A New Structural Motif for μ-Opioid Antagonists

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On the basis of the structural features of the Dmt-Tic pharmacophore, a new motif leading to a fairly potent μ-opioid antagonist is described. This motif contains the 4-amino-1,2,4,5-tetrahydro-2-benzazepine-3-one skeleton as a substitute for the Tic residue, which provides the conformational constraint compatible with the μ-opioid receptor. The stereoselective synthesis of four stereoisomers is performed starting from homochiral 2′,6′-dimethyltyrosine (Dmt) and *o*-aminomethylphenylalanine.

Introduction

The use of the Tyr-Tic pharmacophore provided a powerful means to develop opioid peptide analogues that have selectivity for the μ- or δ-receptor, partial to full agonist or antagonist character, or interesting balanced μ-agonist/δ-antagonist properties.[1,2] These properties are influenced by very subtle structural variations.[3] Substitution of Tyr[1] by 2′,6′-dimethyltyrosine (Dmt) further facilitates μ- and δ-receptor recognition.[4−6] The dipeptide Dmt-Tic-OH is the shortest fragment having potent[5,7] to moderate δ-antagonist properties.[2,8] Modifications on the C-terminus of Dmt-Tic have resulted in analogues that display a variety of μ-/δ-selectivities and agonist/antagonist combinations. (Figure 1)[2,9,10]

From these studies it was concluded that a third aromatic nucleus determines δ-opioid agonist activity when it is located at a prescribed distance from the Dmt-Tic pharmacophore. However, very small modifications such as going from an amide to a urea bond can change drastically the pharmacological profile.[10] Very few modifications of the Tic residue are allowed without loss of potency.[2,8,11−13] Inversion of its chirality (D-Tic) resulted in a μ-selective agonist.[1,7]

We have designed a new structural motif **5** taking into account the observations described above, and we have investigated its binding affinity for and agonist/antagonist character at the opioid receptors. (Figure 2). The structure of **5** differs from the previously reported inactive analogue **6** by a reversal of the 4-amino-1,2,4,5-tetrahydro-2-benzazepine-3-one (Aba) ring: the Dmt chain is attached at the N^2 in **5** rather than at N^4 in **6**, thereby reversing the Aba amide bond orientation.[7]

In **5**, the Dmt carbonyl has been reduced, a modification that was shown to be allowed in TIPP.[14] This feature eliminates the slow cis/trans equilibrium that exists in Dmt-Tic containing peptides and that may influence receptor affinity by lowering the rotational barrier and thereby allowing an easier adaption of the bioactive conformation.[14,15] The central core contains an Aba skeleton, which constrains the conformation of the aromatic residue at position 2 of the peptidomimetic, a feature that was shown to be essential for the antagonist properties of Tic-containing peptides. The exocyclic phenylacetyl chain is intended to provide a three-atom spacer between the central residue and the exocyclic aromatic ring, as in **1** and **4**.

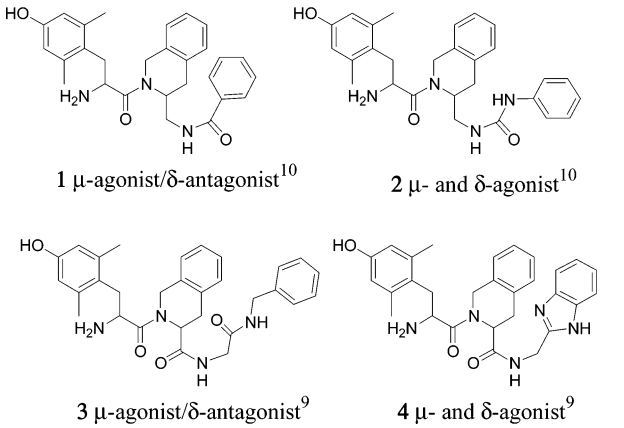

Figure 1. Dmt-Tic derivatives reported in the literature as opioid receptor ligands.

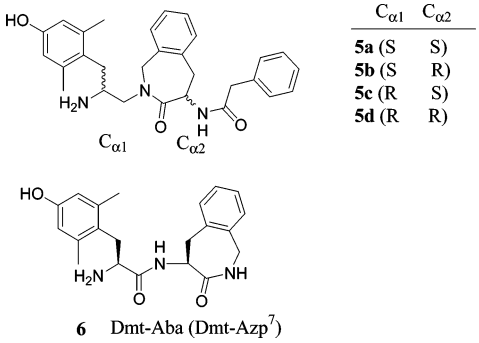

Figure 2. Structural differences between our target structures and the previously reported Dmt-Aba.

