JOC *Article*

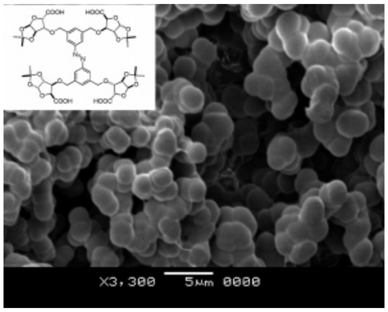
A Tetrameric Sugar-Based Azobenzene That Gels Water at Various pH Values and in the Presence of Salts

Aasheesh Srivastava,[†] Subir Ghorai,[‡] Anup Bhattacharjya,*,[‡] and Santanu Bhattacharya*,[†]

Department of Organic Chemistry, Indian Institute of Science, Bangalore, India, 560012, and
Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Kolkata 700032, India

sb@orgchem.iisc.ernet.in

Received February 16, 2005



A novel low-molecular mass tetrameric sugar derivative containing azobenzene core, **1**, showed pronounced hydrogelation at micromolar concentration. Based on this observation, four related azobenzene based tetrameric sugar derivatives, **4**−**7**, and three tetrameric sugar derivatives with a bis-terephthalamide core, **9**−**11**, were also synthesized. However, none of these closely related analogues of the compound **1** showed effective gelation. The gel formed from **1** was characterized extensively using melting temperature analysis, UV−vis, FT-IR, circular dichroism spectroscopy, and scanning electron microscopy. The resultant gel exhibited impressive tolerance to the pH variation of the aqueous phase and gelated water in the pH range of 4−10. While UV−vis and CD spectroscopy indicated that pronounced aggregation of the azobenzene chromophores in **1** was responsible for gelation, FT-IR studies showed that hydrogen bonding is also a contributing factor in the gelation process. The melting of gel was found to depend on the pH of the aqueous medium in which gel was formed. The gel showed considerable photostability to UV irradiation, indicating tight intermolecular packing inside the gelated state that rendered azobenzene groups in the resultant aggregate refractory to photoisomerization. The electron micrographs of the aqueous gels of **1** showed the existence of spongy globular aggregates in such gelated materials. Addition of salts to the aqueous medium led to a delay in the gelation process and also caused remarkable morphological changes in the microstructure of the gel.

Introduction

Hydrogels, generally prepared from natural or synthetic polymers, represent an important class of materials that offer widespread applications.[1] These polymeric materials entrap a lot of water and show many interesting physical properties such as phase transitions, etc. However, the factors precisely responsible at molecular level for hydrogelation are not often easy to deduce.[2] It is surmised that such polymers form a kind of entangled network in which the solvent molecules are entrapped due to surface tension. In recent past, many low molecular mass compounds have been reported as effective gelators for organic liquids.[3] Low molecular mass gelators are attractive alternatives to their polymeric counterparts as such molecules can be systematically tailored, and it is sometimes possible to delineate various factors at molecular level that are responsible for gelation in preference to crystallization, solubilization, or precipitation of such systems. The gelation involving low molec-

---

[†] Indian Institute of Science.

[‡] Indian Institute of Chemical Biology.

(1) (a) Lee, K. Y.; Mooney, D. J. *Chem. Rev.* **2001**, *101*, 1869. (b) Langer, R. *Nature* **1998**, *392*, 5. (c) Guetnet, G.-M. *Thermoreversible Gelation of Polymers and Biopolymers*; Academic Press: London, 1992.

(2) Nakama, T.; Ooya, T.; Yui, N. *Polym. J.* **2004**, *36*, 338.

(3) Recent reviews: (a) van Esch, J.; Feringa, B. L. *Angew. Chem., Int. Ed.* **2000**, *39*, 2263. (b) Abdallah, J.; Weiss, R. G. *Adv. Mater.* **2000**, *12*, 1237. (c) Terech P.; Weiss, R. G. *Chem. Rev.* **1997**, *97*, 3133.

