

## RESEARCH ARTICLE

# Novel enantioselective fluorescent sensors for tartrate anion based on acridinezswsxa

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**Abstract**

Novel chiral fluorescence sensors **L-1** and **D-1** incorporating *N*-Boc-protected alanine and acridine moieties were synthesized. The recognition ability of the sensors was studied by fluorescence titration, <sup>1</sup>H NMR spectroscopy and density functional theory (DFT) calculations. The sensors exhibited good enantioselective fluorescent sensing ability toward enantiomers of tartrate anion for the selected carboxylate anions and formed 1: 1 complexes by multiple hydrogen bonding interactions.

**KEYWORDS**

enantioselective recognition, sensor, tartrate anion

## 1 | INTRODUCTION

Fluorescence sensors can effectively provide a real-time analytical tool for the direct and simple chiral enantiomeric determination of chiral reagents, catalysts, natural products and drugs.<sup>[1–3]</sup> Because of their sensitivity, selectivity and versatility fluorescence techniques have been used to study the interaction between enantiomers and sensors.<sup>[4]</sup> The principles for constructing chiral fluorescent sensors are based on studies of biological compounds, among which carboxylic acids are one of the most attractive targets in this field because of their central role in many enzyme activities, DNA regulation, hormone transport, peptide synthesis and intracellular communication.<sup>[5–7]</sup> The practical, rapid and accurate methods applied in enantioselective recognition of chiral carboxylate are an important analytically in nature and biological processes.<sup>[8–11]</sup> Our approach to the design of a novel chiral fluorescence sensor relies on the covalent attachment of chiral amino acid substituents to the fluorophore acridine via a short ester linker. Specifically, the chiral amino acid groups provide stereo centers, whereas the oxygen or amino atoms of the sensor form non-covalent

interactions with hydroxyl or carboxylate anions of the guest via hydrogen bonding and electrostatic forces.<sup>[12–15]</sup> Furthermore, when the sensor interacts with the guest, oxygen or nitrogen atoms adjacent to the acridine fluorophore might quench the fluorescence via photo-induced electron transfer.<sup>[16–18]</sup> Here, we report the study of an acridine derivative as an enantioselective fluorescent sensor for recognition of tartrate anions.

## 2 | EXPERIMENTAL

### 2.1 | Materials

The reagents used were of commercial origin and were employed without further purification. Purification by column chromatography was carried out over silica gel (230–400 mesh). Optical rotations were undertaken on a Perkin-Elmer Model 341 polarimeter. The infrared (IR) spectra were performed on a Nicolet 670 FTIR spectrophotometer. High-resolution mass spectra were measured on an Agilent 1290LC-6540 Accurate Mass Q-TOF using electrospray ionization (ESI). <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded on a Bruker AV-400 spectrometer. Fluorescence spectra were obtained with an F-7000 FL spectrophotometer. Anions were used as their tetrabutylammonium

salts. 4,5-Bis(bromomethyl)acridine was prepared as described in the literature.<sup>[19]</sup>

## 2.2 | Syntheses

To a solution of 4,5-bis(bromomethyl)acridine (0.37 g, 1.0 mmol) in dichloromethane (10 ml), 20% NaOH (10 ml), tetrabutylammonium iodide (0.81 g, 2.2 mmol) and L- or D-N-Boc-protected alanine (2.2 mmol) were added consecutively. The mixture was stirred at room temperature for 2 days with monitoring by thin-layer chromatography. After consumption of the starting material, the mixture was poured into a separator funnel over water and extracted with dichloromethane. The combined organic extracts were rinsed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and the solvent evaporated under reduced pressure to yield crude products, which were then purified by column chromatography over silica gel using petroleum ether: ethyl acetate (10: 1) as the eluent to obtain the pure products **L-1** or **D-1** as yellow solids.

