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High Yield Synthesis and Preliminary Spectroscopic Study of Mono-*N*-alkylated Cyclen Derivatives of Salicylic Acid

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Key words: cyclen, metal ion complexes, salicylic acid derivatives

Abstract

Selective and high yield synthesis of *N*-substituted salicylic acid derivatives of cyclen has been achieved by using a direct synthetic procedure under mild reaction conditions. The protonation constants of these compounds were determined by potentiometric titration. The complexing properties of the cyclen derivatives with metal cations were investigated by UV–Vis spectroscopy and ^{1}H NMR. The stability constants of Mg^{2+}, Ca^{2+} and Sr^{2+} complexes with ligands **5** and **6** were determined.

Introduction

Selective *N*-substitution of 1,4,7,10-tetraazacyclododecane remains an interesting challenge owing to their many applications in biochemistry [1], medicine [2], chemistry [3], and environmental protection [4]. Mostly, the cyclen derivatives have side chains of different nature on the nitrogen atoms. For analytical purposes cyclen derivatives are frequently equipped with one chromogenic or fluorescing substituent and three chelating groups. Such compounds show high affinity to lanthanide cations [5]. By contrast, derivatives with one *N*-substituent selectively complex transition metal cations [6]. The complexing ability of such ligands is determined by properties of both macrocycle and substituents. Some substituents show complexing aptitude by themselves. For example, hydroxyacids and their derivatives are known as chelating reagents for alkaline earth and transition metal cations. Such properties represent salicylic acid, which is able to complex Ca^{+2}, Mg^{+2}, Fe^{+3}, Cu^{+2}, Co^{+2} and Al^{+3} cations [7]. Introduction of salicylic acid residue to macrocycle should contribute to the properties of entire molecule.

Experimental and elementary analysis

General

^{1}H NMR spectra were recorded at 200 and 500 MHz on Varian instrument. ^{13}C NMR spectra were recorded at 125 MHz on Varian instrument. Mass spectra were registered on AMD-604 apparatus. IR measurements were taken on Genesis II (Mattson) instrument. Elementary analyses were performed on Carlo Erba CHNS-O EA 1108 apparatus.

Thin layer chromatography (TLC) analyses were carried out on Alufolien covered with silica gel 60-F-254 (0.2 mm thickness) while silica gel 60 (70–230 mesh) was used for column chromatography.

All reagents were of the best grade commercially available and were distilled, crystallized or used without further purification, as appropriate. 1,4,7,10-Tetraazacyclododecane (Strem Co., France), salicylic acid methyl ester and salicylamide (Aldrich, Germany), 1,2-dibromoethane (Aldrich, Germany), magnesium, calcium, strontium and aluminium perchlorates (Aldrich, Germany) were used as received. 1,2-Dibromoethane is toxic substance. It is stable, but may be light sensitive. Incompatible with strong oxidizing agents, magnesium, alkali metals. Environmental hazard – harmful to aquatic organisms. Usage of safety glasses, gloves, good ventilation is required.

2-(1′Bromoetoxy)-benzamide and 2-(1′bromoetoxy)-benzoic acid methyl ester were prepared and have spectral and analytical data identical to those given in the literature [8, 9].

Potentiometric titrations were performed in demineralized water at 25 °C using Teleko pH-meter equipped with Radiometer PHG200 pH-electrode. Water refers to high purity water with conductivity ≤0.03 μS cm^{-1}, obtained from the HYDROLAB purification system.

UV–Vis measurements were carried out with a Unicam UV-330 Spectrophotometer in buffered solution at

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pH = 5 or in water. Buffer solutions were prepared using buffer concentrate (citric acid/sodium hydroxide solution; Aldrich, Germany). All experiments were conducted in a quartz cuvette with a volume of 4 mL and a path length of 1 cm in water or buffer solution (pH = 5), over the range of 190–900 nm.

General synthetic procedure for bromine derivatives of salicylic acid

A mixture of 1,2-dibromoethane (40 mmol), salicylamide (salicylic acid methyl ester) (10 mmol) and K_2CO_3 (10 mmol) in 5 mL of DMF was refluxed for 6 h. After cooling an excess of reagents and solvent was removed under reduced pressure. To the residue methylene chloride (20 mL) was added. Inorganic salts were filtered off. The filtrate was concentrated under vacuum and the obtained oil was purified by gradient column chromatography using petroleum ether/methylene chloride solvent system.

2-(2-Bromoethoxy)-benzoic acid amide [8]

Yield 40%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.68–3.73 (2H, m), 4.37–4.42 (2H, m), 6.25 (1H, br s), 6.87 (1H, d, $J = 7.8$ Hz), 7.00–7.08 (1H, m), 7.36–7.44 (1H, m), 7.75 (1H, br s), 8.14 (1H, dd, $J_1 = 1.9$ Hz, $J_2 = 7.8$ Hz). ESI-MS: m/z 245 $[M+H]^+$. Calculated 245.09 for $C_9H_{11}BrNO_2$. Properties of this compound are identical to those given in literature.

