Replacement of the *N*-terminal Tyrosine Residue in Opioid Peptides with 3-(2,6-Dimethyl-4-carbamoylphenyl)propanoic Acid (Dcp) Results in Novel Opioid Antagonists

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3-(2,6-Dimethyl-4-carbamoylphenyl)propanoic acid (Dcp), a 2′,6′-dimethyltyrosine analogue containing a carbamoyl group in place of the hydroxyl function and lacking the amino group, was synthesized. The replacement of Tyr[1] in an enkephalin analogue and in dynorphin A(1-11)-NH_2 with Dcp resulted in the first opioid peptide-derived antagonists that do not contain a phenolic hydroxyl group at the 1-position residue. The cyclic peptide Dcp-c[D-Cys-Gly-Phe(pNO_2)-D-Cys]NH_2 represents a novel, potent μ opioid antagonist.

Introduction

The *N*-terminal tyrosine residue of naturally occurring opioid peptides plays a key-role in the interaction with opioid receptors. The results of early studies indicated that deletion of the *N*-terminal amino group or of the Tyr[1] hydroxyl group in Met-enkephalin produced compounds with no activity or very weak opioid activity.[1,2] However, a number of recently performed structural modifications of the Tyr[1] residue of certain opioid peptides resulted in potent opioid agonists and antagonists. An interesting discovery was that the 2′,6′-dimethyl substitution of the Tyr[1] residue of opioid agonist peptides and the deletion of the positively charged *N*-terminal amino group resulted in opioid antagonists.[3−5] This required the substitution of Tyr[1] with 3-(2,6-dimethyl-4-hydroxyphenyl)propanoic acid (Dhp[a]) (Figure 1). The replacement of Tyr[1] in opioid peptides with the methylated Dhp analogues (2*S*)-2-methyl-3-(2,6-dimethyl-4-hydroxyphenyl)propanoic acid [(2*S*)-Mdp][6] or (3*S*)-3-methyl-3-(2,6-dimethyl-4-hydroxyphenyl)propanoic acid [(3*S*)-Mdp][7] produced even more potent opioid antagonists.[4,5,7]

Another interesting, recent observation was that the replacement of the Tyr[1] hydroxyl group in a cyclic enkephalin analogue with various substituents (−$CONH_2$, −$COCH_3$, −CH_3) resulted in compounds with high opioid agonist potency.[8] In this series of compounds, the highest potency was displayed by opioid peptide analogues containing *p*-carbamoylphenylalanine, also known as (4-carboxamido)phenylalanine (Cpa),[9] in place of Tyr[1].[8] Cpa[1]-analogues of a number of linear opioid peptides had also been reported to retain opioid agonist potencies comparable to those of their respective parent peptides.[9]

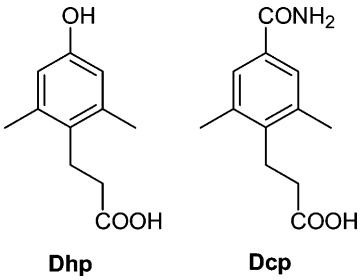

Figure 1. Structural formulas of Dhp and Dcp.

On the basis of these observations, it is of interest to determine how the replacement of the Dhp hydroxyl group in the Dhp[1]-containing opioid antagonist peptides with a carbamoyl group would affect the in vitro opioid activity profile. This can be achieved by substituting 3-(2,6-dimethyl-4-carbamoylphenyl)-propanoic acid (Dcp) (Figure 1) for Dhp. Here, we describe the synthesis of Dcp and the preparation and pharmacological characterization in vitro of Dcp[1]-analogues of the cyclic opioid peptide H-Tyr-c[D-Cys-Gly-Phe(pNO_2)-D-Cys]NH_2[10] and dynorphin A(1-11)-NH_2 (Dyn A(1-11)-NH_2.

The preparation of Dcp (**12**) is depicted in Scheme 1. Iodination of 2,6-dimethylphenol afforded 4-iodo-2,6-dimethylphenol (**5**), which was converted to benzoic acid **6** via metal−halogen exchange. Acid **6** was then protected as ester **7** and further transformed to triflate **8** in excellent yields. Heck coupling of **8** with *tert*-butyl acrylate under microwave conditions afforded alkene **9**, the trans configuration of which was established by the measurement of the NMR coupling constant (16.4 Hz) of the two alkene protons. Ester **9** was converted to amide **10** by treatment with ammonia. Subsequent catalytic hydrogenation, followed by standard TFA removal of *tert*-butyl yielded Dcp **12**.

