph was treated with TFA, as reported for TFA·H-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.30 g (96%); R_f (A) 0.41; HPLC K' 3.28; mp 120−122 °C; $[\alpha]^{20}_D$ −21.3; *m/z* 423 $(M + H)^+$.

Boc-Dmt-Tic-Lys(Ac)-NH-Ph. This compound was obtained by condensation of Boc-Dmt-OH with TFA·H-Tic-Lys(Ac)-NH-Ph via WSC/HOBt, as reported for Boc-Dmt-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.14 g (80%); R_f (B) 0.67; HPLC K' 4.25; mp 133−135 °C; $[\alpha]^{20}_D$ −17.3; *m/z* 715 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.89 (m, 15H), 2.02 (s, 3H), 2.35 (s, 6H), 3.05−3.20 (m, 6H), 4.46−4.53 (m, 3H), 4.92 (m, 3H), 6.29 (s, 2H), 6.96−7.64 (m, 9H).

TFA·H-Dmt-Tic-Lys(Ac)-NH-Ph (5). Boc-Dmt-Tic-Lys(Ac)-NH-Ph was treated with TFA, as reported for TFA·H-Dmt-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.07 g (98%); R_f (A) 0.35; HPLC K' 3.73; mp 130−132 °C; $[\alpha]^{20}_D$ −15.3; *m/z* 615 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.89 (m, 6H), 2.02 (s, 3H), 2.35 (s, 6H), 3.05−3.20 (m, 6H), 3.95−4.53 (m, 4H), 4.92 (m, 3H), 6.29 (s, 2H), 6.96−7.64 (m, 9H).

Boc-Dmt-Tic-Lys-NH-Ph. Boc-Dmt-Tic-Lys(Z)-NH-Ph was treated with H_2 in the presence of Pd/C 10%, as reported for Boc-Dmt-Tic-Lys-NH-CH_2-Ph: yield 0.18 g (94%); R_f (B) 0.64; HPLC K' 4.71; mp 138−140 °C; $[\alpha]^{20}_D$ −17.8; *m/z* 673 $(M + H)^+$.

2TFA·H-Dmt-Tic-Lys-NH-Ph (6). Boc-Dmt-Tic-Lys-NH-Ph was treated with TFA, as reported for TFA·H-Dmt-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.05 g (92%); R_f (A) 0.34; HPLC K' 3.15; mp 149−151 °C; $[\alpha]^{20}_D$ −15.8; *m/z* 573 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.89 (m, 6H), 2.35 (s, 6H), 2.65−3.05 (m, 4H), 3.95−4.53 (m, 4H), 4.92 (m, 1H), 6.29 (s, 2H), 6.96−7.64 (m, 9H).

Benzyl 5-(*tert*-Butyl 3-carbamoyl-3,4-dihydroisoquinoline-2(1*H*)-carboxyloyl)-5-(1*H*-benzo[*d*]imidazol-2-yl)pentylcarbamate {Boc-Tic-NH-CH[$(CH_2)_4$-NH-Z]-Bid}. To a solution of Boc-Tic-OH (0.33 g, 1.20 mmol) and 2TFA·H_2N-CH[$(CH_2)_4$-NH-Z]-Bid [benzyl 5-amino-5-(1*H*-benzo[*d*]imidazol-2-yl)pentylcarbamate;[43] 0.70 g, 1.20 mmol] in DMF (10 mL) at 0 °C, NMM (0.26 mL, 2.40 mmol), HOBt (0.20 g, 1.32 mmol), and WSC (0.25 g, 1.32 mmol) were added. The reaction mixture was stirred for 3 h at 0 °C and 24 h at room temperature. After DMF was evaporated, the residue was dissolved in EtOAc and washed with $NaHCO_3$ (5% in H_2O) and brine. The organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was precipitated from Et_2O/Pe (1:9, v/v): yield 0.59 g (80%); R_f (B) 0.63; HPLC K' 4.94; mp 137−139 °C; $[\alpha]^{20}_D$ −13.3; *m/z* 613 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.84 (m, 15H), 2.92−3.17 (m, 4H), 4.17−4.87 (m, 3H), 4.92−5.34 (m, 3H), 6.96−7.70 (m, 13H).