2-(2-Bromoethoxy)-benzoic acid methyl ester [9]

Yield 45%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.66 (2H, t, $J = 6.6$ Hz), 3.89 (3H, s), 4.34 (2H, $J = 6.6$ Hz), 6.93–7.06 (1H, m), 7.40–7.49 (1H, m), 7.75 (1H, br s), 7.78 (1H, dd, $J_1 = 1.8$ Hz, $J_2 = 7.7$ Hz). ESI-MS: m/z 260 $[M+H]^+$. Calculated 260.10 for $C_{10}H_{12}BrO_3$. Properties of this compound are identical to those given in literature.

General synthetic procedure for 1,4,7,10-tetraazacyclododecane derivatives of salicylic acid

A typical procedure was as follows: a mixture of cyclen (1 mmol) and anhydrous $NaHCO_3$ (1 mmol) in chloroform (20 mL) and alkylating agent (1 mmol) was stirred under reflux for a week at 50 °C. The reaction was monitored by TLC. After cooling down, the inorganic salts were filtered off and the solvent was evaporated under reduced pressure. The residue was purified by gradient column chromatography on silica gel with chloroform/methanol solvent system.

*1-N-(1′-Ethyl-2′-phenoxy-2″-carbamoyl)-1,4,7,10-tetraazacyclododecane (**5**)*

Colourless oil. Yield 94%. ^{1}H NMR (500 MHz, CD_3OD): δ 2.86 (12H, s), 2.93–2.95 (4H, m), 3.08 (2H, t, $J = 4.9$ Hz), 4.28 (2H, t, $J = 4.9$ Hz), 7.07 (1H, t, $J = 7.8$ Hz), 7.20 (1H, d, $J = 8.3$ Hz), 7.50 (1H, t, $J = 8.3$ Hz), 7.78 (1H, d, $J = 7.8$ Hz); ^{13}C NMR (125 MHz, d-DMSO): δ 45.0, 46.6, 47.8, 49.3, 51.5, 67.0, 113.9, 121.3, 123.8, 131.3, 133.1, 156.9, 167.4; ESI-MS: m/z 336 $[M+H]^+$. ESI-HRMS calculated 336.2394 for $C_{17}H_{30}N_5O_2$; found 336.2400. IR (film): ν 3450, 3333, 3066, 2943, 2836, 2359, 1657, 1592, 1458, 1378, 1235, 1156, 1105, 1048, 959, 918, 757 cm^{-1}. Anal. Calcd. for $C_{17}H_{29}N_5O_2$: C, 60.87; H, 8.71; N, 20.88. Found: C, 60.65; H, 8.67; N, 20.83.

*1-N-(1′-Ethyl–2′-phenoxy–2″-methoxycarbonyl)-1,4,7,10-tetraazacyclododecane (**6**)*

Colourless oil. Yield 98%. ^{1}H NMR (500 MHz, CD_3OD): δ 2.88–2.90 (4H, m), 2.94–3.00 (8H, m), 3.05–3.10 (6H, m), 3.90 (3H, s), 4.22 (2H, t, $J = 4.4$ Hz), 7.04 (1H, t, $J = 7.8$ Hz), 7.22 (1H, d, $J = 8.3$ Hz), 7.56 (1H, t, $J = 8.3$ Hz), 7.86 (1H, d, $J = 7.8$ Hz); ^{13}C NMR (125 MHz, d-DMSO): δ 44.3, 45.3, 47.0, 50.1, 53.0, 56.7, 66.8, 114.2, 120.7, 120.9, 131.4, 134.4, 158.1, 166.9; ESI-MS: m/z 351 $[M+H]^+$. ESI-HRMS calculated 351.2391 for $C_{18}H_{31}N_4O_3$; found 351.2400. IR (film): ν 3430, 3156, 2946, 2841, 2775, 2359, 1718, 1633, 1598, 1453, 1367, 1300, 1247, 1134, 1087, 1046, 965, 908, 759 cm^{-1}. Anal. Calcd. for $C_{18}H_{30}N_4O_3$: C, 61.7; H, 8.63; N, 15.97. Found: C, 61.57; H, 8.60; N, 15.92.

Results and discussion

Monosubstituted cyclens can be synthesized in two ways. The first consists in direct substitution that usually requires excess of an expensive cyclen or/and high dilution conditions [10]. The second indirect method is based on tri-*N*-protected cyclen [11]. If very reactive electrophiles are used, especially those, which have other reactive groups, overcoming of the side reaction is impossible. Among such reagents acid chlorides can be pointed out. Particularly interesting are the synthesis of hydroxyacid derivatives. To defeat side reactions involving hydroxyl group and acid chloride, we used acetylsalicylic acid chloride as an acylating reagent. Two reactions were performed: direct acylation of cyclen and acylation of triprotected cyclen. The first reaction allowed only for formation of monosubstituted product (Figure 1) with low yield (27%), the second one was more selective but the yield was still unsatisfactory (48%).

