Research Paper

Journal of Chemical Research

Microwave-assisted synthesis of a novel steroid-derived Schiff base chemosensor for detection of Al³⁺ in aqueous media

Yu Chen

Journal of Chemical Research 1–6 © The Author(s) 2020 Article reuse guidelines: sagepub.com/journals-permissions DOI: 10.1177/1747519820914827 journals.sagepub.com/home/chl



Abstract

A novel steroid-derived Schiff base chemosensor, N'-(2-hydroxybenzylidene)- 3α -hydroxy-cholanhydrazide (LA), has been designed and prepared via microwave irradiation. The sensor LA showed highly selective fluorescent sensing for Al³⁺ with a low detection limit of 34 nM in the pH range from 6.05 to 9.32 in ethanol/water (1:2, v/v) solution. The binding stoichiometry between LA and Al³⁺ was determined as 1:1 by Job's plot and further verified with ¹H NMR studies. Under a UV lamp, the fluorescence color changes could be easily detected by the naked eye. In addition, the sensor LA has been applied in detection of Al³⁺ in real water samples.

Keywords

aluminum(III) ions, chemosensor, microwave irradiation, Schiff base, steroid

Date received: 25 November 2019; accepted: 3 March 2020



Introduction

Aluminum is the most abundant metal in the earth's crust, forms approximately 8% of earth mass. Aluminum has been widely used in modern life, such as water treatment plants, food additives, textiles, and pharmaceuticals. However, with the rapid development of aluminum industry, the content of aluminum in natural water and soil is gradually increasing.¹ As we all know, aluminum is not a biologically essential element, and excessive accumulation of Al³⁺ would be toxic and cause many serious diseases, such as neuronal disorder,² loss of memory, listlessness, and Alzheimer's disease.^{3,4} Therefore, the design and synthesis of chemosensors with high selectivity and excellent sensitivity in detecting aluminum is of great significance.

Schiff bases are good ligands for metal ions, with which they can form strong coordinate bonds. This characteristic has resulted in their extensive use in the design and synthesis of chemosensors.^{5–9} Two possible mechanisms that have been reported, the C=N isomerization and the suppression

of this isomerization, are responsible for fluorescence emission behavior of the Schiff bases.^{10–12} Binding with metal ions, the C=N isomerization would be inhibited and provides a significant change in the fluorescence emission, which is a crucial advantage in practical applications.¹³

"Green chemistry" is the frontier of international chemical science research today and has received widespread concern and attention from governments, business, and chemical circles around the world. As a new technology in line with the concept of "green chemistry," microwave irradiation has attracted considerable interest in recent years.^{14,15} Microwave-assisted organic synthesis is a valuable technology that helps reduce reaction times,

Pingdingshan Polytechnic College, Pingdingshan, P.R. China

Corresponding author:

Yu Chen, Pingdingshan Polytechnic College, Pingdingshan 467001, P.R. China.

Email: pdschenyu@163.com



Scheme I. Synthetic protocol for sensor LA.

Table 1. Optimization of the reaction conditions synthesissensor LA.

Entry	Solvent	Additive	Time/h	Yield/%
I	EtOH	_	5	12ª
2	EtOH	HCI	5	69 ª
3	EtOH	AcOH	5	51ª
4	EtOH	H ₂ SO	5	43 ª
5	AcOH		5	65ª
6	THF	HCI	5	36ª
7	MeOH	HCI	5	51ª
8	DMF	HCI	5	73 ª
9	DMSO	HCI	5	35ª
10	MeOH	HCI	0.17	86 ^b

^aConventional method.

