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Article

Synthesis and Molecular Recognition of Novel Cyclic Pseudopeptides Containing *L*-Glutamic Acid or *L*-Aspartic Acid Backbones†

WANG, Tao(王涛) HUANG, Xiao-Yi(黄小毅) XIA, Chuan-Qin(夏传琴)
YU, Xiao-Qi*(余孝其) XIE, Ru-Gang*(谢如刚)

Department of Chemistry, Sichuan University, Chengdu, Sichuan 610064, China
Key Laboratory of Green Chemistry & Technology, Ministry of Education, Chengdu, Sichuan 610064, China

Novel cyclic pseudopeptides containing *L*-glutamic acid or *L*-aspartic acid backbone structures were efficiently synthesized and characterized. Their chiral recognition properties for *L*- and *D*-amino acid methyl ester hydrochloride were discussed also.

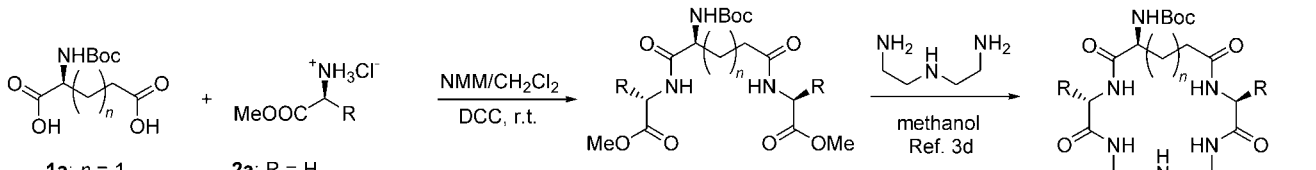
Keywords cyclic pseudopeptide, molecular recognition, synthesis

Cyclic peptides possess a wide range of functions such as molecular recognition, ion-transport, preparation of nanotubes, acting as antibiotics, toxins, and hormones and so on.[1] It was challenging to design novel cyclopeptides as ion-transport receptor and enantioselective artificial receptor. Recent researches indicated that the introduction of non-natural amino acid, *D*-amino acid or some non-amino acid section to the backbone of cyclopeptides either shows high ion-transport activities or improves the properties in molecular recognition.[2]

There are many reports on the synthesis of cyclic peptides,[3] but few examples include the use of the functional groups in the side chain of amino acid to build the ring. In this paper, we used *L*-glutamic acid and *L*-aspartic acid as starting materials to synthesize some novel cyclic pseudopeptides. As shown in Scheme 1, in the first step, DCC was employed to introduce *L*-amino acid methyl esters to *L*-glutamic acid or *L*-aspartic acid to form tripeptide structures. In the second step, the tripeptide reacts with diethylenetriamine in methanol to afford the desired cyclic pseudopeptides. In the last step, the cyclization reaction did not occur at room temperature, while preferable yields were obtained after 3 d under reflux conditions. We also attempted to substitute diethylenetriamine with bis(2-aminoethyl)ether, but unfortunately, no reaction happened in methanol under reflux conditions for 2 d.

UV-visible titration is known to be very useful in determination of bind constant. It was applied in the recognition of the cyclic pseudopeptides **4** for *L*- and *D*-amino acid methyl ester hydrochloride. The hosts, the cyclic pseudopeptides have no UV-visible absorption, so the solution of aromatic amino acid methyl ester hydrochloride in methanol (2×10^{-4} mol•L^{-1}, 30 ℃) was added into them, which resulted in a regular decrease in the maximum UV-visible absorption (Figure 1).

Scheme 1 Synthetic route of cyclic peptide **4**



1a: n = 1
1b: n = 0

2a: R = H
2b: R = CH_3
2c: R = $CH_2CH(CH_3)_2$

3a: R = H, n = 1
3b: R = CH_3, n = 1
3c: R = $CH_2CH(CH_3)_2$, n = 1
3d: R = H, n = 0
3e: R = CH_3, n = 0
3f: R = $CH_2CH(CH_3)_2$, n = 0

4a: R = H, n = 1
4b: R = CH_3, n = 1
4c: R = $CH_2CH(CH_3)_2$, n = 1
4d: R = H, n = 0
4e: R = CH_3, n = 0
4f: R = $CH_2CH(CH_3)_2$, n = 0

* E-mail: schemorg@mail.sc.cninfo.net; Tel. & Fax: 86-28-85415886
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† Dedicated to Professor Chengye Yuan on the occasion of his 80th birthday.