Results and Discussion

Chemistry. The synthesis of each of the four isomers of 4-Boc-amino-1,2,4,5-tetrahydro-2-[2-(Fmoc-amino)-

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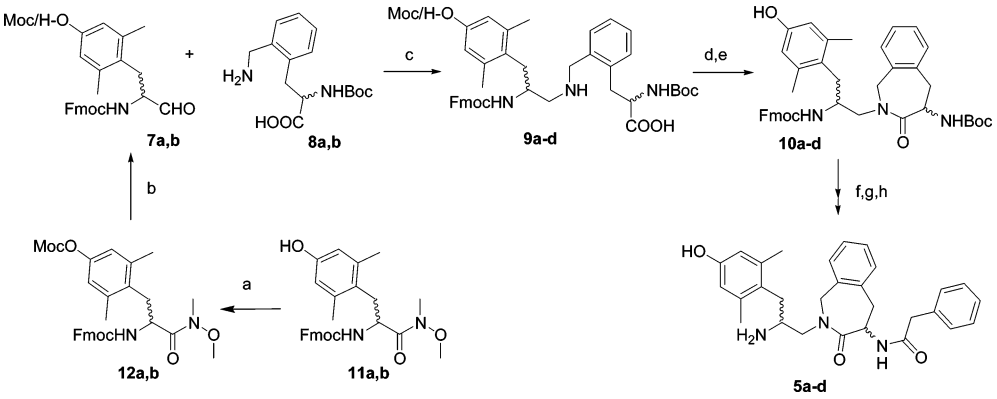
Scheme 1. Synthesis of Stereoisomers of **5**[a]



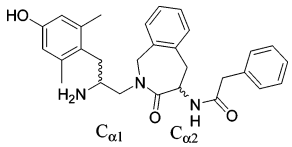
[a] Reagents and conditions: (a) benzene, DIEA (pH 7−8), methyl chloroformate, room temp, 4 h; (b) THF, $LiAlH_4$, 0 °C, 30 min (70% Moc cleavage); (c) $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, NMM, $MgSO_4$, room temp, 4 h; (d) TBTU, NMM, CH_2Cl_2, room temp, o.n.; (e) EtOH, 25% aqueous NH_3, 1 h 20min, room temp: (f) 95% TFA, room temp, 1 h, then $NaHCO_3$; (g) DCC, phenylacetic acid, 1.5 h, room temp; (h) DBU, DTT, THF, 5 h, room temp.

3-(4-hydroxy-2,6-dimethylphenyl)propyl]benzazepin-3-one (**10a**−**d**) having orthogonally protected amines was achieved stereoselectively starting from L- or D-*N*-Boc-*o*-aminomethylphenylalanine (Boc-*o*-Amp) (**8a**,**b**) and L- or D-Fmoc-Dmt(Moc)aldehyde (**7a**,**b**).[16,17] Reductive amination using $NaBH(OAc)_3$ gave intermediates **9a**−**d**, which were cyclized using 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluranium tetrafluoroborate (TBTU) to give four stereoisomers of **10a**−**d** (Scheme 1). The Boc-*o*-Amp enantiomers (**8a**,**b**) were prepared by reduction of Boc-(L or D)-*o*-cyanophenylalanine, which was obtained by stereoselective synthesis as described by us.[16] Fmoc-L- or D-Dmt-aldehyde (**7a**,**b**) was prepared from the corresponding methyloxycarbonyl (Moc)-protected Weinreb amide (**12a**,**b**) by lithium aluminum hydride reduction, which occurred mostly with Moc cleavage.[17] The reduction of the Weinreb amides **11** without phenol protection failed to give pure aldehyde.

After the cyclization and Moc deprotection, two epimeric products (**10**) were observed in a 3/1 ratio on HPLC−MS analysis. As described earlier,[16] this method for the preparation of tetrahydro-2-benzazepine-3-ones does not cause racemization at the α-carbon ($C_{\alpha 2}$) of the benzazepinone. Therefore, racemization at the α-carbon ($C_{\alpha 1}$) of Dmt must have occurred during preparation of the aldehyde[18] or during the reductive amination step.[19] The Moc-deprotected aminobenzazepinones **10** were purified, and the isomers were separated by semipreparative HPLC. After Boc deprotection, phenylacetic acid was coupled and Fmoc removal[20] gave the final products **5a**−**d**, which were purified by preparative HPLC. The purity (>95−99%) and composition of target compounds **5a**−**d** were respectively ascertained by analytical HPLC in two diverse systems and mass spectrometry (Supporting Information). The overall yield from Boc-*o*-Amp **8** to the final compounds (five steps) was 25%.

