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A new fluorescent chemosensor for anion based on an artificial cyclic tetrapeptide

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Abstract—A cyclic tetrapeptide composed of alternating glycine and 8-amino-4-*iso*-butoxyquinoline-2-carboxylic acid was designed and synthesized. Its complexation properties with anions were performed by fluorescence spectroscopy and ^{1}H NMR method.

An area of interest in supramolecular chemistry that continues to attract is the coordination of anions. The rapid growth in this area is due to the realization of many roles that anions play in biology, medicine, catalysis, and the environment.[1]

On account of high sensitivity and simplicity, fluorescent chemosensor can be effectively used as a tool to analyze and measure the amount of anions as well as clarify their function in living system; therefore, the design and synthesis of fluorescent devices for recognition of anion are currently of importance in chemical trace detection.[2,3]

A synthetic anion receptor is generally composed of binding site and covalently linked signaling subunit. Anion binding by protein is most often achieved by the way of neutral amide functions employing the hydrogen-bond acceptor properties of the amido NH group.[4] Recently, increasing interest has been devoted to the synthesis and complexation properties with anions of cyclic peptides.[5] However, to our knowledge, still no cyclic peptide-based fluorescent sensors for anions were reported so far. Herein, we report a new fluorescent anion chemosensor based on an artificial cyclic tetrapeptide, which is composed of alternating glycine and 8-amino-4-*iso*-butoxyquinoline-2-carboxylic acid.

The synthesis of the cyclic tetrapeptide **9** is depicted in Scheme 1. Compound **2** was prepared by refluxing **1**[6] in $SOCl_2$. Reaction of **2** with glycine ethyl ester using NEt_3 as a base in CH_2Cl_2 provided **3** with 90% yield. Compound **4** was obtained by the hydrogenation of **3** in the presence of 10% Pd/C in EtOAc. Hydrolysis of **3** in the presence of 2.5 M KOH and then acidifying with HOAc gave compound **5**. Compound **6** was prepared by the condensation of **4** and **5** in CH_2Cl_2 in the presence of 1.1 M DCC. Compound **6** was hydrolyzed and further hydrogenated to yield the ω-amino acid **8**. Intramolecular condensation of **8** in DMF and CH_2Cl_2 in the presence of DCC produced the cyclic tetrapeptide **9** in 18% yield. The structures of **2**–**9** were confirmed by their ^{1}H and ^{13}C NMR, MS spectroscopy, and elemental analysis.[7]

The complex properties of **9** towards anions were evaluated in CH_3CN. Fluorescence titration experiments were recorded on excitation at 341 nm and emission at 433 nm, respectively. As shown in Figure 1, a decrease in the fluorescence intensity of **9** upon the addition of F^- was observed, which is ascribable to the PET effect from the F^- to quinoline units. When the concentration of F^- increased to 2 equiv, the intensity decreased to about 20% of the initial one. In the cases of AcO^-, $H_2PO_4^-$, the spectral changes of **9** were similar to but smaller than that of F^-, which might be due to their similar basicity but different shapes and different sizes. On the contrary, Cl^- and Br^- induced the increased intensity of **9** under the same conditions. This emission enhancement phenomenon is probably due to the inhibition of the PET quenching mechanism by hydrogen binding or

