The conjugate of adenine–cyclen Zn(II) complex: its synthesis and selective recognition abilities for uracil and uridine

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Abstract

A synthetic route to a novel adenine–cyclen conjugate is described. Preliminary results showed that the adenine–cyclen conjugate can bind Zn^{2+} rapidly in water and the Zn(II) complex can selectively recognize uracil and uridine. The recognition abilities have been demonstrated directly by UV spectrophotometric titration, NMR titration, and ESI-MS. The apparent 1:2 complex for uracil with the complex **5** was determined by spectrophotometric titration and ESI-MS at pH 8.2 with $I = 0.1$ ($NaNO_3$).

Keywords: Conjugate, cyclen, recognition, uracil, uridine

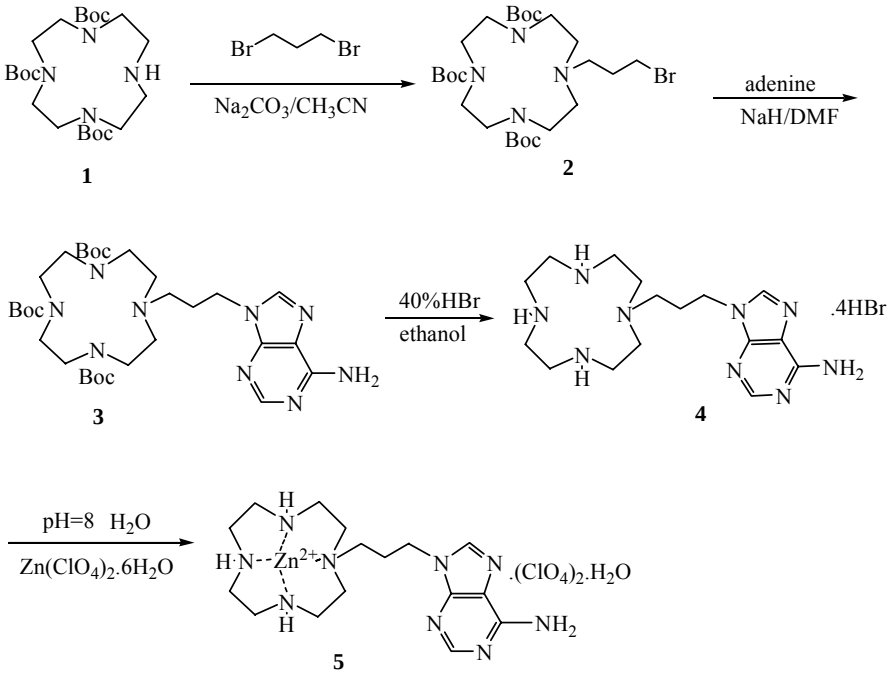
Introduction

The recognition and selective binding of nucleobases and nucleosides play fundamental roles in all of the three stages of genetic information transfer: replication, transcription, and protein synthesis. Hence, the design and selective recognition of nucleobases has been an area of considerable interest.[1–5]

Among the various bases and nucleosides, uracil and uridine have received much attention. Uracil arises in DNA from mis-incorporation during DNA replication (A-U) or as a result of the spontaneous hydrolytic deamination of cytosine (G-U). While the A-U base pair is not directly mutagenic, some transcription factors have significantly reduced binding affinity for A-U compared to A-T base pairs,[6] which can affect gene expression. Thus, although the eukaryotic replicative polymerases have a high fidelity, with mis-incorporation occurring at a rate of approximately 1×10^{-7} per base pair per generation,[7] the finite probability presents undesired biological consequences. In humans, spontaneous deamination of cytosine occurs at a rate of approximately 100–500 events per cell per day.[8] Failure to repair the G-U base pair leads to a G-C transition mutation to A-C during DNA replication. Consequently the presence of uracil in DNA is detrimental, and its recognition and removal are essential. In general, uracil DNA

glycosylase (UDG) is a base- excision repair enzyme that specifically recognizes and removes uracil from double- or single-stranded DNA. So, we need to design artificial receptors to uracil to mimic the biochemical processes. Recently, Kimura reported that cyclen–Zn(II) complexes appended with different side groups can selectively recognize different nucleobases.[9] In particular, the Zn(II)-macrocyclic polyamine complexes which appended aromatic sulfonamides [10–12] have been applied to the recognition of nucleobases, for example, thymine (dT) and uracil (U), which possess similarly weak acidic protons at the "imide"groups.[13] Kalesse reported that the tyrosine-cyclen conjugate could serve as a new tool for the selective cleavage of RNA, with a preference for unpaired uridine.[14]