^bMicrowave irradiation: 300 W, ordinary pressure.

increase yields, achieve cleaner reactions, simplify workups, and design energy-saving protocols.^{16–18}

Although various Schiff base chemosensors have been designed and synthesized, 19-23 to the best of our knowledge and according to the literature survey that few efforts have been made in the efficient synthesis of chemosensors with "green chemistry" strategy. Besides, steroids are rarely used as a skeleton for fluorescent sensor. In previous studies.^{24–27} we reported a microwave mediated, efficient, high yield, and environmentally friendly synthesis of Schiff base derivatives. Herein, a novel steroidal Schiff base fluorescent sensor, N'-(2-hydroxybenzylidene)-3α-hydroxycholanhydrazide (LA), was designed and prepared via microwave irradiation (Scheme 1), and Al³⁺ detection capability of LA has been investigated. The free LA exhibited almost no detectable fluorescence emission due to the C=N isomerization and the photo-induced electron transfer (PET) phenomena; when coordinating with Al³⁺, the C=N isomerization and PET effects were inhibited and the molecular structure changed from flexible to rigid, which dramatically increased the fluorescence intensity.^{12,28-30}

Results and discussion

To develop an optimal reaction condition, 3α -hydroxycholan-24-hydrazide (3) and salicylaldehyde (4) were employed in conventional and microwave-assisted

procedures. The reaction was monitored with or without additives, such as acetic acid, Sulfuric acid (H₂SO₄), or hydrochloride (HCl) under refluxing in Tetrahydrofuran (THF), Dimethyl Sulfoxide (DMSO), N,N-Dimethylformamide (DMF), ethanol, methanol, or acetic acid. The result demonstrated that the yield of sensor LA was in the range of 12%-73%, while longer reaction time was required. With better catalytic property, HCl was used in the synthesis of sensor LA under microwave irradiation. Fortunately, it showed a dramatic improvement of the yield and remarkable reduction in reaction time (0.17h) when we employed a microwave-assisted method. Compared with conventional thermal heating, microwave irradiation technique decreased the reaction time from 5 to 0.17 h and enhanced the yield from 73% to 86%. The study data were given in Table 1.

The UV-Vis titration spectra of **LA** upon addition of various concentrations of Al^{3+} were carried out in ethanol and water (1:2, v/v) at room temperature. As shown in Figure 1, the absorption spectra of receptor **LA** have three absorption maxima, centered at 254, 264, and 294 nm. Upon addition of increasing concentrations of Al^{3+} (0–1.0 equiv.) to the solution of sensor **LA** (50 µM), those three absorption bands were enhanced gradually, which clearly suggests that **LA** participates in a coordination with Al^{3+} .

The fluorescence emissions of sensor LA (50 μ M) on adding different metal ions (1.0 equiv. of LA) including Ag⁺, Al³⁺, Ba²⁺, Ca²⁺, Co²⁺, Cr³⁺, Cu²⁺, Fe³⁺, Hg²⁺, Mg²⁺, Mn²⁺, Ni²⁺, Pb²⁺, and Zn²⁺ were determined in ethanol/water (1:2, v/v). After excitation at 400 nm, the sensor LA exhibited very week fluorescence emission. Upon addition of Al³⁺ (50 μ M), a remarkable fluorescence emission peak at 460 nm was observed (Figure 2). In contrast, no significant fluorescence change was detected after addition of other metal ions under the same conditions. These results indicate that LA exhibits high selectivity for Al³⁺ over other metal ions. Under the 365nm UV lamp, the remarkable light-blue fluorescence emission associated with the reactions between LA and Al³⁺ was observed by the naked eye, but no significant fluorescence change was detected for LA solution with other metal ions.

The quantitative sensing abilities of LA (50 μ M) toward Al³⁺ were studied, and a working curve was obtained (Figure 3). With addition of increasing concentrations of Al³⁺ ions (0, 10, 20, 30, 40, 50, 60, and 70 μ M), the fluorescence



Figure 1. Absorption spectra of sensor **LA** (50μ M) in ethanol and water (1:2, v/v) solution obtained by adding aliquots of [Al³⁺] (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 equiv.).



