Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713649759

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To cite this Article Hennrich, Gunther , David, Wendi M. , Bomble, Yannick J. , Anslyn, Eric V. , Brodbelt, Jennifer S. and Stanton, John F.(2004) 'Self-assembling Dimeric and Trimeric Aggregates Based on Solvophobic and Charge-pairing Interactions', Supramolecular Chemistry, 16: 7, 521 — 528
To link to this Article: DOI: 10.1080/10610270412331295995
URL: http://dx.doi.org/10.1080/10610270412331295995

Supramolecular Chemistry, October–November 2004, Vol. 16 (7), pp. 521–528

Self-assembling Dimeric and Trimeric Aggregates Based on Solvophobic and Charge-pairing Interactions

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Received (in Southampton, UK) 18 May 2004; Accepted 18 June 2004

Self-assembly processes based on shape complementarity and noncovalent binding interactions are widely recognized as a fundamental principle in nature. Besides charge pairing and hydrogen bonding, hydrophobic interactions play a crucial role in water. Here we report the self-assembly of structurally simple monomers to yield defined dimeric and trimeric aggregates in highly polar media, based on ionic and solvophobic interactions. NMR, mass spectrometry and curve fitting were used to characterize these supramolecular assemblies in water–methanol solutions.

Keywords: Self-assembly; Supramolecular; Ion-pairing; Hydrophobic effect; Molecular recognition

INTRODUCTION

In the field of supramolecular chemistry, structurally simplified model compounds are often used to gain insight into complex self-assembly processes occurring in biological systems [1–3]. Alternatively, mimicking nature is often performed to assemble functional supramolecular artificial systems [4–9]. Over the past decade considerable attention had been given to the design and synthesis of self-assembled supramolecular capsules that are structurally stable in solution, because such assemblies mimic biorelevant self-association events that create discrete suprastructures through noncovalent interactions [10,11]. The two most common motifs use calixarenes [12–15] and glycolurils as monomers [16–18].

In most of these cases [19] the self-assembly relies on hydrogen-bond interactions, which function best in aprotic organic solvents. However, it is highly desirable to work in aqueous media as this is the solvent system that can lead to biorelated applications. As one recent example, Schrader and colleagues reported the formation of ball-shaped dimers in water using simple 1,3,5-substituted benzene building blocks held together by charge-pairing interactions between ammonium and phosphonate groups [20]. The 1,3,5-substitution pattern around benzene is one that has recently seen extensive use, and has been exploited by our group for a variety of purposes [21].

In this work we sought to achieve three main goals: to attain controlled self-assembly in protic media; to show that both ionic and hydrophobic interactions can be exploited to acquire this; and to obtain defined aggregates of higher order than dimeric via a self-selecting process. We chose the 2,4,6-substituted 1,3,5-tristhiophenyl benzenes **1**, **2**, **3**, the trisguanidinium host **4** [22], and citrate (a tricarboxylate) as monomeric species (Fig. 1).

RESULTS AND DISCUSSION

Synthesis

The synthesis of **4** has been reported previously [22]. The syntheses of **1**, **2** and **3** are based on a route we introduced recently [23]. In the synthesis of **1**, 1,3,5-tribromo-2,4,6-tribromomethylbenzene reacts smoothly with sodium azide to give **5** (Scheme 1A). This trisazide derivative can be reduced to the triamine derivative **6** using Staudinger conditions. Finally, the aromatic bromides of **6** are replaced

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ISSN 1061-0278 print/ISSN 1029-0478 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/10610270412331295995

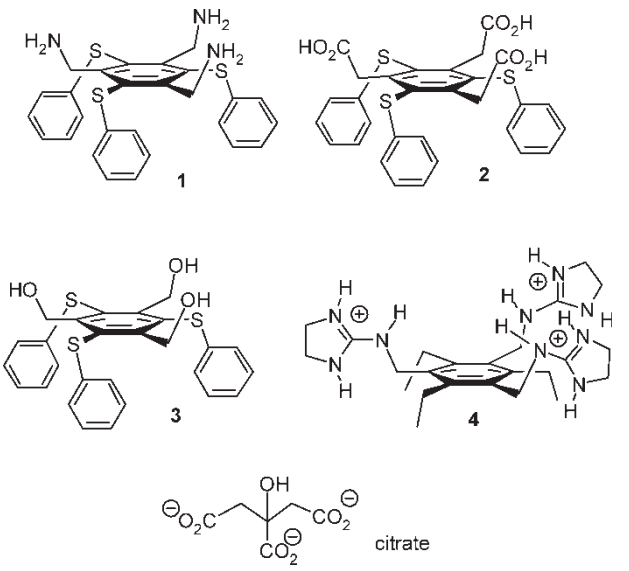
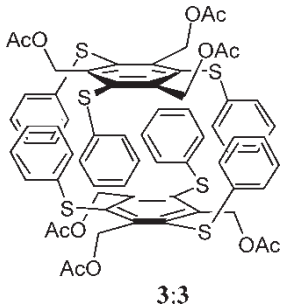