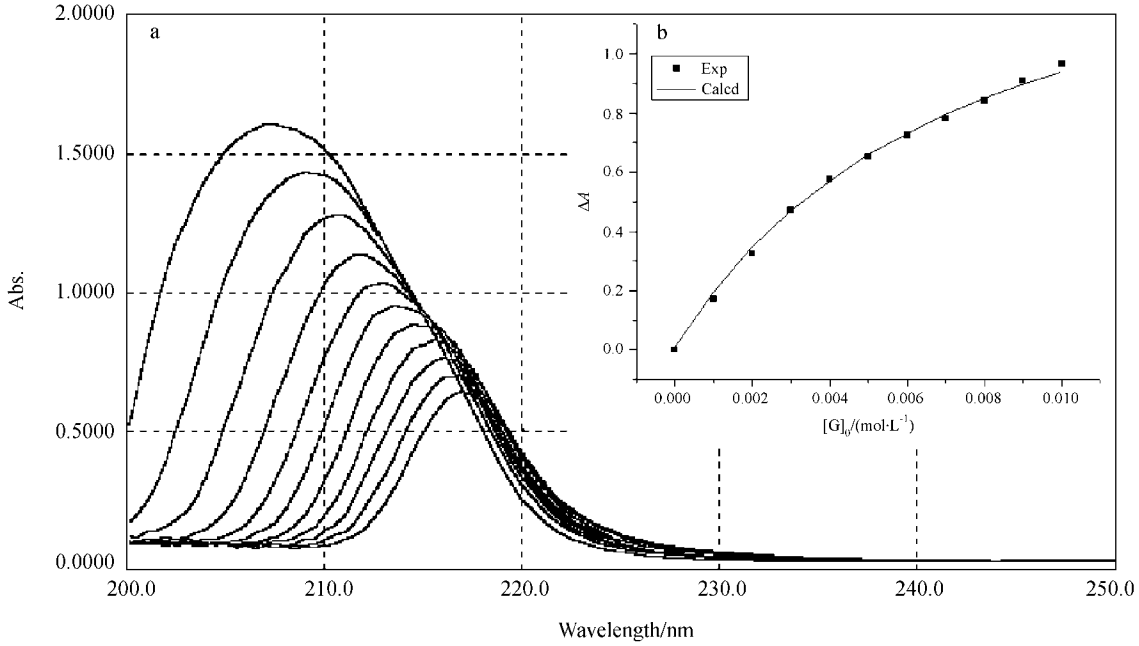

Figure 1 UV-visible spectra of *D*-Phe-OMe•HCl in the presence of **4c** in methanol at 30 ℃ (a) and its curve-fitting plot (b). [*D*-Phe-OMe•HCl]=2×10^{-4} mol•L^{-1}; [**4c**]=0, 1×10^{-4}, 2×10^{-4}, 3×10^{-4}, 4×10^{-4}, 5×10^{-4}, 6×10^{-4}, 7×10^{-4}, 8×10^{-4}, 9×10^{-4}, 1×10^{-3} mol•L^{-1}.

Table 1 Binding constants of cyclopeptides **4a**—**4f** with amino acid methyl esters hydrochloride

