Design, synthesis and pharmacological test of a quinoline based, nonpeptidic analogue of neurotensin(8–13)

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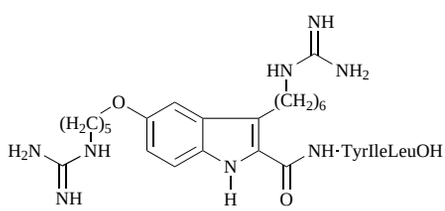
The design, synthesis and pharmacological testing of a quinoline based, nonpeptidic analogue of neurotensin(8–13) using our reported multiple template approach to developing nonpeptidic mimetics of neuropeptides are reported. The newly synthesized quinoline analogue is found to be less active in binding to the neurotensin receptors than previously reported mimetics-1 and -2 which are partial nonpeptidic analogues of neurotensin(8–13). However, the present study led to the discovery of a mistake in a literature procedure for alkylation at position-3 of ethyl indole-2-carboxylate and a consequent mistake in the synthesis of mimics-1 and -2. With the computational and experimental results of the quinoline analogue, along with mimics-1 and -2 with the correct structures, which, accordingly, served as a blind test for the multiple template approach, the concept of this approach has been experimentally validated.

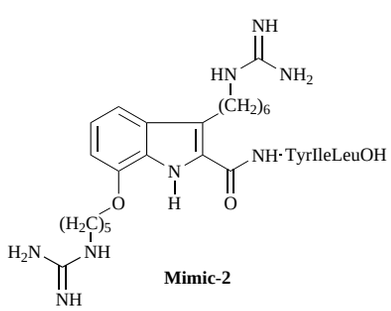
Introduction

We previously reported the development of two partial nonpeptidic neurotensin(8–13) mimetics (mimics-**1** and -**2**, Fig. 1) whose $Arg^{8}Arg^{9}Pro^{10}$ portion is replaced with substituted indole-2-carboxylates as partially flexible non-peptidic equivalents by our multiple template approach.[1–3] Neurotensin(8–13) is a peptide fragment of neurotensin ($pGlu^{1}Leu^{2}Tyr^{3}$-$Glu^{4}Asn^{5}Lys^{6}Pro^{7}Arg^{8}Arg^{9}Pro^{10}Tyr^{11}Ile^{12}Leu^{13}$) and is more biologically relevant than the parent peptide.[4] Full nonpeptidic neurotensin(8–13) mimetics can be used for potential treatments of neuropsychiatric diseases such as schizophrenia and Parkinson's disease and for evaluating further the physiological and putative pathological roles of the neurotensin receptors. The multiple template approach is a method for converting a peptide of interest into a nonpeptidic mimic, which can then be used as a therapeutic drug. The principle of this approach is to convert a vast number of conformers of a peptide to a small number of partially flexible molecules that can individually mimic a different portion of the conformers of the native peptide and altogether mimic all the conformers available to the native peptide. Each of these partially flexible molecules consists of a template of different size responsible for mimicking a certain portion of the conformers of the native peptide. Given the conformational flexibilities of the designed molecules and the conformational flexibility of the native peptide, one could determine how many templates are required to be tested. By testing all the partially flexible molecules, one should arrive at a molecule that covers the receptor-bound conformation of the native peptide and fits to the receptor to achieve the desired biological functions.

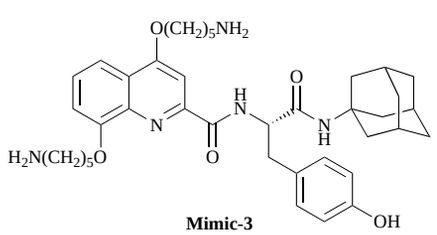
To continue our efforts into developing full nonpeptidic neurotensin(8–13) mimetics by the multiple template approach, we designed, synthesized and tested a full nonpeptidic analogue of mimic-**2** (mimic-**3**, Fig. 1). We report herein the results and implications of the studies of this new mimic.



Mimic-1



Mimic-2



Mimic-3

Fig. 1 Structures of mimics-**1** and -**2** reported previously and newly designed mimic **3**

Design

The notion of replacing the indole template used in mimics-**1** and -**2** with the quinoline template in the structure of mimic-**3** was a further test of the multiple template approach. For this purpose, we wanted to demonstrate that the pharmacological properties of mimic-**2** will be retained or slightly altered when the indole template is replaced by the quinoline template.[1] This is because the difference in the triangular size between the indole and quinoline templates is about 1.2 Å on one side of the triangles (see Fig. 2). According to the multiple template approach, a difference in distance of 1.2 Å can be nullified by the conformational flexibility of the designed molecule.[1] The structures of the templates were optimized by quantum mechanics calculations employing the Gaussian 94 program with the 6-31G* basis set at the Hartree–Fock level.[5]

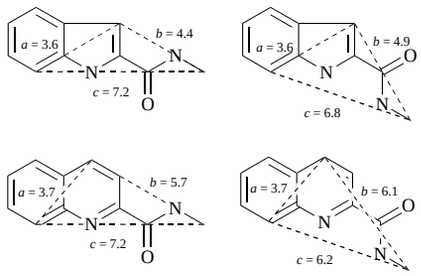
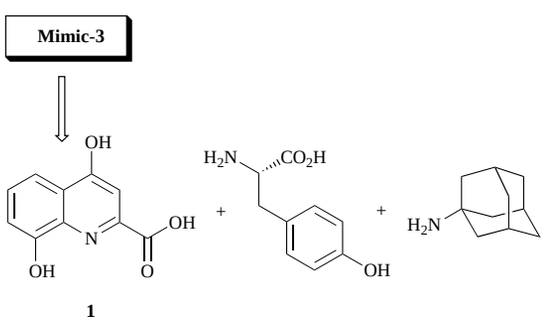