FIGURE 1 Alternating hexasubstituted benzene derivatives **1**, **2**, **3** and **4**, used as monomeric species.

with thiophenyl groups using sodium phenylthiolate and stirring for 36 h, yielding **1**. The synthesis of **2** commences with a reaction between 1,3,5-tribromo-2,4,6-tribromomethylbenzene and KCN to give **7**, followed by a one-pot conversion of the aromatic bromides to aromatic thioethers and the nitriles to carboxylic acids, resulting in **2** (Scheme 1B). The synthesis of **3** involves the reaction of 1,3,5-triacetylmethyl-2,4,6-triphenylthiobenzene with KOH in a water/methanol mixture (Scheme 1C) [23].

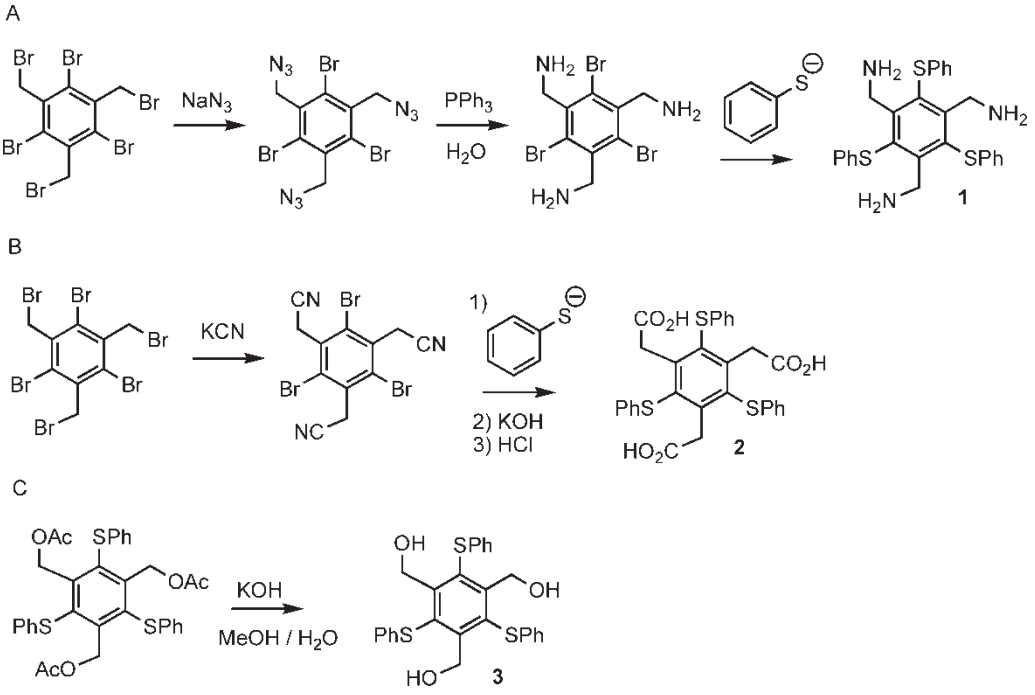
Design Criteria

A previous crystallographic study of a trisacetyl derivative of **3** implicates an edge-to-face interdigitation and attractive interaction between the thiophenyl substituents of two adjacent molecules (**3**:**3**), leading to a tight packing of dimers in the solid state [23]. This led us to investigate this association through a solvophobic effect in highly aqueous media as one type of attractive force for the dimerization of all three monomeric tristhiobenzene species (**1**, **2** and **3**) [24]. We also envisioned charge-pairing interactions for the assembly of **1** and **2**, **2** and **4**, as well as **1** and citrate [25]. These monomers can be mixed to create various dimeric as well as trimer stacks, two of which are reported here.



Binding Studies

Three techniques were used to study some of the possible assemblies that can be created with monomers **1**–**4** and citrate: NMR titrations, curve fitting and, most informative for our analysis,



SCHEME 1 Synthetic routes.

electrospray ionization mass spectrometry (ESI-MS). ESI-MS has been used increasingly to examine noncovalent complexes in the gas phase, including those involving biological macromolecules [26,27], host–guest complexes [28] and supramolecular complexes formed via self-assembly [29]. The ability to obtain molecular weight and stoichiometric information is a significant advantage of mass spectrometry for the evaluation of noncovalent complexes as long as appropriate control experiments are undertaken to differentiate specific noncovalent complexes from nonspecific aggregates that may be formed in the ESI process.