Figure 2. Fluorescent spectra of sensor **LA** (50 μ M) with 1.0 equiv. of various metal ions in ethanol and water (1:2, v/v). Inset: The colors of sensor **LA** and **LA**-Al³⁺ as viewed by the naked eye under a 365-nm UV lamp.

intensity of LA-Al³⁺ enhanced remarkably and showed a maximum fluorescence emission at 460 nm. Besides, a good linear correlation (R2=0.99078) between the emission intensity of LA and the concentration ratio of $[Al^{3+}]/[LA]$ was observed on addition of 0–1.4 equiv. of Al³⁺ ions. According to the titration profile (Figure 4), the binding constant of LA for Al³⁺ could be calculated as $4.13 \times 10^3 \text{ M}^{-1}$ based on the modified Benesi–Hildebrand equation (equation (1)),³¹ in where F, F_{min} , and F_{max} represents the fluorescence intensities of LA-Al³⁺ complex, free LA, and the maximum fluorescence intensity of LA-Al³⁺ complex, respectively.

$$\frac{1}{F - F_{min}} = \frac{1}{K \left(F_{max} - F_{min} \right) \left[A I^{3+} \right]} + \frac{1}{F_{max} - F_{min}}$$
(1)

According to the 3σ method (limit of detection (LOD)= $3\sigma/K$, σ represents the standard deviation of a blank solution and K represents the slope of the calibration curve in Supplemental Figure S1), the LOD of sensor LA



Figure 3. Fluorescence titrations of 50- μ M **LA** (λ_{ex} =400 nm) in ethanol and water (1:2, v/v) in the presence of different equivalents of Al³⁺ ions (0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 equiv.). Inset: Graph of the fluorescence intensity at 460 nm as a function of the concentration ratio of Al³⁺ and **LA**.



Figure 4. Benesi–Hildebrand analysis of the emission changes for the complexation between **LA** and Al³⁺ in ethanol and water (1:2, v/v). (λ_{ex} = 400 nm, λ_{em} = 460 nm).

for Al³⁺ reached 34 nM, which is low for the detection of Al³⁺ comparing with other published Al³⁺ binding sensors and much lower than World Health Organization (WHO) recommended 3.7–7.4 μ M Al³⁺ standard drinking water.^{32,33} These results demonstrate that sensor **LA** could be potentially used for selective detection of Al³⁺ in analytical chemistry, especially in water quality monitoring.

To determine the utility of sensor LA as an Al³⁺-selective receptor in the complex background of competing species, the fluorescence emissions of LA-Al³⁺ were examined in the presence of different metal ions (Figure 5). Upon addition of different metal ions (1 equiv. of Al³⁺), the fluorescence emissions of LA-Al³⁺ were almost not interfered, except for Cu²⁺ and Fe³⁺. The obvious reduction of fluorescence intensities caused by Cu²⁺ and Fe³⁺ might due to an energy or electron transfer.³⁴ As reported in literatures,^{35,36} it was possibly because the newly formed complex LA-Fe³⁺ or LA-Cu²⁺ was too stable to be replaced by Al³⁺.



Figure 5. Relative fluorescence intensity ratio of **LA**-Al³⁺ (50 μ M) in the absence and presence of different metal ions (1.0 equiv.). (λ_{ex} = 400 nm, λ_{em} = 460 nm).



Figure 6. Job's plot for the complexation of LA with Al^{3+} (λ_{ex} = 400 nm, λ_{em} = 460 nm).

To investigate the applicability of sensor LA for detecting Al³⁺ in different environments, the pH effects were studied (see Supplemental Figure S2). The fluorescence emissions of LA (50 μ M) and LA-Al³⁺ complex (50 μ M, 1:1, M/M) were detected in various buffer solutions (see Supplemental Table S1). The free sensor LA exhibited very week fluorescence emissions in the pH range of 3.92-9.84. Upon addition of Al³⁺, a remarkable fluorescence enhancement of LA was observed and the pH value increased from 6.05 to 9.32. On the contrary, in acidic solutions (pH < 6.05), the fluorescence intensities significantly decreased, which might due to the protonation of Schiff base group. Moreover, the fluorescence intensities of LA-Al³⁺ were sharply reduced in strong alkaline solutions (pH>9.32), which mainly result from the decomposition of Schiff base group. Besides, the formation of aluminum hydroxide in strong alkaline solutions could possibly be another reason for this significant inactivation of probing process. These results demonstrate that sensor LA is highly selective toward Al³⁺ in a relatively wide pH range (6.05–9.32).