Fig. 2 Triangular sizes (Å) represented by the indole-2-carboxylic amide (*top*) and quinoline-2-carboxylic amide (*below*) nuclei

Substitution of the last two residues of neurotensin(8–13) by the 1-adamantyl group was based on our finding that the full nonpeptidic analogue of mimic-**2** in which the IleLeu was replaced by the 1-adamantyl group demonstrated a two-fold increase in affinity for human neurotensin receptors relative to mimic-**2**.[6] Use of amino groups in mimic-**3** was based on reported results that the analogues of mimics-**1** and -**2** in which the two guanidino groups are replaced by amino groups demonstrated a two- to three-fold decrease in affinity for the neurotensin receptors.[3] It was our intention to test the quinoline template first and to transform the amino group into a guanidino group later, if the quinoline template was experimentally found to be appropriate. For synthetic efficiency, two ether linkages were used in mimic-**3** since 4,8-dihydroxyquinoline-2-carboxylic acid was commercially available.

Synthesis

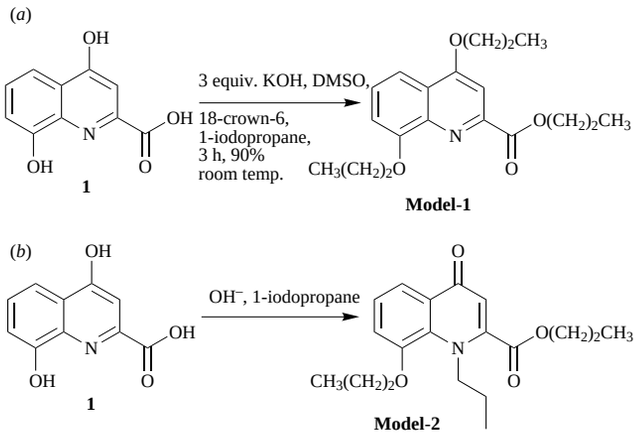
Mimic-**3** was designed to make use of commercially available materials as building blocks for its synthesis (Scheme 1). Assembly of the synthons in Scheme 1 could be easily carried



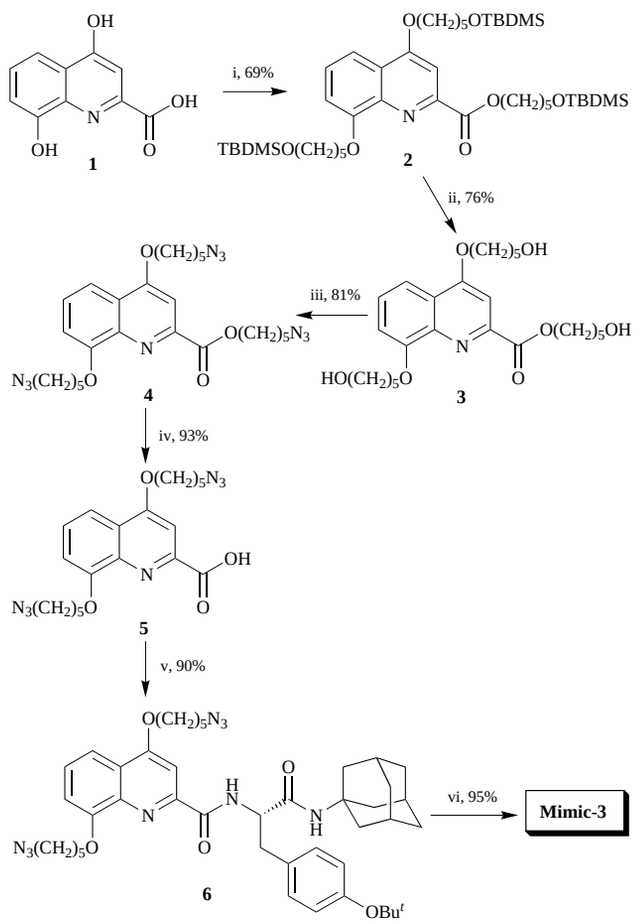
Scheme 1 Retrosynthetic analysis of mimic-**3**

out by standard peptide chemistry. Introduction of the two alkylamino chains of mimic-**3** to 4,8-dihydroxyquinoline-2-carboxylic acid **1** required, however, investigation to establish reaction conditions that could exclusively yield *O*-alkylation of compound **1** and to solve the problem that many *O*-alkylation methods could not be employed because of the poor solubility of compound **1** in most organic solvents.

According to our model study on alkylation with 1-iodopropane, we found that the reaction conditions in Scheme 2(*a*) employing three equivalents of KOH and a catalytic amount of 18-crown-6 at room temperature for three h could yield exclusive *O*-alkylation in high yield. The evidence for exclusive *O*-alkylation of 4,8-dihydroxyquinoline-2-carboxylic acid in our model study is: (i) that the observed chemical shifts of the ten sp^2 carbons ranged from 101 to 166 ppm, and (ii) that the chemical shifts of the three methylene carbons attached to the three heteroatoms of 4,8-dihydroxyquinoline-2-carboxylic acid were 67.3, 70.1 and 70.4 ppm, respectively. One would observe the chemical shift for one of the ten sp^2 carbons in the range of 180 to 200 ppm and the chemical shift for one of the three methylene carbons at less than 50 ppm, if *N*-alkylation occurs as shown in Scheme 2(*b*).