The ^{1}H NMR spectroscopic investigations of the aggregation of **1** and **2** by incremental dilution in D_2O–CD_3OD (1:1, buffered with Tris at pD 7.5) did not indicate the formation of homodimers via face-to-face thiophenyl interactions, or any higher stoichiometry aggregates. Furthermore, ESI-MS of solutions of **1** and **2** indicate the monomers as the species present in solution. Presumably these species do not dimerize because of coulombic repulsion from the like charges.

However, in the case of the trisalcohol **3** at low mM concentrations, ESI-MS indicates formation of a homodimer ($m/z = 988$, negative ion mode) in 100% methanol (**3** is insoluble in water). With increasing concentrations a trimer is also observed ($m/z = 1483$). There is also a concentration-dependent monomer–dimer distribution below 1 mM, which confirms the specific formation of dimers, as opposed to nonspecific aggregation during the mass spectral determination. A similar study with 1,3,5-tris(hydroxymethyl)mesitylene (THM) [30], lacking the appended thiophenyl substituents, did not result in the formation of any defined aggregates of THM at a variety of concentrations (see Supplementary Material). Therefore, **3** clearly dimerizes, most likely through thiophenyl interactions. However, methanol is not a solvent conducive solely to a solvophobic effect. Therefore, we interpret the interaction to support an attraction between the thiophenyls, probably involving edge-to-face interactions as found in the crystal structure [23].

We investigated heterodimerization of compounds **1** and **2** with **3** in D_2O–CD_3OD solutions, driven by solvophobic and attractive interactions between the thiophenyl groups. The titration data, as shown in Figs. 2 and 3, are indicative of a binding interaction between **1** and **3**, as well as **2** and **3**. The fact that **3** is insoluble in the solvent mixture, yet remains soluble until it is nearly one equivalent relative to **1** and **2**, supports the formation of the proposed dimeric aggregates. We wanted to confirm this dimeric aggregate formation in other ways. We first examined curve fitting of the data shown in Figs. 2 and 3.

The NMR titration curves were fit with an algorithm for binding of **1** with **2**, and **1** with **3**, but also incorporating the dimerization of **3**. General equilibria with associated K_{AA} and K_{AB} values are expressed in Eq. (1), where A = **3** and B = **1** or **2** in our analysis. We follow the chemical shift of B (δ_{obs}), hence Eq. (2) results. Using mass balance and equilibria expressions, [AB] and [B] can be related to [A] (Eqs. (3) and (4)). Finally, [A] can be determined by solving Eq. (5). Iteration of the four parameters δ_{AB}, δ_B, K_{AB} and K_{AA} leads to curve fitting, as shown in Fig. 2.

$$A_2 \rightleftharpoons 2A \overset{B}{\rightleftharpoons} AB \tag{1}$$

$$\delta_{obs} = \delta_{AB}([AB]/[B]_t) + \delta_B([B]/[B]_t) \tag{2}$$

$$[AB] = K_{AB}[A][B]_{tot}/(1 + K_{AB}[A]) \tag{3}$$

$$[B] = [B]_{tot} - K_{AB}[A][B]_{tot}/(1 + K_{AB}[A]) \tag{4}$$

$$2K_{AA}K_{AB}[A]^3 + (2K_{AA} + K_{AB})[A]^2 + (K_{AB}[B]_t + 1 - K_{AB})[A] - [A]_t = 0 \tag{5}$$

The curve fits shown are not perfect (Fig. 2). In each case the experimental data level out to a constant chemical shift earlier in the titration than do the calculated points. This is probably due to the precipitation of A, which starts near the addition of one equivalent, and hence the chemical shift of B becomes less sensitive to A than if A remained completely soluble. However, the curve-fitting procedures do show that the mathematics involved in the assumed algorithm can model the general shape of the data.

Further evidence of the predicted aggregation comes from a mass spectral analysis. The mass spectrum of a 1:1.5 mixture of **1** and **3** (using the NMR solutions) clearly indicates the presence of **1**/**3** dimeric structures ($m/z = 989$, Fig. 3A). Likewise, the presence of a **2**/**3** complex ($m/z = 1072$) in solution is confirmed by mass spectrometry (Fig. 3B). Although the solvophobic forces that could hold the **1**/**3** and **2**/**3** complexes together are absent in the gas phase, we still detect these aggregates experimentally. This further supports the hypothesis that there are attractive forces between the thiophenyl groups.