The binding stoichiometry between sensor LA and Al^{3+} (Figure 6) was studied by the method of continuous variation (Job's plot). In the Job's plot, the total concentration of

LA and Al^{3+} was 50 µM, with a continuous variable molar fraction of guest $[Al^{3+}]/([LA+Al^{3+}])$. The maximum fluorescence emission intensity at 460 nm was observed at the molar ratio of 0.51, establishing a 1:1 binding stoichiometry between sensor LA and Al^{3+} .

Additional evidence was given by NMR (nuclear magnetic resonance) studies of sensor LA and LA-Al³⁺ complex. The ¹H NMR spectra of LA in the absence and presence of Al³⁺ were recorded in DMSO- d_6 . Significant spectral changes were observed (Figure 7). Signals for the protons of -NH- (H³) at 11.57 ppm and -OH (H¹) at 11.19 ppm disappeared, which indicates the enolization and deprotonation of LA in the presence of Al³⁺.^{37,38} Besides, the signals of H2 and H4 were downfield shifted 0.08 and 0.3 respectively. These phenomena indicate that the hydroxyl group, carbonyl group, and C=N group of sensor LA participate in a coordination with Al³⁺, which in return changes the electron distribution in the chemosensor.

Based on the above results, a binding mechanism for the fluorescence response of LA toward Al^{3+} is proposed. As shown in Scheme 2, the fluorescence emission of free LA is nearly quenched, which may due to the PET phenomenon causing by lone pair electron from Schiff-base nitrogen atom to the phenol moiety and C=N isomerization process. However, upon addition of Al^{3+} ions (2.0 equiv.), two nitrogen atoms of the azomethine groups and the hydroxyl group on the phenol participate in the coordination with Al^{3+} , forming a 1:1 complex and resulting in a remarkable fluorescence enhancement of LA.

By utilizing a previously reported method,^{39,40} sensor LA was used to measure the Al³⁺ content in actual water samples, including tap water (from our laboratory), domestic sewage (from student residences at our college), and industrial sewage (from industrial areas in Pingdingshan City). All the water samples were filtered through a 0.2-mm filter membrane to remove large particular impurities, followed by removal of remaining organics by extraction processes. The resulting samples were diluted with ethanol and water (1:2, v/v) in a 10.0-mL volumetric flask. Table 2 shows the results acquired using sensor LA with the appropriate concentration gradient of Al³⁺ added. The results indicate that sensor LA had good recovery and demonstrated high accuracy in the analysis of Al³⁺. Therefore, sensor LA can measure the concentration of Al³⁺ in real water samples and has practical value in environmental analysis.

Conclusion

In summary, a highly selective and sensitive Schiff base chemosensor, N'-(2-hydroxybenzylidene)- 3α -hydroxycholanhydrazide (LA), has been designed and synthesized under microwave irradiation. LA shows significant "turnon" fluorescence behavior with Al³⁺ in ethanol/water (1:2, v/v) solution, giving strong light-blue emission. The detection limit was calculated to be 3.4×10^{-8} molL-1 and remained stable, detecting Al³⁺ in the pH range of 6.05–9.32. In addition, the sensor LA exhibits satisfactory results for Al³⁺ detection in the analysis of real water samples and can be further used in potential applications for the detection of



Figure 7. ¹H NMR spectra of sensor LA and LA-Al³⁺ in DMSO-d₆.



Scheme 2. Proposed sensing mechanism between sensor LA and Al³⁺.

Tal	ble	2.	Determination	of A	∖l³+ ir	real	water	samples	with	sensor	L/	4.
-----	-----	----	---------------	------	---------	------	-------	---------	------	--------	----	----

Sample	рНª	Added Al ³⁺ (nM)	Found ^b Al ³⁺ (nM)	Recovery (%)	RSD (%)
Tap water	6.71	40	39.18	97.95	1.25
·		60	60.51	100.85	
		80	79.76	99.70	
		100	100.25	100.25	
Domestic sewage	7.96	40	39.66	99.15	0.60
0		60	60.33	100.55	
		80	80.20	100.25	
		100	99.89	99.89	
Industrial sewage	8.43	40	39.41	98.53	0.98
C C		60	60.39	100.65	
		80	79.67	99.59	
		100	100.52	100.52	

RSD: relative standard deviation.