For the preparation of the cyclic opioid peptide analogues Dcp-c[D-Cys-Gly-Phe(pNO_2)-D-Cys]NH_2 (**1**) and Dhp-c[D-Cys-Gly-Phe(pNO_2)-D-Cys]NH_2 (**2**), the linear precursor peptide of H-c[D-Cys-Gly-Phe(pNO_2)-D-Cys]NH_2 was synthesized by the solid-phase technique with Acm protection of the Cys side chains, and disulfide bond formation was carried out in solution

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[a] Abbreviations: Acm, acetamidomethyl; DAMGO, H-Tyr-D-Ala-Gly-N$^{\alpha}$MePhe-Gly-ol; DCI, 1,3-diisopropylcarbodiimide; Dcp, 3-(2,6-dimethyl-4-carbamoylphenyl)propanoic acid; Dhp, 3-(2,6-dimethyl-4-hydroxyphenyl)-propanoic acid; DIEA, *N*,*N*-diisopropylethylamine; DPDPE, H-Tyr-c[D-Pen-Gly-Phe-D-Pen]OH; DSLET, H-Tyr-D-Ser-Gly-Phe-Leu-Thr-OH; Dyn A, dynorphin A; GPI, guinea pig ileum; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; MVD, mouse vas deferens; NMM, *N*-methylmorpholine; TAPP, H-Tyr-D-Ala-Phe-Phe-NH_2; TFA, trifluoroacetic acid; U50,488, *trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide; U69,593, (5α,7α,8*β*)−(—)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]-dec-8-yl]benzeneacetamide. The symbols and abbreviations are in accordance with recommendations of the IUPAC−IUB Joint Commission on Biochemical Nomenclature and Symbolism for Amino Acids and Peptides. *Biochem J.* **1984**, *219*, 345−373.

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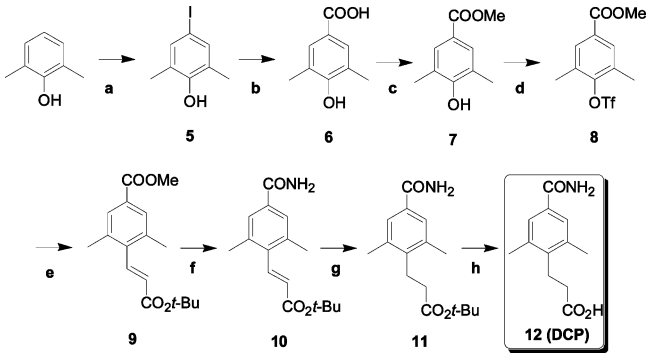
Scheme 1[a]



[a] Reagents and conditions: (a) KI, KIO_3, HCl, H_2O, rt, 2 h, 30%; (b) *n*-BuLi, THF, CO_2(g), −78 °C, 30 min, 41%; (c) H_2SO_4, MeOH, reflux, 15 h, 97%; (d) Tf_2O, Et_3N, CH_2Cl_2, −25 °C, 30 min, 91%; (e) CH_2═$CHCOOtBu$, $PdCl_2(Ph_3)_2$, CuI, KI, Et_3N, DMF, MW 110 °C, 50 min, 94%; (f) NH_3(g), MeOH, rt, 72 h, 76%; (g) H_2(g), Pd/C, EtOAc, 60 °C, 60 psi, 2 h, 98%; (h) TFA, rt, 1 h, 89%.

with iodine as the oxidation reagent. The exocyclic Dcp or Dhp was then attached to the cyclic tetrapeptide in solution. [Dcp[1]]-dynorphin A(1-11)-NH_2 was prepared by solid-phase synthesis. All final products were purified by preparative HPLC