Entry	Host	Guest	K_L/(L•mol^{-1})	K_D/(L•mol^{-1})	K_D/K_L
1	**4a**	His-OMe•2HCl	2.470×10^2	3.494×10^2	1.415
2	**4d**	His-OMe•2HCl	3.274×10^2	4.219×10^2	1.287
3	**4a**	Phe-OMe•HCl	1.387×10^2	1.578×10^2	1.134
4	**4d**	Phe-OMe•HCl	3.124×10^2	3.125×10^2	1.000
5	**4b**	His-OMe•2HCl	2.578×10^2	4.041×10^2	1.567
6	**4e**	His-OMe•2HCl	2.139×10^2	2.985×10^2	1.396
7	**4b**	Phe-OMe•HCl	1.423×10^2	1.598×10^2	1.123
8	**4e**	Phe-OMe•HCl	1.690×10^2	1.666×10^2	0.986
9	**4c**	His-OMe•2HCl	2.905×10^2	3.802×10^2	1.309
10	**4f**	His-OMe•2HCl	2.775×10^2	4.178×10^2	1.506
11	**4c**	Phe-OMe•HCl	1.313×10^2	1.357×10^2	1.033
12	**4f**	Phe-OMe•HCl	1.652×10^2	1.852×10^2	1.121

Changes in the UV spectrum of the guest are recorded and employed to determine the binding constants using nonlinear least-squares curve fitting with a 1∶1 association model according to standard procedure.[4]

The chiral recognition abilities of the cyclic peptides for amino acid esters are showed in Table 1. It was found that the cyclic peptides can associate well with these substrates, affording the K_D/K_L in the range of 0.983—1.567. All these cyclic peptides exhibit poor enantioselectivity for phenylalanine methyl ester hydrochloride and good enantioselectivities for histidine methyl ester hydrochloride. The *D*/*L*-enantioselectivities of cyclic pseudopeptides **4a** and **4b** containing *L*-glutamic acid for histidine methyl ester hydrochloride are better than the ones **4d** and **4e** containing *L*-aspartic acid (Entries 1, 2 and 5, 6), but another cyclic pseudopeptide **4c** containing *L*-glutamic acid show worse enantioselectivities than **4f** (Entries 9, 11).

Experimental

Melting points were measured on a XRC-1 melting point apparatus and uncorrected. UV-vis absorption were measured on Tu-1600 UV/vis spectrophotometer. ^{1}H NMR spectra were recorded on a Varian INOVA-400 spectrometer in $CDCl_3$ with TMS as the internal standard. Mass spectra were measured on a Finnigan MAT 4510 spectrometer.

General procedure for the preparation of peptide 3a—3f

NMM (*N*-methyl morphine, 44 mmol) was added

into the solution of *L*-amino acid methyl ester hydrochloride (44 mmol) in 100 mL of anhydrous CH_2Cl_2. After 1 h, Boc-*L*-Glu (or Boc-*L*-Asp) (20 mmol) was added, then when the temperature of the solution was kept below 0 ℃, DCC (42 mmol) was added into the solution. After stirring for 1 h at 0 ℃, the reaction mixture was left at room temperature for 24 h. The precipitate DCU (dicyclohexylurea) was filtered off and the filtrate was washed with 10% citric acid, saturated $NaHCO_3$ aqueous solution and brine, respectively. The organic phase was dried and concentrated in vacuum, and the residue was recrystallized in a mixture of ethyl acetate and petroleum ether to afford a white solid.

3a: Yield 88%, m.p. 84—85 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.42 (s, 9H, Boc), 2.00—2.17 (m, 2H, β-H-Glu), 2.35—2.44 (m, 2H, γ-H-Glu), 3.76 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.96—4.24 (s, 4H, α-H-Gly), 4.32 (s, 1H, α-H-Glu), 5.36 (s, 1H, NHCO- Glu), 7.22 (s, 1H, NHCO-Gly), 7.68 (s, 1H, NHCO-Gly); MS (EI 70 eV) *m/z*: 390 $(M+1)^+$.

3b: Yield 92%, m.p. 142—144 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.25—1.43 (m, 15H, CH_3), 1.81—1.84 (m, 2H, β-H-Glu), 2.17—2.22 (m, 2H, γ-H-Glu), 3.77 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.01 (s, 1H, α-H-Glu), 4.62—4.73 (s, 2H, α-H-Ala), 5.06 (s, 1H, NHCO-Glu), 7.56 (s, 1H, NHCO-Ala), 7.89 (s, 1H, NHCO-Ala); MS (EI 70 eV) *m/z*: 418 $(M+1)^+$.