As a final goal we were able to observe the controlled formation of trimeric species, consisting of **1**, **3** and citrate, by capping preformed complexes between **1** and **3**, with one equivalent of citrate. A 1:1-binding stoichiometry for citrate using host **4** has been demonstrated [22], and is analogous to that proposed between **1** and citrate. Indeed, both the mass ($m/z = 1186$) and NMR

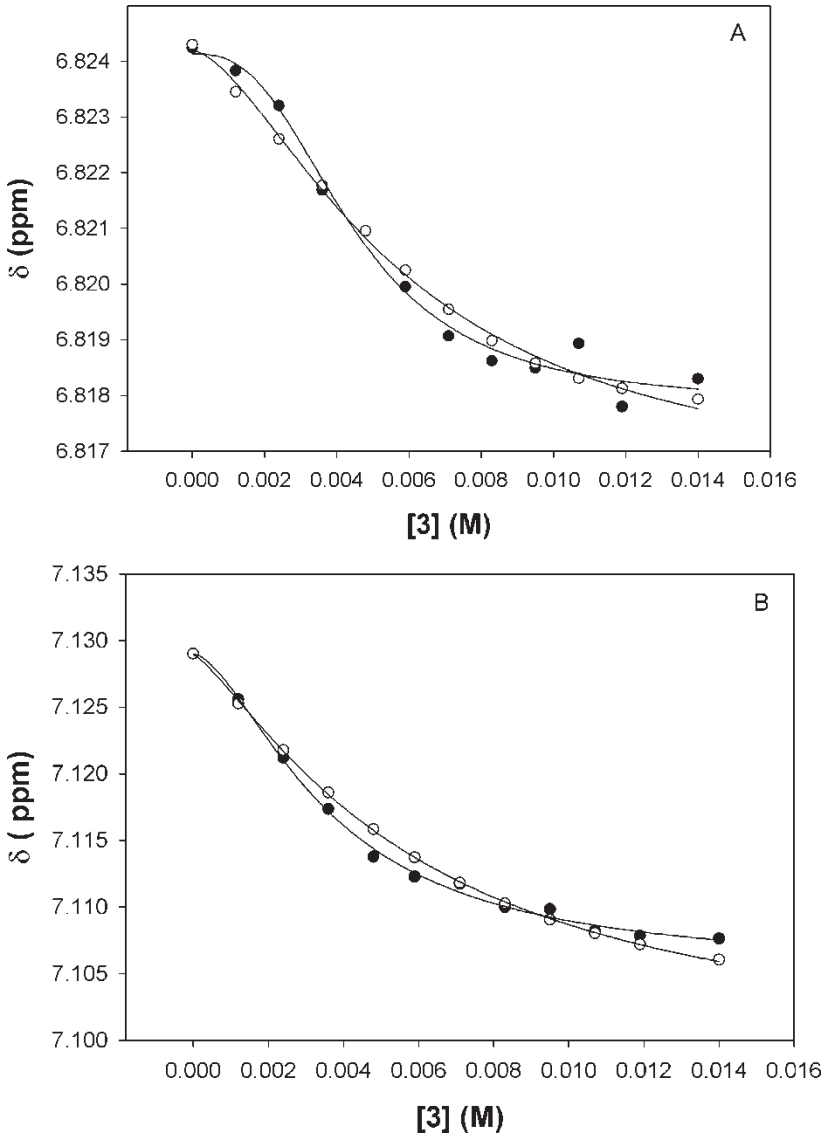


FIGURE 2 Shift of the thiophenyl protons of **1** (*p*) (plot A) and **2** (*o*) (plot B) in D_2O–CD_3OD (1:1, $c = 0.01$ M), buffered at pD 7.5, upon addition of **3** ($c = 0.12$ M in CD_3OD). Closed circles are experimental data, and open circles are calculated data. Plot A: $K_{AB} = 1.9 \times 103\,M^{-1}$, $K_{AA} = 2.3 \times 10^3\,M^{-1}$, $\delta_{AB} = 7.096$, $\delta_B = 7.129$. Plot B: $K_{AB} = 8.6 \times 10^3\,M^{-1}$, $K_{AA} = 2.7 \times 10^3\,M^{-1}$, $\delta_{AB} = 6.817$, $\delta_B = 6.824$. As stated in the text, these affinity constants are not very accurate because of precipitation toward the end of the titrations.

spectra show the formation of a trimer **1**/**3**/citrate aggregate (Figs. 3C and 4). Either the neutral trimeric species is not efficiently ionized or it is not well desolvated in the ESI process, leading to the low spectral intensities observed for this species. Harsher conditions to promote desolvation lead primarily to dissociation of the complex. However, isolation and collisional activated dissociation of the **1**/**3**/citrate species results in both the loss of the citrate cap to generate dimeric **1**/**3** and the loss of monomer **3** to generate a **1**/citrate complex, thus confirming the identity of the trimeric complex.

In a similar fashion, when one equivalent of compound **4** was added to the complex of **2** and **3**, NMR (Fig. 5) as well as mass spectrometry (Fig. 3D) indicate the formation of the trimeric aggregate **2**/**3**/**4** ($m/z = 766$, doubly-charged ion). The identity of the **2**/**3**/**4** complex was confirmed by a collisional activated dissociation experiment in a manner similar to that described above for the **1**/**3**/citrate species. The data of Figs. 4 and 5 were not used to calculate K_a values for trimer formation because, at the concentrations required for the NMR studies, complete dimer aggregate formation cannot be assumed.