**L-1**: 0.23 g, yield, 39.7%; m.p.: 136–138°C;  $[\alpha]_D^{20} = -52.8$  ( $c = 0.50$ , CHCl<sub>3</sub>); **D-1**: 0.24 g, yield, 41.5%; m.p.: 137–139°C;  $[\alpha]_D^{20} = +49.6$  ( $c = 0.50$ , CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.73 (s, 1H), 7.94 (d,  $J = 5.4$  Hz, 2H), 7.75 (d,  $J = 4.4$  Hz, 2H), 7.52 (q,  $J = 5.1$  Hz, 2H), 6.08 (d,  $J = 9.2$  Hz, 2H), 5.95 (d,  $J = 9.2$  Hz, 2H), 5.31 (s, 2H), 5.23 (s, 2H), 1.58 (s, 6H), 1.41 (s, 18H); <sup>13</sup>C NMR (CDCl<sub>3</sub>): 173.40, 155.25, 145.96, 136.29, 134.05, 128.50, 126.31, 125.58, 79.81, 63.88, 49.45, 28.37, 18.86; IR (KBr): 3357, 1741, 1689, 1531, 1220, 761 cm<sup>-1</sup>. ESI-MS calcd for C<sub>31</sub>H<sub>39</sub>N<sub>3</sub>O<sub>8</sub> [M + Na]<sup>+</sup> 604.26, found 604.30; elemental analysis calcd (%) for C<sub>31</sub>H<sub>39</sub>N<sub>3</sub>O<sub>8</sub>: C 64.01, H 6.76, N 7.22, found: **D-1**, C 63.97, H 6.73, N 7.18; **L-1**: C 63.95, H 6.71, N 7.17.

## 3 | RESULTS AND DISCUSSION

### 3.1 | Synthesis

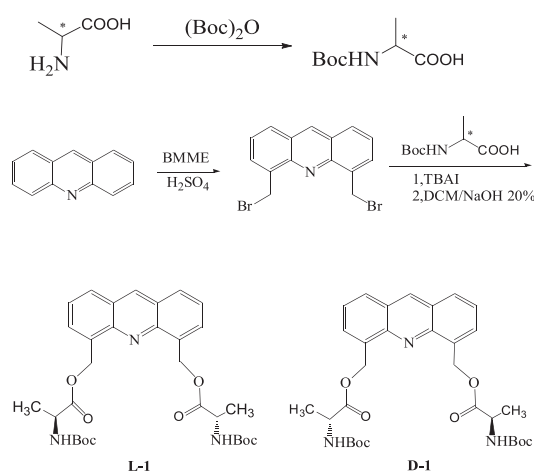
Enantiopure chiral L-N-Boc-alanine was developed from the cheap and commercially available chiral alanine. The product was further reacted

with 4,5-bis(bromomethyl)acridine at room temperature for 24 h to introduce the fluorescence group (Scheme 1). Preparation of compound **D-1**, an enantiomer of **L-1**, was the same as that for **L-1** by starting with D-N-Boc-alanine and 4,5-bis(bromomethyl)acridine. The structures of these compounds were characterized by IR, MS, <sup>1</sup>H NMR spectra and elemental analysis.

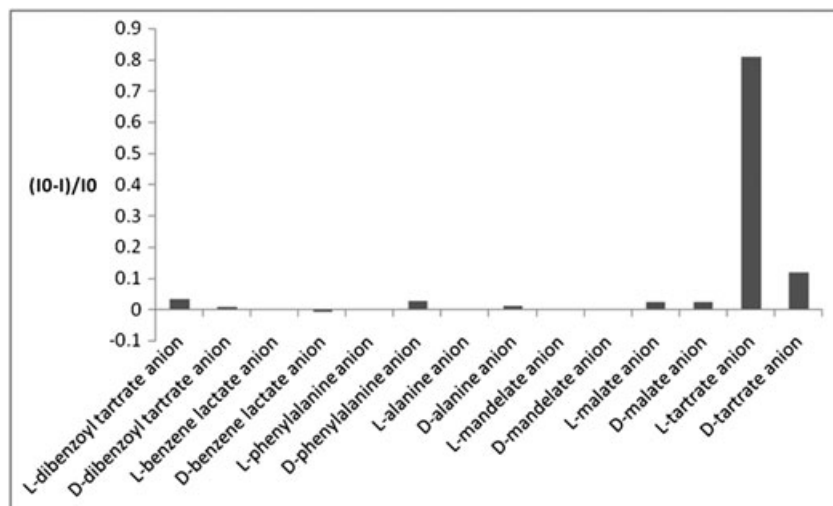
### 3.2 | Fluorescence spectroscopic studies

There was almost no change in the UV/vis spectra of sensor **L-1** or **D-1** upon addition of carboxylate anions such as D- or L-dibenzoyl tartrate, benzene lactate, phenylalanine, alanine, mandelate, malate, tartrate (as tetrabutylammonium salts) in acetonitrile, even when the guests were present in excess ([guest]/[**L-1** or **D-1**] = 50; Figure S1). Therefore, enantioselective recognition was investigated using the fluorescence spectra only. The fluorescence spectra of sensor **L-1** were studied in the presence of the above-mentioned enantiomers; we found that other anions produce a slight change in fluorescence, except for tartrate (Fig. 1).

