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New water-soluble iminecalix[4]arene with a deep hydrophobic cavity

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ABSTRACT

We report the synthesis of a new water-soluble iminecalix[4]arene host **4c** with a deep hydrophobic cavity. The negatively charged four carboxylate functions on the top of the cavity play a major role in the recognition of charged molecular species. The ^{1}H NMR titration experiments revealed that host **4c** binds with cationic (**10–12**) and neutral guests (**6–9**) in water with high binding constants in the order of 10^4–10^5 M^{-1}. Cationic guest **9** showed highest binding constant of 2.81×10^5 M^{-1} with host **4c** amongst all tested guests. Selectivity over anionic guests (**13–17**) is established by the presence of negative charges at the top of the deep hydrophobic cavity, as guests **15** and **17** were not recognized by host **4c**. Neutral pyridine derivatives with hydrophobic chains at *para* positions showed high binding constants of 6.02×10^4–2.23×10^5 M^{-1}. The data obtained for the recognition of the guests by host **4c** revealed that the ionic as well as the hydrophobic–hydrophobic interactions are crucial in the molecular recognition in aqueous medium.

1. Introduction

Calix[4]arene and its functionalized derivatives represent an important class of supramolecules having enormous applications.[1,2] Owing to their relatively easy synthesis and high versatility, Schiff base derivatives of calix[4]arenes have been used as molecular platforms in the development of artificial receptors for recognition of neutral and charged molecular species.[3,4]

Though generally examined for recognition behavior in the organic mediums, the development of calix[4]arenes Schiff base derivatives for recognition of aromatic guests in aqueous medium is comparatively nascent.[5] The recognition of aromatic guest molecules by synthetic hosts in aqueous medium, where the entire biological process takes place, is of particular interest in host–guest chemistry.[6,7]

In this context, a new molecular host with four carboxylate groups on the top of the deep hydrophobic cavity generated by Schiff base functions on the wide rim of calix[4]arene seems to be promising for the recognition of the neutral as well as cationic aromatic guests. Pyridine derivatives have been extensively studied as guest molecules. When protonated, these guest molecules showed strong binding toward hosts, while merely recognized in their neutral forms.[8] We describe herein a deep hydrophobic pocket synthetic receptor **4c** that binds cationic aromatic guests (**10–12**) and neutral pyridine guests (**6–9**) in water with high binding constants. Low binding ability of anionic guests **13–17** was attributed to the negative charges positioned on the top of the deep hydrophobic cavity.

The iminecalix[4]arene derivatives **2a–c**, obtained by selective modification of the wide rim of aminocalix[4]arene[9] keeping narrow rim free for further modifications, were used as precursors.[10]

As shown in Scheme 1, the reaction of iminecalix[4]arenes **2a–c** with ethyl 2-bromoacetate in acetonitrile in the presence of K_2CO_3 at 80 °C afforded compounds **3a–c** with 86.36–87.23% yields.[11]

The synthesis of the target host **4c** is depicted in Scheme 1. The hydrolysis of compound **3c** in THF–water mixture using sodium hydroxide as a base led to the formation of host **4c** as a salt in a good yield.[12] The residual THF in completely dried host **4c** was found difficult to be eliminated and hence the obtained material was used as it is. The imine bonds were reported to be unstable under acidic conditions.[13] Therefore, the whole process to obtain host **4c** was carried out under basic environment at 5–10 °C. The negative charges on the wide and the narrow rim of **4c** enable its solubility in water (0.02 M) at pH/pD 8.5. The products of alkaline hydrolysis of compounds **3a** and **3b** were insoluble in aqueous alkaline solutions.

All the compounds shown in Scheme 1 were characterized by analysis of their ^{1}H NMR, ^{13}C NMR, and MALDI-TOF spectra. The tetra *o*-alkylation of compound **2c** was established from the close observation of ^{1}H NMR signals in compound **3c** for singlet of imine protons, singlet of calixarene aromatic protons, pair of doublets for methylene bridge protons of calix[4]arene, and singlet of methylene protons on 2-ethoxy-2-oxoethoxy functions. These protons