CONCLUSION

In summary, we have demonstrated the formation of self-assembled dimers via thiophenyl

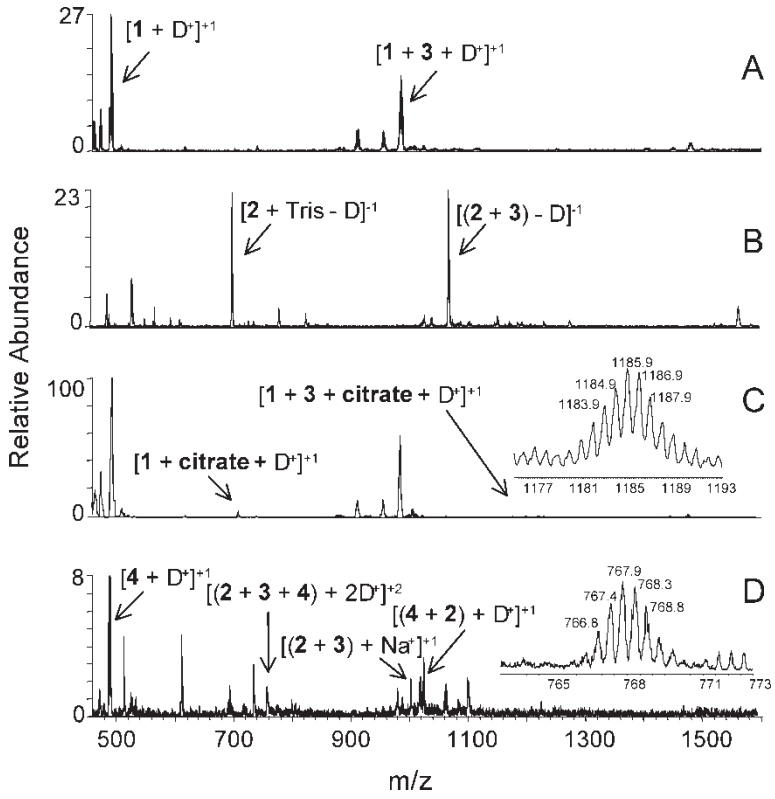


FIGURE 3 ESI-mass spectra of dimers **1**/**3** (spectrum A), **2**/**3** (spectrum B), trimer **1**/**3**/citrate (spectrum C and inset) and trimer **2**/**3**/**4** (spectrum D and inset). Ions of interest are denoted with arrows. As noted in the experimental section, the solutions are buffered with Tris. In B, possibly even higher order aggregation at MW near 1600 can be noted.

solvophobic interactions in a highly competitive solvent system. Furthermore, discrete trimeric assemblies could be created from the dimeric assemblies, thereby combining two different types of noncovalent interactions in the overall controlled aggregation state, namely solvophobic and charge pairing. The ultimate goal is to control higher stoichiometry aggregation states in water leading to function, which is a direction we are currently pursuing.

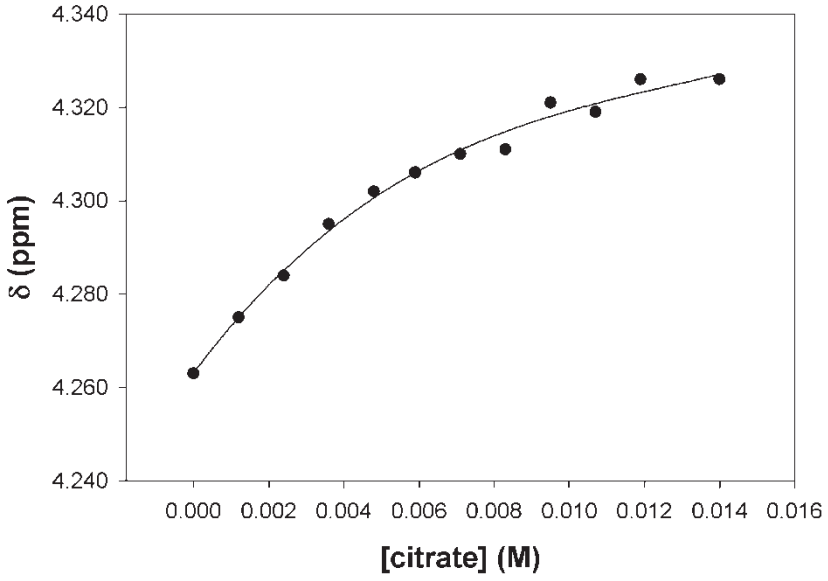


FIGURE 4 Shift of the CH_2-protons of **1** upon addition of citrate ($c = 0.12$ M in D_2O–CD_3OD 1:1) to dimer **1**/**3** in D_2O–CD_3OD (1:1, $c = 0.01$ M), buffered at pD 7.5.