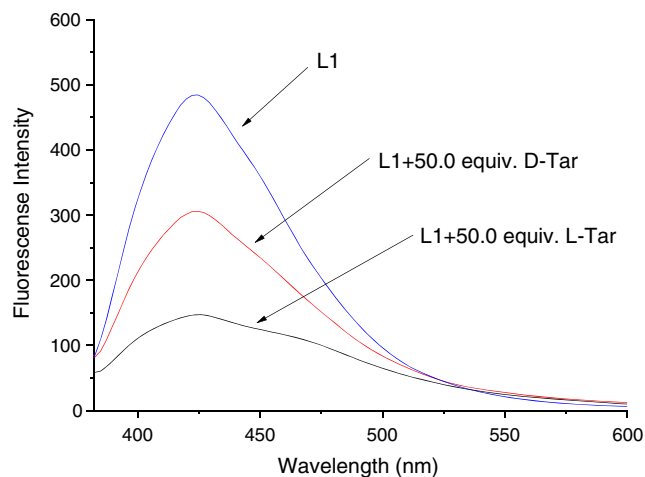
Figure 2 shows the different fluorescence intensity changes when 50 equiv. of L- or D-tartrate anion was added to sensor **L-1**. The



**SCHEME 1** Synthesis of compounds **L-1** and **D-1**



**FIGURE 1** Fluorescent spectra changes of sensor **L-1** ( $3.0 \times 10^{-5}$  M) measured in CH<sub>3</sub>CN upon the addition of 100 equiv. of various anions ( $\lambda_{\text{ex}} = 356$  nm). Fluorescent spectra changes ( $(I - I_0)/I_0$ ) of sensor **L-1** ( $3.0 \times 10^{-5}$  M) at 423 nm upon addition of different carboxylic acid anions (as tetrabutylammonium) in CH<sub>3</sub>CN at 25°C (50 equiv.,  $1.5 \times 10^{-3}$  M). L-Dibenzoyl tartrate anion, D-dibenzoyl tartrate anion, L-benzene lactate anion, D-benzene lactate anion, L-phenylalanine anion, D-phenylalanine anion, L-alanine anion, D-alanine anion, L-mandelate anion, D-mandelate anion, L-malate anion, D-malate anion, L-tartrate anion and D-tartrate anion

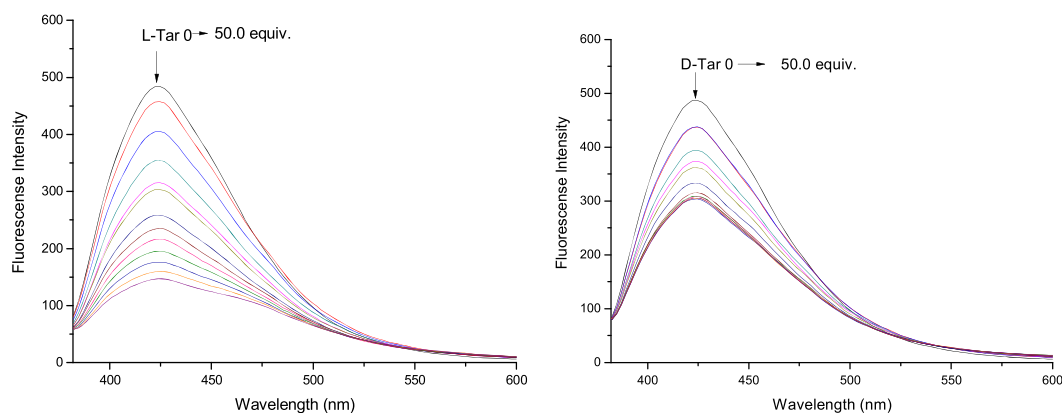


**FIGURE 2** Fluorescent spectra of **L-1** ( $3.0 \times 10^{-5}$  M) with 50.0 equiv. of L- or D-tartrate anion in  $\text{CH}_3\text{CN}$  ( $\lambda_{\text{ex}} = 356$  nm)