Compound **1** does not complex alkali and alkaline earth metal cations. It only interacts with cobalt (II) cations and the formed complex has unusual 2:1 (ligand:metal) stoichiometry [12]. During spectroscopic titration of ligand **1** with cobalt perchlorate in acetonitrile solution, the main changes were found in region that corresponds to acetylsalicylic acid moiety. In this situation, the complexation, which takes place outside the macrocycle, could not be excluded. Unfortunately,

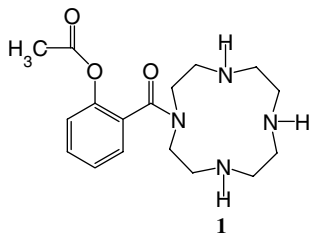

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Figure 1. Acetylsalicylic acid derivative of 1,4,7,10-tetraazacyclododecane [12].

the obtained crystals of complex are of poor quality and could not be examined by X-ray. To elucidate the participation of carbonyl group in complex formation, the C=O group was located on larger distance from the macrocycle. To this purpose two salicylic acid derivatives were synthesized (Scheme 1) [8, 9], and used for cyclen modification (Scheme 2).

Synthesis of ligands **5** and **6** was very simple. The mixture of cyclen, alkylating reagent (used in molar ratio), and $NaHCO_3$ in chloroform was stirred under reflux for a week at 50 °C. The slow reaction progress was controlled by TLC. Even after 7 days same unreacted substrates were detected. An excess of cyclen did not accelerate the reaction progress and did not improve the yield of monosubstitution.

Usage of the macrocycle:alkylating agent ratio 1:1 is the best choice for this reaction. Thus, compared with other methods, in which a large excess of cyclic tetraamines (2–10 equiv.) *versus* alkylating agent are used, this result shows significant improvement. For both reactions the mono-*N*-alkylation is highly selective and the yields are almost quantitative. Interesting is that only tracer amounts of bis-alkylated and tri-alkylated macrocycles were formed. The side products were only characterized by TLC and were not further analysed.

Properties of the synthesized cyclen derivatives were studied by UV–Vis spectrophotometry. The obtained ligands **5** and **6** show absorption band of 200–315 nm with maximum at 231 nm ($\log \varepsilon = 3.92$ and $\log \varepsilon = 3.98$, respectively) and 290 nm ($\log \varepsilon = 3.46$ and $\log \varepsilon = 3.60$, respectively) in aqueous solution.

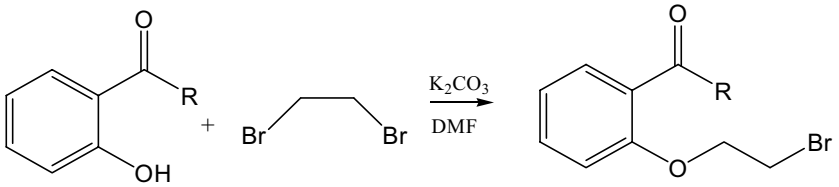
Compounds **5** and **6** have been found sensitive to pH. Figure 2 shows spectral changes in UV–Vis spectra recorded during pH titration in aqueous solution of ligands with 1×10^{-3} M HCl. In both cases slight increase of absorption band at $\lambda = 290$ nm is observed and more pronounced decrease of absorption band at $\lambda = 231$ nm. This technique did not allow as calculating the protonation constants, so potentiometric measurements were carried out.

For this purpose two acidic solutions were prepared by addition of almost stoichiometric amount of HCl (1:3.05) to the aqueous solution of ligands. The deprotonation constants (K_n) of **5** and **6** were determined by potentiometric pH titrations of acidic solutions of **5** and **6** against 0.02 M KOH at 25 °C (Figure 3).

The equilibriums could be described as below:

$$L + H^+ \leftrightharpoons LH^+$$

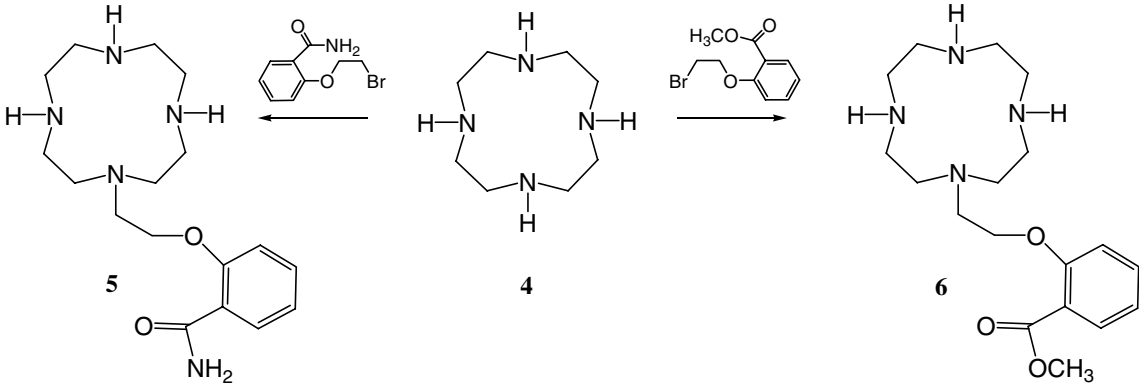
$$LH^+ + H^+ \leftrightharpoons LH_2^{2+}$$



R = NH_2, OCH_3

2. R = NH_2
3. R = OCH_3

Scheme 1. Synthesis of alkylating reagents [8, 9].