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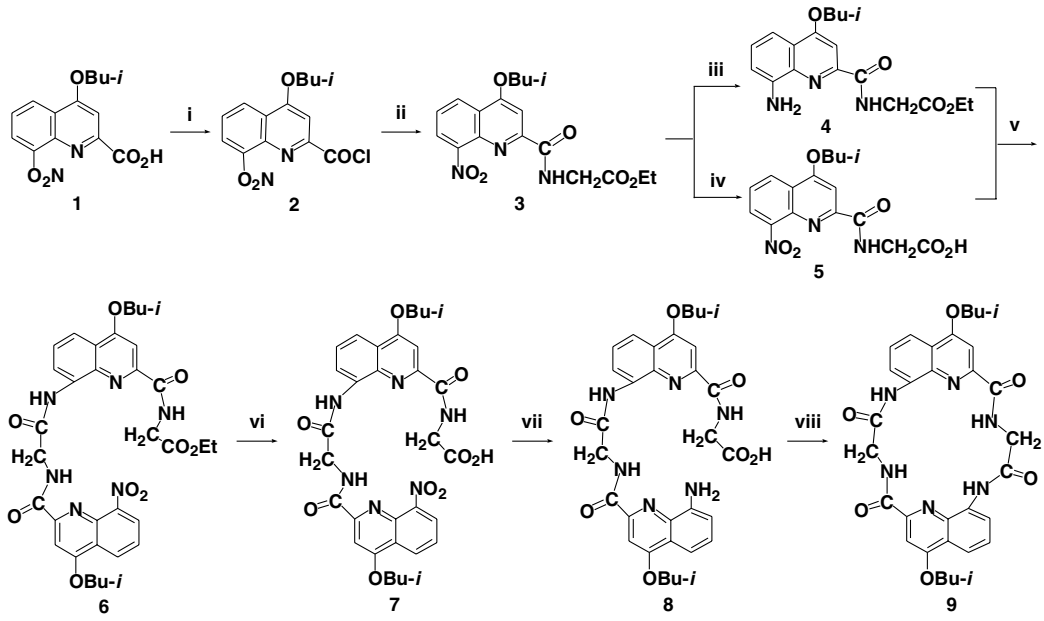


Scheme 1. Reagents and conditions: (i) $SOCl_2$, reflux, 3 h, 100%; (ii) CH_2Cl_2, Et_3N, $NH_2CH_2CO_2C_2H_5$, rt, 24 h, 90%; (iii) ethyl acetate, Pd/C, H_2, rt, 4 h, 98%; (iv) THF, MeOH, 2.5 M KOH, rt, 20 h, 97%; (v) CH_2Cl_2, 1.1 M DCC, rt, 24 h, 89%; (vi) THF, MeOH, 2.5 M KOH, rt, 20 h, 90%; (vii) DMF, Pd/C, H_2, rt, 4 h, 99%; (viii) DMF, CH_2Cl_2, 1.1 M DCC, rt, 24 h, 18%.

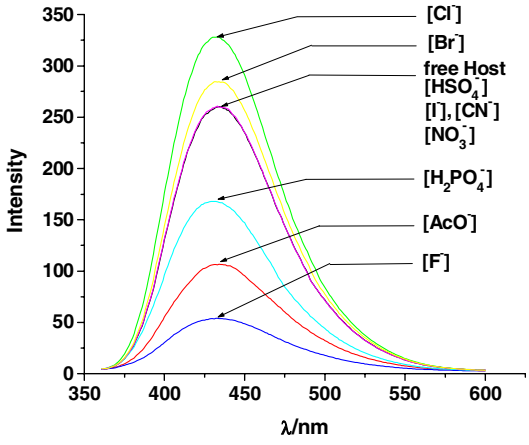


Figure 1. Fluorescent emission spectra of **9** (1×10^{-5} M) upon the addition of 2 equiv of tetrabutylammonium anion salts in acetonitrile; $\lambda_{ex} = 341$ nm.

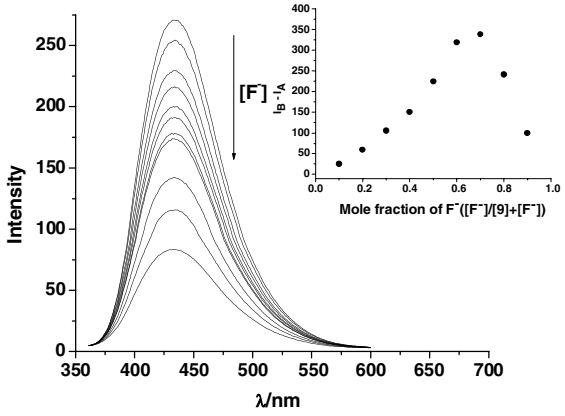


Figure 2. Fluorescent emission spectra of **9** (1×10^{-5} M) in the presence of *n*-$Bu_4N^+F^-$ in CH_3CN. The concentration of F^-: 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0×10^{-5} M; $\lambda_{ex} = 341$ nm. Inset: Job plot for **9** and F^-.

an increase of the rigidity of the receptor.[2c] Moreover, 2 equiv of other anions including HSO_4^-, I^-, CN^-, and NO_3^- caused no obvious spectral changes of **9**.