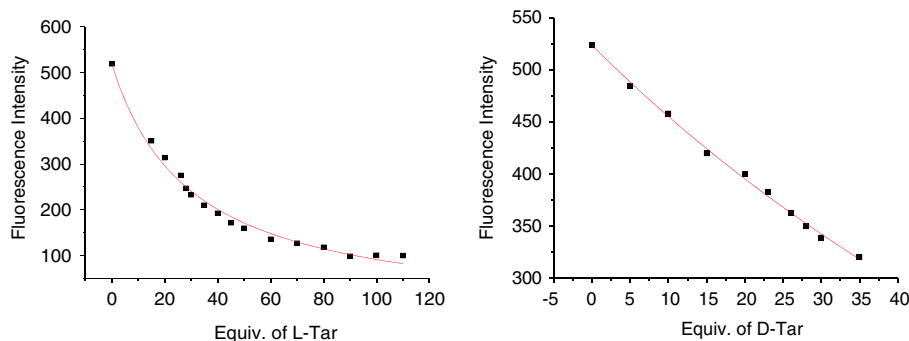
quenching efficiency was  $\sim 70\%$  when L-tartrate was added to the solution of **L-1**, but only  $\sim 38\%$  when D-tartrate was added. The fluorescence quenching efficiencies ( $\Delta I_L / \Delta I_D = 1.90$ ) indicated that sensor **L-1** shows good enantioselective recognition between L- and D-tartrate anions. The decrease in fluorescence was similar to the anion-induced fluorescence quenching previously reported.<sup>[20,21]</sup> When the guest binds with the host, an electron from the anion transfers to the singly

occupied molecular orbital (SOMO) of the excited fluorophore, which in turn prevents the excited-state binaphthyl electron from transferring to the lowest unoccupied molecular orbital (LUMO). Therefore, an anion-induced decrease in fluorescence was observed.<sup>[22,23]</sup>

To further understand the host-guest interaction, fluorescent titration was carried out. Titration of sensor **L-1** ( $3.0 \times 10^{-5}$  M) with 50 equiv. of L- or D-tartrate anion in acetonitrile was undertaken. With the increase in anions, the fluorescence intensity of **L-1** gradually weakened, indicating that a host-guest interaction had happened. The fluorescence quenching efficiency was related to the nonlinear curve fitting (Fig. 3), which was determined by monitoring changes in the fluorescence intensity and applying measurable values to equation (1) below.<sup>[24,25]</sup> In equation (1)  $I_0$  and  $I$  represent the fluorescence intensity of **L-1** in the absence and presence of L- or D-tartrate anion, and  $C_H$  and  $C_G$  are the concentrations of sensor **L-1** and tartrate anion respectively.  $C_0$  is the initial concentration of **L-1**. Satisfactory nonlinear curve fitting (the correlation coefficient is  $>0.99$ ) (Fig. 4) confirmed that a 1: 1 complex formed between sensor **L-1** and the L- or D-tartrate anion. For the 1: 1 stoichiometry complex, the association constant ( $K_{\text{ass}}$ ) was calculated from equation (1). The values of  $K_{\text{ass}}$  were  $1.15 \times 10^3 \text{ M}^{-1}$  for L-tartrate and  $2.29 \times 10^2 \text{ M}^{-1}$  for D-tartrate. This demonstrated that the interaction of sensor **L-1** with the tartrate anion was highly enantioselective ( $K_{\text{ass}}(\text{L})/K_{\text{ass}}(\text{D}) = 4.83$ ).