Results and Discussion

The cyclic peptide analogue Dcp-c[D-Cys-Gly-Phe(*p*NO_2)-D-Cys]NH_2 (**1**) displayed high μ opioid antagonist activity (K_e^{μ} = 3.92 ± 0.48 nM) in the GPI assay (Table 1). It was a relatively weak κ antagonist (K_e^{κ} = 74.1 ± 9.4 nM) in that same assay and a weak δ antagonist (K_e^{δ} = 176 ± 22 nM) in the MVD assay. Thus, it showed quite high μ versus κ and μ versus δ receptor selectivity in the functional assays, as indicated by the K_e ratio. The high μ antagonist activity of **1** is in agreement with the high μ receptor affinity of this compound (K_i^{μ} = 2.84 ± 0.28 nM) determined in the μ receptor binding assay (Table 2). The low κ and δ receptor affinities shown by **1** in the binding assays are also in tune with its low κ and δ antagonist potencies determined in the functional assays. Consequently, this compound also showed considerable μ receptor selectivity in the binding assays (Table 2). Thus, cyclic peptide Dcp-c[D-Cys-Gly-Phe(*p*NO_2)-D-Cys]NH_2 (**1**) represents a novel μ-selective opioid antagonist with high potency.

In comparison with the parent peptide Dhp-c[D-Cys-Gly-Phe(*p*NO_2)-D-Cys]NH_2 (**2**), the Dcp[1]-analogue (**1**) displayed similar μ antagonist activity in the GPI assay and about a 2-fold higher μ receptor binding affinity. However, the Dhp[1]-analogue (**2**) showed about 3-fold higher κ and δ antagonist potencies than **1** in the functional assays and about 3-fold higher κ and δ receptor binding affinities. Therefore, the Dcp[1]-analogue (**1**) turned out to be more μ-selective than the Dhp[1]-parent (**2**), as indicated by the K_e and K_i ratios (Tables 1 and 2). In comparison with the nonselective opioid antagonist naloxone and with the μ-selective antagonist CTOP,[11] compound **1** has about the same high μ receptor binding affinity but is less μ-selective than CTOP.

The Dcp[1]-analogue of Dyn A(1-11)-NH_2 (**3**) showed moderate κ antagonist activity in the GPI assay with a K_e value of 30.7 ± 3.8 nM. It was a weak μ antagonist, and its κ vs μ receptor selectivity ratio was 17 in the functional assay (Table 1). Interestingly, it turned out to be a weak partial δ agonist (*e* = 0.33) in the MVD assay. In comparison with the Dhp[1]-analogue of Dyn A(1-11)-NH_2 (**4**), [Dcp[1]]Dyn A(1-11)-NH_2 (**3**) was a 10-fold weaker κ antagonist but was about equipotent as a μ antagonist. In agreement with these results, **3** showed about 10-fold lower κ receptor binding affinity compared to that of **4** and comparable μ receptor binding affinity (Table 2). In the δ receptor binding assay, **3** and **4** displayed low δ affinities of similar magnitude. As indicated by the K_i ratios (Table 2), [Dcp[1]]-Dyn A(1-11)-NH_2 (**3**) is less κ-selective than [Dhp[1]]Dyn A(1-11)-NH_2 (**4**). Taken together, the results of the functional assays and receptor binding assays indicated that the replacement of Dhp in Dyn A(1-11)-NH_2 parent peptide **4** with Dcp resulted in a 10-fold drop of κ antagonist potency and reduced κ receptor selectivity, mainly as a consequence of the decrease in κ receptor binding affinity. In comparison with the nonpeptide κ antagonist nor-binaltorphimine,[12] compound **3** is less potent and less κ-selective.