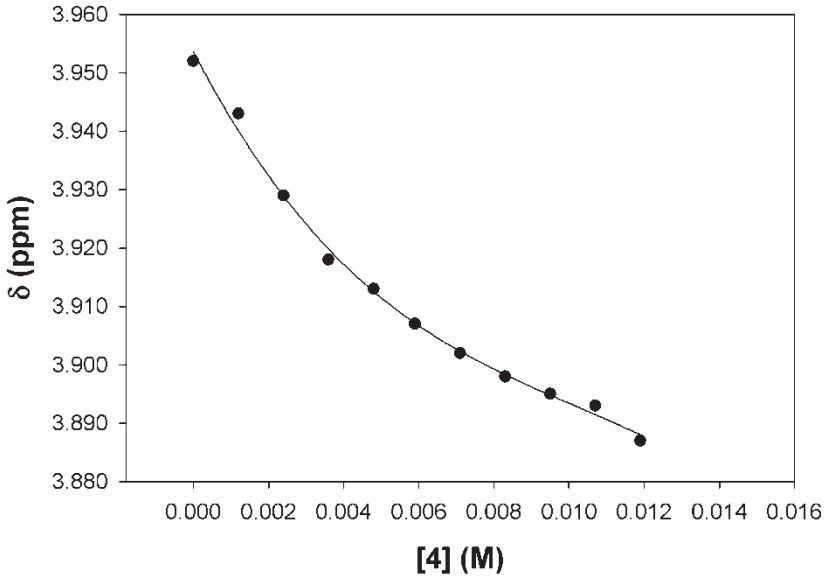


FIGURE 5 Shift of the CH_2-protons of **2** upon addition of **4** ($c = 0.12$ M in D_2O–CD_3OD 1:1) to dimer **2**/**3** in D_2O–CD_3OD (1:1, $c = 0.01$ M), buffered at pD 7.5.

EXPERIMENTAL

General Methods

All reagents and solvents, which were of the highest purity available, were obtained from Aldrich. For characterization of the substances, NMR data were recorded at 25°C on a Bruker AC-250 spectrometer, $CDCl_3$ was used as solvent unless otherwise stated, with chemical shifts reported as ppm and $CDCl_3$ serving as solvent internal standard. Low- and high-resolution mass spectra to confirm identities of compounds were measured on a Finnigan TSQ70 and a VG analytical ZAB2-E mass spectrometer, respectively.

Materials

1,3,5-Trisazidomethyl-2,4,6-trisbromobenzene (5)

A suspension of 1,3,5-trisbromomethyl-2,4,6-trisbromomethylbenzene [22] (1.2 g, 2.02 mmol) and sodium azide (780 mg, 0.012 mol) in DMF (15 ml) was stirred at 45°C for 24 h. The reaction mixture was filtered and the solvent removed. To the remaining residue water (30 mL) was added, and the suspension was extracted with dichloromethane (3×15 mL). The combined organic phases were dried ($MgSO_4$), evaporated to dryness, and the resulting solid was recrystallized from methanol to give pure **5** as colorless needles. Yield: 731 mg (76%); mp 71°C. ^{1}H NMR: $\delta = 4.91$ (s, 6 H; CH_2); ^{13}C NMR: $\delta = 136.24$, 130.05 (Ar), 56.17 (CH_2); CI^+-MS m/z (%): 481 (12, $[M^+]$), 439 (100), 409 (6); HRMS found: 476.8291; calculated for $C_9H_6N_3Br_3$: 476.8296.

1,3,5-Trisaminomethyl-2,4,6-trisbromobenzene (6)

A solution of 1,3,5-trisazidomethyl-2,4,6-trisbromobenzene (**5**, 850 mg, 1.77 mmol) and triphenylphosphine (1.85 g, 7.1 mol) was stirred in THF–water (10:1, 20 mL) at room temperature for 18 h. After the solvent was removed, the reaction mixture was dissolved in dichloromethane (30 mL) and extracted with hydrochloric acid (2 N, 5×15 mL). The combined aqueous layer was brought to pH 12 with sodium hydroxide solution and extracted with ethyl acetate (5×15 mL). The combined organic phase was dried (Na_2SO_4), evaporated to dryness, and the remaining solid was recrystallized from benzene to give pure **6** as a colorless solid. Yield: 298 mg (42%); mp 198°C. ^{1}H NMR: $\delta = 4.21$ (s, 6 H, CH_2), 1.51 (NH_2); ^{13}C NMR: $\delta = 142.18$, 125.69 (Ar), 49.15 (CH_2); CI^+-MS m/z (%): 401 (100, $[M^+]$), 321 (27), 277 (17), 229 (39); HRMS found: 399.8641; calculated for $C_9H_{13}Br_3N_3$: 399.8660.