Nucleobases as supramolecular motifs have the most interesting and intriguing class of molecular architectures.[15] The A-T and C-G interaction has been utilized in a variety of interested supramolecular systems.[16–21] Herein, we present a Zn(II) complex of a novel adenine-cyclen conjugate which may possibly combine the properties of adenine and cyclen in the hope of increasing the specificity for recognition of uracil and uridine. To the best of our knowledge, this is the first example of an adenine-cyclen conjugate which has a flexible spacer to connect adenine and cyclen. The synthetic route is shown in Scheme 1.



Scheme 1

Results and Discussion

ESI-MS studies on the complexation of uracil, uridine with 5. ESI-MS is one of the most promising tools for the characterization of supramolecular complexes.[22] In order to determine the

stoichiometry of the complex formed by uracil and **5**, ESI-MS experiments were performed regularly for a mixture of uracil or uridine and **5** (mM) in H_2O (pH=8.2, I=0.1 ($NaNO_3$)). The ESI-MS signals are detected in the positive mode and the spectrum is shown as Figure 1. The experimental mass spectra for the 1:2 ratio of **5**:uracil (ESI-MS: *m/z* =732.4 (M^+), 620.5 (M^+-uracil)) fit into the theoretical mass distribution spectra of **5**·2 uracil. Its recognition mechanism between **5** and uracil may be as shown in Figure 3. Under the conditions at pH=8.2, the N(3)H in uracil loses a proton, and coordinates with Zn(II) in the conjugate **5**; then adenine in conjugate **5** interacts with the other uracil through the Watson–Crick type of binding. When the pH was adjusted to 8.2, the complex formed by **5** and uridine in 1:1 ratio of **5**:uridine (ESI-MS: m/z=655.8(M^++1), 679.8 $(M+Na)^+$) (Figure 2) should fit the theoretical mass spectrum with the assembly structure **5**·uridine. The assembly between **5** and uridine is shown in Figure 4.

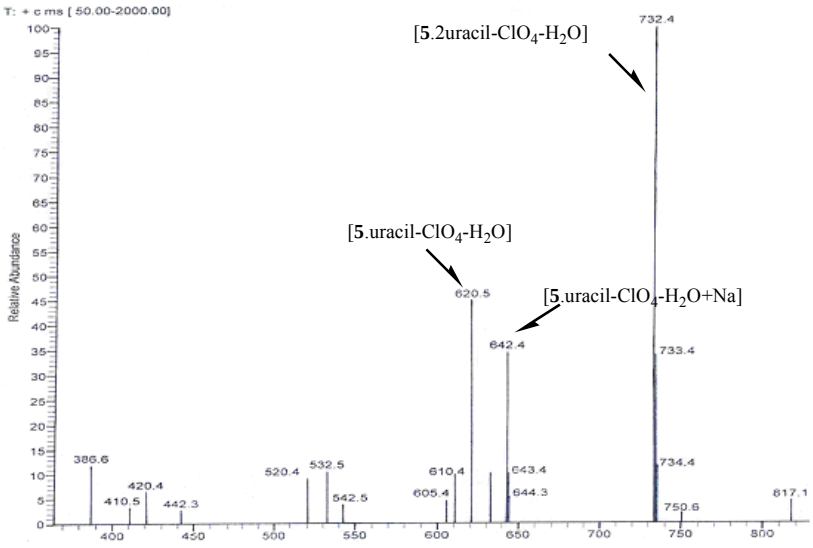


Figure 1. Mass spectrum (ESI) of the mixture of complex **5** and uracil.