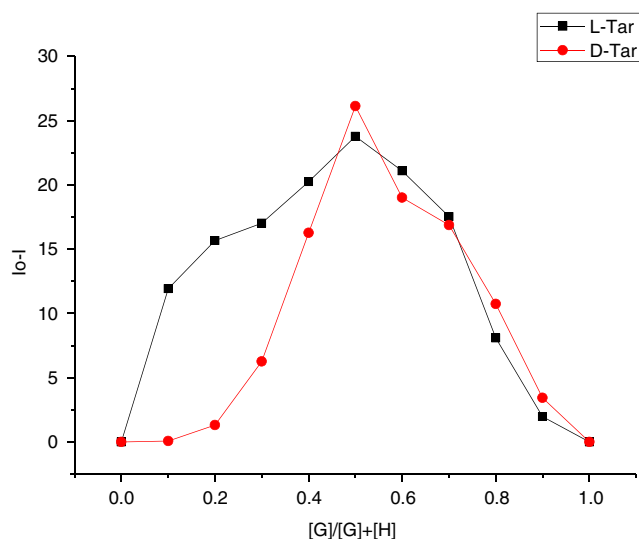


**FIGURE 3** (a) Fluorescence spectra of sensor **L-1** ( $3.0 \times 10^{-5}$  M) with L-tartrate anion in  $\text{CH}_3\text{CN}$ . Equivalents of anion:  $0 \rightarrow 50.0$ . (b) Fluorescence spectra of sensor **L-1** ( $3.0 \times 10^{-5}$  M) with D-tartrate anion in  $\text{CH}_3\text{CN}$ . Equivalents of anion:  $0 \rightarrow 50.0$



**FIGURE 4** (a) Changes in the fluorescence intensity of **L-1** at 423 nm upon addition of L-tartrate anion. The line shown is a non-linear fitted curve. The correlation coefficient ( $R$ ) of the non-linear curve fitting is 0.9949. (b) Changes in the fluorescence intensity of **L-1** at 356 nm upon addition of D-tartrate anion. The line shown is a non-linear fitted curve. The correlation coefficient ( $R$ ) of the non-linear curve fitting is 0.9988

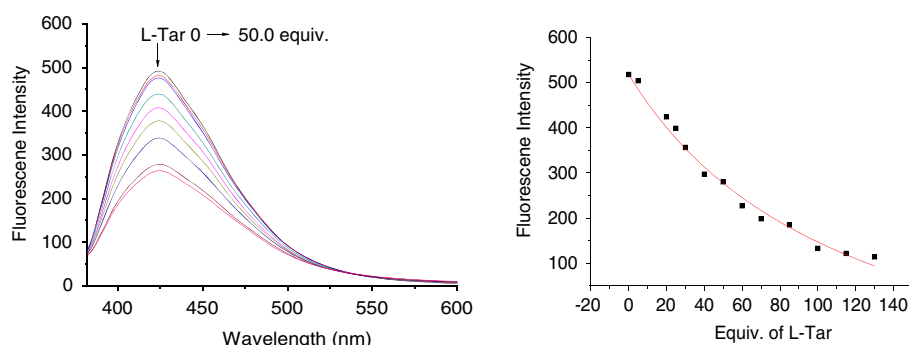
$$I = I_0 + (I_{\text{lim}} - I_0) / 2C_0 \left\{ C_H + C_G + 1/K_{\text{ass}} - \left[ (C_H + C_G + 1/K_{\text{ass}})^2 - 4 C_H C_G \right]^{1/2} \right\} \quad (1)$$



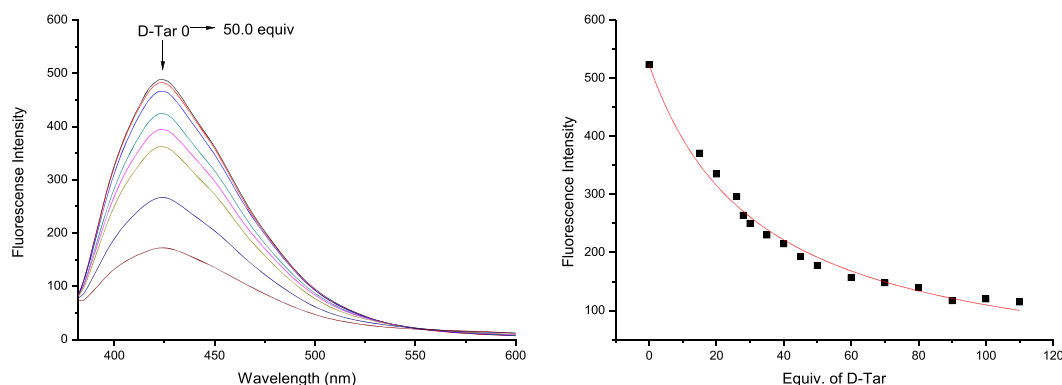
**FIGURE 5** Job's plot for **L-1** and L-tartrate and D-tartrate anion in  $\text{CH}_3\text{CN}$ . The total amount of substance of **L-1** and L-tartrate or D-tartrate anion is  $1.0 \times 10^{-4} \text{ M}$ .  $I_0$  = fluorescent intensity of sensor **L-1** and  $I$  = fluorescent intensity of sensor **L-1** in the presence of tartrate anion