The data described above indicate that the replacement of Dhp with Dcp in the two opioid peptide analogues had no effect on μ receptor binding affinity but resulted in a decrease of κ and δ affinity. These results suggest that at the μ receptor, the −$CONH_2$ group of Dcp contributes to receptor binding to the same extent as the −OH group of Dhp. Both the −$CONH_2$ group and the −OH group have the capability to strengthen binding through H-bond donation to an acceptor site on the receptor. The decrease in κ and δ receptor binding affinity observed with the Dcp[1]-peptide analogues may be due to steric interference at these receptors because the −$CONH_2$ group is bulkier than the −OH group. Furthermore, it is possible that the −$CONH_2$ group may be unable to engage in proper hydrogen bond formation or that hydrogen bonding via one or both −NH_2 hydrogens or via the carbonyl moiety may produce a slight misalignment of the Dcp side chain relative to the position of the Dhp side chain at the κ and δ receptors. It is interesting to note that the replacement of the Dhp hydroxyl group of Dhp-c[D-Cys-Gly-Phe(*p*NO_2)-D-Cys]NH_2 (**2**) with the carbamoyl group had no effect on μ antagonist potency, whereas the corresponding substitution of a carbamoyl group for the Tyr[1] hydroxyl group in the agonist peptide H-Tyr-c[D-Cys-Gly-Phe-(*p*NO_2)-D-Cys]NH_2 produced a 15-fold drop in μ agonist potency.[8] This difference may be explained by slightly different modes of μ receptor binding of the Dcp[1]-antagonist peptide versus the Phe(*p*$CONH_2$)[1]-agonist peptide. The side chain of Dcp has greater conformational flexibility than that of Phe-(*p*$CONH_2$) in terms of rotation around the $C^{\alpha}-C^{\beta}$ and $C^{\alpha}-CO$ bonds, which could result in a different positioning of the aromatic ring moiety at the receptor binding site. Furthermore, the rotation of the 2,6-dimethyl-4-carbamoylphenyl ring in Dcp is impeded by the presence of the two methyl substituents, whereas the 4-carbamoylphenyl ring of Phe(*p*$CONH_2$) enjoys greater rotational freedom. This difference in rotational behavior may result in different hydrogen-bonding patterns of the −$CONH_2$ substituent with receptor moieties. Finally, it is also possible that the structural requirements of the active and inactive receptor conformations differ from one another with regard to the interaction of the *N*-terminal residue of the agonist peptide and the antagonist peptide. In this context, it is of interest to note that the replacement of the phenolic hydroxyl group in cyclazocine,[13] *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines,[14] and morphinan derivatives[15] with the −$CONH_2$ group had little effect on μ receptor binding. Thus, the carbamoyl group was a perfect surrogate for the hydroxyl group in these structurally rigid nonpeptide opioid agonists and antagonists.

In conclusion, we synthesized the first opioid peptide-derived antagonists that do not contain a phenolic hydroxyl group at the 1-position residue. The cyclic peptide Dcp-c-[D-Cys-Gly-Phe(*p*NO_2)-D-Cys]NH_2 represents a novel, potent μ opioid antagonist.

Table 1. Antagonist Potencies (K_e Values) of Dcp[1]- and Dhp[1]-Analogues of Opioid Peptides[a]

		GPI	GPI	MVD	K_e ratio	
no.	antagonist	K_e^{μ} (nM)[b]	K_e^{κ} (nM)[c]	K_e^{δ} (nM)[d]	$\mu/\kappa/\delta$	$\kappa/\mu/\delta$
1	Dcp-c[D-Cys-Gly-Phe(pNO$_2$)-D-Cys]NH$_2$	3.92 ± 0.48	74.1 ± 9.4	176 ± 22	1/19/45	
2	Dhp-c[D-Cys-Gly-Phe(pNO$_2$)-D-Cys]NH$_2$	3.68 ± 0.45	22.6 ± 3.0	63.3 ± 10.5	1/6/17	
3	[Dcp[1]]Dyn A(1-11)-NH$_2$	533 ± 110	30.7 ± 3.8	P. A. (33%)[e]		1/17/-
4	[Dhp[1]]Dyn A(1-11)-NH$_2$[f]	445 ± 102	2.96 ± 0.44	5310 ± 940		1/150/1790

[a] Values represent means of three to six determinations. [b] Determined with TAPP as μ agonist. [c] Determined with U50,488 as κ agonist. [d] Determined with DPDPE as δ agonist. [e] Partial agonist (maximal inhibition of contractions = 33%). [f] Data taken from Lu et al.[5]

Table 2. Binding Affinities of Dcp[1]- and Dhp[1]-Analogues of Opioid Peptides[a]

					K_i ratio	
no.	antagonist	K_i^{μ} (nM)	K_i^{κ} (nM)	K_i^{δ} (nM)	$\mu/\kappa/\delta$	$\kappa/\mu/\delta$
1	Dcp-c[D-Cys-Gly-Phe(pNO$_2$)-D-Cys]NH$_2$	2.84 ± 0.28	980 ± 170	25.8 ± 0.8	1/345/9	
2	Dhp-c[D-Cys-Gly-Phe(pNO$_2$)-D-Cys]NH$_2$	4.79 ± 0.39	299 ± 57	11.6 ± 1.1	1/62/2	
3	[Dcp[1]]Dyn A(1−11)-NH$_2$	44.9 ± 6.7	32.0 ± 5.0	129 ± 7		1/1/4
4	[Dhp[1]]Dyn A(1−11)-NH$_2$[b]	27.9 ± 3.3	3.49 ± 0.12	122 ± 24		1/8/35