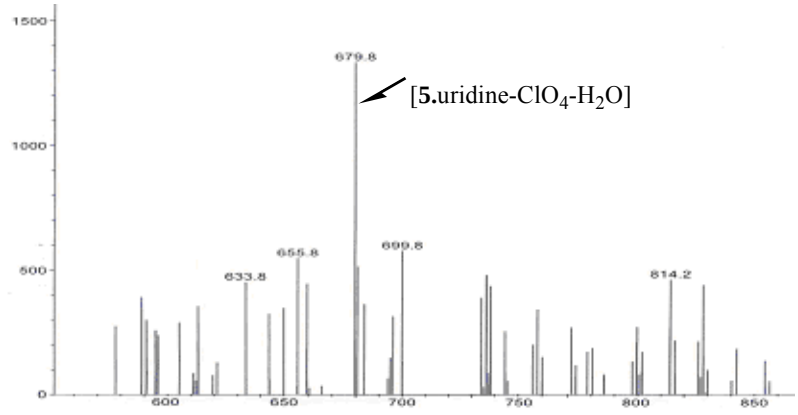


Figure 2. Mass spectrum (ESI) of the mixture of complex **5** and uridine.

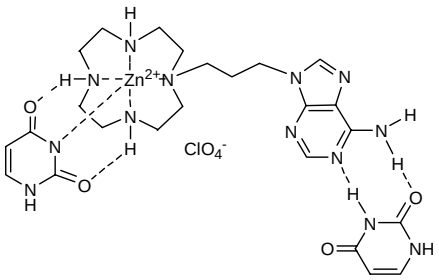


Figure 3. 2:1 Complex formed by uracil and **5**.

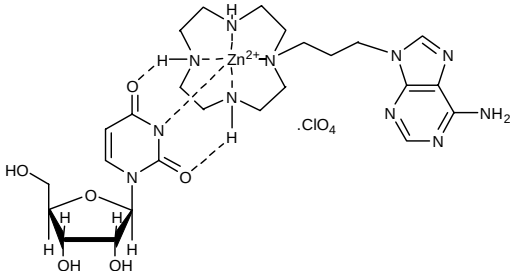


Figure 4. 1:1 Complex formed by uridine and **5**.

UV spectrophotometric titration of uracil with 5. The interactions between **5** and the other nucleobases were also investigated by spectrophotometric titration. The results indicate that the solution absorbance of the mixture of **5** and adenine, guanine, or cytosine was almost unchanged, but the mixture of **5** and uracil resulted in a large change with the addition of guest to **5** (pH=8.2, I=0.1 ($NaNO_3$)). The apparent constants are collected in Tabel 1. The results show that **5** could selectively recognize uracil.

Table 1. Comparison of apparent binding constant (*K*app) of **5** with nucleobases, as determined by the spectrophotometric titration in Tris-HCl (pH 8.2) with *I*=0.1 ($NaNO_3$) at room temperature

Guest	Uracil	Adenine	Cytosine	Guanine
K_{app} (L^2/mol^2)	4.12×10^8	6.41×10^3	3.33×10^3	2.48×10^3

^{1}H- NMR titration of uracil with 5 in DMSO-d_6 solution. The ^{1}H- NMR spectrum of uracil interacting with **5** in DMSO-d_6 might give us more information about the structure of the complex. Figures 5(a) and 5(b) show the ^{1}H- NMR spectrum of uracil and compound **5** respectively.