2TFA·H-Tic-NH-CH[$(CH_2)_4$-NH-Z]-Bid. Boc-Tic-NH-CH-[$(CH_2)_4$-NH-Z]-Bid was treated with TFA, as reported for TFA·

H-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.43 g (97%); R_f (A) 0.45; HPLC K' 3.72; mp 140−142 °C; $[\alpha]^{20}_D$ −14.6; *m/z* 513 $(M + H)^+$.

Boc-Dmt-Tic-NH-CH[$(CH_2)_4$-NH-Z]-Bid. To a solution of Boc-Dmt-OH (0.10 g, 0.32 mmol) and 2TFA·H-Tic-NH-CH[$(CH_2)_4$-NH-Z]-Bid (0.24 g, 0.32 mmol) in DMF (10 mL) at 0 °C, NMM (0.07 mL, 0.64 mmol), HOBt (0.05 g, 0.35 mmol), and WSC (0.07 g, 0.35 mmol) were added. The reaction mixture was stirred for 3 h at 0 °C and 24 h at room temperature. After DMF was evaporated, the residue was dissolved in EtOAc and washed with $NaHCO_3$ (5% in H_2O) and brine. The organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was precipitated from Et_2O/Pe (1:9, v/v): yield 0.23 g (91%); R_f (B) 0.63; HPLC K' 4.88; mp 140−142 °C; $[\alpha]^{20}_D$ −14.8; *m/z* 804 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.84 (m, 15H), 2.35 (s, 6H), 2.92−3.17 (m, 6H), 4.41−4.87 (m, 3H), 4.92−5.34 (m, 4H), 6.29 (s, 2H), 6.96−7.70 (m, 13H).

2TFA·H-Dmt-Tic-NH-CH[$(CH_2)_4$-NH-Z]-Bid (7). Boc-Dmt-Tic-NH-CH[$(CH_2)_4$-NH-Z]-Bid was treated with TFA, as reported for TFA·H-Dmt-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.04 g (96%); R_f (A) 0.31; HPLC K' 3.90; mp 146−148 °C; $[\alpha]^{20}_D$ −18.3; *m/z* 704 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.84 (m, 6H), 2.35 (s, 6H), 2.96−3.05 (m, 6H), 3.95−4.46 (m, 3H), 4.87−5.34 (m, 4H), 6.29 (s, 2H), 6.96−7.70 (m, 13H).

Boc-Tic-NH-(*D*)-CH[$(CH_2)_4$-NH-Z]-Bid. This compound was obtained by condensation of Boc-Tic-OH with 2TFA·H_2N-(*D*)-CH-[$(CH_2)_4$-NH-Z]-Bid via WSC/HOBt, as reported for Boc-Tic-NH-CH[$(CH_2)_4$-NH-Z]-Bid: yield 0.56 g (83%); R_f (B) 0.64; HPLC K' 4.87; mp 139−141 °C; $[\alpha]^{20}_D$ +6.8; *m/z* 613 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.84 (m, 15H), 2.92−3.17 (m, 4H), 4.17−4.87 (m, 3H), 4.92−5.34 (m, 3H), 6.96−7.70 (m, 13H).

2TFA·H-Tic-NH-(*D*)-CH[$(CH_2)_4$-NH-Z]-Bid. Boc-Tic-NH-(*D*)-CH[$(CH_2)_4$-NH-Z]-Bid was treated with TFA, as reported for TFA·H-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.39 g (92%); R_f (A) 0.47; HPLC K' 3.71; mp 143−145 °C; $[\alpha]^{20}_D$ +7.5; *m/z* 513 $(M + H)^+$.