10.1021/jo050297p CCC: $30.25

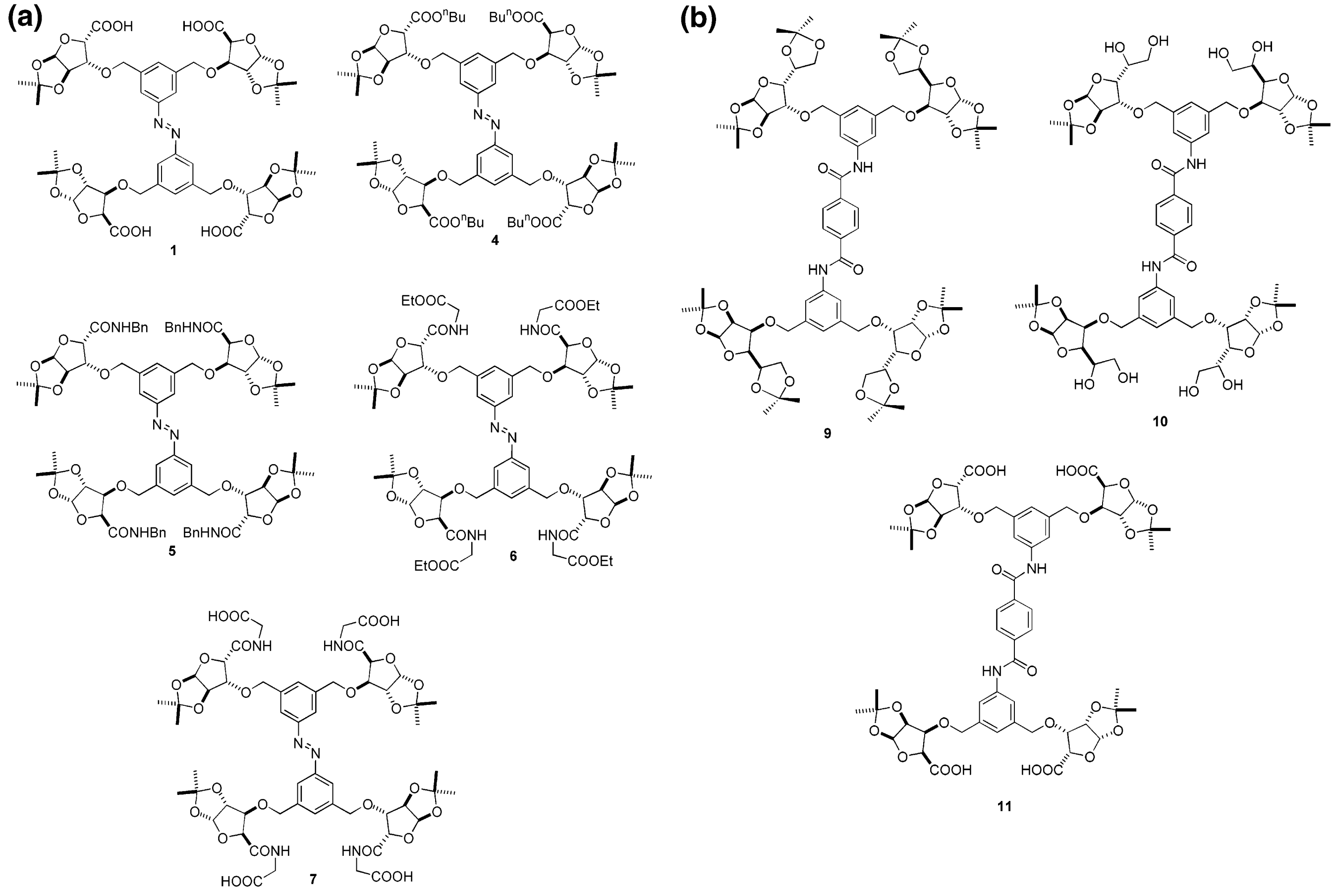


FIGURE 1. (a) Chemical structures of compounds based on azobenzene core. (b) Chemical structures of compounds based on bis-terephthalamide core.

ular mass gelators has been probed using various spectroscopic techniques such as FT-IR,[4a] NMR,[4b] UV–vis,[6a] CD,[4c] fluorescence,[4d] differential scanning calorimetry (DSC),[4a] and rheology[4e,f] to obtain an insight into the gelation process. Such studies have provided useful insight for the rational design of low molecular weight organic gelators.

The design of small molecular weight hydrogelators has proved more challenging and has received attention only recently.[4,5] Discovering new structural motifs for hydrogelation is an important objective of much research. Some of these molecules have shown properties such as efficient gelation at low concentration range, tolerance to additives such as surfactants, and gelation that can be triggered by changes in pH, etc.[4c,6,7] Many of these molecules are derivatives of naturally occurring compounds such as nucleotides, amino acids, or sugars.[8] Similarly, peptide-based hydrogelators have also been reported and have shown interesting properties: control of the gelation process through variation in pH, temperature, or ionic strength.[9] These naturally derived molecules hold significant promise toward the development of gels that can be used in biological systems, and in applications involving biomedical and tissue engineering without the danger of bioincompatibility.[10] Because of their importance as scaffolds for various biomaterials

---

(4) (a) Yun, Y. J.; Park, S. M.; Kim, B. H. *Chem. Commun.* **2003**, 254. (b) Kumar, D. K.; Jose, D. A.; Dastidar, P.; Das, A. *Langmuir* **2004**, *20*, 10413. (c) Estroff, L. A.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2000**, *39*, 3447. (d) Mukhopadhyay, S.; Krishnamoorthy, I. G.; Maitra, U. *J. Phys. Chem. B* **2003**, *107*, 2189. (e) Menger, F. M.; Caran, K. L. *J. Am. Chem. Soc.* **2000**, *122*, 11679. (f) Bhattacharya, S.; Acharya, S. N. G. *Chem. Mater.* **1999**, *11*, 3504.

(5) (a) Estroff, L. A.; Hamilton, A. D. *Chem. Rev.* **2004**, *104*, 1201. (b) Oda, R.; Huc, I.; Candau, S. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 2689. (c) van Bommel, K. J. C.; van der Pol, C.; Muizebelt, I.; Friggeri, A.; Heeres, A.; Meetsma, A.; Feringa, B. L.; van Esch, J. *Angew. Chem., Int. Ed.* **2004**, *43*, 1663. (d) Makarević, J.; Jokić, M.; Frkaneć, L.; Katalenić, D.; Žinić, M. *Chem. Commun.* **2002**, 2238. (e) Gronwald, O.; Shinkai, S. *J. Chem. Soc., Perkin Trans. 2* **2001**, 1933. (f) Bhattacharya, S.; Maitra, U.; Mukhopadhyay, S.; Srivastava, A. Advances in Molecular Hydrogels. In *Molecular Gels: Materials with Self-Assembled Fibrillar Networks*; Weiss, R. G., Terech, P., Eds.; Springer: Dordrecht, 2005; Chapter 17, p 605.

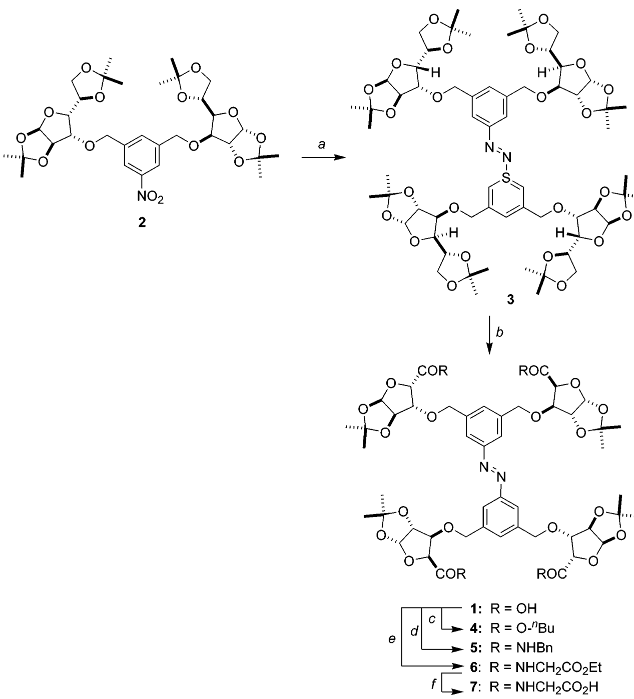
(6) (a) Kobayashi, H.; Friggeri, A.; Koumoto, K.; Amaike, M.; Shinkai, S.; Reinhoudt, D. N. *Org. Lett.* **2002**, *4*, 1423. (b) Kobayashi, H.; Amaike, M.; Jung, J. H.; Friggeri, A.; Shinkai, S.; Reinhoudt, D. N. *Chem. Commun.* **2001**, 1083. (c) Murata, K.; Aoki, M.; Suzuki, T.; Harada, T.; Kawabata, H, Komori, T.; Ohseto, F.; Ueda, K.; Shinkai, S. *J. Am. Chem. Soc.* **1994**, *116*, 6664.

(7) Heeres, A.; van der Pol, C.; Stuart, M.; Friggeri, A.; Feringa, B. L.; van Esch, J. *J. Am. Chem. Soc.* **2003**, *125*, 14252.

(8) (a) Suzuki, M.; Owa, S.; Yumoto, M.; Kimura, M.; Shirai, H.; Hanabusa, K. *Tetrahedron Lett.* **2004**, *45*, 5399. (b) Kiyonaka, S.; Shinkai, S.; Hamachi, I. *Chem.-Eur. J.* **2003**, *9*, 976. (c) Wang, G.; Hamilton, A. D. *Chem. Commun.* **2003**, 310. (d) Iwaura, R.; Yoshida, K.; Masuda, M.; Yase, K.; Shimizu, T. *Chem. Mater.* **2002**, *14*, 3047. (e) McWatt, M.; Boons, J. *Eur. J. Org. Chem.* **2001**, 2535. (f) Amanokura, N.; Yoza, K.; Shinmori, H.; Shinkai, S.; Reinhoudt, D. N. *J. Chem. Soc., Perkin Trans. 2* **1998**, 2585.