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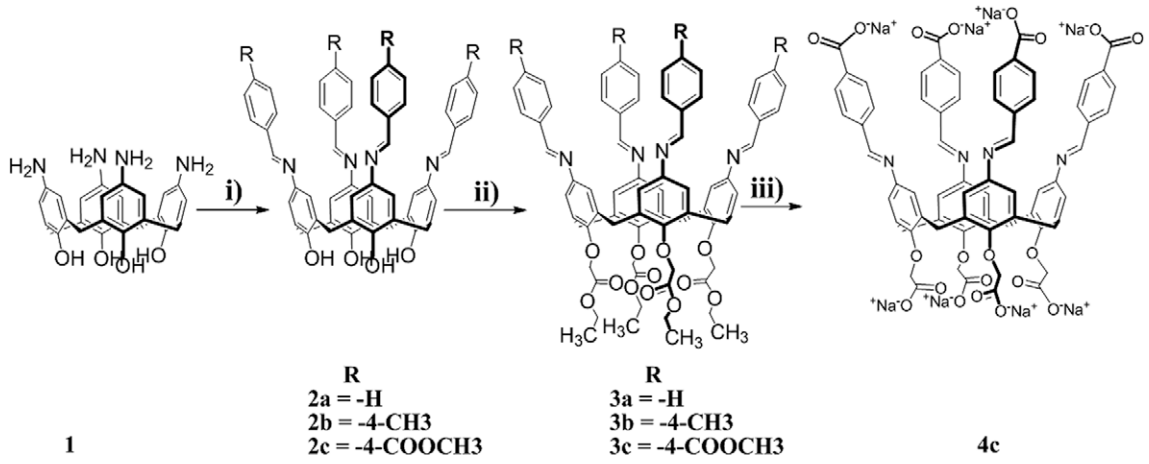


Scheme 1. Reagents and conditions: (i) R–Ph–CHO, CH_3CN, N_2, 2 h, 92.45–95.72%; (ii) Br–$CH_2COOCH_2CH_3$, K_2CO_3, CH_3CN, N_2, 80 °C, 8 h, 86.36–87.23%; (iii) aq NaOH, THF–water (2:1).

appeared at δ = 8.47 ppm, δ = 7.16 ppm, δ = 4.36, and δ = 3.59 ppm, respectively, in a ratio of 1:2:1:1:2. The tetra *o*-alkylation was also confirmed from ^{1}H NMR spectrum of **3c** which showed the absence of phenolic hydroxyl group peak at δ = 10.15 ppm observed in starting compound **2c**. Similar patterns were found for compounds **3a** and **3b.**

The ^{1}H NMR spectrum of compound **3c** showed a typical AB pattern for methylene bridge protons represented by two pairs of doublets at δ = 3.59 and δ = 4.36 ppm for the axial and equatorial protons, respectively, which indicated that compound **3c** existed in a symmetrical cone conformation. This was further confirmed by the observation of a distinct signal at δ = 30.24 ppm for the methylene carbon in the ^{13}C NMR spectrum.[14]

Host **4c** obtained by the complete hydrolysis of esters in compound **3c** was characterized by ^{1}H NMR spectroscopy. The absence of methyl peak at δ = 3.91 ppm for four methyl ester moieties of benzylimido functions on the wide rim, the methylene and methyl peaks at δ = 4.38 ppm and δ = 1.41 ppm, respectively, for four ester moieties on the narrow rim ascertained complete hydrolysis of esters and formation of carboxylate salts. The ^{1}H NMR signal for four imine protons in host **4c** at δ = 8.49 ppm exhibited stability of imine bonds during hydrolysis. Imine linkage on the top of wide rim not only extends the conjugated aromatic system but also increases the depth of the hydrophobic cavity. Similar to **3c**, host **4c** showed a typical AB pattern for methylene bridge protons represented by two pairs of doublets at δ = 3.61 ppm and δ = 4.74 ppm for the axial and equatorial protons, respectively, which indicated that host **4c** existed in a vase-shaped conformation.

Preliminary recognition characteristic of host **4c** was investigated by ^{1}H NMR experiments in deuterated sodium phosphate buffer (20 mM, pH 8.5) using guests **6–17** as neutral, cationic, and anionic guests and the results were compared with those of host **5**. Host **5** as shown in Scheme 2 was obtained by previously reported method.[15] These experiments revealed significant complexation-induced upfield shifts (CIUS) for proton signals in guests, reasonably the averaged signal because of the fast exchange on NMR timescale between the free and complexed guest.