5 **4** **6**

Scheme 2. Synthesis of monosubstituted 1,4,7,10-tetraazacyclododecane derivatives.

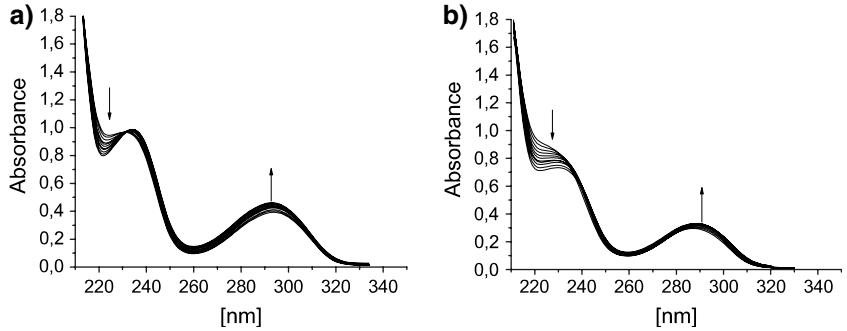


Figure 2. Spectroscopic pH titration against HCl (1×10^{-3} M) in aqueous solution: (a) compound **5** (1×10^{-4} M); (b) compound **6** (1×10^{-4} M).

$$LH_2^{2+} + H^+ \Longleftrightarrow LH_3^{3+}$$

The titration data were analysed for the acid–base equilibriums 1 and 2, where a_{H^+} is the activity of H^+.

$$K_1 = [L]/[H_{-1}L]a_{H^+} \quad (1)$$

$$K_n = [H_{n-1}L]/[H_{n-2}L]a_{H^+} \quad (n = 2, 3) \quad (2)$$

In comparison to the parent cyclen, which showed two nitrogen atoms of high basicity (pK_a 11.0 ± 0.1 and 9.9 ± 0.1) and two nitrogen atoms of low basicity ($pK_a < 2$) [13], presented ligand **5** has one amino group with $pK_a = 9.37 \pm 0.3$, one of reduced basicity ($pK_a = 3.24 \pm 0.2$) and one with low basicity ($pK_a < 2$). Similar acid–base properties have been found for the second compound **6** ($pK_a = 9.42 \pm 0.2$; $pK_a = 3.10 \pm 0.2$ and $pK_a < 2$).

Complexation studies of compound **5** and **6** with alkali metal cations performed in aqueous solution at wide range of pH show no spectral changes. Further studies were performed in buffered solution at pH = 5. Comparing to compounds **1** the new designed ligands **5** and **6** do not interact with Co^{2+} or with other transition metal cations. These ligands form only complexes with Mg^{+2}, Ca^{+2} and Sr^{+2} cations. That may suggest that complexation takes place outside the cyclen and only salicylic acid moiety is involved in this process. However, in UV spectra major changes are observed only in region, which corresponds to cyclen. Figure 4a–c show absorption spectra recorded during titration of ligand **5** with magnesium, calcium and strontium perchlorates.

Formation of complexes was studied by molar-ratio method. Figure 4d–f show successive complex development. Here the total concentration of ligand was kept constant while the total concentration of the metal was increased. Formation of weak complexes of 1:1 stoichiometry is observed.

Figure 5a–c presents data obtained for ligand **6**. The band at 290 nm does not change at all and only decrease of band at 231 nm is observed. Again, only 1:1 (ligand:metal) complexes are formed Figure 5d–f).

The stability constants of those complexes determined by molar-ratio method are collected in Table 1.

It was found that compounds **5** and **6** recognize aluminium cations. Results of spectroscopic titration of **5** and **6** against $Al(ClO_4)_3$ in buffered solutions are shown in Figure 6. In this case several equilibriums of very strong complexes were found and calculation of complexation constants was impossible.