^aDetermined with a pH meter before treating with Al³⁺.

^bResults are based on three measurements.

nanomolar concentrations of Al³⁺ in chemical and environmental systems.

Experimental

All chemicals and solvents were obtained from commercial sources and used without further purification, except when specified. Solvents used in fluorescent experiments were of spectroscopic grade. The ¹H NMR and ¹³C NMR spectra of the ligand were recorded on an Agilent 400-NMR DDR2 spectrometer. The chemical shifts (δ) are recorded in ppm, relative to tetramethylsilane (SiMe₄). The Fourier transform

infrared spectrum was recorded on a Perkin-Elmer 1700 FTIR spectrophotometer. Mass spectra were obtained using a Finnigan LCQ DECA mass spectrometer in electrospray ionization (ESI) positive mode. A Mapada UV-6100 UV-Vis spectrophotometer was used for recording the UV-Vis spectra. Fluorescence emission spectra were recorded on a Dual-FL fluorescence spectrophotometer. Elemental analysis (C, H, and N) was performed with an Elementar Vario MICRO cube, and the results are within $\pm 0.4\%$ of the calculated values. All the reactions were performed in a commercial microwave apparatus (XH-100A, 100–1000W; Beijing Xianghu Science and Technology Development Co.

Preparation of N'-(2-hydroxybenzylidene)- 3α -hydroxy-cholanhydrazide (sensor LA)

To a solution of compound 3 (1 mmol) in methanol (5 mL), salicylaldehyde (0.95 mmol) and acetic acid (2 drops) were added successively. The mixture was heated to reflux in the microwave oven at 300 W for 10 min. After completion, the mixture was cooled and filtered to afford crude product. The crude product was recrystallized from ethanol to give the desired product LA (428 mg) as yellow solid. Yield 86%; m.p. 254.5–254.9°C (EtOH). IR (KBr, cm)⁻¹: 3453, 3221, 3070, 2941, 2872, 1687, 1624, 855, 750, 627. ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.57 (s, 1H, NH), 11.19 (s, 1H, Ar-OH), 8.33 (s, 1H, =CH), 7.48 (d, J=8.0Hz, 1H, ArH), 7.25-7.29 (m, 1H, ArH), 6.87-6.91 (m, 2H, ArH), 4.45 (d, J=1.2 Hz, 1H, 3 α -OH), 0.92 (t, J=8.0 Hz, 3H, 21-CH₂), 0.87 (s, 3H, 19-CH₂), 0.61 (s, 3H, 18-CH₂). ¹³C NMR (100 MHz, DMSO-d₆): δ 173.98, 162.46, 151.48, 134.61, 131.73, 125.24, 123.78, 121.45, 75.04, 61.26, 60.71, 60.54, 47.46, 46.70, 41.48, 40.56, 40.33, 40.13, 39.39, 36.34, 36.08, 35.56, 34.18, 32.92, 32.07, 31.34, 29.03, 28.46, 25.59, 23.45, 17.07. ESI-MS, m/z: 495.86 [M+1]⁺. Anal. calcd for C₃₁H₄₆N₂O₃: C, 75.26; H, 9.37; N, 5.66; found: C, 75.06; H, 9.34; N, 5.68%.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Yu Chen 🕩 https://orcid.org/0000-0002-2711-6535

Supplemental material

Supplemental material for this article is available online.

References

- 1. He T, Xin Q, Zhen LZ, et al. *Spectro Chim Acta A* 2019; 207: 31.
- Campbell A, Becaria A, Lahiri DK, et al. J Neurosci Res 2004; 75: 565.
- 3. Flaten TP. Brain Res Bull 2001; 55: 187.