The guest concentration was kept constant (1×10^{-3} M) while the host concentration was varied from 8×10^{-4} to 3×10^{-3} M, and the chemical shifts of the protons of guest were recorded at each concentration. The obtained ^{1}H NMR data were analyzed by the well-known method of nonlinear least square regression analysis,[16] which allowed the calculation of association constant (K_a). The results of ^{1}H NMR experiments run for hosts **4c** and **5** with guests **6–17** have been presented in Table 1.

The ^{1}H NMR titration experiments indicated that the signals for the protons of guests **6–12**, **14**, **16**, and **17** were markedly affected by the addition of host **3c**, whereas there was no change in the protons of guests **13** and **15**. Host **5** showed significant recognition properties for guests **10**, **11**, **12,** and **16** amongst all tested guests.

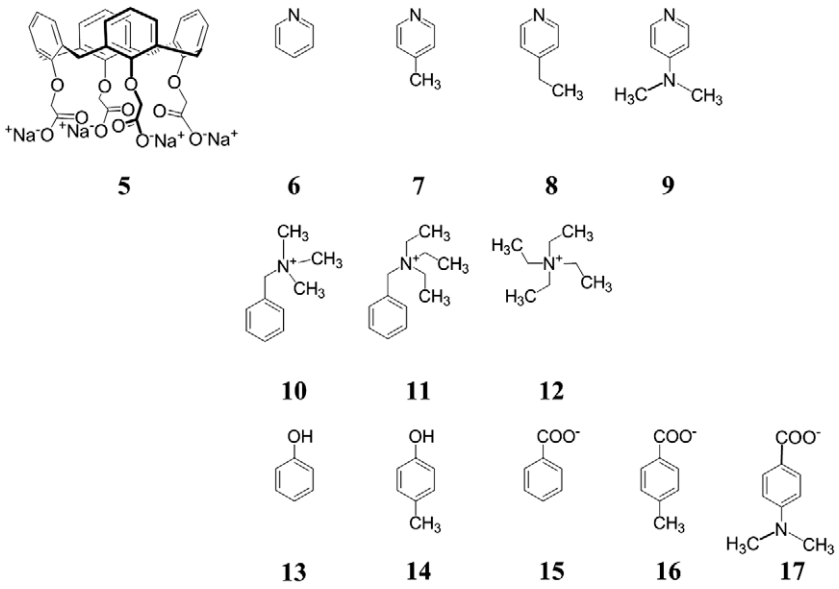
As shown in Figure 1, the signals for the methyl and methylene protons at δ = 1.13 ppm and δ = 2.61 ppm, respectively, in guest **8** and the signal for the proton *para* to the trimethylammoniomethyl moiety at δ = 7.51 ppm in guest **10** were markedly affected by the addition of host **4c**; they shifted to upfields and $\Delta\delta$ ($\delta_{complex} - \delta_{free\ guest}$) reached the maximum values of −2.70, −1.29, and −2.19 ppm, respectively, upon the addition of 1 equiv of host **4c**. There was no change in the protons of guest **8** upon addition of host **5**.

A close comparison of the $\Delta\delta$ values of protons of the guest **8** after complexation with host **4c** shows that the aromatic proton (or methyl, or methyl and methylene protons) at the 4-position of pyridine guests gives the highest $\Delta\delta$ value, followed by the aromatic protons at the 3- and 2-positions, which indicates that the pyridine guests penetrate into the cavity of host **4c** from the *p*-position of the N atom as illustrated in Figure 2a. In case of guest **10**, after complexation with host **4c**, only a slight upfield shift is observed for the protons of the trimethylammoniomethyl moiety while the aromatic protons experience a CIUS that follows the order $H_{para} > H_{meta} > H_{ortho}$. This indicates that the aromatic nucleus of the guest is selectively included in the cavity of host **4c** as illustrated in Figure 2b.

As shown in Table 1, host **4c** showed maximum association constant K_a of 2.81×10^5 for guest **10**, the least association constant of 2.90×10^2 for guest **16** while the association constant for guests **6–9** were in the above-mentioned range. **10**, **11**, **12**, and **16** were the only guests recognized by the host **5** amongst all tested guests and the binding constants were comparable to the values reported in the literature.[17] The high binding ability of host **4c** for the guests **6–12** is attributed to its deep cavity generated by tetra benzylimido groups on the wide rim of calix[4]arene. This proposition can be supported by the recognition behavior of host **5** which showed limited or no recognition ability for the guests **6–17**.