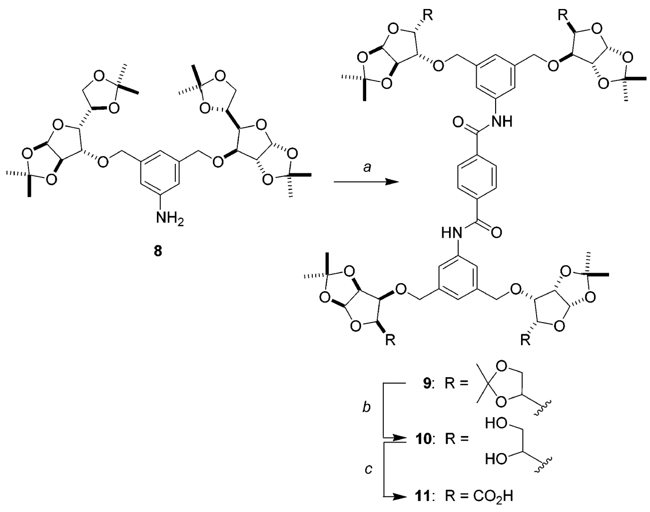
(9) (a) Ozbas, B.; Kretsinger, J.; Rajagopal, K.; Schneider, J. P.; Pochan, D. J. *Macromolecules* **2004**, *37*, 7331. (b) Pochan, D. J.; Schneider, J. P.; Kretsinger, J.; Ozbas, B.; Rajagopal, K.; Haines, L. *J. Am. Chem. Soc.* **2003**, *125*, 11802. (c) Pochan, D. J.; Schneider, J. P.; Ozbas, B.; Rajagopal, K.; Pakstis, L.; Kretsinger, J. *J. Am. Chem. Soc.* **2002**, *124*, 15030.

SCHEME 1[a]



[a] Reagents and conditions: (a) Zn, NaOH, EtOH−H_2O, reflux, 3 h, 50%; (b) (i) 75% aq AcOH, 25 °C, 12 h; (ii) $NaIO_4$, MeOH−H_2O, 0−25 °C, 1 h; (iii) $AgNO_3$, KOH, EtOH−H_2O, 0−25 °C, 6 h, 55% from **3**; (c) *n*-BuOH, HOBT, DCC, DMAP, CH_2Cl_2, 25 °C, 18 h, 75%; (d) $PhCH_2NH_2$, HOBT, DCC, CH_2Cl_2, 25 °C, 24 h, 55%; (e) $H_2NCH_2CO_2C_2H_5{\cdot}HCl$, HOBT, DCC, Et_3N, CH_2Cl_2, 25 °C, 24 h, 88%; (f) $LiOH{\cdot}H_2O$, MeOH−H_2O, 25 °C, 24 h, 83%.

SCHEME 2[a]



[a] Reagents and conditions: (a) terephthaloyl chloride, Et_3N, THF, reflux, 12 h, 70%; (b) 75% aq AcOH, 25 °C, 12 h, 96%; (c) (i) $NaIO_4$, MeOH−H_2O, 0−25 °C, 1 h, 93%; (ii) $NaClO_2$, DMSO, NaH_2PO_4, H_2O, 0−25 °C, 1.5 h, 52%.

design, it is useful to develop hitherto unknown classes of low molecular mass systems that ensue strong gelation of water under ambient conditions. Herein, we report the synthesis of a series of new tetrameric sugar derivatives (**1**, **4**−**7**, **9**−**11**), one of which demonstrates highly pronounced hydrogelation properties. We include herein the detailed spectroscopic, thermal, and microscopic characterization of the resultant gel, and the effect of pH and addition of salts to it.

Results and Discussion

Synthesis. The synthesis of **1** was achieved from the known[11] nitro derivative **2** according to Scheme 1 (for details, see Supporting Information). Treatment of **2** with Zn and NaOH in aqueous ethanol under reflux afforded the azobenzene **3** in 50% yield. A sequence of reactions involving selective removal the 5,6-isopropylidene groups by 75% aqueous acetic acid, vicinal diol cleavage with $NaIO_4$, and oxidation of the resulting tetraaldehyde with Ag_2O gave **1** in 55% overall yield from **3**. Several derivatives, **4**, **5**, and **7**, were prepared from **1** by standard procedures as shown in Scheme 1. In a separate route, the terephthaloyl-linked dendritic tetracarboxylic acid, **11**, was prepared from the known amine **8**[11] by reaction with terephthaloyl chloride followed by a sequence of reactions similar to that used for the conversion of **3** to **1** (Scheme 2). The structures of all of the compounds were secured by FT-IR, NMR, mass spectral data, and elemental analyses.

Structural Variation. Various lipophilic derivatives of sugar are known to form gels in water.[4f,12] Here, we have synthesized **1** and its other analogues (Figure 1a and b) and studied their properties upon solubilization in water. In this design, we decided to examine tetrameric derivatives of glucose. These can be classified into two broad categories. The first category belongs to the derivatives with azobenzene as the core, while the second category has bis-terephthalic amide as the core. This allowed us to examine the effects of the "core" structure on aggregation properties while keeping the number of sugar molecules on the periphery constant. Within each class, the individual members had structural variation of the functional groups on the peripheral glucose moieties. Accordingly, while **1** has a carboxylic acid moiety and isopropylidene protections on the glucose derivative, **4** had the carboxylic acid converted to its butyl ester. It is known in the literature that carboxylic acids can promote gelation by intermolecular hydrogen bonding while this opportunity is lost when the acid is converted into an ester.[4e] Molecules **5**, **6**, and **7** were various amide derivatives of the pendant carboxylic acid moiety present in the sugar units of **1**. Many amides are known to form gels with the amide group helping in the aggregate formation.[13] From our laboratory, hydrogelators based on long hydrocarbon chain derivatives of sugars have elucidated the importance of such functional groups in the gelation process.[4f]

Replacement of the azobenzene core and changing it to the bis-amide derivative of terephthalic acid resulted in another class of tetrameric sugar molecules. This core ensured that the number of sugar moieties is maintained at four, similar to their number in molecules **1**−**7**. Consequently, compounds **9**−**11** were synthesized. Compound **9** has the hydroxyls protected by isopropylidene

---

(10) Frigerri, A.; Feringa, B. L.; van Esch, J. *J. Controlled Release* **2004**, *97*, 241.