Host **9** formed 1:2 stoichiometric complex with F^- and 1:1 complexes with AcO^-, $H_2PO_4^-$, Cl^-, and Br^-, which were determined by Job's method.[8] From the fluorescence titration experiments (Fig. 2), the association constant (K_a) between **9** and F^- was calculated to be 4.8×10^9 M^{-2} by Stern–Volmer equation.[9] Similarly, the K_a values for AcO^-, $H_2PO_4^-$, Cl^-, and Br^- were estimated to be 2.34×10^4, 1.59×10^3, 3.0×10^4, and 1.9×10^3 M^{-1}, respectively.

In order to study the nature of anion coordination, ^1H NMR titrating experiments were further carried out. When F^- was added to the solution of **9** in DMSO-d_6, the signals of H_a and H_b shift downfield and become a broad peak. Meanwhile, no obvious changes for the aromatic proton signals were observed (Fig. 3A). The results suggest that the complexation between **9** and F^- only involves hydrogen bonding interactions between the anion and the amide groups of **9** (Scheme 2). In the case of Cl^-, the chemical shift changes of the amide protons in **9** are similar to those of F^-, but H_b shift is obviously lower than that of H_a (Fig. 3C).

In summary, we have presented a new fluorescent anion sensor based on an artificial cyclic tetrapeptide, which showed a highly binding affinities for anions, particularly, for fluoride ion.

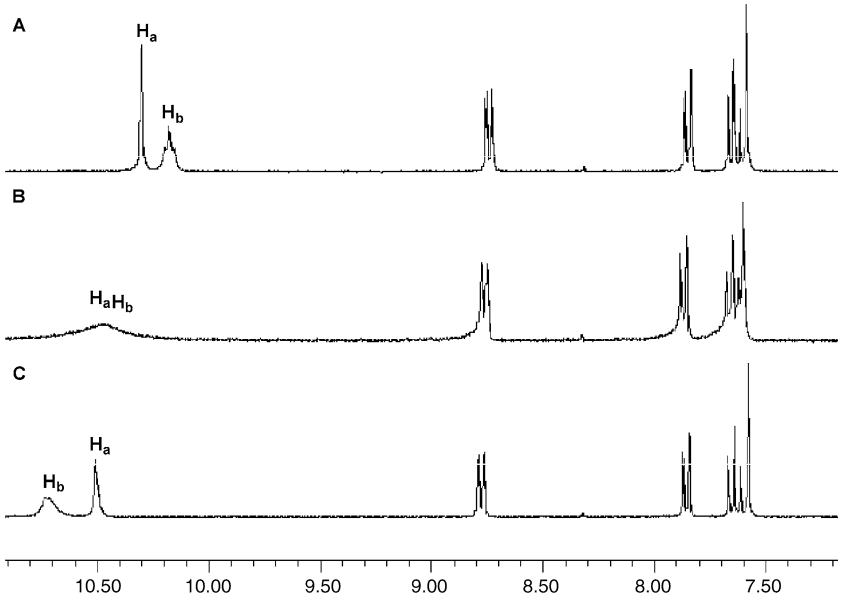
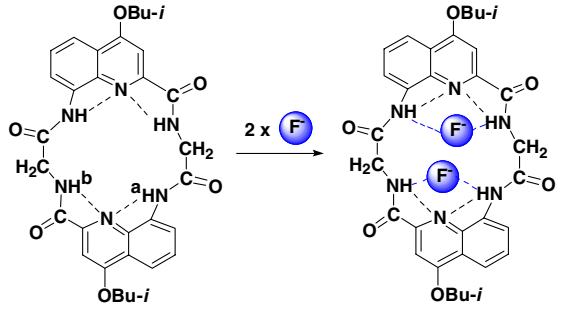


Figure 3. Partial ^{1}H NMR (300 MHz) spectra of host **9** in DMSO-d_6. (A) **9**; (B) **9**+0.5 equiv of F^-; (C) **9**+0.5 equiv of Cl^-; anions used were in the form of their n-Bu_4N^+ salts.