As shown in Figure 2, the plausible special orientation of complex between host **4c** and molecules of guests **8** and **10** is presented by the energy-minimized structure generated by Spartan® (MM$^+$Force Field).

Host **4c** showed high binding affinity for cationic guests **10–12** with lower or no binding affinity for anionic guests **13–17**. The binding constants of host **5** for cationic guests **10–12** as well as anionic guests **13–17** were relatively lower than those recorded



Scheme 2. Host **5** and guests **6–17**.

Table 1
Association constants (K_a M^{-1}) for binding of guests by hosts **4c** and **5**; pD 8.5 (20 mM sodium phosphate buffer)

Guest	6	7	8	9	10	11	12	13	14	15	16	17
Host **4c**	6.02×10^4	1.47×10^5	2.23×10^5	1.70×10^5	2.81×10^5	9.00×10^4	4.46×10^4	[a]ns	5.22×10^2	ns	2.90×10^2	8.91×10^2
Host **5**	ns	ns	ns	ns	1.29×10^2	0.52×10^2	1.09×10^3	ns	ns	ns	8.542×10^2	ns

[a] ns indicates no change in chemical shift.

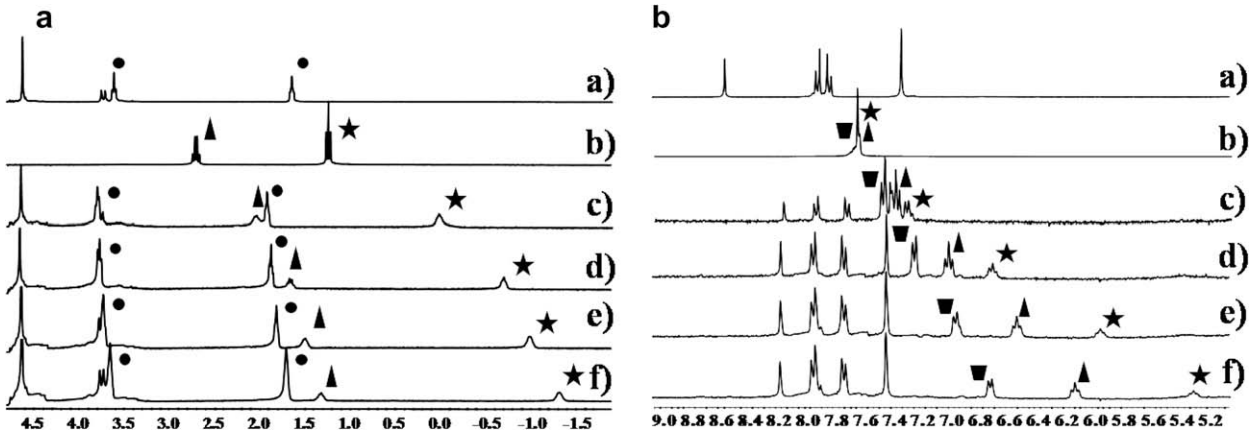


Figure 1. Partial ^1H NMR (D_2O at 298 K) spectra of guests **8** and **10** upon titration with host **4c**. (1a) (a) host **4c**, (b) guest **8**, (c) **8** + 0.25 equiv of **4c**, (d) **8** + 0.5 equiv of **4c**, (e) **8** + 0.75 equiv of **4c**, (f) **8** + 1 equiv of **4c**, residual THF (●), methyl protons (★), methylene protons (▲) in guest **8**; (1b) (a) host **4c**, (b) guest **10,** (c) **10** + 0.25 equiv of **4c**, (d) **10** + 0.5 equiv of **4c**, (e) **10** + 0.75 equiv of **4c**, (f) **10** + 1 equiv of **4c**, *ortho* protons (▼), *meta* protons (▲), *para* protons (★) in guest **9**.

for host **4c**. From the recognition behavior of host **4c** for all the guests, it is clearly seen that the four carboxylate groups on the top of the deep hydrophobic pocket play an important role. As can be seen in Figure 2b, the positively charged trimethylammoniomethyl moiety in guest **10** comes in the vicinity of negatively charged carboxylate functions on the top of the hydrophobic cavity burying phenyl group in the deep gorge. Such orientation of guest **10** in the cavity of the host **4c** leads to the favorable electrostatic interaction along with hydrophobic interactions in deep cavity. In the case of anionic guests, such favorable electrostatic interaction is not possible, hence host **4c** showed poor or no binding affinity for guests **13–17**.