3c: Yield 78%, m.p. 128—130 ℃; ^{1}H NMR ($CDCl_3$, 400 M) δ: 0.90—0.95 (m, 12H, $CH(C\mathbf{H}_3)_2$), 1.41 (s, 9H, Boc), 1.47—1.67 (m, 6H, $C\mathbf{H}_2C\mathbf{H}(CH_3)_2$), 1.74—1.88 (m, 2H, β-H-Glu), 2.19—2.36 (m, 2H, γ-H-Glu), 3.73 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.94 (s, 1H, α-H-Glu), 4.56—4.73 (m, 2H, α-H-Leu), 4.98 (s, 1H, NHCO-Glu), 7.69 (s, 1H, NHCO-Leu), 7.99 (s, 1H, NHCO-Leu); MS (EI 70 eV) *m/z*: 502 $(M+1)^+$.

3d: Yield 87%, m.p. 106—108 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.45 (s, 9H, Boc), 2.60—2.66 (d, J=14.8 Hz, 1H, β-H-Asp), 2.96—2.99 (d, J=14.8 Hz, 1H, β-H-Asp), 3.74 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 4.00—4.03 (s, 4H, α-H-Gly), 4.54 (s, 1H, α-H-Asp), 6.09 (s, 1H, NHCO-Gly), 6.66 (s, 1H, NHCO-Gly), 7.36 (s, 1H, NHCO-Asp); MS (EI 70 eV) *m/z*: 376 $(M+1)^+$.

3e: Yield 87%, m.p. 143—146 ℃, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.34—1.41 (m, 6H, $CHC\mathbf{H}_3$), 1.45 (s, 9H, Boc), 2.55—2.61 (m, 1H, β-H-Asp), 2.86—2.90 (d, J=15.2 Hz, 1H, β-H-Asp), 3.75 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 4.47—4.55 (m, 3H, α-H), 6.09 (s, 1H, NHCO-Ala), 6.40 (s, 1H, NHCO-Ala), 7.40 (s, 1H, NHCO-Asp); MS (EI 70 eV) *m/z*: 404 $(M+1)^+$.

3f: Yield 78%, m.p. 128—130 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.90—0.94 (m, 12H, $CH(C\mathbf{H}_3)_2$), 1.49—1.72 (m, 6H, $C\mathbf{H}_2C\mathbf{H}(CH_3)_2$), 1.45 (s, 9H, Boc), 2.17—2.58 (d, J=14.8 Hz, 1H, β-H-Asp), 2.60—2.85 (d, J=14.8 Hz, 1H, β-H-Asp), 3.71 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 4.47 (s, 1H, α-H-Asp), 4.53—4.58 (m, 2H, α-H-Leu), 6.10 (s, 1H, NHCO-Leu), 6.37 (s, 1H, NHCO-Leu), 7.31 (s, 1H, NHCO-Asp); MS (EI 70 eV) *m/z*: 488 $(M+1)^+$.

General procedure for the preparation of pseudopeptide 4a—4f

To a stirred solution of **3** (4 mmol) in 150 mL of anhydrous methanol, diethylenetriamine (4 mmol) was added under N_2 atmosphere. The solution was heated to reflux for 3 d. The residue was concentrated and purified on a column of silica gel using a mixed solution of $CHCl_3$/methanol/aqueous ammonia ($V:V:V$=5:1:0.1) as eluent to afford a white solid.

4a: Yield 12%, $[\alpha]_D^{20}$ −10.2 (*c* 0.5, CH_3OH); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.42 (s, 9H, Boc), 1.67—1.72 (m, 1H, β-H-Glu), 1.96—2.02 (m, 1H, β-H-Glu), 2.09—2.27 (m, 2H, γ-H-Glu), 2.55—2.69 (m, 4H, $NHC\mathbf{H}_2CH_2$), 2.92—2.95 (s, 1H, $CH_2N\mathbf{H}CH_2$), 3.12—3.43 (m, 4H, $CONHC\mathbf{H}_2$), 3.50—3.94 (m, 5H, α-H), 7.36 (s, 1H, NHCO), 7.40 (s, 1H, NHCO), 7.86 (s, 1H, NHCO), 8.36 (s, 1H, NHCO), 8.55 (s, 1H, NHCO); MS (EI 70 eV) *m/z*: 429 $(M+1)^+$. Anal. calcd for $C_{18}H_{32}N_6O_6$: C 50.46, H 7.53, N 19.61; found C 50.71, H 7.91, N 19.65.