Complex formation has also been studied by ^1H NMR. The experiment was conducted in D_2O at pH = 5. Firstly, ^1H NMR spectra of free ligands **5** and **6** were recorded; next solid magnesium, calcium, or strontium perchlorates were added gradually. Upon small salt doses addition, the ^1H NMR spectra were recorded, and then the next amount of salt was added. The salt addition was provided until 1:1 molar ratio was achieved. Minor changes were observed in aromatic region of spectra; however they are shown only in the shapes of multiplates if ligand **5** is studied in ^1H NMR spectra of free ligand **5** broad multiplets are observed. Similar shapes of multiplates are observed upon equimolecular addition of $Mg(ClO_4)_2$. While well pro-

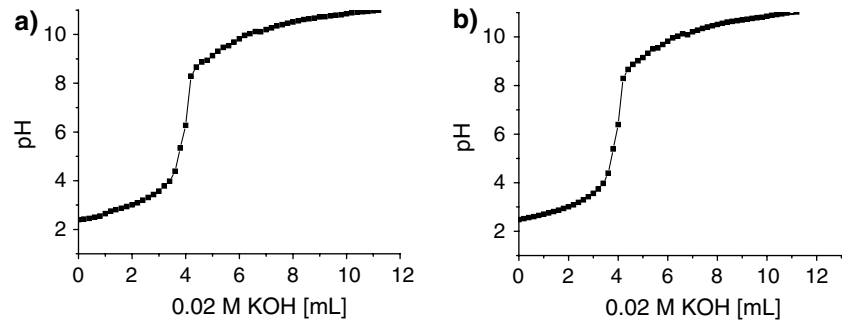


Figure 3. pH Titration against KOH (0.02 M) in aqueous solution: (a) compound **5** ($c_L = 2.04 \times 10^{-3}$ M; $c_{H^+} = 6.45 \times 10^{-3}$ M HCl, $I = 0.1$ (KNO_3)), (b) compound **6** ($c_L = 2.03 \times 10^{-3}$ M, $c_{H^+} = 6.45 \times 10^{-3}$ M HCl, $I = 0.1$ (KNO_3)).