(11) Ghorai, S.; Bhattacharjya, A.; Basak, A.; Mitra, A.; Williamson, R. T. *J. Org. Chem.* **2003**, *68*, 617.

(12) Pfannemuller, B.; Welte, W. *Chem. Phys. Lipids* **1985**, *37*, 227.

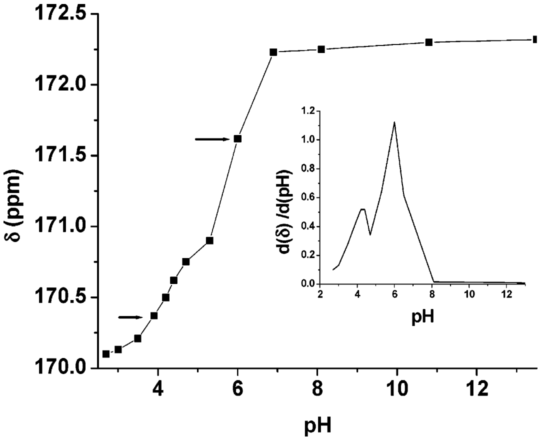


FIGURE 2. δ (ppm) versus pH profile for the carbonyl carbon of **1** in a d_6-DMSO/water mixture. Arrows indicate the pK_a values. Inset shows the derivative plot [d(δ)/d(pH) vs pH].

groups; in **10**, the 5,6-hydroxyls are deprotected, and in **11**, the 5,6-hydroxyls are converted to carboxylic acid groups.

pK_a Calculation for 1. The presence of four carboxylic acid moieties in **1** prompted us to measure their pK_a values. ^{13}C NMR is ideally suited for studying this.[14a] The carbonyl carbon exhibits a downfield shift when the carboxylic acid group is converted to the carboxylate form. This shift has been exploited for measuring the pK_a values of many biologically relevant compounds, for example, bilirubin, a molecule that has two carboxylic groups and two lactam groups.[14] It has been found further that the pK_a does not get affected much even when a mixture of solvents is used.[14b,c] This is because the aqueous phase is the main solvent and the base on molar scale for the compound.

Similar to the bilirubin case, **1** exhibited a downfield shift when 200 μL of 191 mM NaOH solution was added to a d_6-DMSO solution the compound. Thus, the carbonyl carbon in **1** exhibited a chemical shift of 168.65 ppm in d_6-DMSO, while in the presence of excess base, the value had shifted to 172.32 ppm. When 116 mM HCl was incrementally added to this, the chemical shift value of the carbonyl carbon kept shifting progressively to lower values. The apparent pH of the solution was noted after each addition of the acid. The variation in the chemical shift value with the apparent pH of the solution has been plotted in Figure 2. It can be observed that the sample exhibits a bi-sigmoidal behavior, indicating that at least two of the four theoretical pK_a's are discernible. The respective pK_a values were calculated by differentiating the spectra (inset of Figure 2). It was found that the apparent pK_as for **1** are 4.2 and 6.0, respectively. As can be seen from Figure 2, the δ value did not shift much after pH 6.5; hence, this corresponds to the completely ionized (tetracaboxylate) state of **1**. It has been noted in the case of mesobilirubin-XIIIα that closely lying pK_a values are not clearly discernible by ^{13}C NMR even for isotopically enriched samples.[14b] In the present instance, the ^{13}C NMR from **1** was obtained from the natural abundance of ^{13}C present in the sample. Nevertheless, the breaks were reasonably sharp, although the resolution of all four pK_a values was not possible.

Gelation. We found that for 1 mg of **1** dissolved in 10 μL of DMSO, the resulting solution could instantaneously gel up to 1 mL of water. This corresponds to a gelation capacity of 0.1 wt %. In other words, as many as 55 500 water molecules can be trapped by one gelator molecule. Similar gelation was also achieved when water was injected into a solution of **1** in MeOH. The gelation was thus affected in around 1 μM range. Clearly, **1** ranks among one of the most potent low-molecular mass synthetic hydrogelators in the class of sugar-based gelators.[6a,15]

Our investigation showed that while **1** was a highly efficient gelator of water, other structurally closely related compounds failed to show any hydrogelation. This indicates the importance of the presence of both $-CO_2H$ moiety and the azobenzene core for gel formation. Thus, **4**, which is a butyl ester derivative of **1**, failed to exhibit gelation ability. Similarly, the amide derivatives of **1** were incapable of hydrogelation. Compounds **9**−**11**, with the terephthaloyl backbone, also did not show gelation and precipitated out from water. These compounds, however, did not produce crystals of satisfactory quality to enable the structural studies using single-crystal X-ray diffraction.

The process of gelation occurs when a delicate balance between solubility and precipitation is maintained.[4e] Thus, an adequate blending of the hydrophobicity and hydrophilicity is important for hydrogelation to occur. The extended planar surface of the azobenzene core provides sufficient stacking interactions for molecules of **1** to assemble. The aggregation of azobenzene moieties was also evidenced from UV−vis and CD experiments (see below).

We also attempted the gelation of a number of organic solvents such as acetonitrile, 1,4-dioxane, chloroform, propan-2-ol, acetone, ethyl acetate, toluene, and hexane. However, none of the compounds investigated herein elicited gelation of any of these solvents. The compounds were found to be soluble in polar solvents such as acetonitrile, 1,4-dioxane, chloroform, and propan-2-ol, and were either insoluble or precipitated out of the solutions in less polar solvents such as ethyl acetate, toluene, and hexane.

Thermal Stability of the Hydrogel. Thermal stability of the resultant hydrogel was analyzed by the inverted-tube method as described earlier.[6a] The gel for the aqueous phase at pH 7 indicated a "melting" in the range of 55−60 °C whereupon the gelated mass progressively changed to sol form and finally fell down from the inverted test tube. Increasing the concentration of the gelator is known to increase the thermal stability of the gel.[6a,15b] In our studies with **1**, we also found a similar trend wherein the melting temperature of the resultant hydrogel was found to increase with increasing gelator concentration. The effect of concentration of the gelator

---

(13) (a) Suzuki, M.; Yumoto, M.; Kimura, M.; Shirai, H.; Hanabusa, K. *Chem.-Eur. J.* **2003**, *9*, 348. (b) Kumar, D. K.; Jose, D. A.; Dastidar, P.; Das, A. *Chem. Mater.* **2004**, *16*, 2332. (c) Wuang, G.; Hamilton, A. D. *Chem. Commun.* **2003**, 310.

(14) (a) Hansen, P. E.; Thiessen, H.; Brodersen, R. *Acta Chem. Scand. B* **1979**, *33*, 281. (b) Lightner, D. A.; Holmes, D. L.; McDonagh, A. F. *J. Biol. Chem.* **1996**, *271*, 2397. (c) Holmes, D. L.; Lightner, D. A. *Tetrahedron* **1995**, *51*, 1607.