[a] Values represent the means of three to six determinations. [b] Data taken from Lu et al.[5]

Experimental Section

Chemistry. Synthesis of Dcp (12). 4-Iodo-2,6-dimethylphenol (5). Concentrated HCl (35 mL, 420 mmol) was added slowly to 2,6-dimethylphenol (5.1 g, 42 mmol) in methanol (85 mL) in a cooling bath while keeping the temperature of the resulting solution below 30 °C. KI (4.6 g, 28 mmol) and KIO_3 (2.8 g, 13 mmol) in water (42 mL) were then added. The pale yellow solution immediately turned to a black precipitate upon addition. The flask was then kept in a fridge overnight. The precipitate was filtered, and the filtrate was purified with flash chromatography, eluting with hexanes and ethyl acetate (20:1) to give **5** as white fluffy needles (3.1 g, 30%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (s, 2H), 2.19 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.1, 137.0, 125.6, 82.3, 15.5. HRMS (ESI) *m*/*e* cacld for C_8H_9IO [M − H]$^-$ 246.9698; obsd, 246.9613.

4-Hydroxy-3,5-dimethylbenzoic Acid (6). A solution of **5** (3.32 g, 13.4 mmol) in anhydrous THF (48 mL) was cooled to −78 °C and treated with *n*-butyllithium (∼1.6 M) in *n*-hexanes (19 mL, 29.5 mmol) over 10 min. The reaction mixture was maintained at −78 °C for an additional 20 min and then bubbled with dry CO_2 for another 30 min. The reaction mixture was carefully quenched with saturated Na_2CO_3 (2.0 mL). The volatiles and solvent were removed under reduced pressure and diluted with water (35 mL) and ethyl acetate (50 mL). The aqueous phase was washed with ethyl acetate (50 mL × 2) and then slowly acidified to pH 1 with concentrated HCl. The aqueous phase was extracted with ethyl acetate (3 × 50 mL), and the combined organic phases were dried over Na_2SO_4 and concentrated to give **6** as a white solid (0.90 g, 41%). ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ 7.67 (s, 2H), 2.28 (s, 6H). ^{13}C NMR (75 MHz, $(CD_3)_2CO$) δ 166.8, 157.4, 130.2, 123.5, 121.3, 15.4. HRMS (ESI) *m*/*e* cacld for $C_9H_{10}O_3$ [M − H]$^-$ 165.0630; obsd, 165.0555.

Methyl 4-hydroxy-3,5-dimethylbenzoate (7). A solution of **6** (1.71 g, 10.3 mmol) in methanol (34 mL) and concentrated H_2SO_4 (1.92 mL) was held at reflux for 15 h. The volatiles and solvent were removed under reduced pressure and diluted with ethyl acetate (50 mL). The aqueous phase was washed with water (30 mL), saturated $NaHCO_3$ (30 mL), and brine (30 mL). The aqueous phase was extracted with ethyl acetate (3 × 50 mL), and the combined organic phases were dried over Na_2SO_4 and concentrated to give **7** as a light beige solid (1.86 g, 97%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (s, 2H), 3.87 (s, 3H), 2.27 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.3, 156.5, 130.4, 122.9, 121.7, 51.8, 15.8. HRMS (ESI) *m*/*e* cacld for $C_{10}H_{12}O_3$ [M − H]$^-$ 179.0786; obsd, 179.0707.

Methyl 3,5-dimethyl-4-(trifluoromethylsulfonyloxy)benzoate (8). To a solution of **7** (2.00 g, 11.1 mmol) and Et_3N (3.1 mL, 22.2 mmol) in anhydrous CH_2Cl_2 (24 mL) at −78 °C was slowly added a solution of Tf_2O (2.45 mL, 14.5 mmol) in CH_2Cl_2 (4 mL). The reaction mixture was stirred for 30 min at −25 °C before CH_2Cl_2 (25 mL) was added. The resultant solution was washed with saturated $NaHCO_3$ (40 mL) and brine (40 mL). The organic layer was dried over $MgSO_4$ and concentrated to give a residue that was purified by column chromatography, eluting with hexanes and ethyl acetate (20:1) to give **8** as a colorless oil (3.15 g, 91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (s, 2H), 3.87 (s, 3H), 2.38 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.7, 149.7, 131.8, 131.0, 129.5, 118.5 (q, J = 320 Hz) 52.2, 16.9. HRMS (EI) *m*/*e* cacld for $C_{11}H_{11}F_3O_5S$ [M]$^+$ 312.0279; obsd, 312.0271.