The binding characteristics of **L-1** towards D- or L-tartrate anions were further examined using Job's plot (Fig. 5). A maximum change in fluorescence was observed when the molar fraction of sensor **L-1** vs.  $[\text{L-1}] + [\text{tartrate anion}]$  was 0.5, indicating 1:1 binding stoichiometry between **L-1** and D- or L-tartrate anions.<sup>[26,27]</sup>

To ascertain whether the change in fluorescence of **L-1** toward the enantiomers of tartrate anion was due to enantioselective recognition, we investigated use of the enantiomeric compound **D-1**. It was found that the fluorescence intensity of **D-1** was slightly quenched upon addition of L-tartrate, but D-tartrate had a much greater fluorescence quenching effect on D-tartrate anions under the same conditions (Figures 6 and 7). Values of the association constant ( $K_{\text{ass}}$ ) and correlation coefficient ( $R$ ) obtained by nonlinear least squares analysis are listed in Table 1. The values of  $K_{\text{ass}}$  were  $2.86 \times 10^2 \text{ M}^{-1}$  for L-tartrate and  $1.05 \times 10^3 \text{ M}^{-1}$  for D-tartrate anions. This indicated that **D-1** exhibited remarkable fluorescence enantioselectivity towards tartrate anions ( $K_{\text{ass(D)}}/K_{\text{ass(L)}} = 0.27$ ) (Table 1). The results of fluorescence spectra titrations indicated that enantiomers of the guest anion interacted with **L-1** and **D-1** in a similar fashion.



**FIGURE 6** (a) Fluorescence spectra of sensor **D-1** ( $3.0 \times 10^{-5} \text{ M}$ ) with L-tartrate anion in  $\text{CH}_3\text{CN}$ . Equivalents of anion: 0  $\rightarrow$  50.0.  $\lambda_{\text{ex}} = 356 \text{ nm}$ . (b) Changes in the fluorescence intensity of **D-1** at 423 nm upon addition of L-tartrate anion. The line shown is a non-linear fitted curve. The correlation coefficient ( $R$ ) of the non-linear curve fitting is 0.9938



**FIGURE 7** (a) Fluorescence spectra of sensor **D-1** ( $3.0 \times 10^{-5} \text{ M}$ ) with D-tartrate anion in  $\text{CH}_3\text{CN}$ . Equivalents of anion: 0  $\rightarrow$  50.0.  $\lambda_{\text{ex}} = 356 \text{ nm}$ . (b) Changes in the fluorescence intensity of **D-1** at 423 nm upon addition of D-tartrate anion. The line shown is a non-linear fitted curve. The correlation coefficient ( $R$ ) of the non-linear curve fitting is 0.9941

**TABLE 1** The association constant ( $K$ ) of sensor **L-1** and **D-1** ( $3.0 \times 10^{-5}$  M) with L-tartrate anion and D-tartrate anion in acetonitrile at 25°C

Entry	Host	Guest <sup>a</sup>	$K_{\text{ass}}$ [ $\text{M}^{-1}$ ] <sup>b,c</sup>	$K(\text{L})/K(\text{D})$	$R$
1	L-1	L-tartrate	$1.15 \times 10^3$	4.83	0.9949
2	L-1	D-tartrate	$2.39 \times 10^2$		0.9988
3	D-1	L-tartrate	$2.86 \times 10^2$	0.27	0.9938
4	D-1	D-tartrate	$1.05 \times 10^3$		0.9941

<sup>a</sup>Data were calculated from the results of the results of the fluorescent titrations in acetonitrile.

<sup>b</sup>All error values were obtained from nonlinear curve fitting.