Scheme 2. Proposed binding mode for **9** with fluoride ions.

Acknowledgements

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7. Synthesis of compounds **3**–**9**. *Ethyl[(4-iso-butoxy-8-nitro-quinoline-2-carbonyl)amino]acetate* (**3**). A solution of **1** (1 mmol) in $SOCl_2$ (10 mL) was refluxed for 3 h, and the excess $SOCl_2$ was then removed under reduced pressure. The resulting acid chloride (**2**) was dissolved in CH_2Cl_2 (20 mL) and added dropwise to an ice-cold solution of glycine ethyl ester (1.1 equiv) and Et_3N (2.2 equiv) in CH_2Cl_2 (20 mL). The reaction mixture was stirred for 24 h, then treated with 1 N HCl (10 mL), and extracted with CH_2Cl_2. The combined layers washed with 5% $NaCO_3$ solution twice, then washed with water twice, and dried over anhydrous $NaSO_4$. The solvent was subsequently evaporated in vacuo, and the residue was subjected to a silica gel column (petroleum ether/ethyl acetate, 3:1). It gave a light yellow solid after recrystallization from CH_2Cl_2–MeOH. Yield: 0.337 g (90%). Mp: 113–114 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.60 (t, $J = 5.4$ Hz, 1H, N*H*), 8.49 (dd, $J = 1.3$, 8.5 Hz, 1H, Ar*H*), 8.13 (dd, $J = 1.3$, 7.5 Hz, 1H, Ar*H*), 7.70 (s, 1H, Ar*H*), 7.64 (t, $J = 7.5$ Hz, 1H, Ar*H*), 4.28 (q, $J = 6.1$ Hz, 2H, OCH_2CH_3), 4.27 (q, $J = 6.1$ Hz, 2H, $NHCH_2$), 4.11 (d, $J = 6.5$ Hz, 2H,

$OCH_2CH(CH_3)_2$), 2.35–2.28 (m, 1H, $CH_2CH(CH_3)_2$), 1.33 (t, $J = 7.2$ Hz, 3H, OCH_2CH_3), 1.14 (d, $J = 6.7$ Hz, 6H, $OCH_2CH(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.0, 163.9, 163.1, 152.4, 147.7, 138.9, 126.5, 125.2, 125.0, 123.2, 99.8, 77.4, 61.4, 41.6, 28.0, 19.0, 14.1. MS (EI): *m/z* 375 (M^+). Anal. Calcd for $C_{18}H_{21}N_3O_6$: C, 57.59; H, 5.64; N, 11.19. Found: C, 57.54; H, 5.66; N, 11.19.

Ethyl[(8-amino-4-iso-butoxy-quinoline-2-carbonyl)amino]-acetate (**4**). A solution of **3** (1 mmol) in ethyl acetate (20 mL) was hydrogenated at room temperature in the presence of 10% Pd/C (10 mg) for 4 h. The reaction mixture was filtered and evaporated to give a yellow solid. Yield: 0.340 g (98%). Mp: 157–158 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 8.60 (t, $J = 5.0$ Hz, 1H, N*H*), 7.58 (s, 1H, Ar*H*), 7.56 (d, $J = 1.1$ Hz, 1H, Ar*H*), 7.35 (t, $J = 7.6$ Hz, 1H, Ar*H*), 6.99 (dd, $J = 1.1$, 7.5 Hz, 1H, Ar*H*), 4.29 (d, $J = 5.8$ Hz, 2H, OCH_2CH_3), 4.25 (d,$J = 7.1$ Hz, 2H, $NHCH_2$), 4.01 (d, $J = 6.5$ Hz, 2H, $OCH_2CH(CH_3)_2$), 2.30–2.21 (m, 1H, $CH_2CH(CH_3)_2$), 1.32 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 1.11 (d, $J = 6.7$ Hz, 6H, $OCH_2CH(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.2, 165.1, 162.9, 147.7, 143.8, 137.4, 127.7, 122.8, 111.3, 110.3, 98.5, 74.9, 61.6, 41.5, 28.1, 19.2, 14.2. MS (EI): *m/z* 345 (M^+). Anal. Calcd for $C_{18}H_{23}N_3O_4$: C, 62.59; H, 6.71; N, 12.17. Found: C, 62.24; H, 6.53; N, 11.93.