Scheme 2 Model study of *O*-alkylation



Scheme 3 Synthesis of mimic-**3**. *Reagents and conditions:* i, $I(CH_2)_5OTBDMS$, KOH, a trace of 18-crown-6, DMSO, room temp., 12 h; ii, TBAF, THF, room temp., 2 h; iii, HN_3, PPh_3, DEAD, CH_2Cl_2, 0 °C, 1.5 h; iv, 2 M KOH, MeOH, THF, room temp., 1 h; v, O-Bu^t-Tyr-NH-Ada, DCC, HOBt, DMF, 40 °C, 12 h; vi, H_2, 10% Pd–C, EtOAc–MeOH (2:1), conc. HCl, room temp. 12 h.

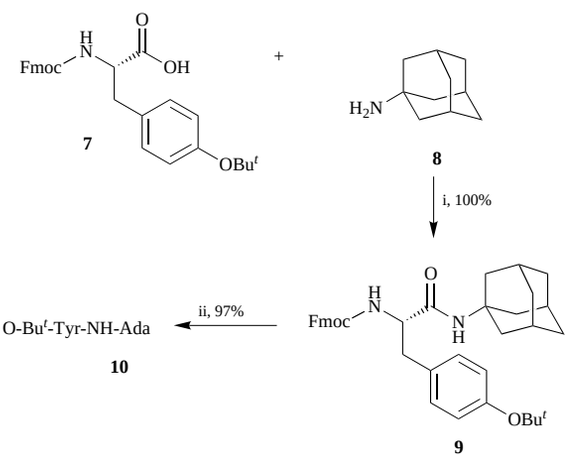
The synthesis of mimic-**3** as outlined in Scheme 3 thus started from *O*-alkylation of 4,8-dihydroxyquinoline-2-carboxylic acid with 1-(*tert*-butyldimethylsilyloxy)-5-iodopentane.[7] Similar reaction conditions using 1-(*tert*-butyldimethylsilyloxy)-5-iodopentane were accordingly used to prepare intermediate **2**. The same evidence for the exclusive *O*-alkylation was observed in the NMR spectra of intermediate **2**. Intermediate **2** was then transformed to intermediate **5** according to the published protocol.[4] Additional support for the exclusive *O*-alkylation was also observed in (i) the carbon NMR spectrum of the subsequent intermediate **5** where the chemical shifts of the two methylene carbons directly attached to the two ethereal oxygen atoms of the 4,8-dihydroxyquinoline moiety are almost identi-

Table 1 Comparison of binding affinities for neurotensin, neurotensin(8–13) and mimics-**2** and -**3** at human and mouse neurotensin receptors

Compound	K_d [nM]	
	Human neurotensin receptor in CHO–K1 membranes	Mouse neurotensin receptor in N1E-115 intact cells
Neurotensin(8–13)	0.14 ± 0.01 (4)	0.61 ± 0.02 (3)[11]
Neurotensin	1.6 ± 0.06 (93)	8.2 ± 0.6 (15)
Mimic-**2**	14 500 ± 1 200 (4)	2 600 ± 300 (6)
Mimic-**3**	76 900 ± 6 600 (4)	nd

K_d = apparent equilibrium dissociation constant. Values are geometric mean ± S.E.; each value is the mean of duplicate determinations made in independent experiments; values in parentheses indicate *n* value; nd = no data.

cal at 70.0 ppm. Coupling of intermediate **5** to the intermediate of O-Bu^t-Tyr-NH-Ada **10** in the presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) in DMF afforded the precursor of mimic-**3** (**6**) in good yield. Intermediate **10** was synthesized from two commercially available starting materials in a high yield according to Scheme 4.

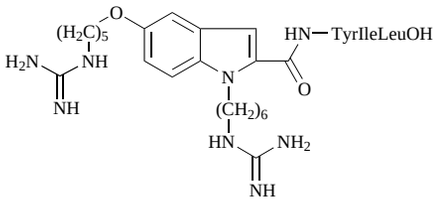


Scheme 4 Synthesis of O-Bu^t-Tyr-NH-Ada (**10**). *Reagents and conditions:* i, DCC, HOBt, DMF, room temp., 12 h; ii, piperidine, THF, room temp., 2 h.

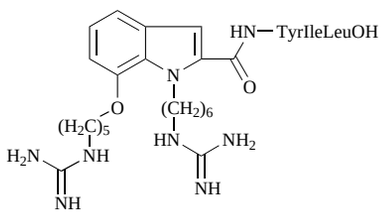
Precursor **6** was efficiently converted into mimic-**3** in its tris-hydrochloric acid salt form by catalytic hydrogenation in the presence of drops of concentrated HCl, thus reducing the azido groups into amine groups and deprotecting the *tert*-butyl group. Purification by reversed-phase HPLC eluting with MeCN–TFA yielded mimic-**3** in its tris(trifluoroacetate) salt form.

Pharmacology

Mimic-**3** in its tris(trifluoroacetate) salt form was tested for its ability to compete for [^{3}H]neurotensin binding at the human neurotensin receptors (NTR) that had been stably transfected in CHO–KI cells. Cell culture and radioligand binding assays were performed using previously described methods.[8–10] The binding affinities (K_d) of neurotensin, neurotensin(8–13) and mimics-**2** and -**3** are listed in Table 1. We present values previously derived at the mouse neurotensin receptors in intact N1E-115 cells for comparison. Neurotensin(8–13) was the most potent at both the human and mouse NTR. Additionally, the order of potency for the remaining compounds stayed the same in both cell lines. An examination of the K_d values for mimics-**3** and -**2** reveals a more than five-fold decrease in binding affinity of mimic-**3** over mimic-**2**. Additionally, mimic-**2** exhibited a decreased affinity at the human neurotensin receptor in comparison to the mouse neurotensin receptors.