Methyl (*E*)-4-(3-*tert*-butoxy-3-oxoprop-1-enyl)-3,5-dimethylbenzoate (9). To a solution of **8** (0.802 g, 2.57 mmol) in anhydrous and pre-degassed DMF (11.0 mL) were added $PdCl_2(Ph_3)_2$ (0.182 g, 0.26 mmol), CuI (0.162 g, 0.85 mmol), KI (1.15 g, 7.71 mmol), and Et_3N (2.2 mL). After degassing the resultant solution for another 5 min, *tert*-butyl acrylate (1.32 g, 10.3 mmol) was added into the sealed microwave tube and irradiated at 110 °C for 50 min. After dilution with ethyl acetate (15 mL) and water (15 mL), the organic phase was washed with water (20 mL), 1 N HCl (20 mL), and brine (20 mL). The organic layer was dried over $MgSO_4$ and concentrated to give a residue that was purified by column chromatography, eluting with hexanes and ethyl acetate (20:1) to give **9** as a pale yellow oil (0.702 g, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (s, 2H), 7.70 (d, 1H, J = 16.4 Hz), 6.00 (d, 1H, J = 16.4 Hz), 3.90 (s, 3H), 2.37 (s, 6H), 1.54 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.9, 165.6, 141.1, 138.8, 136.7, 129.1, 129.0, 126.8, 80.8, 52.0, 28.1, 20.9. HRMS (EI) *m*/*e* cacld for $C_{17}H_{22}O_4$ [M]$^+$ 290.1518; obsd 290.1517.

Butyl (*E*)-*tert*-3-(2,6-dimethyl-4-carbamoylphenyl)acrylate (10). A solution of **9** (2.86 g, 9.86 mmol) in methanol (60 mL) was saturated with NH_3 at 0 °C in a sealed tube. The resultant solution was stirred at room temperature for 72 h. The solution was filtered, and the solvent was removed under reduced pressure to give a white residue. The residue was purified by column chromatography, eluting with hexanes and ethyl acetate (1:1) to give **10** as a white flaky solid (2.06 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67 (d, 1H, J = 16.4 Hz), 7.48 (s, 2H), 6.38 (br s, 2H), 5.98 (d, 1H, J = 16.4 Hz), 2.34 (s, 6H), 1.53 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.5, 165.7, 141.1, 137.9, 137.0, 132.4, 126.9, 126.7, 80.9, 28.1, 21.0. HRMS (ESI) *m*/*e* cacld for $C_{16}H_{21}NO_3$ [M − H]$^-$: calcd, 275.1521; obsd, 275.1430.

***tert*-Butyl 3-(2,6-dimethyl-4-carbamoylphenyl)propanoate (11).** An argon purged reaction vessel was charged with **10** (550 mg, 2 mmol) and Pd/C (10%, 100 mg) in EtOAc (30 mL). The reaction vessel was then carefully pressurized to 60 psi with H_2 and heated at 60 °C for 2 h. The mixture was cooled to room temperature, vented with argon, and filtered through Celite. The filtrate was collected, and the solvent was removed under reduced pressure to yield **11** as a pure white solid (540 mg, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (s, 2H), 6.18 (s, 2H), 2.95−2.91 (t, 2H), 2.35 (s, 6H), 2.33−2.30 (t, 2H), 1.44 (s, 9H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 172.3, 170.0, 142.2, 136.9, 131.0, 127.3, 80.8, 34.3, 31.9, 28.3,

25.4, 20.0. HRMS (ESI) *m*/*e* calcd for $C_{16}H_{24}NO_3$ $[M + H]^+$ 278.1756; obsd, 278.1762.