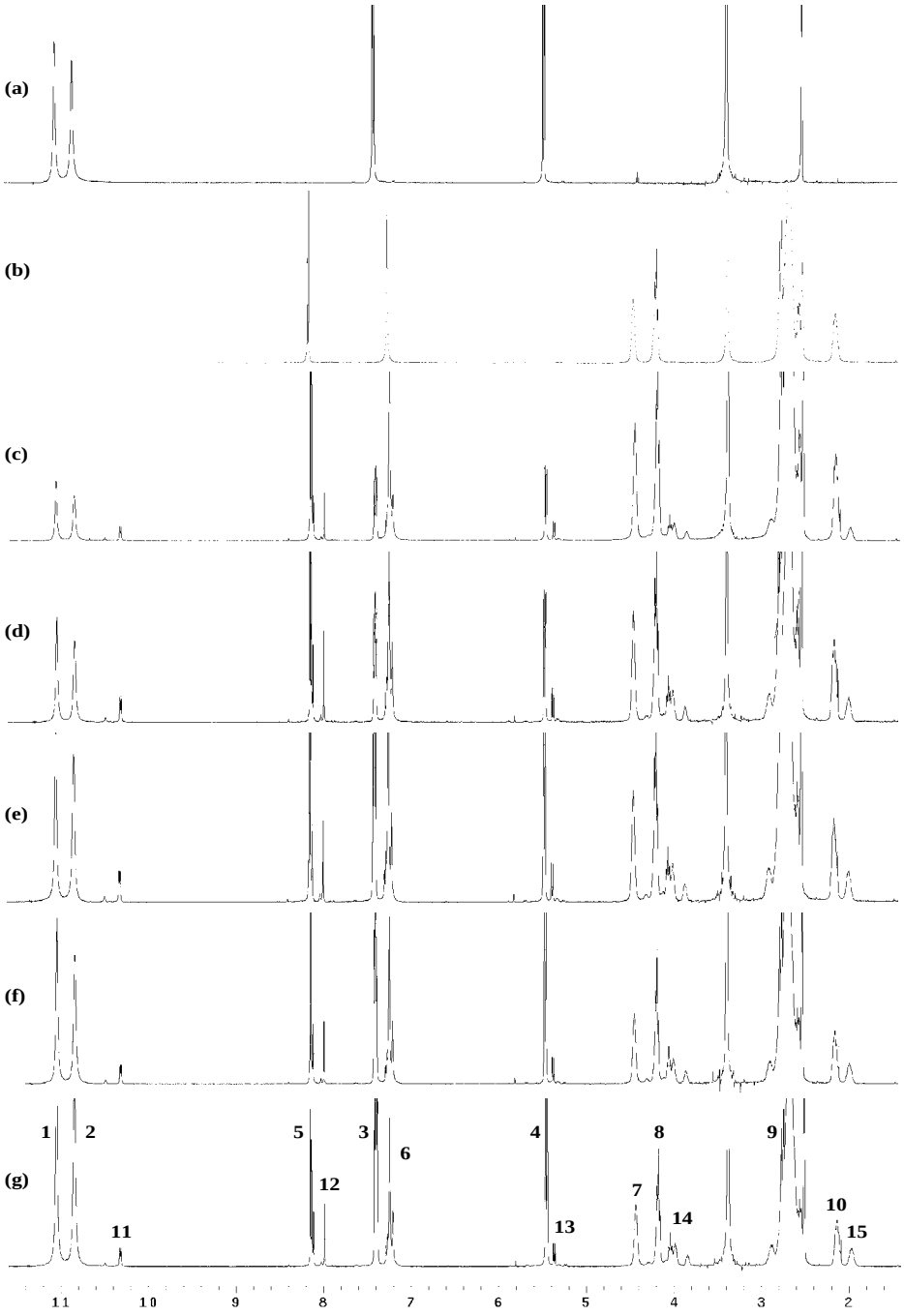


Figure 5. ^{1}H- NMR (400 MHz) spectrum changes of uracil in DMSO with increasing concentration of uracil at room temperature. The ratios of **5**:uracil are (a) 0:1, (b) 1:0, (c) 1:0.5, (d) 1:1, (e) 1:1.5, (f) 1:2, (g) 1:2.5, respectively.

Peaks 1–4 respectively belong to N(3)-H, N(1)-H, H(6) and H(5) of uracil, and peaks 6–11 correspond to H(8), H(2), NH_2, adenine-CH_2, cyclen-NH, cyclen-CH_2 and $CH_2CH_2CH_2$ in **5**. With the addition of uracil to **5**, new peaks 12–16 appeared and indicate that the supramolecular

recognition of **5** and uracil exists in the mixed system. Furthermore, the initial peaks 1 and 2 of the N(3)-H, N(1)-H in uracil shifted to peak 11, peak 4 to peak 13, and implied that N(3)-H, N(1)-H in uracil attended coordination or formed a hydrogen bond with adenine; peak 5 to peak 10 implied that adenine interacted with uracil; peak 10 to peak 15 implies that Zn^{2+}-cyclen moieties of **5** became non-equivalent in the complex of **5**/uracil. So, the conjugate of adenine-Zn^{2+}-cyclen can interact with uracil through the coordination between Zn^{2+} -cyclen and N(3)H in uracil and the Watson–Crick type of binding (Figure 5).