Mimic 1



Mimic 2

Fig. 3 The correct structures of Mimics **1**–**2**

Discussion

The observed relatively low affinity of mimic-**3** suggested that the multiple template approach was incorrect. As explained for the design of mimic-**3**, if the theory is correct, mimic-**3** would show a similar affinity for the neurotensin receptors as mimic-**2**.

To confirm this implication, we re-examined all the experimental results of mimics **1**–**3** and found that this implication was not relevant. We first found a mistake in the literature procedure for alkylation at position-3 of ethyl indole-2-carboxylate with ethyl 3-iodopropanoate using K_2CO_3 as base in CH_3CN.[12] According to such reagents and reaction conditions along with the reported NMR data of '2-carboxyindole-3-propanoic acid',[12] we think that the alkylation actually occurred at the nitrogen atom, and not at position-3. This is evident from the reported chemical shift (a triplet at 4.77 ppm) of the two protons of the methylene group attached to the indole ring of the '2-carboxyindole-3-propanoic acid'. The correct structure prepared from indole-2-carboxylate by the reported procedure should be 2-carboxyindole-1-propanoic acid. Inevitably, employing this literature procedure for the synthesis of mimics-**1** and -**2**, alkylations of the indole intermediates with 1-(*tert*-butyldimethylsilyloxy)-6-iodohexane actually occurred at position-1, and not at position-3 as reported previously.[1–3,13] Evidence for the *N*-alkylation of mimics-**1** and -**2** is (i) disappearance of the NH stretch peaks of the indole ring of mimics-**1** and -**2** in the IR spectra, (ii) disappearance of the signals of protons attached to the nitrogen atoms of mimics-**1** and -**2** in the ^{1}H NMR spectra, and (iii) four triplets for four sets of protons of the methylene groups attached to the heteroatoms observed in the range of 4.97 to 3.57 ppm. One would observe a strong NH stretch peak around 3335 cm^{-1} in the IR spectrum, a signal for the nitrogen proton in the NMR spectrum, and only three triplets in that range of chemical shift, if the alkylations had occurred at the indole position-3. The correct structures of mimics-**1** and -**2** are now shown in Fig. 3.

Given the correct structures of mimics-**1** and -**2**, we recalculated the sizes of the triangles of the indole template (see Fig. 4). The sizes of the new triangles represented by the indole template with correct linkages as depicted in Fig. 4 reveal that differences in the triangular size between the indole and quinoline templates are more than 1.2 Å[1] on one side of the triangles (see Figs. 2 and 4). This explains why mimic-**3** is significantly less active in binding than mimic-**2**. The sizes of the new triangles of mimics-**1** and -**2** (Fig. 4) also provided an important *blind* test of the multiple template approach. If the sizes of the triangles in the correct structures of mimics-**1** and -**2** are beyond the range of the triangles (*i.e.* a = 2.9 to 3.8 Å,

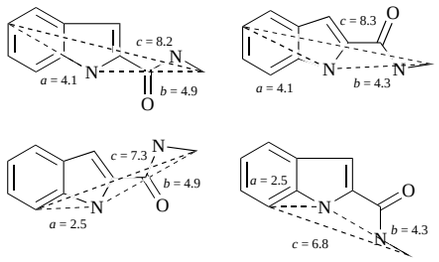


Fig. 4 Triangular sizes represented by the indole-2-carboxylic amide nucleus**.** Distances (Å) were measured in the structures optimized by quantum mechanics calculations. Hydrogen atoms are not shown for clarity.

$b = 4.2$ to 6.9 Å and $c = 3.4$ to 10.3 Å) governed by the entire conformers of neurotensin(8–13), as calculated from the peptide,[1] one could conclude that the multiple template approach is wrong. In this case mimics-**1** and -**2** would mimic conformers that are not available to neurotensin(8–13) and yet were experimentally found to be moderately active. As apparent in Fig. 4, we found that none of the triangular sizes is beyond the entire conformational range of neurotensin(8–13). Therefore, new calculations of the template size and the binding data of mimics-**1** to -**3** support the theory of the multiple template approach. With the data obtained on these mimics, we have experimentally demonstrated that in the multiple template approach some templates (*i.e.* the indole template) can lead to active mimics and others (*i.e.* the quinoline template) cannot, although finding the indole template for active mimics-**1** and -**2** turned out to be serendipitous.

Conclusion

We have found that the literature procedure for alkylation at the 3-position of ethyl indole-2-carboxylate employing K_2CO_3 as base in CH_3CN is in error, and that the related alkylated structures of mimics-**1** and -**2** have been mistakenly reported. The newly synthesized nonpeptidic mimic-**3**, which was designed on the basis of the incorrect structure of mimic-**2**, was therefore less active in binding than mimic-**2**. With the results of mimic-**3** and mimics-**1** and -**2** with the correct structures, which accordingly served as a blind test for the used approach, we experimentally validated the concept of the multiple template approach. Further studies are required to demonstrate the practicality of the multiple template approach and will be reported in due course.