The neutral pyridine derivatives (guests **6–9)** showed high binding constants with host **4c**, while no effects on chemical shifts were observed for these guests upon titration with host **5**. Host **4c** showed highest K_a value of 2.23×10^5 M^{-1} for guest **8** amongst neutral guests **6–9**. From Table 1, the increasing order of affinity of the guests **6–8** for host **4c** can be presented as **6** < **7** < **8**. Guest **8** with a longer alkyl chain at the *para* position had the maximum association constant. The association constant decreases with decrease in the chain length at the *para* position of the pyridine guests, hence pyridine (**6**) showed less binding affinity than 4-methyl pyridine (**7**) and 4-ethyl pyridine (**8**). This indicates that deep hydrophobic cavity of host **4c** welcomes a hydrophobic open-chain substituent at the 4-position of pyridine guests. It is interesting to notice that without any ionic interactions, these neutral pyridine derivatives (guests **6–8**) showed strong binding characteristics with the deep hydrophobic pocket of host **4c**. As can be

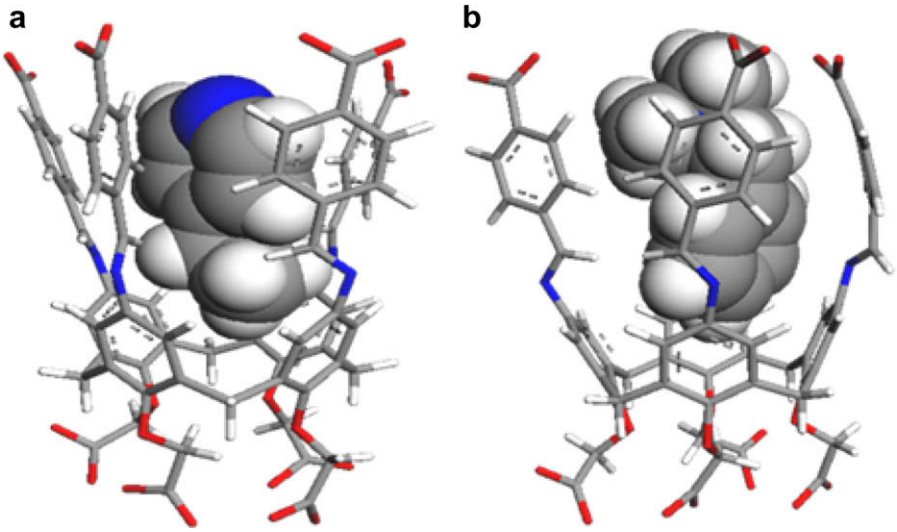


Figure 2. Energy-minimized structure of complex between host **4c** and molecule of guests **8** and **10** by Spartan® (MM⁺ Force Field) (a) complex between host **4c** and guest **8** (side view); (b) complex between host **4c** and guest **10** (side view).

seen in Figure 2a, the alkyl moiety at the *para* position of the guest **8** is buried deep into the hydrophobic cavity of host **4c**, thus enhancing hydrophobic interactions along with –CH···π interactions and π–π stacking interactions. Relatively more hydrophilic guest **9** showed low binding constant with host **4c** than guest **8**. Comparable K_a values for neutral and cationic guests were found because the hydrophobic interactions are predominant and the electrostatic interaction only enhances the binding of the cationic guests in the cavity of host **4c**.

In this Letter, we have shown that the water-soluble iminecalix[4]arene derivative bearing tetra carboxylate groups on the top of the deep hydrophobic cavity has been successfully synthesized in prominent yield. From the complexation studies of host **4c** for the tested guests, it can be concluded that the deep hydrophobic cavity of host **4c** plays a major role in recognition of the guests in aqueous medium. This phenomenon is supported by comparing the results obtained for host **4c** with host **5**, which did not recognize most of the tested guests. As can be seen from the recognition behavior of cationic guests and neutral pyridine derivatives, hydrophobic interactions along with –CH···π and π–π stacking interactions proved to be crucial in the molecular recognition process in aqueous medium. The electrostatic interaction also plays a major role in molecular recognition, as it imparts high binding affinity to the host for cationic guests as compared to anionic guests. Based on our knowledge, it is the first time to report huge K_a values for neutral pyridine guests. We are currently working on the molecular recognition of host **4c** with pyridine derivatives and structurally similar aromatic compounds.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.10.058.