Experimental Section

General Procedures. ESI-MS spectra data were recorded on a Finnigan LCQ^{DECA} mass spectrometer. ^{1}H- NMR spectra were measured on a Varian INOVA-400 spectrometer. Chemical shifts in ppm are reported relative to internal Me_4Si ($CDCl_3$, DMSO-d_6) or 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (D_2O). Melting points were determined using a micro-melting point apparatus without any corrections. Cyclen was prepared by a previously reported method.[23] 1,4,7-Tris-(tert-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (3-Boc-cyclen) was prepared according to the literature.[24] All other chemicals and reagents were obtained commercially and used without further purification.

1-{1-[4,7,10-Tris-(*tert*-butyloxycarbonyl)]-1,4,7,10-tetraazacyclododecane}-3-bromopropane (2). Under N_2 atmosphere, a solution of 1,3-dibromopropane (1 mL, 9.85 mmol) and Na_2CO_3 (0.25 g, 2.17 mmol) in 50 mL of dry CH_3CN was stirred at room temperature; 1,4,7-Tris-(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane **1** (0.50 g, 1.06 mmol) was added slowly and then the reaction mixture was stirred at 80 ^oC for another 72h. The insoluble inorganic salt was filtered off and the filtrate was concentrated *in vacuo*. The residue was dissolved in $CHCl_3$ and then purified by silica gel column chromatography (ethyl acetate:petroleum ether = 1:4). A colorless amorphous solid was obtained in 50.9% yield. M.p: 58–60 ^oC; ESI-MS: *m/z* = 617 $(M+Na)^+$.

1-{1-[4,7,10-Tris-(*tert*-butyloxycarbonyl)]-1,4,7,10-tetraazacyclododecane}-3-(9-adenine)-propane (3). NaH (40 mg, 1 mmol) was added to a suspension of adenine (2.2 mmol, 297.3 mg) and KI (60 mg, a catalytic amount) in dry, degassed N, N-dimethylformamide (15 mL), and stirred for 0.5h at room temperature. A solution of **2** (2 mmol, 1.04g) in dry DMF (10 mL) was added slowly to this mixture and the reaction then stirred under N_2 at 50 ^oC overnight. Water (30 mL) was added to the mixed solution, and then the mixture was extracted with ethyl acetate (3×15 mL). The organic layer was dried with anhydrous Na_2SO_4. The residue after evaporating the solvent *in vacuo* below 40 ^oC was purified by silica gel chromatography (methanol: chloroform = 1:8) to afford a colorless amorphous solid **3** (yield 80%). Mp 118-120 ^oC, ^{1}H NMR ($CDCl_3$, 400 MHz) : δ 1.42–1.46 (m, 27H, $OC(CH_3)_3$), 2.06–2.11 (m, 2H, $CH_2CH_2CH_2$), 2.62–2.65 (t, 2H, CH_2-cyclen, *J*=16Hz), 3.21–3.52 (m, 16H, CH_2NCH_2), 4.20–4.23 (t, 2H, CH_2-

adenine, *J*=12Hz), 5.89 (m, 2H, adenine-NH_2), 7.84 (s, 1H, H(2) in adenine); 8.35 (s, 1H, H(8) in adenine). IR (KBr pellet): 3422, 2976, 1666, 1416, 1250, 1168, 774 cm^{-1}. ESI-MS: *m/z* = 648.3 $(M+H)^+$. HRMS (ESI) calcd. for $C_{31}H_{54}N_9O_6$ $[M+H]^+$: *m/z* = 648.4192. Found: 648.4165.

1-[1-(1,4,7,10-Tetraazacyclododecane)] -3-(9-adenine)propane tetrahydrobromide (4). To a solution of **3** (0.647 g, 1.0 mmol) in dry EtOH (5 mL) at 0 °C, 40% aqueous HBr (1 mL) was added slowly. After being stirred overnight at room temperature, the reaction mixture was concentrated *in vacuo* below 40 °C to give a solid. This was crystallized from EtOH/24% aqueous HBr to afford **4**•4 HBr as a white powder (0.490g, 78%). Mp: 178–179 °C. ^{1}H- NMR (D_2O, 400 MHz): δ 2.13–2.21 (m, 2H, $CH_2CH_2CH_2$), 2.78–2.80 (t, 2H, CH_2-adenine, *J*=8Hz), 2.86–3.17 (m, 16H, NCH_2CH_2N), 4.35–4.38 (t, 2H, $CH_2CH_2CH_2$, *J*=12Hz), 8.40 (s, 1H, H(2) in adenine), 8.47 (s, 1H, H(8) in adenine). IR (KBr pellet): 3748, 3446, 2917, 1665, 1541, 1109, 961, 671, 529 cm^{-1}. ESI-MS (*m/z*) 348 (M^++1-4 HBr).