Experimental

Tetrahydrofuran and diethyl ether were distilled from sodium benzophenone ketyl prior to use. DMSO was dried with CaH_2. Methylene chloride was distilled from P_2O_5 prior to use. Solvents used for chromatography were purchased in 5 gallon drums, redistilled from an all-glass apparatus and stored in glass bottles. Hexanes refers to light petroleum with bp 64–69 °C. Silica gel 60 (Merck, 230–400 mesh ASTM for flash chromatography) was used for column chromatography. TLC was performed on Merck silica gel 60F-254 (0.25 mm, precoated on glass). Other reagents were used as supplied by the Aldrich Chemical Co. and Lancaster Synthesis Inc. NMR spectra were taken on a Bruker AC-300 instrument. Chemical shifts are reported in δ units with reference to Me_4Si ($\delta = 0.00$ ppm) for ^1H spectra or $CDCl_3$ ($\delta = 77.00$ ppm) for ^{13}C spectra as internal standards. *J* Values are recorded in Hz. Ar, φ and Fn denote quinoline, phenyl and fluorene rings, respectively. Mass spectra were obtained on a FINNIGAN MAT-900 instrument. High resolution mass data were collected by employing EI at 70 eV with PFK reference or by using ESI with a reference material of PEG 400. Melting points were determined in open capillary tubes on a Gallenkamp capillary melting point apparatus and are uncorrected.

(5-*tert*-Butyldimethylsilyloxy)pentyl 4,8-bis[(5-*tert*-butyldimethylsilyloxy)pentyloxy]quinoline-2-carboxylate 2

Dry DMSO (20 ml) was added to a mixture of 4,8-dihydroxyquinoline-2-carboxylic acid (1025 mg, 5 mmol), KOH powder (1.0 g, 15.2 mmol) and a catalytic amount of 18-crown-6 under N_2. After the mixture was stirred at room temp. for 3 h, 1-iodo-5-(*tert*-butyldimethylsilyloxy)pentane (5.9 g, 18 mmol) was introduced to the mixture *via* a syringe. The reaction was quenched with cold water after 12 h stirring at room temp. The ethyl acetate extract was washed with water and then with brine, dried (Na_2SO_4) and evaporated. Flash chromatography on silica gel eluting with ethyl acetate–hexanes (1:9) gave intermediate **2** (2.8 g, 69%) as a light yellow oil; ν_{max}(neat)/cm^{-1} 1746, 1715, 1256 and 1100; δ_H(300 MHz; $CDCl_3$) 0.03–0.04 (18 H, m, $SiCH_3$), 0.87–0.90 [27 H, m, $C(CH_3)_3$], 1.45–1.68 (12 H, m, 2-H_2 + 4-H_2), 1.85–2.12 (6 H, m, 3-H_2), 3.62–3.70 (6 H, m, 5-H_2OSi), 4.19 (2 H, t, *J* 7.0, 1-H_2OAr), 4.27 (2 H, t, *J* 6.3, 1-H_2OAr), 4.42 (2 H, t, *J* 7.2, CO_2CH_2), 7.07 (1 H, d, *J* 7.2, 5-ArH), 7.47 (1 H, t, *J* 8.1, 6-ArH), 7.56 (1 H, s, 3-ArH) and 7.77 (1 H, d, *J* 7.5, 7-ArH); δ_C(75.46 MHz; $CDCl_3$) 6.7, 18.0, 22.0, 22.2, 25.7, 28.1, 28.5, 32.2, 32.4, 62.5, 62.6, 62.7, 65.7, 68.5, 68.8, 100.8, 109.5, 112.9, 123.2, 127.5, 140.4, 147.8, 155.3, 162.2 and 165.7; *m/z* (ESI) 806 $[M + H]^+$ {Found (HRMS): *m/z* 806.5242. Calc. $[M + H]^+$ for $C_{43}H_{80}NO_7Si_3$: 806.5243}.

5-Hydroxypentyl 4,8-bis(5-hydroxypentyloxy)quinoline-2-carboxylate 3

To a solution of intermediate **2** (400 mg, 0.48 mmol) in 5 ml of THF was added TBAF (1 M in THF; 2.2 ml) at room temp. under N_2. The reaction was quenched with water after 2 h stirring at room temp. The ethyl acetate extract was washed with water and then with brine, dried (Na_2SO_4) and concentrated. Flash chromatography on silica gel eluting with ethyl acetate–hexane (85:15) yielded intermediate **3** (170 mg, 76%) as a colourless oil: ν_{max}(neat)/cm^{-1} 3373br, 1720, 1269 and 1068; δ_H(300 MHz; $CDCl_3$) 1.55–1.80 (12 H, m, 2-H_2 + 4-H_2), 1.85–2.14 (6 H, m, 3-H_2), 2.55 (3 H, s, OH), 3.58–3.75 (6 H, m, 5-H_2O), 4.19 (2 H, t, *J* 6.1, 1-H_2OAr), 4.25 (2 H, t, *J* 7.5, 1-H_2OAr), 4.43 (2 H, t, *J* 6.3, CO_2CH_2), 7.06 (1 H, d, *J* 7.4, 5-ArH), 7.47 (1 H, t, *J* 8.0, 6-ArH), 7.55 (1 H, s, 3-ArH) and 7.76 (1 H, d, *J* 8.4, 7-ArH); δ_C(75.46 MHz; $CDCl_3$) 22.3, 22.4, 28.1, 28.4, 28.6, 32.2, 62.2, 62.4, 66.0, 68.7, 69.0, 101.1, 109.7, 113.1, 123.4, 127.8, 140.4, 147.9, 155.3, 162.5 and 165.9; *m/z* (ESI) 464 $[M + H]^+$ {Found (HRMS): *m/z* 464.2649. Calc. $[M + H]^+$ for $C_{25}H_{38}NO_7$: 464.2648}.