(15) Jung, J. H.; Shinkai, S.; Shimizu, T. *Chem.-Eur. J.* **2002**, *8*, 2684.

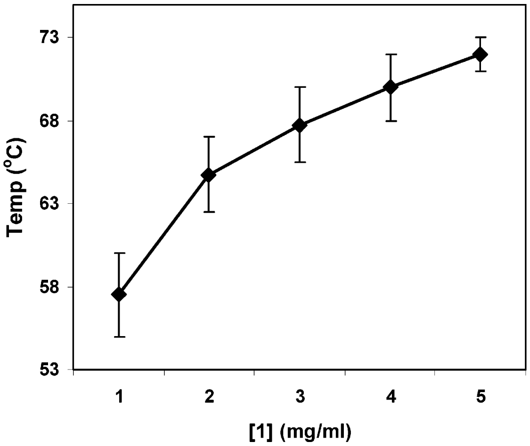


FIGURE 3. Dependence of thermal stability of gel on the concentration of **1**.

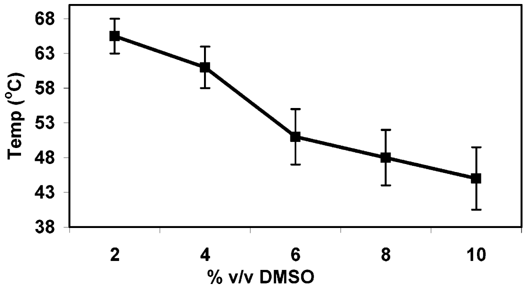


FIGURE 4. Effect of amount of cosolvent on the thermal stability of gel. [**1**] = 2 μM.

on the thermal stability of the gel is shown in Figure 3. In the figure, the vertical bars indicate the temperature range over which the gel melts and finally falls down from the inverted tube. We also found that the range over which the gels melt becomes narrower as the concentration of the gelator was increased. It is possible that with higher concentration of gelators in the hydrogel, a larger number of molecules is accommodated in the aggregate; and, under these circumstances, the packing of gelator molecules become more pronounced due to enhanced $\pi-\pi$ and hydrogen-bonding interactions, resulting in a higher thermal stability.

Effect of Cosolvent on the Thermal Stability of Gels. Further, we analyzed the effect of amount of cosolvent on the thermal stability of the resultant gels. Thus, increasing amounts of DMSO were added to 1 mg of **1**. Upon addition of 500 μL of water to this, gel formation was observed until around 6% cosolvent (30 μL of cosolvent in a 500 μL mixture). For >6% DMSO, sample needed to be kept at 4 °C to effect gel formation. Thus, gel formation became more difficult as the amount of cosolvent was increased. Also, the thermal stability of the resultant gel was highly compromised as the amount of DMSO was increased in the medium. Thus, for ca. 2% v/v DMSO/water mixture, the gel melting occurred at 60 °C; for a 10% mixture, the gelation was occurring at 45 °C with the range of melting having increased considerably (Figure 4).

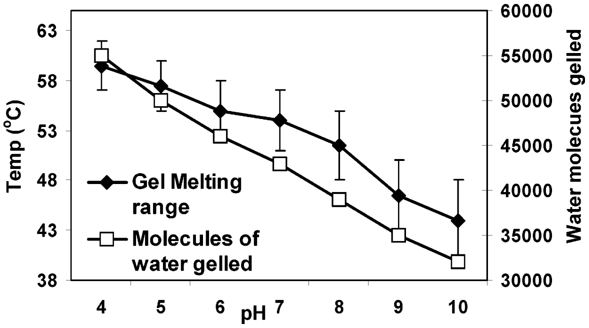


FIGURE 5. The effect of pH of medium on the thermal stability of gel, and number of water molecules entrapped per molecule of **1**. For thermal stability studies, [**1**] = 1 μmol.

Effect of pH on Gelation. The gel melting as well as the maximum gelation capacity (MGC) of **1** at room temperature were also studied at various pH values. Gelation was attempted using water maintained at various pH values. The gelation was abolished in the presence of various buffers. A 0.1 M KCl solution was used to maintain the ionic strength, and the pH of the solution varied by the addition of ca. 1 mM HCl or NaOH. The hydrogelation ability of **1** was preserved in the pH range from 4 to 10. This indicates that **1** is a versatile gelator unaffected by changes in pH of the aqueous medium over a wide range. However, acidification of gel near pH 2 converted the gel into a sol. At such highly acidic conditions, the cleavage of acetal residues takes place, rendering the resulting product water-soluble. This also demonstrates the importance of protection of OH functional groups of the carbohydrate residues in **1** for optimized gelation of water. It was observed that the thermal stability as well as the number of water molecules gelled per molecule of **1** were dependent on the pH of the medium gelated. The gel was thermally more stable at the pH ranges 4−5. Moreover, a lesser amount of gelator was required to achieve gelation at these pHs. As the pH of the solution to be gelled was increased, there was a gradual decrease in the stability of the gel as well as the capacity to entrap the amount of water molecules (Figure 5). This indicates that the carboxylic acid-mediated hydrogen bonding is an important contributor for gel formation. This parallels the observation that when the CO_2H groups in **1** are blocked by esterification, the resulting compound did not induce gelation of water, and suggests that the availability of CO_2H is essential for gelation of water.

FT-IR studies indicate that at pH 4−5 the CO_2H groups in **1** are virtually un-ionized and mostly associate via intermolecular hydrogen bond-mediated −COOH··· HOOC− type stabilization. While a MeOH solution of **1** showed a carbonyl stretch at 1737 cm^{-1}, the corresponding xerogel of **1** showed it at 1743 cm^{-1} when recorded as a KBr pellet. This is indicative of the existence of hydrogen bonding in the xerogel. Under similar conditions, a similar value was found for the solid form of **1** too, indicating that in both the xerogel and the solid form, the molecules associate via hydrogen bonding. Yet at higher pH values, the $-CO_2H$ groups in **1** ionize to CO_2^- ions. A FT-IR of xerogel formed by drying of a gel sample at pH 10 elicited the carbonyl stretch at around 1650 cm^{-1}. This is characteristic of a compound having an ionized carboxylate moiety, and in turn rules out the possibility of intermolecular hydrogen bonding. It is possible that the gelation under these conditions most likely originate from $\pi-\pi$ stacking interactions. Aggrega-