3-(2,6-Dimethyl-4-carbamoylphenyl)propanoic Acid (12, Dcp). Compound **11** (400 mg, 1.43 mmol) was dissolved in TFA (5 mL) at 0 °C, and the solution was warmed to room temperature and stirred for 1 h. TFA was then removed, and the residue was triturated with dry ether. Compound **12** was obtained as a white solid (280 mg, 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.80 (s, 1H), 7.49 (s, 2H), 7.34 (s, 1H), 2.86−2.82 (t, 2H), 2.34−2.29 (t, 2H), 2.29 (s, 6H). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 174.5, 168.6, 141.5, 136.4, 132.3, 128.8, 127.7, 33.2, 25.3, 20.1, 20.0. HRMS (ESI) *m*/*e* calcd for $C_{12}H_{16}NO_3$ $[M + H]^+$ 222.1130; obsd, 222.1138.

Peptide Synthesis. H-c[D-Cys-Gly-Phe(*p*NO_2)-D-Cys]NH_2. The linear precursor peptide of H-c[D-Cys-Gly-Phe(*p*NO_2)-D-Cys]NH_2 was prepared by the manual solid-phase technique using Boc-protection of the α-amino group and Acm protection of the Cys side chain and DIC/HOBt as coupling agents. The peptide was assembled on a *p*-methylbenzhydrylamine resin (1.16 mM/g titratable amine, Bachem Bioscience, King of Prussia, PA) according to a published protocol.[5] The peptide was cleaved from the resin by HF/anisole treatment in the usual manner. After evaporation of the HF, the resin was extracted three times with Et_2O and subsequently three times with glacial AcOH. The crude peptide was obtained in solid form through lyophilization of the acetic acid extract in 95% yield. Disulfide bond formation was carried out with the crude peptide using a 10-fold excess of iodine in MeOH/H_2O (4:1) as the oxidation agent. After 30 min at room temperature, Dowex 2 × 8-400 ion-exchange resin (CH_3COO^- form) was added, and the reaction mixture was stirred for an additional 15 min. The mixture was then filtered, and the colorless filtrate was evaporated in vacuo to dryness. The residue was dissolved in glacial AcOH, filtered, and lyophilized. The crude peptide was obtained in solid form (55% yield) and was purified by preparative HPLC. HPLC *K*′ 4.73; TLC R_f 0.15 (I), R_f 0.70 (II); MS $[M + H]^+$ 471.

Dcp-c[D-Cys-Gly-Phe(*p*NO_2)-D-Cys]NH_2 (1) and Dhp-c[D-Cys-Gly-Phe(*p*NO_2)-D-Cys]NH_2 (2). To a solution of Dcp or Dhp (0.1 mmol), HBTU (38 mg, 0.1 mmol), and *N*,*N*-diisopropylethylamine (DIEA) (16.5 μL, 0.15 mmol) in 5 mL DMF were added H-c[D-Cys-Gly-Phe(*p*NO_2)-D-Cys]NH_2 × TFA (58.9 mg, 0.1 mmol) and NMM (11 μL, 0.1 mmol). After stirring for 30 min, the solvent was evaporated to dryness in vacuo, and the residue was extracted with 25 mL of AcOEt. After washing with 5% $KHSO_4$, saturated $NaHCO_3$, and brine, the organic phase was dried ($MgSO_4$), filtered, and evaporated to dryness in vacuo. The peptide was purified by reversed-phase HPLC.

Dcp-c[D-Cys-Gly-Phe(*p*NO_2)-D-Cys]NH_2 (1). HPLC *K*′ 2.95: TLC R_f 0.74 (I), R_f 0.82 (II); MS $[M + H]^+$ 674.

Dhp-c[D-Cys-Gly-Phe(*p*NO_2)-D-Cys]NH_2 (2). HPLC *K*′ 4.54; TLC R_f 0.81 (I), R_f 0.90 (II); MS $[M + H]^+$ 647.

[Dcp^1]Dyn A(1-11)-NH_2 (3). The peptide was prepared by the manual solid-phase technique by using the protocol described above for the synthesis of the linear precursor peptide of H-[D-Cys-Gly-Phe(*p*NO_2)-D-Cys]NH_2. Side chain protection was as follows: tosyl (Arg) and 2-chlorobenzyloxycarbonyl (Lys). HPLC *K*′ 5.77; TLC R_f 0.21 (I), R_f 0.46 (II); MS $[M + H]^+$ 1402.

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Supporting Information Available: Experimental details and refs 16−22. This material is available free of charge via the Internet at http://pubs.acs.org.

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