[(4-iso-Butoxy-8-nitro-quinoline-2-carbonyl)amino]acetic acid (**5**). To a solution of **3** (1 mmol) in THF (20 mL) was added potassium methoxide solution (25% in methanol, 2.5 equiv). The mixture was stirred at room temperature for 20 h and then excess acetic acid was added. The solvent was subsequently evaporated in vacuo, extracted with CH_2Cl_2 (3×15 mL). The combined layers was washed with water (10 mL) and dried over anhydrous $NaSO_4$. Removal of the solvent left a solid which gave yellow needles after recrystallization from CH_2Cl_2–MeOH. Yield: 0.338 g (97%). Mp: 231–233 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 8.60 (d, $J = 7.6$ Hz, 1H, Ar*H*), 8.50 (d, $J = 7.6$ Hz, 1H, Ar*H*), 8.13 (t, $J = 5.4$ Hz, 1H, N*H*), 7.38 (s, 1H, Ar*H*), 7.63 (m, 1H, Ar*H*), 4.35 (d, 2H, $J = 3.6$ Hz, $OCH_2CH(CH_3)_2$), 4.11 (d, $J = 5.0$ Hz, 2H, $NHCH_2$), 2.37–2.24 (m, 1H, $CH_2CH(CH_3)_2$), 1.13 (d, $J = 5.8$ Hz, 6H, $OCH_2CH(CH_3)_2$). *^{13}C NMR (75 MHz, $CDCl_3$):* δ 219.2, 172.9, 164.4, 163.4, 152.3, 147.8, 139.1, 126.7, 125.5, 125.2, 123.4, 100.1, 77.2, 75.9, 78.0, 41.4, 28.1, 19.2. MS (EI): *m/z* 329 (M^+-H_2O). Anal. Calcd for $C_{16}H_{17}N_3O_6$: C, 55.33; H, 4.93; N, 12.10. Found: C, 55.32; H, 4.96; N, 11.19.

Ethyl[(4-iso-butoxy-7-{2-[(4-isobutoxy-7-nitro-quinoline-2-carbonyl)-amino]-acetylamino}-quinoline-2-carbonyl)amino]acetate (**6**). To a mixture of **4** (1 mmol) and **5** (1 mmol) suspended in CH_2Cl_2 (20 mL) at 0 °C was added DCC (1.1 mmol). The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 24 h. The mixture was filtered and evaporated. The precipitate was collected by filtration and washed with hot methanol to give a yellow solid (0.602 g, 89%). Mp: 246 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 10.74 (s, 1H, CON*H*Ar), 9.37 (t, $J = 5.9$ Hz, 1H, $CONHCH_2$), 8.97 (t, $J = 6.5$ Hz, 1H, $CONHCH_2$), 8.70 (dd, $J = 1.1$, 6.6 Hz, 1H, Ar*H*), 8.48 (dd, $J = 1.3$, 8.6 Hz, 1H, Ar*H*), 8.13 (dd, $J = 1.3$, 7.5 Hz, 1H, Ar*H*), 7.89 (dd, $J = 1.2$, 8.5 Hz, 1H, Ar*H*), 7.52 (s, 1H, Ar*H*), 7.68 (s, 1H, Ar*H*), 7.64 (t, $J = 8.0$ Hz, 1H, Ar*H*), 7.52 (t, $J = 8.0$ Hz, 1H, Ar*H*), 4.45 (d, $J = 6.6$ Hz, 2H, CH_2), 4.37 (d, $J = 6.0$ Hz, 2H, CH_2), 4.26 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 4.15 (d, $J = 6.3$ Hz, 2H, CH_2), 4.05 (d, $J = 6.5$ Hz, 2H, CH_2), 2.30–2.28 (m, 1H, $CH_2CH(CH_3)_2$), 1.31 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 1.15 (d, $J = 6.7$ Hz, 6H, $OCH_2CH(CH_3)_2$) 1.11 (d, $J = 6.7$ Hz, 6H, $OCH_2CH(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.8, 167.3, 165.4, 165.3, 163.6, 163.3, 152.2, 148.9, 147.7, 137.8, 134.6, 127.6, 126.7, 125.6, 125.3, 123.4, 122.0, 117.7, 116.1, 99.9, 99.3, 99.2, 77.2, 76.9, 61.4, 46.5, 41.9, 28.2, 28.1, 19.22, 19.17, 14.3. MS (EI): *m/z* 674 (M^+). Anal. Calcd for $C_{34}H_{38}N_6O_9$: C, 60.53; H, 5.68; N, 12.46. Found: C, 60.63; H, 5.74; N, 12.34.