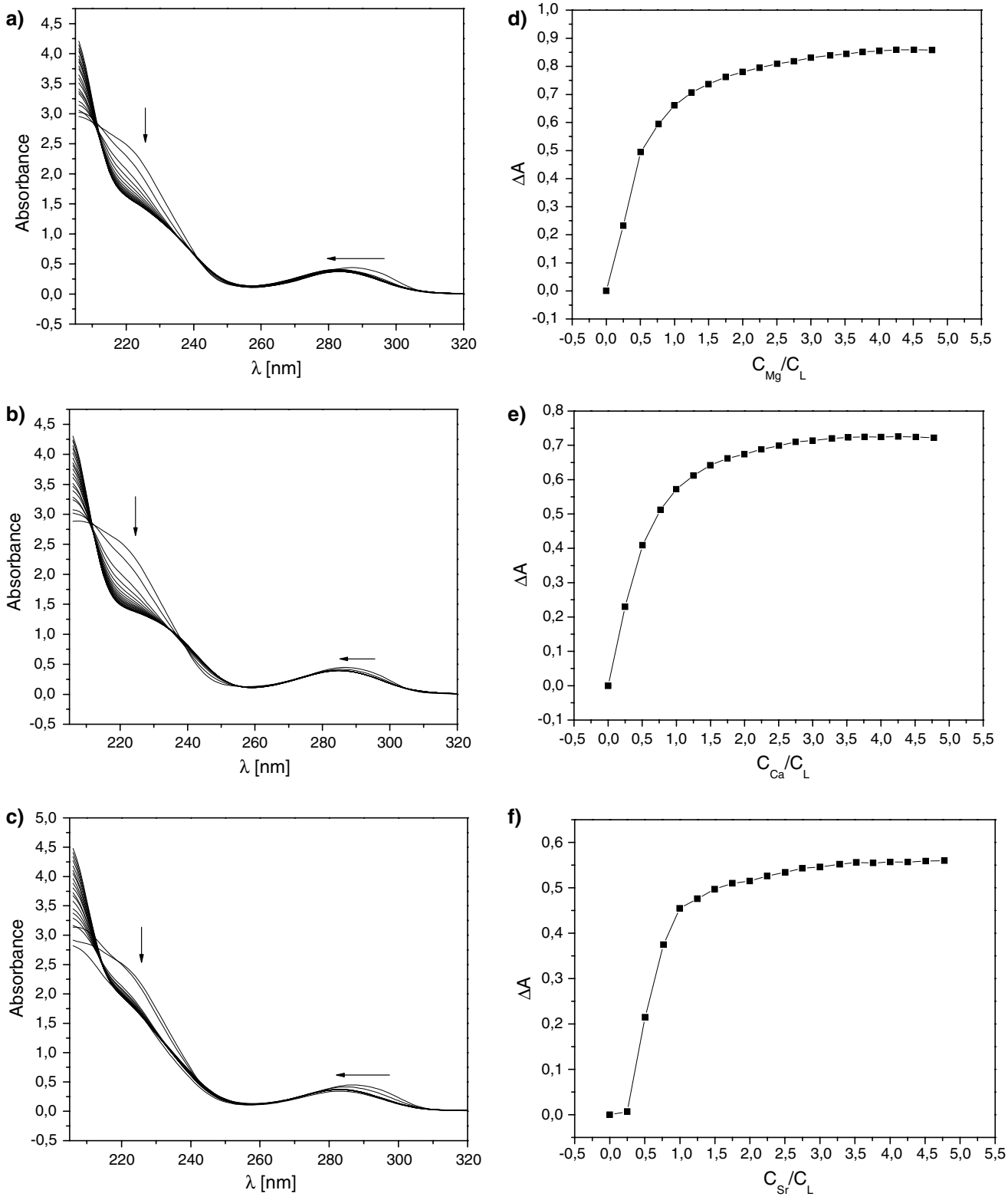


Figure 4. Spectroscopic titration of compound **5** ($c_L = 1.67 \times 10^{-4}$ M) in buffered solution at pH = 5 (a) with $Mg(ClO_4)_2$ ($c_M = 0–8.35 \times 10^{-4}$ M), (b) with $Ca(ClO_4)_2$ ($c_M = 0–8.35 \times 10^{-4}$ M), (c) $Sr(ClO_4)_2$ ($c_M = 0–8.35 \times 10^{-4}$ M); Dependence of absorbance at 225 nm for ligand **5** *versus* molar ratio c_M/c_L: (d) $Mg(ClO_4)_2$, (e) $Ca(ClO_4)_2$, (f) $Sr(ClO_4)_2$.

nounced multiplates are typical for complexes of ligand **5** and $Ca(ClO_4)_2$, and $Sr(ClO_4)_2$ in aromatic region. Major changes were detected in aliphatic region (Figures 7 and 8). In the spectra of free ligand **5** five multiplets (Figure 7a) are observed in aliphatic region. Salt addition causes chemical shift of multiplet at 2.78 ppm and change of the shapes of muliplets. Ligand **6** exhibits analogous properties. Obtained results show that in complexation mainly cyclen is involved, but the changes are insignificant.