1-[1-(1,4,7,10-Tetraazacyclododecane)] -3-(9-adenine)propane zinc (II) diperchlorate (5). The hydrobromide salt of **4** (0.671g, 1.0 mmol) was dissolved in 5 mL of water. After adjusting the aqueous solution to alkaline (pH ≥ 8), $Zn(ClO_4)_2$•$6H_2O$ (0.408g, 1.1 mmol) in EtOH (5 mL) was added. The mixture was stirred overnight at room temperature. The solution was gradually concentrated to obtain a white powder of diperchlorate salts **5** in 88.3% yield. Mp 174–175 °C. ^{1}H- NMR (DMSO-d_6, 400 MHz): δ 2.09–2.15 (m, 2H, $CH_2CH_2CH_2$), 2.50–2.77 (m, 18H, $NHCH_2CH_2NH$), 4.15–4.19 (t, 2H, $CH_2CH_2CH_2$, *J*=16Hz), 4.43 (s, 2H, adenine-NH_2). 7.24 (s, H(2) in adenine), 8.13–8.15 (d, H(8) in adenine, *J*=8Hz), IR (KBr pellet): 3751, 3423, 3244, 2926, 1649, 1475, 1119, 993, 628 cm^{-1}. ESI-MS (*m/z*) 413.5 (M^+ +1–2ClO_4-H_2O).

ESI-MS. Uracil or uridine was added into a solution of **5** (5 mmol) in Tris-HCl buffer solution at pH=8.2 with *I* = 0.1 ($NaNO_3$), and ESI-MS spectra data were recorded on a Finnigan LCQ^{DECA} mass spectrometer.

^{1}H- NMR titrations. The NMR spectra were measured on a Varian INOVA-400 spectrometer, with chemical shifts in ppm reported relative to internal Me_4Si ($CDCl_3$, DMSO-d_6) or 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt (H_2O). Into a DMSO solution of **5** (5 mmol), uracil was added to retain the ratio of **5**:uracil as (a) 0:1, (b) 1:0, (c) 1:0.5; (d) 1:1; (e) 1:1.5, (f) 1:2; (g) 1:2.5, and ^{1}H- NMR spectra were measured.

UV–Visible titrations. UV spectra were recorded on a Hitachi U-3500 spectrophotometer at 25.0±0.1 °C. The solution of **5** (3 mL, 5.0×10^{-5} mol/L) in Tris–HCl buffer (pH=8.2) with I=0.1 ($NaNO_3$) was put into a quartz cell. The solution of uracil (10μL, 2×10^{-2} mol/L) in Tris–HCl buffer (pH=8.2) with I=0.1 ($NaNO_3$) was added in portions. The titrations were run at least twice. The decreases in their absorption at $\lambda_{max.}$=262 nm were measured, and the apparent binding constants K_{app} of **5** with uracil were determined from the plots of $1/\Delta A$ versus $1/[G]_0^2$ where $[G]_0$ is the concentration of uracil, $\Delta A = A_f - A_b$ where A_f, A_b correspond to uracil-bound and uracil-unbound form of the tested compound, respectively. The data fitted to the Bensi-Hildebrand Equation (1), wherein the slope is equal to $1/K_{app}\cdot a$ and the y-intercept equal to 1/a. K_{app} was determined from the ratio of the slope to the y- intercept.

$$1/\Delta A = 1/K_{app}\cdot a\cdot 1/[G]_0 + 1/a \quad (a=\Delta\varepsilon\cdot[H]_0) \qquad (1)$$

Conclusions

In conclusion, we have synthesized the conjugate of Zn(II)-cyclen and adenine which could be a highly selective receptor for uracil and uridine in aqueous solution at pH=8.2 with I=0.1 ($NaNO_3$). Furthermore, the formation of the 1:2 complex with uracil indicates that this work can be extended to design receptors for the site-specific binding of nucleic acids.

Acknowledgements

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