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Intermolecular Interactions of Sulfonated Azo Dyes: Crystal Structures of the Diammonium, Dilithium, Magnesium and Calcium Salts of 7-Hydroxy-8-(phenylazo)-1,3-naphthalenedisulfonic Acid (Orange G)

WILLIAM H. OJALA, LINH KHANH LU, KEVIN E. ALBERS AND WILLIAM B. GLEASON

Department of Laboratory Medicine and Pathology and the Biomedical Engineering Center, University of Minnesota, Minneapolis, MN 55455, USA

TIMOTHY I. RICHARDSON AND REX E. LOVRIEN

Department of Biochemistry, University of Minnesota, St Paul, MN 55108, USA

AND ELISE A. SUDBECK

*Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, USA*

Abstract

The crystal structures of four salts containing the Orange G dianion have been determined, three at low temperature. Diammonium Orange G, $C_{16}H_{10}N_2O_7S_2(NH_4)_2.4H_2O$, forms triclinic crystals, space group $P\bar{1}$ (No. 2), with $a = 9.165$ (1), $b = 10.149$ (4), $c = 12.623$ (1) Å, $\alpha = 87.43$ (2), $\beta = 88.07$ (1), $\gamma = 71.00$ (2)°, $V = 1108.8$ (8) Å^3, $Z = 2$, $T = 173$ K, Cu $K\alpha$, $\lambda = 1.54178$ Å, $M_r = 514.52$, $D_x = 1.541$ g cm^{-3}, $\mu = 27.24$ cm^{-1}, $F(000) = 540$, $R = 0.048$ for 3471 observed reflections. Dilithium Orange G, $C_{16}H_{10}N_2O_7S_2Li_2.8H_2O$, forms triclinic crystals, space group $P\bar{1}$ (No. 2), with $a = 9.589$ (1), $b = 10.495$ (1), $c = 14.208$ (2) Å, $\alpha = 99.61$ (1), $\beta = 100.58$ (1), $\gamma = 115.81$ (1)°, $V = 1215.2$ (8) Å^3, $Z = 2$, $T = 173$ K, Cu $K\alpha$, $\lambda = 1.54178$ Å, $M_r = 564.39$, $D_x = 1.542$ g cm^{-3}, $\mu = 26.25$ cm^{-1}, $F(000) = 588$, $R = 0.030$ for 3862 observed reflections. Magnesium Orange G, $C_{16}H_{10}N_2O_7S_2Mg.8H_2O$, forms triclinic crystals, space group $P\bar{1}$ (No. 2), with $a = 8.844$ (1), $b = 10.179$ (1), $c = 13.901$ (1) Å, $\alpha = 79.18$ (1), $\beta = 79.49$ (1), $\gamma = 79.49$ (1)°, $V = 1194.2$ (5) Å^3, $Z = 2$, $T = 173$ K, Cu $K\alpha$, $\lambda = 1.54178$ Å, $M_r = 574.81$, $D_x = 1.598$ g cm^{-3}, $\mu = 29.46$ cm^{-1}, $F(000) = 600$, $R = 0.041$ for 4148 observed reflections. Calcium Orange G, $C_{16}H_{10}N_2O_7S_2Ca.9H_2O$, forms monoclinic crystals, space group $P2_1/n$ (No. 14), with $a = 9.395$ (4), $b = 27.492$ (9), $c = 10.368$ (2) Å, $\beta = 111.62$ (3)°, $V = 2489$ (3) Å^3, $Z = 4$, $T = 297$ K, Mo $K\alpha$, $\lambda = 0.71073$ Å, $M_r = 608.60$, $D_x = 1.624$ g cm^{-3}, $\mu = 4.82$ cm^{-1}, $F(000) = 1272$, $R = 0.039$ for 3083 observed reflections. Molecular geometry and conformation of the dye molecule are closely similar from structure to structure. In each case, the molecule exists as the hydrazo tautomer rather than as the azo tautomer. A recurring close intermolecular contact is a bridging interaction in which two water

molecules coordinated to a given metal atom are hydrogen bonded to two O atoms of a single sulfonate group.