4b: Yield 20%, $[\alpha]_D^{20}$ −44.6 (*c* 0.5, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.41 (s, 6H, $C\mathbf{H}_3CH$), 1.44 (s, 9H, Boc), 1.78—1.80 (m, 1H, β-H-Glu), 2.12—2.72 (m, 1H, β-H-Glu), 2.30—2.37 (m, 2H, γ-H-Glu), 2.44—2.47 (m, 2H, $CONHCH_2C\mathbf{H}_2$), 2.78 (s, 1H, $CH_2N\mathbf{H}CH_2$), 2.82—2.89 (m, 2H, $CONHCH_2C\mathbf{H}_2$), 3.20—3.69 (m, 4H, $CONHC\mathbf{H}_2CH_2$), 3.79—3.96 (m, 1H, α-H), 4.27—4.30 (m, 1H, α-H), 4.51—4.56 (t, J=7.4 Hz, 1H, α-H), 5.81 (s, 1H, NHCO), 7.22 (s, 2H, NHCO), 7.36 (s, 1H, NHCO), 7.84 (s, 1H, NHCO); MS (EI 70 eV) *m/z*: 457 $(M+1)^+$. Anal. calcd for $C_{20}H_{36}N_6O_6$: C 52.62, H 7.95, N 18.64; found C 52.73, H 8.28, N 18.72.

4c: Yield 18%, $[\alpha]_D^{20}$ −36.2 (*c* 0.5, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.91—0.96 (m, 18H, CH-side chain-Leu), 1.44 (s, 9H, Boc), 1.73—1.82 (m, 2H, β-H-Glu), 2.08—2.30 (m, 3H, $CH_2N\mathbf{H}CH_2$, γ-H-Glu), 2.67—2.83 (m, 4H, $CONHCH_2C\mathbf{H}_2$), 3.27—3.78 (m, 4H, $CONHC\mathbf{H}_2CH_2$), 3.92—3.94 (m, 1H, α-H), 4.38—4.40 (m, 1H, α-H), 4.55—4.69 (m, 1H, α-H), 5.14 (s, 1H, NHCO), 7.78 (s, 1H, NHCO), 8.01 (s, 1H, NHCO), 8.50 (s, 1H, NHCO), 8.90 (s, 1H, NHCO); MS (EI 70 eV) *m/z*: 541 $(M+1)^+$. Anal. calcd for $C_{26}H_{48}N_6O_6$: C 57.75, H 8.95, N 15.54; found C 57.87, H 9.11, N 14.88.

4d: Yield 15%, $[\alpha]_D^{20}$ −7.0 (*c* 0.5, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.40 (s, 9H, Boc), 2.07 (s, 1H, $CH_2N\mathbf{H}CH_2$), 2.50—2.53 (m, 4H, $CONHCH_2C\mathbf{H}_2$), 2.54—2.68 (m, 2H, β-H-Asp), 3.15—3.34 (m, 4H, $CONHC\mathbf{H}_2CH_2$), 3.47—3.74 (m, 4H, α-H), 4.30 (t, J=8.4 Hz, 1H, α-H), 6.98 (s, 1H, NHCO), 7.76 (s, 1H, NHCO), 7.80 (s, 1H, NHCO), 8.35 (s, 1H, NHCO), 8.60 (s, 1H, NHCO); MS (EI 70 eV) *m/z*: 415 $(M+1)^+$. Anal. calcd for $C_{17}H_{30}N_6O_6$: C 48.26, H 7.30, N 20.28; found C 49.36, H 7.46, N 19.52.