5-Azidopentyl 4,8-bis(5-azidopentyloxy)quinoline-2-carboxylate 4

To a solution of intermediate **3** (800 mg, 1.72 mmol) and triphenylphosphine (1.6 g, 6.1 mmol) in CH_2Cl_2 (20 ml) was added HN_3 (1.6 M in CH_2Cl_2; 5 ml) at 0 °C under argon. Diethylazodicarboxylate (DEAD) (1 ml, 6.4 mmol) was then introduced dropwise to the mixture with stirring at the same temperature. The reaction was quenched with H_2O after 1.5 h stirring at 0 °C. The CH_2Cl_2 extract was washed with brine, dried (Na_2SO_4) and concentrated. Flash chromatography on silica gel eluting with ethyl acetate–hexanes (3:7) gave intermediate **4** (754 mg, 81%) as a light yellow oil; ν_{max}(neat)/cm^{-1} 2099, 1800, 1723, 1265 and 1078; δ_H(300 MHz; $CDCl_3$) 1.55–1.78 (12 H, m, 2-H_2 + 4-H_2), 1.85–2.15 (6 H, m, 3-H_2), 3.25–3.35 (6 H, m, 5-H_2N_3), 4.20 (2 H, t, *J* 8.4, 1-H_2OAr), 4.27 (2 H, t, *J* 6.3, 1-H_2OAr), 4.43 (2 H, t, *J* 6.9, CO_2CH_2), 7.07 (1 H, d, *J* 7.4, 5-ArH), 7.48 (1 H, t, *J* 8.1, 6-ArH), 7.56 (1 H, s, 3-ArH) and 7.77 (1 H, d, *J* 8.7, 7-ArH); δ_C(75.46 MHz; $CDCl_3$) 23.2, 23.3, 23.4, 28.1, 28.4, 28.5, 28.53, 28.6, 51.2, 65.6, 68.4, 68.7, 101.0, 109.7, 113.2, 123.4, 127.8, 140.5, 147.9, 155.3, 162.3 and 165.9; *m/z* (ESI) 539 $[M + H]^+$ {Found (HRMS): *m/z* 539.2846. Calc. $[M + H]^+$ for $C_{25}H_{35}N_{10}O_4$: 539.2843}.

4,8-Bis(5-azidopentyloxy)quinoline-2-carboxylic acid 5

To a solution of intermediate **4** (700 mg, 1.3 mmol) in 6 ml of MeOH and 4 ml of THF was added aqueous KOH (2 M; 6 ml) at room temp. The mixture was stirred at room temp. for 1 h and then acidified to pH 3 with 20% aqueous HCl. The ethyl acetate extract was washed with H_2O and then with brine, dried (Na_2SO_4) and concentrated to give intermediate **5** (517 mg, 93%) as a yellow oil without further purification; ν_{max}(neat)/cm^{-1} 3533, 2097, 1765, 1728, 1267 and 1076; δ_H(300 MHz; $CDCl_3$) 1.60–1.82 (8 H, m, 2-H_2 + 4-H_2), 1.98–2.12 (4 H, m, 3-H_2), 3.36 (4 H, t, *J* 6.2, 5-H_2N_3), 4.25 (2 H, t, *J* 6.4, 1-H_2OAr), 4.39 (2 H, t, *J* 6.9, 1-H_2OAr), 7.20 (1 H, d, *J* 7.2, 5-ArH), 7.60 (1 H, t, *J* 7.8, 6-ArH), 7.74 (1 H, s, 3-ArH), 7.84 (1 H, d, *J* 8.1, 7-ArH) and 9.56 (1 H, s, CO_2H); δ_C(75.46 MHz; $CDCl_3$) 23.1, 23.2, 28.1, 28.4, 28.5, 51.1, 69.0, 69.8, 99.8, 111.3, 113.7, 122.9, 128.5, 134.9, 147.9, 152.3, 162.7 and 165.5; *m/z* (ESI) 428 $[M + H]^+$ {Found (HRMS): *m/z* 428.2050. Calc. $[M + H]^+$ for $C_{20}H_{26}N_7O_4$: 428.2046}.

***N*-[(2*S*)-1-Adamantylamino-3-(4-*tert*-butoxyphenyl)-1-oxopropan-2-yl]-4,8-bis(5-azidopentyloxy)quinoline-2-carboxamide 6**