Receptor Binding Activity. Radioreceptor binding assays have been performed as described previously.[21] Binding affinities for μ- and δ-opioid receptors were determined by displacing, respectively, [^{3}H]naloxone and [^{3}H][Ile^{5,6}]deltorphin I from adult male Wistar rat brain membrane binding sites. Naltrexone hydrochloride was used to define nonspecific tissue binding. The data were analyzed by a nonlinear least-squares regression analysis program Prism Graph Pad.

Table 1. Binding Affinities of Stereoisomers of **5**



		K_i (nM)[a]		
compd	configuration $C_{\alpha 1}$, $C_{\alpha 2}$	[^{3}H]naloxone (μ)	[^{3}H][Ile^{5,6}]-deltorphin I (δ)	K_i ratio μ/δ
5a	*S,S*	3.3 ± 0.1	25.8 ± 2.3	1/7.8
5b	*S,R*	15.1 ± 1.2	684 ± 58	1/45.2
5c	*R,S*	23.5 ± 0.8	1096 ± 90	1/46.6
5d	*R,R*	41.7 ± 1.4	2895 ± 392	1/69.4

[a] Mean of three to five determinations ± SEM.

The obtained results are summarized in Table 1. Compounds **5a** and **5b**, having a L-Dmt-derived configuration at $C_{\alpha 1}$, display better affinities for the μ- and δ-opioid receptors than the epimers **5c**,**d**, which is in agreement with previous observations in Dmt-Tic compounds.[7] However, **5c**,**d** still have substantial affinity for the μ-receptor. The configuration at $C_{\alpha 2}$ also influences receptor affinity, the isomers **5b** and **5d** having a lower μ- and δ-affinity than **5a**,**c**. Compound **5a** is clearly the most potent analogue, with a K_i of 3.3 for the μ-receptor and of 25.8 nM for the δ-receptor. Its selectivity is, however, the lowest of all isomers.

Functional Bioactivity. To determine their in vitro opioid activities, compounds were tested in bioassays based on inhibition of electrically evoked contractions of the guinea pig ileum (GPI) and mouse vas deferens (MVD) as reported in detail elsewhere.[22] The GPI contains μ- and κ-opioid receptors and permits determination of μ- and κ-receptor-mediated agonist or antagonist effects. In the MVD assay, opioid effects are primarily mediated by δ-receptors; however, μ- and κ-receptors also exist in this tissue.[22] In both assays, none of the compounds showed agonist activity at concentrations up to 10 μM.

To determine the μ-antagonist potencies of the compounds, their K_e values against the specific μ-agonist TAPP (H-Tyr-D-Ala-Phe-Phe-NH_2) were measured in the GPI assay. K_e values of compounds with δ-receptor antagonist properties were determined in the MVD

Table 2. Antagonist Potencies of Stereosiomers of **5** Determined in the GPI and MVD Assays[a]

		GPA	MVD	
compd	configuration $C_{\alpha1}$, $C_{\alpha2}$	K_e (nM) TAPP	K_e (nM) DPDPE	K_e ratio μ/δ
	TIPP		4.8 ± 0.2	
5a	*S,S*	38.6 ± 4.2	318 ± 52	1/8
5b	*S,R*	207 ± 14	185 ± 20	1/0.6
5c	*R,S*	823 ± 86	1590 ± 180	1/2
5d	*R,R*	85.1 ± 4.9	1440 ± 30	1/17

[a] Mean of three to five determinations ± SEM.

assay against the δ-agonist DPDPE (H-Tyr-*c*[D-Pen-Gly-Phe-D-Pen]OH) (Table 2). All compounds showed antagonist properties in the GPI and in the MVD assay. Compound **5a**, with (*S*)-stereochemistry at both chiral carbons, is the most potent one and is a fairly potent μ-antagonist with an 8-fold selectivity versus the δ-receptor. In general, a good correlation between antagonist potencies and receptor binding affinities was observed except for **5d**, which showed higher μ-antagonist activity in the GPI assay than would be expected on the basis of its relatively weak μ-receptor binding affinity. This could be due to a possible difference in the structural requirements for ligand interactions between central (rat brain) and peripheral (GPI) μ-receptors.