- 4. Good PF, Olanow CW and Perl DP. *Brain Res* 1992; 593: 343.
- Wang R, Jiang GQ and Li XH. *Inorg Chim Acta* 2017; 455: 247.
- 6. Supriti S, Titas M, Basab C, et al. Analyst 2012; 137: 3975.
- 7. Upadhyay KK and Ajit K. Org Biomol Chem 2010; 8: 4892.
- Wei HD, Dan W, Xiang JZ, et al. Sens Actuators B 2015; 209: 359.
- 9. Onder A and Serkan E. Sens Actuators B 2015; 208: 159.
- 10. Zhao CX, Ju YY and David RS. *Chem Soc Rev* 2010; 39: 1996.
- 11. Lei L, Yong QD, Hong WL, et al. *Tetrahedron Lett* 2010; 51: 618.
- 12. Jun Y, Long F, Jing CQ, et al. *Tetrahedron Lett* 2016; 57: 2910.
- 13. Ece E, Ümit E and Özgür Muhammed İFK. Spectrochim Acta A 2018; 203: 273.
- 14. Li MX, Chen CL, Ling CS, et al. *Bioorg Med Chem Lett* 2009; 19: 2704.
- 15. Kappe CO. Angew Chem Int Ed 2004; 43: 6250.
- 16. Polshettiwar V and Varma RS. Acc Chem Res 2008; 41: 629.
- 17. Katrizky AR and Angrish P. Steroids 2006; 71: 660.
- Ramesh E and Raghumathan R. *Tetrahedron Lett* 2008; 49: 1812.
- 19. Yu LX, Shan SM, Hong PP, et al. J Lumin 2017; 192: 56.
- Kalyani R, Amit KM, Meman S, et al. *Inorg Chim Acta* 2019; 492: 156.
- 21. Xin S, Ya WW and Yu P. Org Lett 2012; 14: 3420.
- 22. Zohreh S, Mehdi A and Soraia M. J Lumin 2019; 207: 78.
- 23. Das B, Jana A, Mahapatra AD, et al. *Spectrochim Acta A* 2019; 212: 222.
- 24. Shi ZC, Zhao ZG, Liu M, et al. C R Chim 2013; 16: 977.
- 25. Shi ZC, Zhao ZG, Liu XL, et al. *Chin Chem Lett* 2011; 22: 405.
- 26. Shi ZC, Zhao ZG, Liu XL, et al. J Chem Res 2011; 35: 198.
- 27. Li GH, Shi ZC, Li XR, et al. J Chem Res 2011; 35: 278.
- Tang KC, Chang MJ, Lin TY, et al. J Am Chem Soc 2011; 133: 17738.
- 29. Wu JS, Liu WM, Zhuang XQ, et al. Org Lett 2007; 9: 33.
- Fujikawa Y, Urano Y, Komatsu T, et al. J Am Chem Soc 2008; 130: 14533.
- 31. Liu SR and Wu SP. Sens Actuators B 2012; 171: 1110.
- 32. Sinha S, Koner RR, Kumar S, et al. RSC Adv 2013; 3: 345.
- Sen B, Mukherjee M, Banerjee S, et al. *Dalton Trans* 2015; 44: 8708.
- 34. Martinez R and Molina P. Org Lett 2005; 7: 5869.
- Burdette SC, Frederickson CJ, Bu W, et al. *J Am Chem Soc* 2003; 125: 1778.
- 36. Liu W, Xu L, Sheng R, et al. Org Lett 2007; 9: 3829.
- Monfared HH, Falakian H, Bikas R, et al. *Inorg Chim Acta* 2013; 394: 526.
- 38. Xiao W, Lu ZL, Wang XJ, et al. Polyhedron 2000; 19: 1295.
- 39. Qu SZ, Zheng CH, Liao GM, et al. RSC Adv 2017; 7: 9833.
- 40. Wu X, Chen J and Zhao JX. Analyst 2013; 138: 5281.
- Zhou CE, Zhao ZG and Tang XL. Chin J Org Chem 2007; 27: 513.