To a solution of intermediate **5** (200 mg, 0.47 mmol) in anhydrous DMF (10 ml) were added (2*S*)-*N*-adamantyl-2-amino-3-[(4-*tert*-butoxy)]propanamide **10** (210 mg, 0.52 mmol), DCC (116 mg, 0.56) and HOBt (80 mg, 0.56). The mixture was quenched with cold H_2O after 12 h stirring at 40 °C. The ethyl acetate extract was washed with H_2O and then with brine, dried (Na_2SO_4) and concentrated. Flash chromatography on silica gel eluting with ethyl acetate–hexanes (4:6) gave intermediate **6** (329 mg, 90%) as a light yellow oil; ν_{max}(neat)/cm^{-1} 3337, 2098, 1660, 1504 and 1080; δ_H(300 MHz; $CDCl_3$) 1.31 [9 H, s, $C(CH_3)_3$], 1.55–2.15 (27 H, m, AdaH + 2-H_2 + 3-H_2 + 4-H_2), 3.05 (1 H, dd, *J* 9.1 and 13.3, φCH_2R), 3.23 (1 H, dd, *J* 5.4, and 13.4, φCH_2R), 3.34 (2 H, t, *J* 6.6, 5-H_2N_3), 3.42 (2 H, t, *J* 6.4, 5-H_2N_3), 4.22 (2 H, t, *J* 6.2, 1-H_2OAr), 4.29 (2 H, t, *J* 6.3, 1-H_2OAr), 4.56–4.65 (1 H, m, COCH), 5.21 (1 H, s, CON*H*Ada), 6.94 (2 H, d, *J* 8.3, 3,5-φH), 7.08 (1 H, d, *J* 8.6, 5-ArH), 7.25 (2 H, d, *J* 8.3, 2,6-φH), 7.46 (1 H, t, *J* 8.0, 6-ArH), 7.65 (1 H, s, 3-ArH), 7.77 (1 H, d, *J* 8.3, 7-ArH) and 8.97 (1 H, d, *J* 7.9, ArCON*H*); δ_C(75.46 MHz; $CDCl_3$) 23.3, 23.5, 28.3, 28.5, 28.7, 28.8, 29.2, 36.1, 38.4, 41.3, 51.1, 51.3, 51.8, 55.9, 68.4, 68.7, 78.1, 98.6, 110.0, 113.4, 123.3, 124.2, 127.1, 129.9, 132.1, 139.7, 149.2, 154.1, 154.9, 162.6, 164.5 and 168.8; *m/z* (ESI) 781 $[M + H]^+$ {Found (HRMS): *m/z* 780.4583. Calc. $[M + H]^+$ for $C_{43}H_{58}N_9O_5$: 780.4561}.

***N*-[(2*S*)-1-Adamantylamino-3-(4-hydroxyphenyl)-1-oxopropan-2-yl-4,8-bis(5-aminopentyloxy)quinoline-2-carboxamide tris(trifluoroacetate) salt (mimic-3 in its salt form)**

To a solution of intermediate **6** (100 mg, 0.13 mmol) in ethyl acetate (10 ml) and methanol (5 ml) was added 10% Pd–C (20 mg) followed by six drops of concentrated HCl. The mixture was filtered through Celite after 12 h stirring under a H_2 atmosphere at room temp. The residue concentrated from the filtrate was dissolved in 0.1% TFA (2 ml) and 80% acetonitrile in 0.1% TFA (1 ml), and purified by reversed-phase HPLC on a Vydak C_8 (15–20 μm particle size, 250 mm length × 22 mm id) column using gradient elution; starting with 90% of buffer A (0.1% TFA in H_2O) and then linearly increasing the concentration of buffer B (prepared from 20% of buffer A in MeCN) at t = 1 min to 90% over 30 min, at a flow rate of 8 cm^3 min^{-1} with the detector wavelength set at 220 nm, gave mimic-**3** as its tris(trifluoroacetate) salt (>95% pure). The retention time of mimic-**3** was 25.94 min. After lyophilization, a yellow powder was isolated; mp 164.4–166.5 °C; ν_{max}(neat)/cm^{-1} 3422br, 1655, 1508 and 1078; δ_H(300 MHz; [$^2H_6$]DMSO) 1.55–1.75 [14 H, m, CH_2(Ada) + 2-H_2 + 4-H_2], 1.86–2.0 [10 H, m, CH_2(Ada) + 3-H_2], 2.02 [3 H, s, CH(Ada)], 2.80–3.01 (6 H, m, 5-H_2N^+ + φCH_2R), 4.21 (2 H, t, *J* 5.8, 1-H_2OAr), 4.32 (2 H, t, *J* 5.7, 1-H_2OAr), 4.71 (1 H, q, *J* 7.0, COCH), 6.64 (2 H, d, *J* 8.1, 3,5-φH), 7.04 (2 H, d, *J* 8.2, 2,6-φH), 7.30 (1 H, d, *J* 7.8, ArH), 7.49–7.58 (2 H, m, ArH), 7.65–7.90 (8 H, m, N^+H_3 + CON*H*Ada + ArH), 8.59 (1 H, d, *J* 8.4, ArCON*H*) and 10.07 [1 H, s, 1-Ar^+H or PhOH (not likely but not impossible)]; δ_C(75.46 MHz; [$^2H_6$]DMSO) 22.4, 22.5, 26.7, 26.8, 27.8, 28.2, 28.8, 36.0, 38.4, 40.9, 51.0, 54.1, 68.4, 98.5, 111.1, 113.0, 114.9, 122.6, 127.0, 127.7, 130.3, 139.1, 149.2, 154.6, 155.9, 162.2, 163.0 and 169.6; *m/z* (ESI) 672 $[M + H]^+$ {Found (HRMS): *m/z* 672.4145. Calc. $[M + H]^+$ for $C_{39}H_{54}N_5O_5$: 672.4125}.