Conclusions

On the basis of structural features of the Dmt-Tic pharmacophore, we have identified a new structural motif leading to a fairly potent μ-opioid antagonist. Similar structural considerations have led Pagé et al. to another new scaffold for μ-opioid ligands in which the Tic residue is still present.[24] In the stuctural motif described here, all stereoisomers of **5** are interacting more effectively with the μ-opioid receptor than with the δ-opioid receptor (Tables 1 and 2). Therefore, the 4-amino-1,2,4,5-tetrahydro-2-benzazepin-3-one ring imposes a conformational constraint that is more compatible with the μ-receptor than with the δ-receptor. It is important to note that the two amide bonds in **5** are reversed compared to those in the Dmt-Tic analogues. As a result, in the most potent isomer **5a**, the (*S*)-stereochemistry at $C_{\alpha2}$ corresponds to a topological arrangement of a D-amino acid in a normal peptide sequence.[25] The corresponding Dmt-Aba analogue **6**, having the usual orientation of the amide bond and of the Aba heterocycle and lacking the third aromatic nucleus, is reported by Lazarus (and named Dmt-Azp) to be completely inactive.[7] We believe that this new structural motif can lead to novel opioid peptide mimetics with interesting pharmacological profiles by replacing the phenylacetyl chain as reported by Pagé[10] and Balboni.[9]

Experimental Section

General Experimental Procedures. ^{1}H NMR were recorded on a Bruker Advance DRX 250 spectrometer at a ^{1}H frequency of 250 MHz. Chemical shifts are given in parts per million (ppm) relative to Me_4Si. The solvent used is indicated. Abbreviations used are as follows: s, singlet; bs, broad singlet; d, doublet; dd, double doublet; t, triplet; q, quadruplet; m, multiplet. Mass spectra were obtained on a VG Quattro II spectrometer (EPS ionization, cone voltage 70 V, capillary voltage 3.5 kV, source temperature 80 °C). Data collection was done with Masslynx software. LC−MS was performed by coupling of a Kontron 325 system to the mass spectrometer. This system was equipped with a UV detector (model 332, λ = 215 nm), automatic injector (model 465), and LC-6A type pumps. The runs were performed on a Supelco Discovery Bio Wide Pore column (C_{18}, 5 μm, d = 0.46 cm, l = 25 cm) at a flow rate of 1 mL/min and with a gradient from 97:3 to 20:80 water/CH_3CN containing 0.1% TFA over 30 min, followed by an isocratic run of 20:80 water/CH_3CN containing 0.1% TFA for 10 min, further referred to as system 1. A different HPLC system was equipped with a Agilent Zorbax Eclipse XDB column (C_8, 5 μm, d = 0.46 cm, l = 15 cm) with a gradient from 97:3 to 20:80 water/CH_3OH containing 0.1% TFA over 30 min, followed by an isocratic run at 20:80 water/CH_3OH containing 0.1% TFA for 10 min, further referred to as system 2. All final products were purified by reversed-phase chromatography on a Gilson system provided with a 322-H pump and run by a Unipoint software package and were isolated as their TFA salts. A Supelco Discovery Bio Wide Pore column (C_{18}, 10 μm, d = 21.2 mm, l = 25 cm) was used at a flow rate of 20 mL/min with a gradient from 97:3 to 20:80 water/CH_3CN containing 0.1% TFA over 30 min, followed by a 10 min isocratic run with 100% CH_3CN/0.1% TFA. Fmoc-L-Dmt was supplied by RSP (One Innovation Drive, Worcester, MA) and was 99% ee. Fmoc-D-Dmt was prepared as described in ref 26 with an ee of 95%, determined by N_α-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (FDAA) analysis.[27]