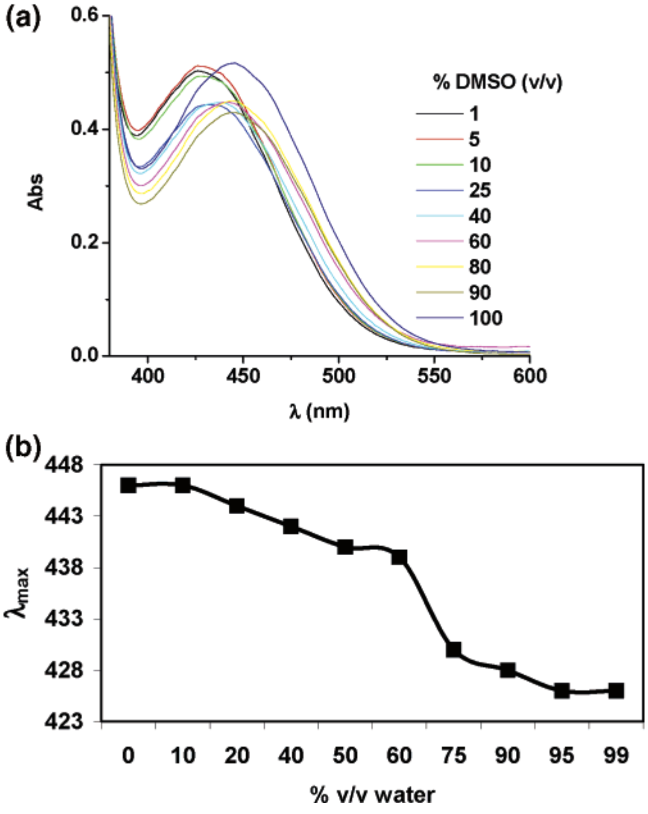


FIGURE 6. (a) UV−visible spectra of **1** in a DMSO/water mixture ([**1**] = 0.1 mg/mL). (b) Variation in the λ_{max} of the azobenzene core with change in % v/v of water in a DMSO/water mixture. [**1**] = 0.1 mg/mL of the solution.

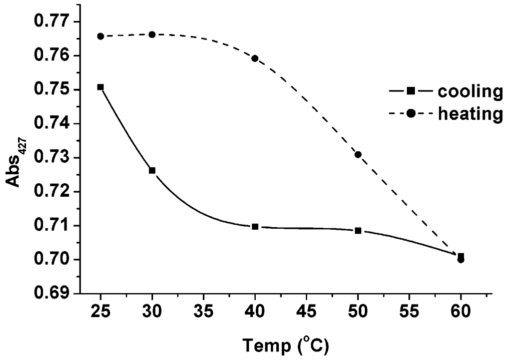


FIGURE 7. Hysteresis in the A_{427} during heating and cooling scans.

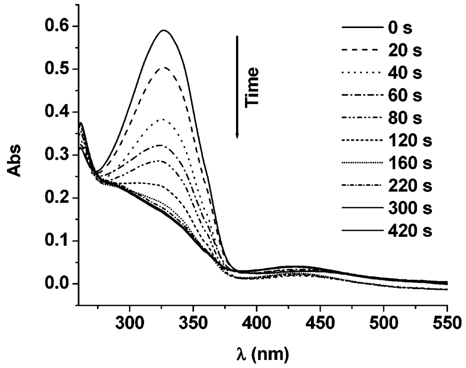


FIGURE 8. UV−vis changes in the DMSO solution of **1** (0.1 mg/mL) upon irradiation with 350 nm UV light for different intervals of time.

tion of the azobenzene moiety present in **1** could be inferred from the hyperchromicity of the peak at 427 nm observed in the UV−vis spectrum **1** when the “sol” form **1** was allowed to cool and form gel (vide infra).

UV−Vis Studies on the Hydrogels. The hydrogels prepared from **1** were optically transparent. The presence of azobenzene moiety in **1**, and its optical clarity, prompted us to study the UV−vis spectral changes during the course of aggregation. Solutions of **1** at the concentration of 0.1 mg/mL in different v/v ratios of water/DMSO were analyzed. It was observed that as the amount of water in the solvent mixture increased, there was a progressive blue shift in the λ_{max} around 450 nm (Figure 6a). As can be seen in Figure 6b, this change became pronounced only when the percentage of water exceeded 60% v/v. With the increase in water content, the shift in the UV−vis spectra toward lower wavelength indicates that $\pi-\pi$ stacking occurs in water with the formation of H-type or related aggregates.[6a]

We also examined the effect of temperature variation in the UV−vis profile of the hydrogels. Decreasing the temperature of aqueous solution of **1** (above MGC) led to a progressive increase in the absorbance at 427 nm. The change in absorbance at 427 nm also showed clear hysteresis during the heating/cooling cycle (Figure 7). This indicates that the gelation process is kinetically controlled and that the aggregation of the chromophores occurs in slightly different fashion after each heating/cooling cycle.

Effect of Photoirradiation. The *trans*-to-*cis* photoisomerization of the azobenzene moiety in solution phase could be achieved by UV irradiation of **1**.[16] Thus, when a 0.1 mg/mL solution of **1** in DMSO was irradiated with a UV light of ca. 350 nm, a progressive change in the UV−vis profile of the sample was observed (Figure 8). The band at around 325 nm exhibited gradual hypochromism as the sample was irradiated with the UV light. This is indicative of the *trans*-to-*cis* isomerization of the azobenzene chromophore. After irradiation for around 7 min, the sample did not show any further change in the UV−vis, indicating the achievement of the photostationary state (PSS). In other systems where azobenzene units are present, it is known that the photostationary state in azobenzene derivatives is achieved quite rapidly.[17] It may be noted that there was no perceptible change in the peak near 425 nm at this concentration, probably due to the small absorption coefficient of this peak. Yet, when a 2 mg/mL solution of **1** in DMSO was irradiated for 15 min at 25 °C, it led to a shift in the UV−vis absorption λ_{max} from 444 to 428 nm (Figure 9, left panel). In contrast, the gel formed by **1** was found to be quite photo resistant. Upon irradiation of the hydrogel under same conditions for up to 20 min, the gel retained its integrity and the color of the sample virtually did not change (Figure 9, right panel). This suggests that in the gelated state, the azobenzene moieties are aggregated so strongly that even UV irradiation for 20 min is insufficient to lead to any significant photoisomerization. These types of observa-

---

[16] Wang, G.; Tong, X.; Zhao, Y. *Macromolecules* **2004**, *37*, 8911.

[17] (a) Kay, K.-Y.; Han, K.-J.; Yua, Y.-J.; Park, Y. D. *Tetrahedron Lett.* **2002**, *43*, 5053. (b) Murakami, H.; Kawabuchi, A.; Kotoo, K.; Kunitake, M.; Nakashima, N. *J. Am. Chem. Soc.* **1997**, *119*, 7605.