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10. *Typical procedure for the synthesis of compound* **2**: 0.5 g of **1** (1.03 mmol) and 150 mL of acetonitrile were taken in a dried round-bottomed flask, and the mixture was stirred at room temperature under nitrogen atmosphere. Then benzaldehyde (0.87 g, 8.26 mmol) was injected to the reaction mixture with stirring and the reaction was continued for 2 h. After completion, hexane was added to the reaction mixture, stirred for 5 min, and then filtered to obtain the solid product; the filter cake was washed three times with hexane and dried under vacuum. The final compound **2a** was recrystallized from 1:50 mixture of dichloromethane and hexane (100 mL). Using this method, compounds **2b** and **2c** were also obtained by reacting **1** with suitable aldehydes. Compound **2a**: light pink solid (95.72% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, 298 K) δ (ppm): 10.15 (s, 4H, OH), 8.34 (s, 4H, N=CH), 7.82–7.78 (m, 8H, Ar), 7.40–7.38 (m, 12H, Ar), 7.01 (s, 8H, Ar), 4.31 (d, 4H, $ArCH_2Ar$, J = 13.0 Hz), 3.63 (d, 4H, $ArCH_2Ar$, J = 13.0 Hz). ^{13}C NMR (300 MHz, $CDCl_3$): 159.26, 147.53, 146.28, 136.40, 131.32, 128.87, 128.86, 128.82, 121.90, 32.47. MALDI-TOF: m/z = 837.37 $[M+H]^+$. Compound **2b**: light pink solid (94.75% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, 298 K) δ (ppm): 10.20 (s, 4H, OH), 8.31 (s, 4H, N=CH), 7.69 (d, 8H, Ar), 7.20 (d, 8H, Ar), 7.00 (s, 8H, Ar), 4.30 (d, 4H, $ArCH_2Ar$, J = 14.2 Hz), 3.58 (d, 4H, $ArCH_2Ar$, J = 13.7 Hz), 2.38 (s, 12H, Ar-CH_3). ^{13}C NMR (300 MHz, $CDCl_3$): 159.23, 147.34, 146.39, 141.71, 133.84, 129.61, 128.85, 128.77, 121.84, 32.48, 21.38. MALDI-TOF: m/z = 893.39 $[M+H]^+$. Compound **2c**: dark khaki solid

(92.45% yield). ^{1}H NMR ($CDCl_3$, 300 MHz, 298 K) δ (ppm): 10.71 (s, 4H, OH), 8.41 (s, 4H, N=CH), 8.05 (d, 8H, Ar), 7.86 (d, 8H, Ar), 7.05 (s, 8H, Ar), 4.32 (d, 4H, $ArCH_2Ar$, J = 14.2 Hz), 3.61 (d, 4H, $ArCH_2Ar$, J = 13.1 Hz), 3.98 (s, 12H, Ar-$COOCH_3$). ^{13}C NMR (300 MHz, $CDCl_3$): 166.66, 157.85, 147.95, 145.68, 140.10, 132.27, 130.09, 128.77, 128.60, 122.05, 52.61, 32.39. MALDI-TOF: m/z = 893.39 $[M]^+$.