Syntheses and Characterizations. Boc-L-*o*-Amp (8a) or Boc-D-*o*-Amp(8b). Boc-L-*o*-cyano-Phe (99% ee)[16] or Boc-d-*o*-cyano-Phe (98.5% ee)[16] (2.0 g, 6.89 mmol) was dissolved in EtOH−H_2O (3:1, 70 mL) to which a 0.1 M AcOH solution (2 mL) was added, as well as 10% Pd on C (0.4 g, 20 wt %). After hydrogenation in a Parr apparatus (50 psi, room temp, 4 h), the mixture was filtered over dicalite and rinsed with EtOH. The filtrate was evaporated, and the residue was crystallized from hot EtOH (1.9 g, 95%, white crystals): mp 171.9−173.9 °C; MS *m/z* 295 $(M + H)^+$, calcd 295.35; HPLC system 1, t_R = 14.7 min; ^{1}H NMR (D_2O) δ 1.41 (9H, s, CH_3 Boc), 3.06 (1H, dd, CHβ), 3.34 (1H, dd, CHβ'), 4.27 (1H, dd, CHα), 4.40 (2H, s, CH_2N), 7.31−7.51 (4H, M, H_{arom}).

Fmoc-Dmt-*N*,*O*-Me$_2$ (11). Fmoc-L-Dmt-OH or Fmoc-D-Dmt-OH (0.5 g, 1.16 mmol) was dissolved in acetonitrile (60 mL). TBTU (0.372 g, 1.16 mmol), *N*,*O*-dimethylhydroxylamine·HCl (0.123 g, 1.28 mmol), and *N*-methylmorpholine (NMM) (0.351 g, 3.48 mmol) were added, and the mixture was stirred at room temperature. After 2 h, more *N*,*O*-dimethylhydroxylamine·HCl (0.246 g, 0.26 mmol) was added, and stirring was continued overnight. Then the volatiles were removed in vacuo, and a yellow oil was obtained. This oil was dissolved in $CHCl_3$ (60 mL) and was successively extracted with saturated $NaHCO_3$ (3 × 60 mL), 1 N HCl (3 × 60 mL), and saturated NaCl (1 × 20 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated in vacuo until an oil remained. The product was immediately used in the next reaction (**11a**, 550 mg, 100% yield; **11b**, 543 mg, 98% yield).**11a** and **11b**: MS *m/z* 475 $(M + H)^+$, calcd 474.22; HPLC system 1, t_R = 25.0 min; ^{1}H NMR ($CDCl_3$) δ 2.32 (6H, s, CH_3 Dmt), 2.90−3.14 (2H, m, $CH_{2\beta}$), 3.14 (3H, s, N−CH_3), 3.52 (3H, s, O−CH_3), 4.14−4.48 (4H, m, CH Fmoc, CH_2 Fmoc, CH_α), 5.07 (1H, m), 5.55 (1H, m), 6.25 (2H, s, H_{arom} Dmt), 7.30 (2H, t, H_{arom} Fmoc), 7.40 (2H, t, H_{arom} Fmoc), 7.55 (2H, m, H_{arom} Fmoc), 7.73 (2H, d, H_{arom} Fmoc).

Fmoc-Dmt(Moc)-*N*,*O*-Me$_2$ (12). Fmoc-L-Dmt-*N*,*O*-Me_2 (**11a**) or Fmoc-D-Dmt-*N*,*O*-Me_2 (**11b**) (0.55 g, 1.16 mmol) was dissolved in dry benzene (60 mL). DIEA (0.1557 g, 1.97 mmol) (pH 7−8) and methyl chloroformate (0.1199 g, 1.276 mmol) were added, and the mixture was stirred for 4 h. The solvent was evaporated, and the residue was dissolved in CH_2Cl_2. The organic layer was extracted with 1 N HCl (3 × 30 mL), dried over $MgSO_4$, and filtered. After evaporation in vacuo, a foam remained (**12a**, 597 mg, HPLC purity 90%, 97% yield; **12b**, 560 mg, HPLC purity 90%, 91% yield). **12a** and **12b**: MS *m/z* 533 $(M + H)^+$, calcd 532.22; HPLC system 1, t_R = 28.3 min; ^{1}H NMR ($CDCl_3$) δ 2.34 + 2.37 (6H, 2s, CH_3 Dmt), 2.91−3.14 (2H, m, $CH_{2\beta}$), 3.12 (3H, s, N−CH_3), 3.46 (3H, s, O−CH_3), 3.86 (3H, s, (C=O)OCH_3), 4.09−4.37 (4H, m, CH Fmoc, CH_2 Fmoc,

CH_α), 5.35 (1H, m), 6.81 + 6.86 (2H, s, H_{arom} Dmt), 7.25−7.45 (4H, m, H_{arom} Fmoc), 7.56 (2H, m, H_{arom} Fmoc), 7.76 (2H, d, H_{arom} Fmoc).