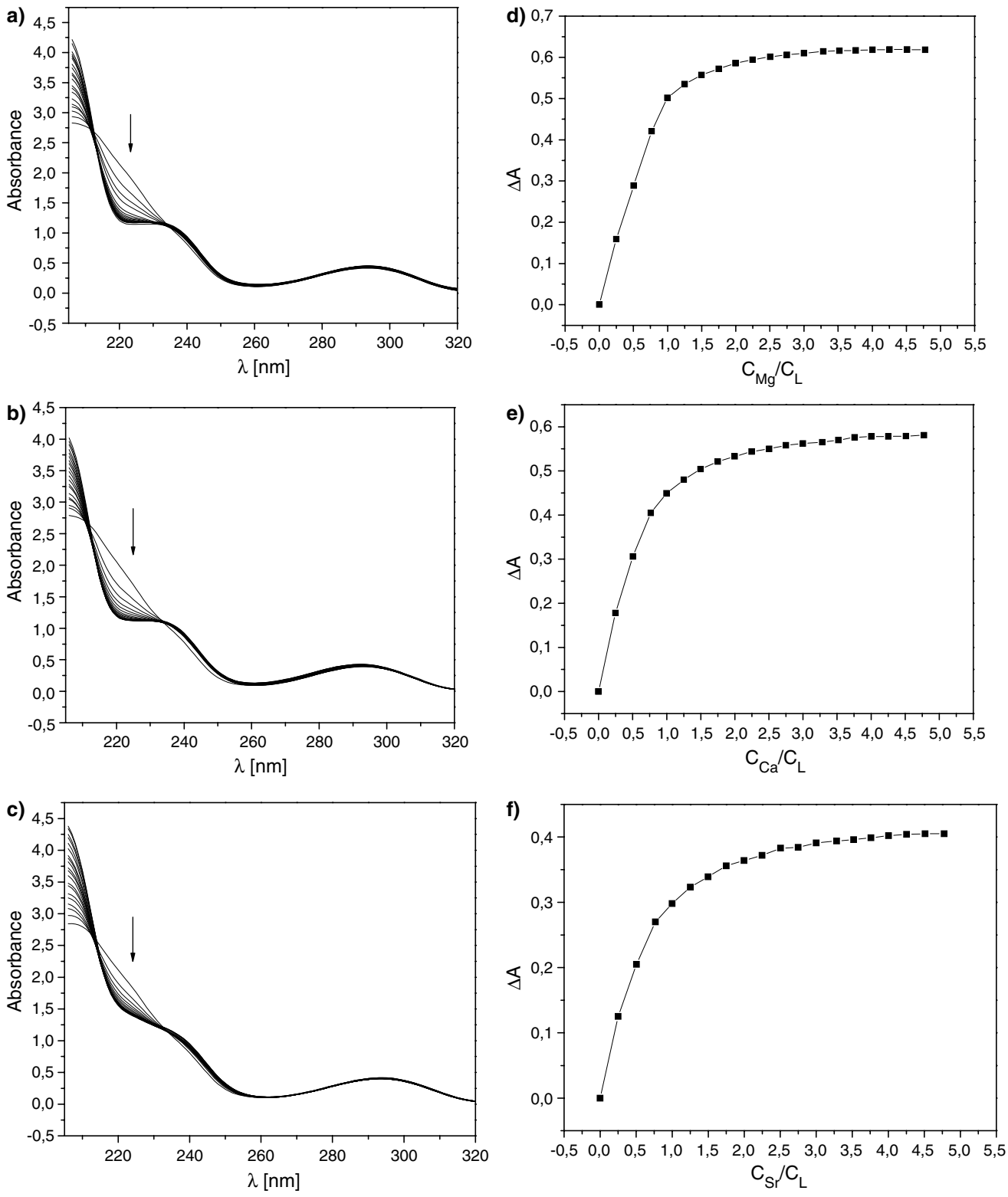


Figure 5. Spectroscopic titration of compound **6** ($c_L = 1.68 \times 10^{-4}$ M) in buffered solution at pH = 5 (a) with $Mg(ClO_4)_2$ ($c_M = 0$–8.45×10^{-4} M), (b) with $Ca(ClO_4)_2$ ($c_M = 0$–8.45×10^{-4} M), (c) $Sr(ClO_4)_2$ ($c_M = 0$–8.45×10^{-4} M); Dependence of absorbance at 225 nm for ligand **6** *versus* molar ratio c_M/c_L: (d) $Mg(ClO_4)_2$, (e) $Ca(ClO_4)_2$, (f) $Sr(ClO_4)_2$.

Table 1. Stability constants for 1:1 ligand complexes of alkali earth ions in buffered solution at pH = 5

Compound	$\log\beta_{Mg}$	$\log\beta_{Ca}$	$\log\beta_{Sr}$
5	5.65 ± 0.13	5.80 ± 0.57	6.59 ± 0.22
6	5.91 ± 0.26	5.86 ± 0.78	6.15 ± 0.55

Conclusions

Synthesis of monosubstituted cyclen derivatives **5** and **6** by direct alkylation of cyclen was described. Both compounds have been found as sensitive molecules to pH. Their acid–base properties have been studied by

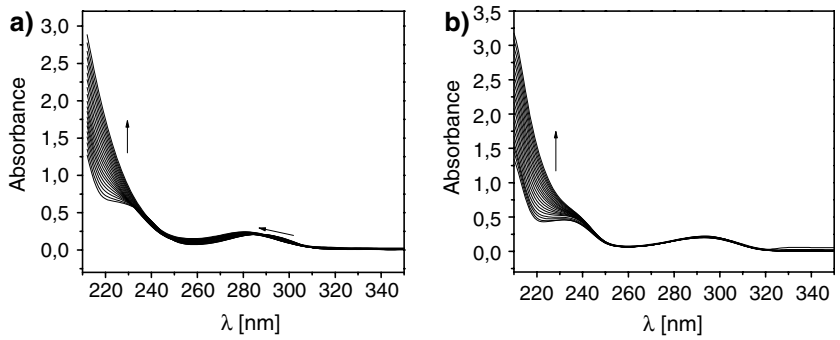


Figure 6. Spectroscopic titration of ligands in buffer solution (pH = 5) with $Al(ClO_4)_3$ ($c_M = 0–1 \times 10^{-3}$; (a) ligand **5** ($c_L = 2 \times 10^{-4}$ M); (b) ligand **6** ($c_L = 2 \times 10^{-4}$ M).