(2*S*)-*N*-Adamantyl-3-[(4-*tert*-butoxy)phenyl]-2-(9-fluorenylmethoxycarbonylamino)propanamide 9

A solution of *N*-Fmoc-*tert*-butyl-L-tyrosine (1.4 g, 3.05 mmol), 1-adamantylamine (468 mg, 3.1 mmol), DCC (693 mg, 3.4 mmol) and HOBt (455 mg, 3.4 mmol) in dry DMF (10 ml) was stirred at room temp. under N_2 for 12 h. The mixture was poured into saturated aqueous NH_4Cl and extracted with ethyl acetate. The extract was washed with H_2O and then with brine, dried (Na_2SO_4) and concentrated. Flash chromatography on silica gel eluting with EtOAc–hexanes (3:7) afforded intermediate **9** (1.8 g, 100%) as a white solid; mp 92.1–93.6 °C; ν_{max}($CHCl_3$)/cm^{-1} 3310br, 1694, 1665, 1536, 1257 and 1043; δ_H(300 MHz; $CDCl_3$) 1.33 [9 H, s, $C(CH_3)_3$], 1.57 [6 H, s, CH_2(Ada)], 1.80 [6 H, s, CH_2(Ada)], 2.01 [3 H, s, CH(Ada)], 2.78–2.88 (1 H, m, φCH_2R), 3.08–3.15 (1 H, m, φCH_2R), 4.10–4.24 (1 H, m, 9-FnH), 4.22 (1 H, t, *J* 6.9, COCH), 4.32–4.45 (2 H, m, CO_2CH_2), 4.93 (1 H, s, CON*H*Ada), 5.54 (1 H, d, *J* 6.9, FmocN*H*), 6.94 (2 H, d, *J* 6.0, 3,5-φH), 7.13 (2 H, d, *J* 6.0, 2,6-φH), 7.32 (2 H, t, *J* 6.5, FnH), 7.41 (2 H, t, *J* 7.3, FnH), 7.59 (2 H, d, *J* 7.4, FnH) and 7.76 (2 H, d, *J* 7.5, FnH); δ_C(75.46 MHz; $CDCl_3$) 28.7, 29.2, 36.1, 38.9, 41.3, 47.1, 52.0, 56.9, 66.9, 78.3, 119.9, 124.2, 125.0, 127.0, 127.6, 129.9, 131.6, 141.2, 143.7, 154.2, 155.7 and 169.3; *m/z* (ESI) 593 $[M + H]^+$ {Found (HRMS): *m/z* 593.3364. Calc. $[M + H]^+$ for $C_{38}H_{45}N_2O_4$: 593.3379}.

(2*S*)-*N*-Adamantyl-2-amino-3-[(4-*tert*-butoxy)phenyl]propanamide 10

To a solution of intermediate **9** (1.8 g, 3.04 mmol) in THF (15 ml) was added piperidine (1 ml). The solvent was evaporated after 2 h stirring at room temp. Flash chromatography on silica gel eluting first with ethyl acetate–hexanes (1:1) and then with ethanol–ethyl acetate (15:85) gave intermediate **10** (1.1 g, 97%) as a white solid; mp 102.5–103.6 °C; ν_{max}(neat)/cm^{-1} 3334, 1657, 1516, 1166 and 905; δ_H(300 MHz; $CDCl_3$) 1.34 [9 H, s, $C(CH_3)_3$], 1.5 (2 H, s, NH_2), 1.64–1.70 [6 H, m, CH_2(Ada)], 1.94–1.99 [6 H, m, CH_2(Ada)], 2.04–2.08 [3 H, m, CH(Ada)], 2.71 (1 H, dd, *J* 8.5 and 13.7, φCH_2R), 3.09 (1 H, dd, *J* 4.6 and 13.7, φCH_2R), 3.43 (1 H, dd, *J* 4.6 and 8.4, COCH), 6.80 (1 H, s, CON*H*Ada), 6.93 (2 H, d, *J* 8.4, 3,5-φH) and 7.10 (2 H, d, *J* 8.3, 2,6-φH); δ_C(75.46 MHz; $CDCl_3$) 28.8, 29.4, 36.3, 40.4, 41.4, 51.0, 56.8, 78.3, 124.2, 129.7, 132.7, 154.0 and 173.1; *m/z* (ESI) 371 $[M + H]^+$ {Found (HRMS): *m/z* 371.2703. Calc. $[M + H]^+$ for $C_{23}H_{35}N_2O_2$: 371.2699}.

Propyl 4,8-bis(propyloxy)quinoline-2-carboxylate (model-1)

Dry DMSO (20 ml) was added to a mixture of 4,8-dihydroxyquinoline-2-carboxylic acid **1** (248 mg, 1.2 mmol), KOH powder (270 mg, 3.96 mmol) and a catalytic amount of 18-crown-6 under N_2. The mixture was stirred at room temp. for 1 h and then 1-iodopropane (734 mg, 4.32 mmol) was added. The reaction was quenched with cold water after 2 h stirring at room temp. The ethyl acetate extract was washed with water and then with brine, dried (Na_2SO_4) and evaporated to dryness. Flash chromatography on silica gel eluting with ethyl acetate–hexanes (3:7) gave the product (330 mg, 83%) as a light yellow solid; mp 65.8–66.4 °C; ν_{max}(neat)/cm^{-1} 1742, 1713, 1265, 1119 and 1072; δ_H(300 MHz; $CDCl_3$) 1.05–1.16 (9 H, m, 3-H_3), 1.86–2.13 (6 H, m, 2-H_2), 4.15 (2 H, t, *J* 7.1, OCH_2), 4.23 (2 H, t, *J*

6.5, OCH_2), 4.38 (2 H, t, *J* 6.9, CO_2CH_2), 7.07 (1 H, d, *J* 7.8, 5-ArH), 7.47 (1 H, t, *J* 8.5, 6-ArH), 7.57 (1 H, s, 3-ArH) and 7.79 (1 H, d, *J* 8.5, 7-ArH); δ_C(75.46 MHz; $CDCl_3$) 21.8, 22.1, 22.2, 67.3, 70.1, 70.4, 100.9, 109.5, 113.0, 123.4, 127.6, 140.5, 147.9, 155.4, 162.4 and 165.9.

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