Boc-Dmt-Tic-NH-(*D*)-CH[$(CH_2)_4$-NH-Z]-Bid. This compound was obtained by condensation of Boc-Dmt-OH with 2TFA·H-Tic-NH-(*D*)-CH[$(CH_2)_4$-NH-Z]-Bid via WSC/HOBt, as reported for Boc-Dmt-Tic-NH-CH[$(CH_2)_4$-NH-Z]-Bid: yield 0.17 g (87%); R_f (B) 0.65; HPLC K' 5.17; mp 142−144 °C; $[\alpha]^{20}_D$ +4.9; *m/z* 804 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.84 (m, 15H), 2.35 (s, 6H), 2.92−3.17 (m, 6H), 4.41−4.87 (m, 3H), 4.92−5.34 (m, 4H), 6.29 (s, 2H), 6.96−7.70 (m, 13H).

2TFA·H-Dmt-Tic-NH-(*D*)-CH[$(CH_2)_4$-NH-Z]-Bid (8). Boc-Dmt-Tic-NH-(*D*)-CH[$(CH_2)_4$-NH-Z]-Bid was treated with TFA, as reported for TFA·H-Dmt-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.06 g (91%); R_f (A) 0.33; HPLC K' 3.90; mp 149−151 °C; $[\alpha]^{20}_D$ +5.6; *m/z* 704 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.84 (m, 6H), 2.35 (s, 6H), 2.96−3.05 (m, 6H), 3.95−4.46 (m, 3H), 4.87−5.34 (m, 4H), 6.29 (s, 2H), 6.96−7.70 (m, 13H).

Boc-NH-CH[$(CH_2)_4$-NH-Ac]-Bid. A solution of Boc-Lys(Ac)-OH (0.3 g, 1.04 mmol) and NMM (0.11 mL, 1.04 mmol) in DMF (10 mL) was treated at −20 °C with IBCF (0.14 mL, 1.04 mmol). After 10 min at −20 °C, *o*-phenylendiamine (0.11 g, 1.04 mmol) was added. The reaction mixture was allowed to stir while slowly warming to room temperature (1 h) and was then stirred for an additional 3 h. The solvent was evaporated, and the residue was partitioned between EtOAc and H_2O. The EtOAc layer was washed with 5% $NaHCO_3$ and brine and dried over Na_2SO_4. The solution was filtered, the solvent was evaporated, and the residual solid was dissolved in glacial AcOH (10 mL). The solution was heated at 65 °C for 1 h. After the solvent was evaporated, the residue was precipitated from Et_2O/Pe (1:9, v/v): yield 0.3 g (80%); R_f (B) 0.71; HPLC K' 3.03; mp 141−143 °C; $[\alpha]^{20}_D$ −7.6; *m/z* 362 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.84 (m, 15H), 2.02 (s, 3H), 3.20 (m, 2H), 4.87 (m, 1H), 7.26−7.70 (m, 4H).

2TFA·H_2N-CH[$(CH_2)_4$-NH-Ac]-Bid. Boc-NH-CH[$(CH_2)_4$-NH-Ac]-Bid was treated with TFA as reported for TFA·H-Lys(Z)-NH-CH_2-Ph: yield 0.16 g (90%); R_f (A) 0.48; HPLC K' 2.08; mp 149−151 °C; $[\alpha]^{20}_D$ −9.8; *m/z* 262 $(M + H)^+$.

Boc-Tic-NH-CH[$(CH_2)_4$-NH-Ac]-Bid. This compound was obtained by condensation of Boc-Tic-OH with 2TFA·H_2N-CH[$(CH_2)_4$-NH-Ac]-Bid via WSC/HOBt, as reported for Boc-Tic-NH-CH-[$(CH_2)_4$-NH-Z]-Bid: yield 0.45 g (85%); R_f (B) 0.57; HPLC K' 4.09; mp 143−145 °C; $[\alpha]^{20}_D$ −14.2; *m/z* 521 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.84 (m, 15H), 2.02 (s, 3H), 3.05−3.20 (m, 4H), 4.22−4.92 (m, 5H), 6.96−7.70 (m, 8H).