1,3,5-Trisaminomethyl-2,4,6-thiophenyl benzene (1)

Compound **6** (234 mg, 0.58 mmol) and sodium thiophenolate (380 mg, 2.9 mmol) were stirred in DMI (5 mL) under argon at room temperature for 36 h. The solvent was removed by Kugelrohr distillation. To the remaining solid, water (20 mL) was added, and the suspension was extracted with dichloromethane (3×10 mL). The organic phase was dried ($MgSO_4$). Compound **1** was precipitated as the trisammoniumchloride salt by bubbling HCl gas through the dichloromethane solution for 2 min. The precipitate was filtered and recrystallized from ethanol–benzene (1:1) to give pure

1-trishydrochloride as a colorless solid. Yield: 184 mg (53%); mp > 280°C (decomp.). ^{1}H NMR [MeOH-d_4]: $\delta = 7.33$ (t, $J = 7$ Hz, 2 H; *m*-Ph), 7.22 (t, $J = 7$ Hz, 1 H; *p*-Ph), 7.03 (d, $J = 7$ Hz, 2 H; *o*-Ph), 4.53 (s, 6 H; CH_2); ^{13}C NMR: $\delta = 149.83$, 140.03, 139.31, 136.94, 136.84, 131.10, 128.03, 127.64, 127.55 (Ar), 43.21 (CH_2); CI$^+$-MS *m/z* (%): 490 (28) $[M - (3 \times HCl)]^+$, 462 (13), 416 (9), 391 (12), 307 (9), 291 (16), 279 (73), 229 (32), 215 (16), 187 (100); HRMS found: 490.1445; calculated for $C_{27}H_{28}N_3S_3$: 490.1445.

1,3,5-Triscyanomethyl-2,4,6-trisbromobenzene (7)

1,3,5-Trisbromomethyl-2,4,6-trisbromomethylbenzene (1.5 g, 1.69 mmol) and potassium cyanide (1.06 g, 16.3 mmol) were stirred in DMF (10 mL) at room temperature for 16 h. After the solvent was removed *in vacuo*, water (30 mL) was added to the remaining residue, and the suspension was extracted with dichloromethane (3 × 15 mL). The combined organic phases were dried ($MgSO_4$), evaporated to dryness, and the resulting solid was recrystallized from to give pure **7** as colorless crystals. Yield: 1.04 g (95%); mp 114°C. ^{1}H NMR: $\delta = 4.24$ (s, 6 H, CH_2); ^{13}C NMR: $\delta =$ 132.52, 128.37 (Ar), 114.05 (C≡N), 28.27 (CH_2); CI$^+$-MS *m/z* (%): 431 (100, $[M^+]$), 406 (12), 392 (9), 353 (50); HRMS found: 429.8190; calculated for $C_{12}H_7Br_3N_3$: 429.8190.

1,3,5-Triscarboxymethyl-2,4,6-thiophenyl benzene (2)

Compound **7** (730 mg, 1.70 mmol) and sodium thiophenolate (1.10 g, 8.4 mmol) were stirred in DMI (10 mL) under argon at room temperature for 36 h. The solvent was removed by Kugelrohr distillation. To the remaining solid, water (20 mL) was added, and the suspension was extracted with dichloromethane (3 × 10 mL). The organic phase was dried ($MgSO_4$). The solvent was removed *in vacuo* to give crude 1,3,5-triscyanomethyl-2,4,6-thiophenylbenzene (356 mg) as a yellow solid. The solid material was refluxed in ethanol–potassium hydroxide solution (10 N, 10 mL, 1:1) for 18 h. After cooling and removal of ethanol, the solution was brought to pH 2 with hydrochloric acid (2 N). The precipitate was filtered and recrystallized from ethanol–benzene (1:1) to give pure **2** as colorless crystals. Yield: 41 mg (11%); mp 272–274°C (decomp.). ^{1}H NMR [MeOH-d_4]: $\delta = 7.23$ (t, $J = 7$ Hz, 2 H; *m*-Ph), 7.14 (t, $J = 7$ Hz, 1 H; *p*-Ph), 7.01 (d, $J = 7$ Hz, 2 H; *o*-Ph), 4.24 (s, 6 H; CH_2); ^{13}C NMR: $\delta = 174.05$ (C═O), 149.60, 137.62, 136.93, 130.35, 127.67, 127.07 (Ar), 41.61 (CH_2); CI$^+$-MS *m/z* (%): 577 (12) $[M^+]$, 560 (66), 533 (38), 516 (100), 490 (6), 229 (9); HRMS found: 576.0738; calculated for $C_{30}H_{24}O_6S_3$: 576.0735.