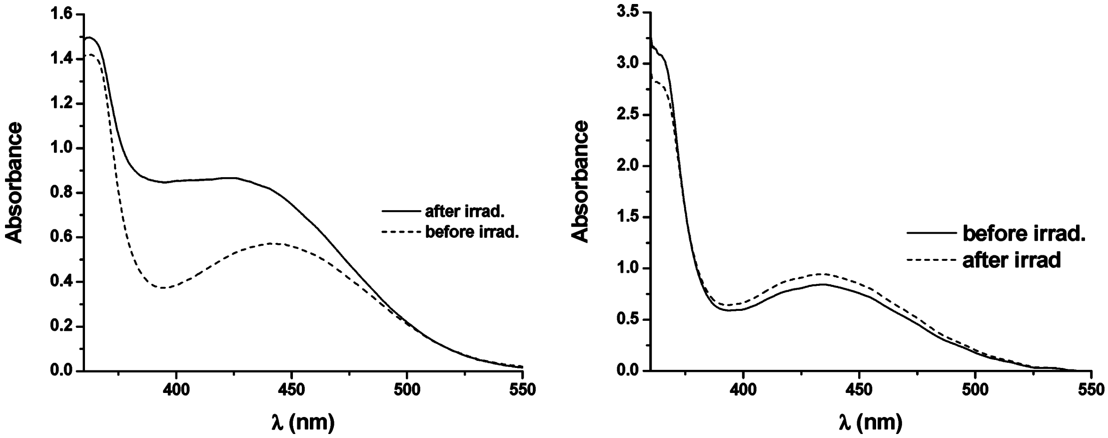


FIGURE 9. UV–vis changes upon irradiation of **1** (2 mg/mL) with 350 nm radiation for 15 min – as DMSO solution (left panel) and hydrogel (right panel).

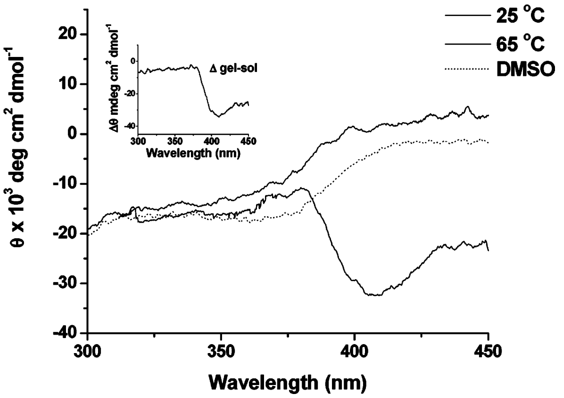


FIGURE 10. CD spectra of **1** (2 μM) as DMSO solution and as hydrogel at different temperatures. (Inset) Difference spectra of gel-sol.

tions have also been reported for other self-assembled systems in which azobenene moieties have been part of gel networks or bilayer membranous aggregates.[18]

It was further found that when pre-irradiated **1** was employed, gel formation was not achieved. Thus, when a DMSO solution of **1** (2 mg in 15 μL of solvent) was pre-irradiated for 10 min and 500 μL of water was added to it, gelation did not occur. This indicates the importance of the *trans*-azobenzene form of **1** for effecting hydrogelation. This is presumably due to the *trans*-form of **1** being more planar, and, hence, greater π-surface is available in this form for aggregation as compared to the *cis*-form.

Chiral Organization in Gels. To gain further insight into the molecular basis of gel formation by **1** in water, circular dichroism (CD) studies were undertaken. Figure 10 shows the CD profiles of the gelator at temperatures below (25 °C) and above (65 °C) the T_{gel}, and as a DMSO solution of identical concentration at 25 °C. As can be seen from the figure, the sol form of **1** (at 65 °C) has a CD profile quite similar to that of its solution in DMSO. The CD profile in the gel form at 25 °C is, however, markedly different. This is further clear from the difference spectrum shown in the inset of Figure 9. It indicates that the aggregation is the primary mode of gel formation. In a similar study involving aggregation of azobenzene-containing glucose derivative, Kobayashi et al. found an analogous CD profile and explained the CD signatures in the gel as arising due to the formation of right-handed helix.[6a] We believe a similar mechanism is operative in our case as well, although it may be pointed out that we did not see any macroscopic chirality in the aggregates of **1**.

Gelation of Salt Solutions. The prior presence of different ions in the water also did not affect the gel formation induced by **1**. Gel formation occurred in the presence of salts such as NaCl (1 mM), KCl (1 mM), $CaCl_2$ (1 mM), and $MgCl_2$ (1 mM). Inclusion of salts influenced the kinetics of the gel formation and the nature of its organization in gelated state. Thus, the presence of different ions in water (pH ≈ 7) led to an increase in the time required for the onset of gelation and resulted in the formation of aggregates of different morphologies. While water could be instantaneously gelled by **1** and SEM studies showed the existence of spongy globular aggregates in the hydrogels of **1** (Figure 11a), the presence of 1 mM $CaCl_2$ led to “fusion” of these globules into “rodlike” fibers (Figure 11b) with gelation starting to occur only after ~15 min. With 1 mM $MgCl_2$ solution, the gel formation by **1** occurred after around 15 min and the resulting gel consisted of fibrillar globules (Figure 11c). In NaCl solution, it took ~7 min for gelation to occur, and the formation of the “aggregated spheres” kind of aggregate was evidenced (Figure 11d). The relevant information summarizing these experiments has been presented in Table 1. The binding of metal cations to the COO^- ions of **1** presumably induces this kind of morphological transition. Thus, the superstructure of the aggregate in gel and the gelation kinetics can be evidently regulated by the choice of metal ions. The presence of even as low as 0.5 mM of $BaCl_2$ in water, however, inhibited the gel formation completely. This led to the precipitation of **1** due to the formation of the Ba^{2+}-salt of **1**.

Conclusions

In conclusion, a low molecular-weight hydrogelator has been synthesized that exhibits pronounced gelation of water. This is the first time such a motif is used for achieving hydrogelation. The versatility of this gelator

---

[18] Koumura, N.; Kudo, M.; Tamaoki, N. *Langmuir* **2004**, *20*, 9897.