[(4-iso-Butoxy-7-{2-[(4-isobutoxy-7-nitro-quinoline-2-carbonyl)-amino]-acetylamino}-quinoline-2-carbonyl)-amino]-acetic acid (**7**). To a solution of **6** (1 mmol) in THF (20 mL) was added potassium methoxide solution (25% in methanol, 2.5 equiv). The reaction mixture was stirred at room temperature for 20 h and then added excess acetic acid. The product was collected by filtration. Yield: 0.581 g (90%). Mp: 189–190 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 12.70 (s, 1H, CO_2H), 10.50 (s, 1H, CON*H*Ar), 9.84 (t, $J = 6.1$ Hz, 1H, $CONHCH_2$), 8.97 (t, 1H, $J = 5.7$ Hz, $CONHCH_2$), 8.76 (dd, $J = 1.1$, 7.8 Hz, 1H, Ar*H*), 8.51 (dd, $J = 1.3$, 8.4 Hz, 1H, Ar*H*), 8.40 (dd, $J = 1.2$, 7.5 Hz, 1H, Ar*H*), 7.91 (dd, $J = 1.2$, 8.4 Hz, 1H, Ar*H*), 7.84 (t, $J = 7.6$ Hz, 1H, Ar*H*), 7.74 (s, 1H, Ar*H*), 7.65 (t, $J = 7.8$ Hz, 1H, Ar*H*), 7.65 (s, 1H, Ar*H*), 4.55 (d, $J = 5.7$ Hz, 2H, CH_2), 4.22 (d,$J = 6.4$ Hz, 2H, CH_2), 4.16 (d, $J = 6.4$ Hz, 2H, CH_2), 4.05 (d, $J = 6.3$ Hz, 2H, CH_2), 2.28–2.18 (m, 1H, $CH_2CH(CH_3)_2$), 1.11 (d, $J = 1.1$ Hz, 6H, $OCH_2CH(CH_3)_2$) 1.09 (d, $J = 1.1$ Hz, 6H, $OCH_2CH(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.2, 167.9, 164.2, 163.6, 162.7, 162.5, 152.9, 149.5, 147.7, 138.1, 137.6, 134.7, 127.5, 126.5, 125.8, 125.0, 122.3, 121.4, 115.4, 100.1, 99.2, 75.1, 74.6, 117.8, 44.2, 41.4, 39.0, 38.7, 27.6, 27.6, 18.9, 18.8. MS (MALDI-TOF): *m/z* 669.4 $(M+Na)^+$, 685.3 $(M+K)^+$. Anal. Calcd for $C_{32}H_{34}N_6O_9 \cdot H_2O$: C, 57.83; H, 5.46; N, 12.64. Found: C, 57.89; H, 5.37; N, 12.48.