Fmoc-Dmt(Moc)-H (7). Fmoc-L-Dmt(Moc)-*N*,*O*-Me_2 (**12a**) or Fmoc-D-Dmt(Moc)-*N*,*O*-Me_2 (**12b**) (0.597 g, 1.12 mmol) was dissolved in THF (20 mL), and the solution was cooled to 0 °C. $LiAlH_4$ (63.8 mg, 1.68 mmol) was added portionwise over 5 min, and the mixture was stirred for 40 min at 0 °C. The reaction was quenched with 10% $KHSO_4$ (20 mL). The phases were separated, and the aqueous layer was extracted with EtOAc (70 mL). The EtOAc and THF layer were combined and were extracted successively with 1 N HCl (3 × 30 mL), saturated $NaHCO_3$ (3 × 30 mL), and saturated NaCl (1 × 20 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated in vacuo (**7a**, 484 mg, 91%; **7b**, 478 mg, 90%). The crude aldehyde was partially (70%) Moc-deprotected. MS *m/z* 474 $(M + H)^+$, 416 $(M + H)^+ - Moc$) calcd 473.18 and 415.18; HPLC gradient 1, t_R = 26.3 min + 23.6 min (−Moc). The aldehydes were used immediately in the reductive amination.

2-Boc-amino-3-(2-{[2-(Fmoc-amino)-3-(4-methoxycarbonyloxy-2,6-dimethylphenyl)propylamino]methyl}-phenyl)propionic acid (9a−d). Boc-(*S*)-*o*-Amp (**8a**) (99% ee)[16] or Boc-(*R*)-*o*-Amp (**8b**) (98.5% ee)[16] (0.150 g, 0.511 mmol) was dissolved in 1,2-dichloroethane (30 mL), and Fmoc-L-Dmt-(Moc)-H (**7a**) or Fmoc-D-Dmt(Moc)-H (**7b**) (0.242 g, 0.511 mmol), $Na(OAc)_3BH$ (0.152 g, 0.715 mmol), NEt_3 (0.051 g, 0.511 mmol), and $MgSO_4$ (0.060 g, 30 wt %) were added. The mixture was stirred at room temperature for 4 h. The reaction was quenched with saturated $NaHCO_3$ (30 mL), and the aqueous layer was extracted with EtOAc (3 × 30 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. **9a** ($C_{\alpha1}$ *S*, $C_{\alpha2}$ *S*): 0.339 g, 89% yield. **9b** ($C_{\alpha1}$ *S*, $C_{\alpha2}$ *R*): 0.327 g, 85% yield. **9c** ($C_{\alpha1}$ *R*, $C_{\alpha2}$ *S*): 0.329 g, 88% yield. **9d** ($C_{\alpha1}$ *R*, $C_{\alpha2}$ *R*): 0.299 g, 80% yield. MS *m/z* 752 $(M + H)^+$ calcd 751.35; HPLC system 1, t_R = 26.5 min for all isomers. The obtained products were immediately cyclized.[16]