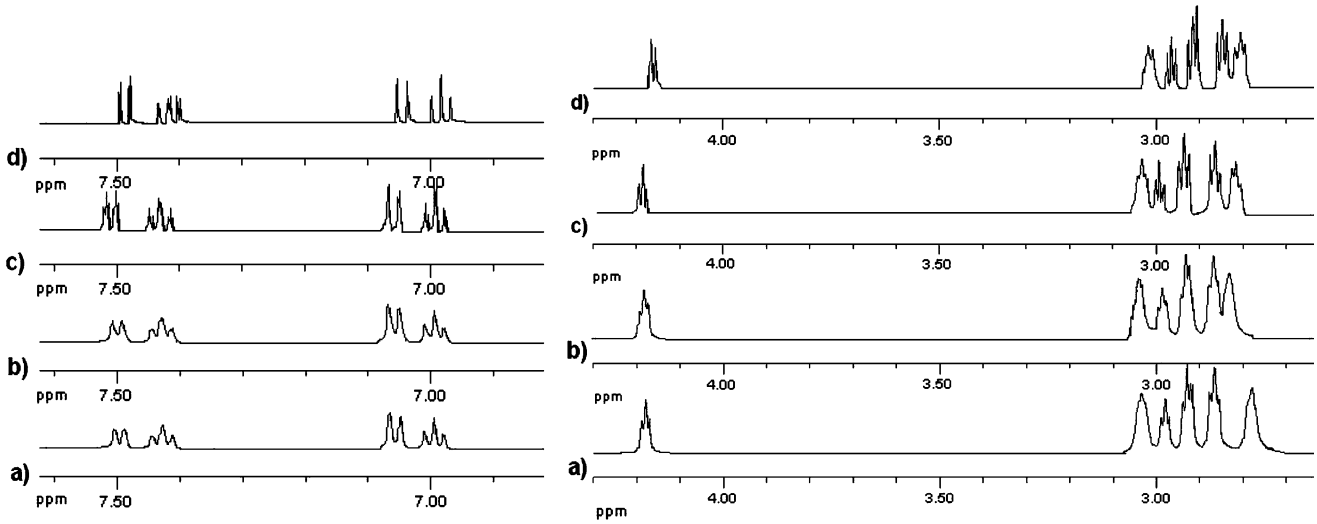


Figure 7. ^{1}H NMR in D_2O at pH = 5: (a) free ligand **5**; (b) upon addition of equimolecular $Mg(ClO_4)_2$; (c) upon addition of equimolecular $Ca(ClO_4)_2$; (d) upon addition of equimolecular $Sr(ClO_4)_2$.

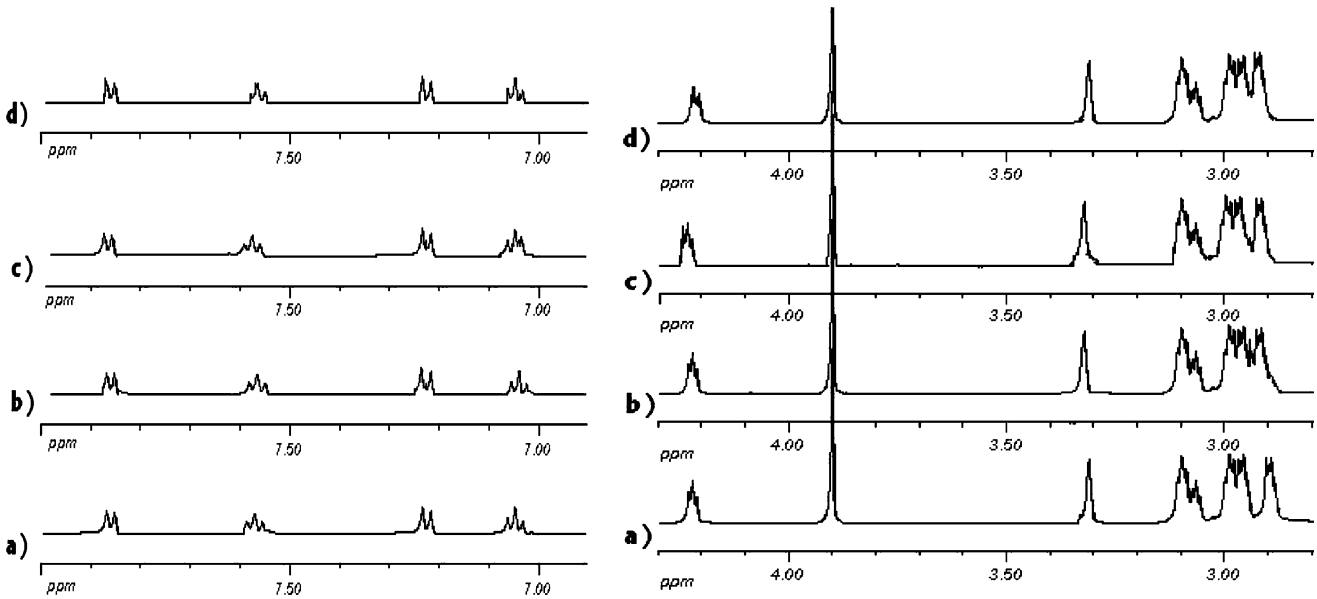


Figure 8. ^{1}H NMR in D_2O at pH = 5: (a) free ligand **6**; (b) upon addition of equimolecular $Mg(ClO_4)_2$; (c) upon addition of equimolecular $Ca(ClO_4)_2$; (d) upon addition of equimolecular $Sr(ClO_4)_2$.

potentiometry. Their protonation constants have been determined. Both compounds have light-harvesting moieties but they exhibit only weak fluorescence. Salt addition does not cause any significant changes of luminescence of examined ligands. UV–Vis spectroscopic studies show that both compounds form complexes with Mg^{+2}, Ca^{+2}, and Sr^{+2}. The stoichiometry of obtained complexes is 1:1. Complexes formation were additionally confirmed by ^{1}H NMR studies. Comparing to formerly published ligand **1**, the properties of compounds **5** and **6** are completely different. It can be explained by the fact that incorporation of salicylic acid moiety linked by ethylene spacer made the molecule more flexible and, of course, there is not withdrawing effect of carbonyl group typical for **1**. Wondering is the fact that newly designed ligands do not show any response for transition metal cation.

Acknowledgements

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