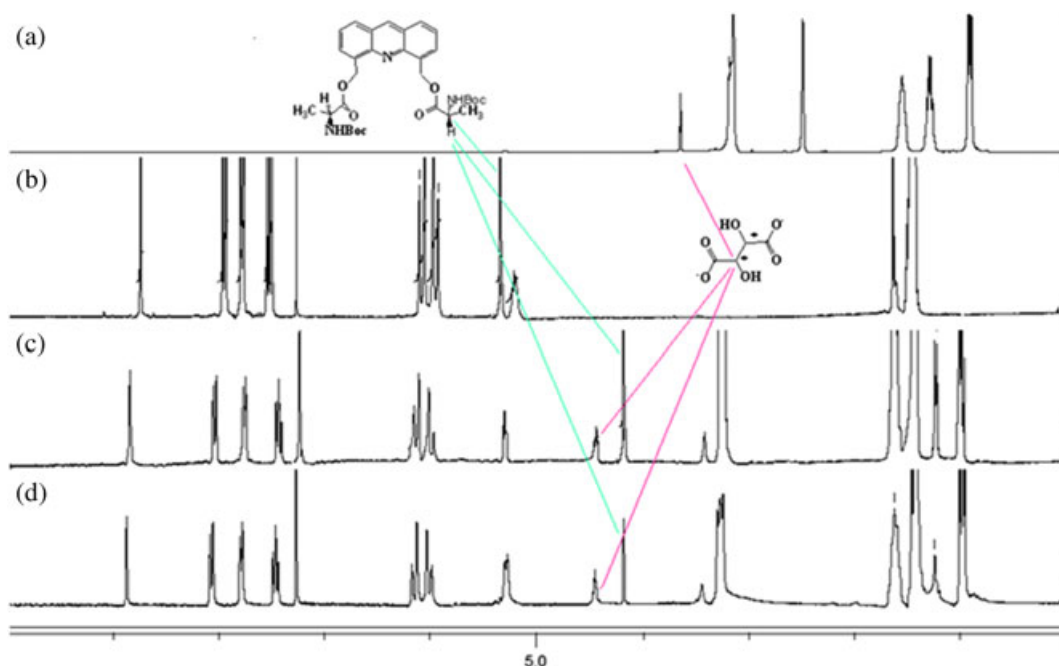
<sup>c</sup>Tartrate anions were used as their tetrabutylammonium salts.

### 3.3 | <sup>1</sup>H NMR studies

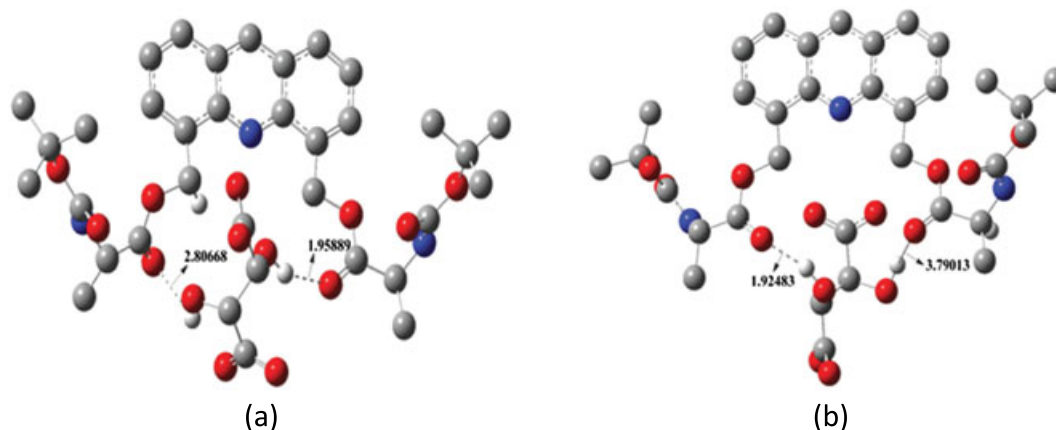
<sup>1</sup>H NMR experiments were applied to investigate the stability of complexes of sensor **L-1** with enantiomers of tartrate anion because this is one of the most effective tools in studying host-guest supramolecular chemistry.<sup>[28]</sup> Figure 8 shows the spectra of **L-1** in DMSO- $d_6$  solution without (Figure 8b) and on addition of 1.0 equiv. D- or L-tartrate anion (Figure 8c,d). We found that on addition of D- or L-tartrate, the chiral CH proton of **L-1** was downshifted from 5.31 p.p.m. to 4.20 or 4.10 p.p.m. ( $\Delta\delta = 1.11$  or 1.21 p.p.m.), whereas the CH proton of tartrate appeared at 4.47 or 4.50 p.p.m. ( $\Delta\delta = 0.80$  or 0.83 p.p.m.) (Figure 8c,d). The interaction of sensor **L-1** with L-tartrate showed that the CH proton had a larger downfield shift than the chiral CH proton of the D-enantiomer. We also found that the -OH protons of tartrate at 3.30 p.p.m. had weakened and downshifted to 3.42 and 3.49 p.p.m. indicating that the -OH of tartrate interacted with the sensor (Figure 8b-d). Therefore, as the proposed modes show in Figure 9, the hydroxy group of tartrate may form hydrogen bonds with the oxygens of **L-1**.