4e: Yield 24%, $[\alpha]_D^{20}$ −47.4 (*c* 0.5, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.17—1.39 (m, 6H, $C\mathbf{H}_3$-CH), 1.40 (s, 9H, Boc), 2.31—2.34 (s, 2H, β-H-Asp), 2.62—2.71 (m, 5H, $CH_2N\mathbf{H}CH_2$, $CONHC\mathbf{H}_2C\mathbf{H}_2$), 3.02

—3.24 (m, 4H, CONHC**H**$_2$CH$_2$), 3.94—4.05 (m, 2H, α-H), 4.25 (t, J=8.8 Hz, 1H, α-H), 7.08 (s, 1H, NHCO), 7.78 (s, 1H, NHCO), 7.95 (s, 1H, NHCO), 8.23 (s, 1H, NHCO), 8.42 (s, 1H, NHCO); MS (EI 70 eV) *m/z*: 443 $(M+1)^+$. Anal. calcd for $C_{19}H_{34}N_6O_6$: C 51.57, H 7.74, N 18.99; found C 51.67, H 7.99, N 18.58.

4f: Yield 21%, $[\alpha]_D^{20}$ −43.6 (*c* 0.5, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.82—0.98 (m, 18H, CH-Leu), 1.40 (s, 9H, Boc), 1.54—1.84 (m, 4H, CONH-CH_2C**H**$_2$), 2.44—2.75 (m, 3H, CH_2N**H**CH_2, β-H-Asp), 3.03—3.82 (m, 4H, CONHC**H**$_2$CH$_2$), 4.24—4.36 (m, 2H, α-H), 4.45 (s, 1H, α-H), 5.30 (s, 1H, NHCO), 7.31 (s, 1H, NHCO), 7.65 (s, 1H, NHCO), 7.90 (s, 1H, NHCO), 8.07 (s, 1H, NHCO); MS (EI 70 eV) *m/z*: 527 $(M+1)^+$. Anal. calcd for $C_{25}H_{46}N_6O_6$: C 57.01, H 8.80, N 15.96; found C 57.14, H 9.00, N 15.65.

UV-visible titration procedure

All solutions were prepared in methanol. The spectra were collected at (25±0.1) ℃ with a 1 cm quartz cell. A 3.0 mL methanol solution of amino acid methyl ester hydrochloride (2.0×10^{-4} mol•L^{-1}) was put into the cell. After the cell temperature was kept at 25 ℃ with a thermostatic cell compartment, the solution of cyclic pseudopeptide (3×10^{-2} mol•L^{-1}) was added in portions via a microsyringe. To avoid the influence of UV-vis absorption of the guest, we could record the spectra by use of the corresponding solution of the guest as reference solution. The whole volume of guest solution added to the cell did not exceed 100 μL to dispel the effect of volume change.

The modified Benesi-Hildebrand equation is as follows,

$$\Delta A = \frac{1}{2}\left\{\Delta\varepsilon\left([H]_0+[G]_0+\frac{1}{K_\alpha}\right)\pm\sqrt{\Delta\varepsilon^2\left([H]_0+[G]_0+\frac{1}{K_\alpha}\right)^2-4\Delta\varepsilon[H]_0[G]_0}\right\}$$

where $[H]_0$ represents the total concentration of host, $[G]_0$ denotes the total concentration of guest; $\Delta\varepsilon$ is the difference between the molar extinction coefficient for the host and host-guest complex; ΔA denotes the changes in the absorption of the host on adding guest. We can use nonlinear least-squares curve fitting with 1∶1 association model to get the binding constant K_α and $\Delta\varepsilon$. For example, when the concentration of *D*-Phe-OMe•HCl was 2.0×10^{-4} mol•L^{-1}, the maximum absorption wavelength is at 213.0 nm, and the absorbance A_0 is 1.089. When the host cyclic pseudopeptide **4c** was portion-wisely added to the cell to make its concentration be 1×10^{-4}, 2×10^{-4}, 3×10^{-4}, 4×10^{-4}, 5×10^{-4}, 6×10^{-4}, 7×10^{-4}, 8×10^{-4}, 9×10^{-4} and 1×10^{-3} mol•L^{-1}, respectively, the maximum absorption decreased orderly and gave the corresponding ΔA (A_0-A) values of 0.017, 0.034, 0.081, 0.147, 0.205 and 0.256. The association constant $K_D=1.357\times10^2$ L•mol^{-1} was obtained by nonlinear least-squares curve fitting program.

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