3-[4-(Boc-amino)-1,2,4,5-tetrahydro-2-benzazepine-3-one-2-yl]-2-Fmoc-amino-1(2,6-dimethyl-4-hydroxyphenyl)-propane (10a−d). The crude open product (**9a**−**d**) (0.327 g, 0.435 mmol) was dissolved in 25 mL of CH_2Cl_2. TBTU (0.139 g, 0.435 mmol) and NMM (0.109 g, 1.087 mmol) were added, and the solution was stirred at room temperature overnight. The mixture was then diluted with 20 mL of CH_2Cl_2 and extracted successively with saturated $NaHCO_3$ (3 × 50 mL), 1 N HCl (1 × 100 mL), and saturated NaCl (1 × 50 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated in vacuo until an oil (283 mg, 89% crude yield) remained. The cyclic products (0.233 g, 0.318 mmol) were dissolved in 15 mL of EtOH. An aqueous ammonia solution (1/1, 5 mL) was added, and the mixtures were stirred for 1 h 20 min. The volatiles were evaporated, and HPLC−MS analysis revealed two peaks in a 3/1 ratio, corresponding to two epimeric compounds **10**. Purification and separation of the epimers were performed by semipreparative HPLC (purity, >99%), giving 15−25% yield of the major isomer over three steps. MS *m/z* 676 $(M + H)^+$, calcd 675.33; HPLC system 1, **10a** ($C_{\alpha1}$ *S*, $C_{\alpha2}$ *S*), **10d** ($C_{\alpha1}$ *R*, $C_{\alpha2}$ *R*), t_R = 33.7 min; **10b** ($C_{\alpha1}$ *S*, $C_{\alpha2}$ *R*), **10c** ($C_{\alpha1}$ *R*, $C_{\alpha2}$ *S*), t_R = 34.2 min; HPLC system 2, **10a** ($C_{\alpha1}$ *S*, $C_{\alpha2}$ *S*), **10d** ($C_{\alpha1}$ *R*, $C_{\alpha2}$ *R*), t_R = 38.0 min; **10b** ($C_{\alpha1}$ *S*, $C_{\alpha2}$ *R*), **10c** ($C_{\alpha1}$ *R*, $C_{\alpha2}$ *S*), t_R = 37.5 min; ^{1}H NMR ($CDCl_3$) **10a**,**d**, δ 1.41 (9H, s, CH_3 Boc), 2.19 (6H, s, CH_3 Dmt), 2.61 (1H, m, C^5H benzazep), 2.89 (1H, m, C^5H' benzazep), 3.12 (1H, m, CH_β Dmt), 3.40 (1H, m, CH'_β Dmt), 3.63−4.4 (6H, m, CH_2−N + CH_α Dmt + C^1H_2 benzazep + CH Fmoc), 4.93−5.28 (3H, m, OH + CH_2 Fmoc), 5.82 (1H, m, C^4H benzazep), 6.39 (2H, s, H_{arom}), 6.74−7.75 (12H, m, H_{arom}); ^{1}H NMR ($CDCl_3$) **10b**,**c**, δ 1.41 (9H, s, CH_3 Boc), 2.21 (6H, s, CH_3 Dmt), 2.60 (1H, m, C^5H benzazep), 2.96 (1H, m, C^5H' benzazep), 3.23 (1H, m, CH_β Dmt), 3.47 (1H, m, CH'_β Dmt), 3.70−4.50 (6H, m, CH_2−N + CH_α Dmt + C^1H_2 benzazep + CH Fmoc), 5.05−5.36 (3H, m, OH + CH_2 Fmoc), 5.91 (1H, m, C^4H benzazep), 6.47 (2H, s, H_{arom}), 6.88−7.73 (12H, m, H_{arom}).

3-[4(*R* or *S*)-Amino-1,2,4,5-tetrahydro-2-benzazepine-3-one-2-yl]-2(*R* or *S*)-Fmoc-amino-1(2,6-dimethyl-4-hydroxyphenyl)propane (13a−d). 10a (52.6 mg, 0.078 mmol), **10b** (65 mg, 0.096 mmol), **10c** (18.6 mg, 0.027 mmol), or **10d** (15 mg, 0.022 mmol) in a minimal amount of 95% TFA/5% H_2O was stirred at room temperature for 1 h. The mixtures were evaporated until a yellow paste remained. The residue was redissolved in EtOAc (50 mL) and extracted with saturated $NaHCO_3$ (3 × 50 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated until a yellow oil remained (89−94% yield). MS *m/z* 576 $(M + H)^+$, calcd 575.28; HPLC system 1, **13a** ($C_{\alpha1}$ *S*, $C_{\alpha2}$ *S*), **13d** ($C_{\alpha1}$ *R*, $C_{\alpha2}$ *R*), t_R = 22.3 min; **13b** ($C_{\alpha1}$ *S*, $C_{\alpha2}$ *R*), **13c** ($C_{\alpha1}$ *R*, $C_{\alpha2}$ *S*), t_R = 22.0 min; ^{1}H NMR (DMSO) **13a**,**d**, δ 2.30 (6H, s, CH_3 Dmt), 2.64−2.78 (2H, m, $CH_{2\beta}$ Dmt), 3.10−3.45 (3H, m, C^5H_2 benzazep + CH−N), 3.80 (1H, m, CH_α Dmt), 3.88−4.47 (5H, m, CH′−N + Fmoc CH_2 + Fmoc CH + C^1H benzazep), 5.13−5.20 (2H, m, C^4H benzazep + C^1H' benzazep), 6.49 (2H, s, CH_{arom}), 8.34−7.59 (12H, m, CH_{arom}); ^{1}H NMR (DMSO) **13b**,**c**, δ 2.24 (6H, s, CH_3 Dmt), 2.66−2.80 (2H, m, $CH_{2\beta}$ Dmt), 3.12−3.50 (2H, m, C^5H_2 benzazep), 3.71 (1H, m, CH_α Dmt), 3.90−4.50 (6H, m, CH_2−N + Fmoc CH_2 + Fmoc CH + C^1H benzazep), 5.23−5.30 (2H, m, C^4H benzazep + C^1H' benzazep), 6.49 (2H, s, CH_{arom}), 8.30−7.60 (12H, m, CH_{arom}).