### 3.4 | DFT calculations recognition properties of L-1 and tartrate anion

The enantioselective recognition mechanism and binding energy between chiral **L-1** and tartrate anions can be obtained by calculation. In this study, All density functional theory (DFT)/ time-dependent DFT (DFT/time dependent density functional theory (TDDFT)) calculations based on the hybrid exchange correlation functional B3LYP<sup>[29]</sup> with 6-31G\*\*basis set<sup>[30]</sup> were carried out using Gaussian 03 program.<sup>[31]</sup> For all calculations, the solvent effect of acetonitrile was considered using Cossi and Barone's conductor-like polarizable continuum model.<sup>[32]</sup> To investigate the electronic properties of singlet excited states, TDDFT was performed in the ground state geometries of **L-1** and **L-1** with D- or L-tartrate anion. Bond lengths were calculated and are shown in Figure 9. There was weak interaction between **L-1** and tartrate anion, with hydrogen bond sites between **L-1** and L-tartrate anion and bond lengths of 1.96 and 2.81 Å, respectively. However, the hydrogen bond lengths between **L-1** and D-tartrate anion were only 1.92 and 3.79 Å, with only one bond length < 3.50 Å; this indicated that the complex of **L-1** and L-tartrate anion was more stable. From the binding energy, it was possible to judge the interaction strength and enantiomer complex ability, which could provide enantioselective recognition. The results of the binding energy calculation for the interaction between **L-1** and tartrate anion enantiomers were  $\Delta E = -12.27$  and  $-8.44$  kJ/mol, respectively. It was found that the negative binding energies between **L-1** and tartrate anion enantiomers were  $\sim 3.83$  kJ/mol. In other words, stable complexes between **L-1** and tartrate enantiomers could be formed by molecular weak interactions.  $|\Delta E_{\text{L-1-L}}| > |\Delta E_{\text{L-1-D}}|$  showed that the complex of **L-1** and L-tartrate anion was more stable than that of **L-1** and D-tartrate anion, which made the enantioselective recognition of salbutamol enantiomers feasible. Where  $|\Delta E_{\text{L-1-L}}|$  and  $|\Delta E_{\text{L-1-D}}|$  represented the binding energy between **L-1** and L-tartrate anion and **L-1** and D-tartrate anion, respectively.



**FIGURE 8** <sup>1</sup>H NMR spectra (400 MHz, DMSO- $d_6$  25°C) of **L-1** and tartrate anion complexes at  $[\text{L-1}] = [\text{guest}] = 4.0$  nM in DMSO- $d_6$  at 400 MHz. (a) Racemic tartrate anion, (b) sensor **L-1**, (c) sensor **L-1** + D-tartrate anion, (d) sensor **L-1** + L-tartrate anion



**FIGURE 9** (a) Optimized geometry of the complex: **L-1** and L-tartrate anion. (b) Optimized geometry of the complex: **L-1** and D-tartrate anion

The above results illustrated that the nature of the sensor, multiple hydrogen-bonding interactions and complementary stereogenic center interactions may be responsible for the enantioselective recognition of tartrate.

## 4 | CONCLUSION

We have demonstrated that upon the introduction of *N*-Boc-protected alanine to the acridine, acridine-based chiral fluorescence sensors **L-1** and **D-1** act as excellent enantioselective fluorescent sensors for tartrate anions.

## ACKNOWLEDGEMENTS

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## SUPPORTING INFORMATION

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