1,3,5-Trishydroxymethyl-2,4,6-thiophenylbenzene (3)

1,3,5-Triacetylmethyl-2,4,6-triphenythiobenzene (400 mg, 0.65 mmol) was refluxed in methanol–potassium hydroxide solution (10 N, 10 mL, 1:1) for 2 h. After cooling and removal of methanol, the solution was neutralized with hydrochloric acid (2 N) and extracted with dichloromethane (3 × 15 mL). The organic phase was washed with saturated sodium bicarbonate solution, dried (Na_2SO_4), and evaporated to dryness to give pure **3** as an amorphous colorless solid. Yield: 324 mg (98%). ^{1}H NMR: $\delta = 7.17$ (t, $J = 7$ Hz, 2 H; *m*-Ph), 7.08 (t, $J = 7$ Hz, 1 H; *p*-Ph), 6.96 (d, $J = 7$ Hz, 2 H; *o*-Ph), 5.07 (s, 6 H; CH_2), 2.91 (s, 3 H; O*H*); ^{13}C NMR: $\delta = 152.35$, 136.96, 135.27, 129.08, 126.29, 125.78 (Ar), 62.63 (CH_2); CI$^+$-MS *m/z* (%): 521 (7), 492 (16, $[M^+]$), 475 (100), 439 (12); HRMS found: 492.0887; calculated for $C_{27}H_{24}O_3S_3$: 492.0888.

NMR Studies

Monomers

Dilution series of compounds **1**, **2** [in D_2O–CD_3OD (1:1), 20 equiv Tris-buffer, adjusted to pD 7.5] and **3** (in CD_3OD) were carried out ($c = 20$, 10, 5, 1 mmol/L), and changes in the aromatic and also methylene ^{1}H signals were followed.

Dimers

A methanolic solution of **3** ($c = 0.12$ M) was titrated into a D_2O–CD_3OD solution (1:1) of compounds **1** and **2** ($c = 0.01$ M, 20 equiv Tris-buffer, adjusted to pD 7.5). The upfield shifts of the thiophenyl protons of **2** (*p*-position) or **3** (*o*-position) were followed.

Trimers

Solutions of sodium citrate or compound **3** in D_2O–CD_3OD (1:1; $c = 0.012$ M, 20 equiv Tris-buffer, adjusted to pD 7.5) were titrated into buffered, pD-adjusted D_2O–CD_3OD solutions of the preformed dimers **1**/**3** or **2**/**3**, respectively. The upfield shifts of the thiophenyl protons of **2** (*p*-position) or **3** (*o*-position) were observed.

ESI-MS Studies

All mass spectra were obtained using a Thermo-Finnigan *LCQ Duo* quadrupole ion trap. Solutions were admitted to the *LCQ* via a Harvard syringe pump at 3–5 μL/min and analyzed in positive and negative ion mode. After initial tuning, the same tune files were subsequently used for all acquisitions. Typical conditions used a source voltage of

±4 kV, heated capillary temperature of 100 or 200°C, low tube lens voltage, and acquisition of 10–20 scans, each consisting of 5 μscans. To establish the identity of dimers and trimers, the ions of interest were isolated and collisional activated dissociation (CAD) was performed ($q = 0.25$, 30 ms) to generate the appropriate fragments.

Monomers

NMR solutions of compounds **1** and **2** (10 mM in D_2O–CD_3OD (1:1), 20 equiv Tris-buffer) were diluted 10-fold in D_2O–CD_3OD (1:1) for ESI-MS analysis. Compound **3** (120 mM in CD_3OD) was diluted 1000-fold in D_2O–CD_3OD (1:1) prior to ESI-MS. Studies of the concentration-dependent dimerization of **3** and control compound THM were carried out at 30, 60, 120 and 240 μM concentration of compound in D_2O–CD_3OD (1:1), 2 mM Tris-buffer (Fig. 2A,B).

Dimers

NMR solutions of dimers **1/2**, **1/3** and **2/3** (1:1.5 in D_2O–CD_3OD (1:1), 20 equiv Tris-buffer) were diluted 5- or 10-fold for ESI-MS analysis. Solutions of **1**/citrate and **2**/**4** were prepared by mixing the appropriate monomers in a 1:1 ratio (500 μM in D_2O–CD_3OD (1:1), 10 mM Tris-buffer).

Trimers

NMR solutions of **1**/**3**/citrate and **2**/**3**/**4** (10 mM **1**, **2** and **3**, 1.25 equiv citrate and **4**, in D_2O–CD_3OD (1:1), 20 equivalents Tris-buffer) were diluted 10-fold D_2O–CD_3OD (1:1) for ESI-MS analysis.

Acknowledgements

We acknowledge support for this work from the National Institutes of Health (GM 65515-2) and the Welch Foundation (F-1155).

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