[1-(4-Hydroxy-2,6-dimethylbenzyl)-2-(3-oxo-4-phenylacetylamino-1,2,4,5-tetrahydrobenzo[*c*]azepin-2-yl)-ethyl]carbamic Acid 9*H*-Fluoren-9-ylmethyl Ester (14a−d). To a solution of **13a**−**d** (11.6 mg, 0.020 mmol) in dry CH_2Cl_2 (20 mL) were added DCC (6.24 mg, 0.030 mmol) and phenylacetic acid (2.75 mg, 0.020 mmol). The mixture was stirred for 90 min at room temperature. The mixture was filtered and extracted with 1 N HCl (3 × 20 mL), saturated $NaHCO_3$ (3 × 20 mL), and saturated NaCl (1 × 20 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was redissolved in toluene and filtered to remove remaining *N*,*N*′-dicyclohexylurea (DCU). After evaporation of the toluene, a yellow paste was obtained (100% yield, HPLC purity >95%). MS *m/z* 694 $(M + H)^+$, calcd 693.32; HPLC system 1, **14a** ($C_{\alpha1}$ *S*, $C_{\alpha2}$ *S*), t_R = 29.0 min; **14b** ($C_{\alpha1}$ *S*, $C_{\alpha2}$ *R*), t_R = 28.4 min; **14c** ($C_{\alpha1}$ *R*, $C_{\alpha2}$ *S*), t_R = 28.6 min; **14d** ($C_{\alpha1}$ *R*, $C_{\alpha2}$ *R*), t_R = 28.9 min.

3-[4-(2-Phenylacetylamino)-1,2,4,5-tetrahydro-2-benzazepine-3-one-2-yl]-2-amino-1(2,6-dimethyl-4-hydroxyphenyl)propane (5a−d). To a solution of Fmoc-protected product (**14a**−**d**) (17.7 mg, 0.025 mmol) in THF (10 mL) were added dithiothreitol (DTT) (39.4 mg, 0.256 mmol) and 1,8 diazabicyclo[5.4.0]undec-7-ene (DBU) (0.118 mg, 0.0007 mmol) as a 1% solution in DMF. The mixture was stirred for 4 h at room temperature. The mixture was evaporated, and the residue was triturated with Et_2O. Purification of the final end products was done by semipreparative HPLC to obtain the pure (>95−99%) TFA salts after lyophilization (45−65% yield over two steps). ^{1}H NMR (DMSO) **5a**,**d**, δ 2.14 (6H, s, CH_3 Dmt), 2.68−2.88 (3H, m, $CH_{2\beta}$ Dmt + C^5H benzazep), 3.15−3.30 (2H, m, C^5H' benzazep + CH_α Dmt), 3.48−3.62 (4H, m, CH_2−N + CH_2CO), 4.20 (1H, d, C^1H benzazep), 5.19−5.35 (2H, m, C^1H' benzazep + C^4H benzazep), 6.42 (2H, s, H_{arom}), 6.99−7.31 (9H, m, H_{arom}), 7.99 (m, NH_3^+), 8.30 (1H, d, NH); ^{1}H NMR (DMSO) **5b**,**c**, δ 2.08 (6H, s, CH_3 Dmt), 2.70−3.00 (4H, m, $CH_{2\beta}$ Dmt + C^5H_2 benzazep), 3.06−3.23 (3H, m, CH_2−N + CH_α Dmt), 3.60 (2H, dd, CH_2CO), 3.78−3.96 (1H, m, C^1H benzazep), 5.07(1H, d, C^1H' benzazep), 5.28−5.37 (1H, m, C^4H benzazep), 6.42 (2H, s, H_{arom}), 6.91−7.32 (9H, m, H_{arom}), 7.83 (m, NH_3^+), 8.22 (1H, d, NH). For HPLC and MS characterization, see Supporting Information.

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Supporting Information Available: Characterization and purity of target compounds **5a**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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