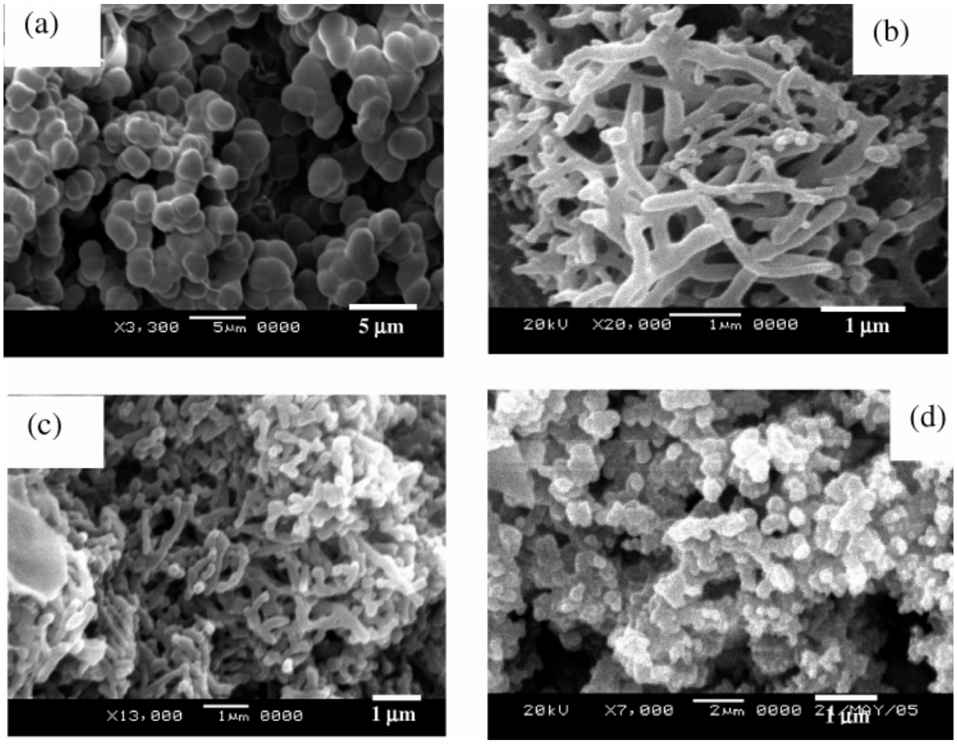


FIGURE 11. SEM of xerogels of **1** in (a) water, (b) 1 mM $CaCl_2$, (c) 1 mM $MgCl_2$, and (d) 1 mM NaCl.

TABLE 1. Effect of Metal Salts on the Hydrogelation by 1[a]

metal salt (concn)	state	gelation time[b]	morphology[c]	T_{gel} (°C)[d]	MGC (μmol)
none	gel	instantaneous	"spongy globules"	65	1
NaCl (1 mM)	gel	7 min	"aggregated spheres"	56	2
KCl (1 mM)	gel	12 min	"wrinkled cloth"	55	2
$MgCl_2$ (1 mM)	gel	15 min	"fused globules"	54	2
$CaCl_2$ (1 mM)	gel	15 min	"fused globules"	57	2
$BaCl_2$ (1 mM)	ppt				

[a] The gelation was performed at 25 °C using 2 μmol of **1**. [b] The time required for complete gelation of the sample. [c] As examined by SEM. [d] At [**1**] = 2 μmol.

is shown by its tolerance to varied pH changes as well as the presence of different salts in the aqueous medium. Gelation was observed between pH 4 and 10 as well as in high salt concentrations. The gelation by **1** was found to be driven by a combination of hydrogen bonding and hydrophobic forces such as $\pi-\pi$ stacking of the azobenzene groups, which arrange in a right-handed helical manner in the aggregate, as indicated by the CD studies. Further studies to utilize such aggregates as scaffolds to encapsulate bioactive macromolecules are in progress.

Experimental Section

General. All reagents were purchased from best-known commercial sources and used without further purification. Silica gel 60−120 mesh size was used for chromatography. FT-IR was performed as KBr pellets of solid **1**, and xerogel of **1**, as well as a neat solution of **1** in MeOH. Melting points are recorded in open capillaries and are uncorrected. UV−vis experiments were performed with the spectrophotometer whose cell holders were connected to a water bath for maintenance of desired temperature. UV irradiation of the gel was done by a UV lamp with emission around 350 nm. pH measurements were done via a precalibrated pH meter.

Methods. Synthesis. See Supporting Information for details.

Determination of pK_a by ^{13}C NMR. 15 mg of **1** was dissolved in 400 μL of perdeuterated dimethyl sulfoxide (d_6-DMSO). The proton-decoupled ^{13}C NMR was recorded on the spectrometer operated at 75 MHz frequency. This gave a carbonyl chemical shift at 168.65 ppm. To this was added 190 μL of 0.2 M NaOH solution in water. The apparent pH of this mixture was measured using a precalibrated Schott Gerate pH meter, and it indicated a pH of 13.5. To this was added incremental amounts of 116 mM HCl aqueous solution until the observed pH was 2.7. The apparent pH as well as the chemical shift of the carbonyl group were noted after each addition. A total of 300 μL of the HCl solution was required to achieve this pH. The result was plotted, and it indicated a bisigmoidal curve. The derivative of the plot gave the apparent pK_a's for the compound.

Gelation of Water. Various tetrameric sugar derivatives having an azobenzene core were studied for their capacity to entrap water. While most of the derivatives were either insoluble in water or precipitated on cooling, **1** showed the ability to entrap water. Gelation was studied by dissolving 1 mg of **1** in a vial using 10 μL of dimethyl sulfoxide (DMSO), and adding a requisite amount of water or aqueous solutions of salts to it. In some cases, rigidification of the resultant mixture into an optically clear gel was instantaneous, while in others the mixture was heated at around 70 °C to get a clear sol that was allowed to cool under ambient conditions.

The rigidification and resistance to flow on inversion of vial by the resulting mixture indicated gelation of the solvent.

Gel Melting Studies. The "melting" temperature of the resultant gel was studied by inverted-tube experiment wherein the vial containing hydrogel was sealed on top and inverted in a temperature-controlled water bath. The temperature of the bath was gradually increased at the rate of 3 °C/min, and temperature range was noted where the gelated mass progressively converted into sol and finally fell down. The experiment was done twice, and it was found that the melting temperature did not vary by $>\pm 1$ °C on repeating the experiment. Similar studies were done for dependence of the gel melting on the concentration of gelator wherein five samples containing the different amounts of gelator were simultaneously put inside the water bath. Gel melting studies were also done in the presence of different salts by using a similar methodology.

UV−Vis Spectrophotometry. The UV−vis spectra for the samples were recorded with water as reference. For temperature dependence studies, the temperature-controlled water bath attached to the instrument was set at required temperatures and the sample was allowed to equilibrate at that temperature for 5 min before the spectra were recorded.

Circular Dichroism. The sample was allowed to equilibrate at the required temperature (65 and 25 °C) for 5 min before the ellipticity was recorded in the wavelength range of 300−450 nm where the gelator was found to have finite absorbance.

Photoirradiation Study. A UV lamp of wavelength ca. 350 nm was employed to irradiate the gel/solution of **1**. The light passed through a water-jacketed cell holder in which a quartz cuvette containing the DMSO solution of **1** or the hydrogel of **1** was kept for the requisite time. The same cuvette was then put in the UV−vis spectrophotometer, and its UV−vis profiles were monitored with respect to the appropriate reference solvent.

Electron Microscopy. For scanning electron microscopy, small samples of the prepared gel were scooped and loaded on brass stubs and allowed to dry in air for about 3 h. These were then coated with gold vapors for ca. 3 min prior to viewing.

Acknowledgment. This work is supported by a grant from the Department of Biotechnology to S.B., and a senior research fellowships of the CSIR, India, to A.S. and S.G.

Supporting Information Available: Detailed experimental procedures and characterization details of compounds **1**, **3**−**7**, and **9**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO050297P