11. *Typical procedure for the synthesis of compound* **3**: To a solution of **2a** (0.5 g, 0.59 mmol), K_2CO_3 (0.83 g, 5.9 mmol), NaI (1.33 g, 8.9 mmol), and acetonitrile (30 mL), ethyl 2-bromoacetate (0.99 g, 5.9 mmol) was added and then the solution was refluxed with stirring under argon atmosphere for 12 h. The reaction mixture was then evaporated in vacuo and the residue was dissolved in 50 mL of dichloromethane and filtered. The filtrate was concentrated and hexane was added. The precipitate was filtered and dried under vacuum to obtain compound **3a**. Using this method, compounds **3b** and **3c** were obtained from **2b** and **2c**, respectively. Compound **3a**: Light yellow solid (86.36%) ^{1}H NMR (300 MHz, $CDCl_3$, 298 K) δ (ppm): 8.33 (s, 4H, N=CH), 7.76 (d, 8H, ArH), 7.41–7.39 (t, 12H, ArH), 7.05 (s, 8H, Ar), 4.50 (s, 8H, O–CH_2–COO), 4.42–4.37 (q, 8H, $COOCH_2$), 4.33 (d, 4H, Ar-CH_2-Ar, J = 11.5 Hz), 3.52 (d, 4H, Ar-CH_2-Ar, J = 11.5 Hz), 1.42 (t, 12H, –CH_3).^{13}C NMR (300 MHz, $CDCl_3$, 298 K): 171.35, 161.12, 150.37, 149.99, 135.94, 134.70, 131.70, 129.19, 129.09, 122.04, 73.79, 62.50, 30.38, 14.67. MALDI-TOF: m/z = 1181.38 $[M]^+$, m/z = 1203.37 $[M+Na]^+$. Compound 3**b**: bright yellow solid (87.27%) ^{1}H NMR (300 MHz, $CDCl_3$, 298 K) δ (ppm): 8.29 (s, 4H, N=CH), 7.65 (d, 8H, ArH), 7.18 (d, 8H, ArH), 7.04 (s, 8H, Ar), 4.51 (s, 8H, O–CH_2–COO), 4.41–4.35 (q, 8H, $COOCH_2$), 4.30 (d, 4H, Ar-CH_2-Ar, J = 12.0 Hz), 3.50 (d, 4H, Ar-CH_2-Ar, J = 12.0 Hz), 2.38 (s, 12H, Ar-CH_3),1.43 (t, 12H, –CH_3).^{13}C NMR (300 MHz, $CDCl_3$, 298 K): 171.25, 160.88, 150.06, 142.16, 140.07, 135.41, 133.27, 129.81, 128.97, 121.86, 73.66, 62.40, 30.28, 21.93, 14.58. MALDI-TOF: m/z = 1260.52 $[M+Na]^+$, m/z = 1259.52 $[M+Na-1]^+$. Compound **3c**: yellow solid (87.03%) ^{1}H NMR (300 MHz, $CDCl_3$, 298 K) δ (ppm): 8.47 (s, 4H, N=CH), 8.02 (d, 8H, ArH), 7.83 (d, 8H, ArH), 7.16 (s, 8H, Ar), 4.53 (s, 8H, O–CH_2–COO), 4.43–4.38 (q, 8H, $COOCH_2$), 4.36 (d, 4H, Ar-CH_2-Ar, J = 13.1 Hz), 3.91 (s, 12H, Ar-$COOCH_3$), 3.59 (d, 4H, Ar-CH_2-Ar, J = 13.1 Hz), 1.41 (t, 12H, –CH_3).^{13}C NMR (300 MHz, $CDCl_3$, 298 K): 171.28, 166.53, 159.92, 150.71, 149.46, 139.75, 135.58, 132.37, 129.98, 128.79, 122.24, 73.68, 62.36, 52.508, 30.24, 14.56. MALDI-TOF: m/z = 1413.44 $[M]^+$, m/z = 1436.42 $[M+Na]^+$.
12. Typical procedure for the synthesis of compound **4**: Compound **3c** (0.1 g, 0.070 mmol) was dissolved in a 2:1 mixture of THF and water (6 mL), and a solution of NaOH (0.045 g, 1.1 mmol) in water (4 mL) was added. The reaction mixture was kept at room temperature overnight and at 5 °C for another 12 h. The solution was filtered and the filter cake (crystalline compound) was dried under vacuum to obtain crystalline compound **4c** with a yield of 99.00%. ^{1}H NMR (300 MHz, D_2O, 298 K) δ (ppm): 8.49 (s, 4H, N = CH), 7.87, 7.76 (dd, 16H, ArH), 7.27 (s, 8H, Ar), 4.74 (d, 4H, Ar-CH_2-Ar, J = 13.2 Hz), 4.53 (s, 8H, O–CH_2–COO), 3.64 (d, 4H, Ar-CH_2-Ar, J = 13.2 Hz). ^{13}C NMR (300 MHz, D_2O, 298 K) 176.08, 167.54, 151.50, 146.56, 142. 72, 139.17, 136. 91, 130.05, 129.03, 128.28, 127. 74, 61.34, 29.67.
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