[(7-{2[(7-Amino-4-iso-butoxy-quinoline-2-carbonyl)amino]-acetylamino}-4-iso-butoxy-quinoline-2-carbonyl)-amino]acetic acid (**8**). A solution of **7** (1 mmol) in DMF (20 mL) was hydrogenated at room temperature in the presence of 10% Pd/C (10 mg) for 4 h. The reaction mixture was filtered and evaporated to give a yellow solid (0.615 g, 99%), which was used for the subsequent reaction without further purification. Mp: 195–196 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 10.60 (s, 1H, CON*H*Ar), 9.96 (t, $J = 5.7$ Hz, 1H, $CONHCH_2$), 9.88 (t, $J = 5.2$ Hz, 1H, $CONHCH_2$), 8.74 (d, $J = 7.7$ Hz, 1H, Ar*H*), 7.95 (s, 1H, Ar*H*), 7.88 (d, $J = 8.4$ Hz, 1H, Ar*H*), 7.64 (t, $J = 2.9$ Hz, 1H, Ar*H*), 7.49 (s, 1H, Ar*H*), 7.33 (t, $J = 7.8$ Hz, 1H, Ar*H*), 7.25 (d, $J = 8.1$ Hz, 1H, Ar*H*), 6.86 (d, $J = 7.5$ Hz, 1H, Ar*H*), 4.47 (d, $J = 6.1$ Hz, 2H, CH_2), 4.15 (d, $J = 6.1$ Hz, 2H, CH_2), 4.11 (d, $J = 6.1$ Hz, 2H, CH_2), 4.06 (d, $J = 6.3$ Hz, 2H, CH_2), 2.24–2.17 (m, 1H, $CH_2CH(CH_3)_2$), 1.11–1.07 (m, 12H, $OCH_2CH(CH_3)_2$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 171.1, 168.5, 165.2, 164.3, 162.5, 162.0, 149.4, 146.9, 146.1, 137.4, 136.4, 134.8, 128.5, 127.5, 122.2, 121.3, 117.5, 115.3, 109.2, 106.3, 99.1, 97.9, 74.6, 74.1, 64.8, 44.2, 41.4, 27.6, 27.6, 18.9, 15.1. MS (MALDI-TOF): *m/z* 639.3 $(M+Na)^+$, 655.3 $(M+K)^+$. Anal. Calcd for $C_{32}H_{36}N_6O_7 \cdot 2.5H_2O$: C, 58.08; H, 6.20; N, 12.70. Found: C, 58.09; H, 6.26; N, 12.27.

Cyclic tetrapeptide (**9**). To a mixture of **8** (0.1 mmol) in DMF (20 mL) and CH_2Cl_2 (80 mL) at 0 °C was added DCC (0.11 mmol). The reaction mixture was stirred at 0 °C for 30 min and at room temperature for 24 h, and then filtered. The solvent was subsequently evaporated in vacuo to give a residue, which was subjected to a silica gel column with petroleum ether and ethyl acetate (3:1) as eluent to afford the product (12 mg, 18%). Mp: >300 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 10.45 (s, 2H, CON*H*Ar), 10.00–9.98 (m, 2H, $CONHCH_2$), 8.68–8.67 (m, 2H, Ar*H*), 7.87–7.84 (m, 2H, Ar*H*), 7.64 (s, 2H, Ar*H*), 7.34–7.29 (m, 2H, Ar*H*), 4.34–4.33 (m, 2H, CH_2), 3.62–3.61 (m, 2H, CH_2), 2.18–2.07 (m, 2H, CH_2CH-$(CH_3)_2$), 1.01 (d, $J = 5.8$ Hz, 12H, $OCH_2CH(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.0, 163.6, 149.6, 138.4,

133.5, 127.6, 122.3, 118.6, 116.8, 99.5, 76.3, 75.2, 44.9, 28.0, 19.0. MS (MALDI-TOF): *m/z* 598.8 $(M+H)^+$, 620.7 $(M+Na)^+$. Anal. Calcd for $C_{32}H_{34}N_6O_6{\cdot}2CH_3CO_2C_2H_5$: C,62.00; H, 6.50; N, 10.85. Found: C, 62.15; H, 6.68; N, 10.79.

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