2TFA·H-Tic-NH-CH[$(CH_2)_4$-NH-Ac]-Bid. Boc-Tic-NH-CH-[$(CH_2)_4$-NH-Ac]-Bid was treated with TFA, as reported for TFA·H-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.31 g (97%); R_f (A) 0.42; HPLC K' 3.38; mp 146−148 °C; $[\alpha]^{20}_D$ −15.5; *m/z* 421 $(M + H)^+$.

Boc-Dmt-Tic-NH-CH[$(CH_2)_4$-NH-Ac]-Bid. This compound was obtained by condensation of Boc-Dmt-OH with 2TFA·H-Tic-NH-CH[$(CH_2)_4$-NH-Ac]-Bid via WSC/HOBt, as reported for Boc-Dmt-Tic-NH-CH[$(CH_2)_4$-NH-Z]-Bid: yield 0.13 g (78%); R_f (B) 0.57; HPLC K' 4.31; mp 150−152 °C; $[\alpha]^{20}_D$ −15.7; *m/z* 712 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.84 (m, 15H), 2.02 (s, 3H), 2.35 (s, 6H), 3.05−3.20 (m, 6H), 4.46 (s, 2H), 4.87−4.92 (m, 3H), 6.29 (s, 2H), 6.96−7.70 (m, 8H).

2TFA·H-Dmt-Tic-NH-CH[$(CH_2)_4$-NH-Ac]-Bid (9). Boc-Dmt-Tic-NH-CH[$(CH_2)_4$-NH-Ac]-Bid was treated with TFA, as reported for TFA·H-Dmt-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.06 g (93%); R_f (A) 0.28; HPLC K' 3.16; mp 153−155 °C; $[\alpha]^{20}_D$ −19.2; *m/z* 612 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.84 (m, 6H), 2.02 (s, 3H), 2.35 (s, 6H), 3.05−3.20 (m, 6H), 3.95−4.46 (m, 3H), 4.87−4.92 (m, 3H), 6.29 (s, 2H), 6.96−7.70 (m, 8H).

Boc-Dmt-Tic-NH-CH[$(CH_2)_4$-NH_2]-Bid. Boc-Dmt-Tic-NH-CH-[$(CH_2)_4$-NH-Z]-Bid was treated with H_2 in the presence of Pd/C 10%, as reported for Boc-Dmt-Tic-Lys-NH-CH_2-Ph: yield 0.17 g (92%); R_f (B) 0.52; HPLC K' 4.02; mp 147−149 °C; $[\alpha]^{20}_D$ −16.3; *m/z* 670 $(M + H)^+$.

3TFA·H-Dmt-Tic-NH-CH[$(CH_2)_4$-NH_2]-Bid (10). Boc-Dmt-Tic-NH-CH[$(CH_2)_4$-NH_2]-Bid was treated with TFA, as reported for TFA·H-Dmt-Tic-Lys(Z)-NH-CH_2-Ph: yield 0.03 g (93%); R_f (A) 0.29; HPLC K' 2.98; mp 156−158 °C; $[\alpha]^{20}_D$ −20.4; *m/z* 570 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.29−1.84 (m, 6H), 2.35 (s, 6H), 2.65−3.05 (m, 6H), 3.95−4.46 (m, 3H), 4.87−4.92 (m, 2H), 6.29 (s, 2H), 6.96−7.70 (m, 8H).

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Supporting Information Available: Chemistry general methods, biological general methods, and elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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(1) Abbreviations. In addition to the IUPAC−IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1985**, *260*, 14−42), this paper uses additional symbols and abbreviations, which are listed in Abbreviations.

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