Introduction

Sulfonated azo dyes are compounds of considerable interest by virtue of their importance and versatility in biomedical applications. For many years these compounds have been known as common and useful biological staining agents, but more recently it has become apparent that some of these dyes also possess important pharmaceutical properties. The sulfonated azo dye Congo Red, for example, binds to a variety of amyloid proteins, including those implicated in such neurodegenerative conditions as scrapie and Alzheimer's disease. This binding interaction with amyloid has made Congo Red a compound of active biomedical interest (Caughey & Race, 1992; Klunk, Pettegrew & Abraham, 1989). Structural studies, such as a recent description of the Congo Red–amyloid porcine insulin complex (Turnell & Finch, 1992), have sought to determine the nature of Congo Red binding as a prerequisite to the design of drugs based on the Congo Red structure and targeted specifically to amyloid deposits. Another sulfonated azo dye, Evans Blue, demonstrates antiviral activity and has been examined as a potential anti-HIV agent (Weaver, Pine, Anand, Bell & Aszalos, 1992). Both Evans Blue and Congo Red, in addition to several other sulfonated azo dyes, have been shown to inhibit HIV-1 protease (Brinkworth & Fairlie, 1992). In addition to possessing these potentially useful pharmaceutical properties, sulfonated azo dyes have recently been used systematically for cocrystallizing and coprecipitating peptides and other biomolecules (Conroy & Lovrien, 1992).
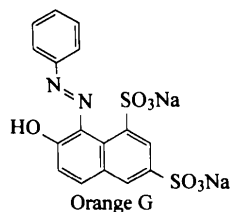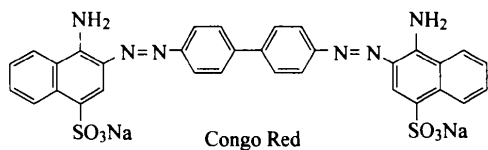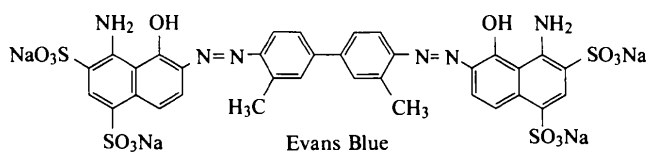
It is ironic that sulfonated azo dyes can be used for the crystallization of other compounds but often prove extremely difficult to crystallize alone. Published examples of their crystal structures with or without cocrystallized molecules are relatively few. These include the structures of Methyl Orange (sodium 4'-dimethylaminoazobenzene-4-sulfonate) as the monohydrate monoethanolate (Hanson, 1973) and as the sodium and potassium salts each complexed with α-cyclodextrin (Harata, 1976). More recently the crystal structure of 3-[(2-arsonophenyl)-azo]-4,5-dihydroxy-2,7-naphthalenedisulfonic acid, a metal complexing agent commonly known as 'Arsenazo I', has been described (Zenki, Shibahara, Yamasaki & Kushi, 1990). In our studies of the biomolecular recognition of sulfated compounds such as heparin by peptides and proteins, we have been investigating the crystal structures of a variety of sulfonated compounds in order to determine the extent to which the sulfonate group can serve as a mimic for the sulfate group and thus help clarify the role played by sulfate groups in molecular recognition. We are also investigating the role of the sulfonate group in its own right, in light of studies showing that certain polymers bearing sulfonate groups can act as heparin-like antithrombogenic materials (Ito, Iguchi, Kashiwagi & Imanishi, 1991; Jozefowicz & Jozefonvicz, 1984). As part of our study of intermolecular interactions in cocrystals of sulfonated azo dyes and biomolecules, we have determined the crystal structures of four salts of the dye that so far has been our most effective cocrystallizing agent. In this paper we present the crystal structures of the diammonium, dilithium, magnesium and calcium salts of 7-hydroxy-8-(phenylazo)-1,3-naphthalenedisulfonic acid, the compound which in the form of its disodium salt is commonly known as 'Orange G'. Of these four salts, three contain metal ions of clear physiological interest and may be considered models for interactions between sulfonates or sulfates and these cations in biological systems. The crystal structure of the diammonium salt may be considered a model for interactions between sulfonate or sulfate groups and the charged N-terminals of peptides or the protonated amino terminals of the side chains of lysine residues. Three of the four structures reported here, those of the diammonium, dilithium and magnesium salts, have been determined at low temperature (173 K).

Evans Blue

Congo Red

Orange G

Experimental

The four salts [designated structures (1), (2), (3) and (4) in Tables 1–7] crystallized from aqueous solutions containing the Orange G disodium salt and excess ammonium sulfate, lithium chloride, magnesium chloride or calcium chloride. Data collection and cell

determination were accomplished using an Enraf–Nonius CAD-4 diffractometer and its associated software. Data reduction and structure refinement were carried out using the *TEXSAN* software package (Molecular Structure Corporation, 1985). Reflections with $I > 3\sigma(I)$ were considered 'observed'. Structure solution was accomplished using *SHELXS86* (Sheldrick, 1985). In all four crystal structures, the non-H atoms were refined anisotropically and the H atoms were refined with fixed thermal parameters (approximately 1.2 times the iso-

tropic equivalents of the atoms to which they were bonded).* The function minimized was $\Sigma w(|F_o| - |F_c|)^2$, where $w = 4F_o^2/\sigma^2(F_o^2)$. In the magnesium salt structure (structure 3), the magnesium cations were located on two different inversion centers, giving a total of one Mg atom per asymmetric unit; positional parameters for the Mg atoms were fixed. For this

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* Lists of structure factors, anisotropic displacement parameters, least-squares planes data and complete geometry have been deposited with the IUCr (Reference: GR0362). Copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Fig. 1. View of the diammonium Orange G asymmetric unit, showing atom numbering. For non-H atoms, 50% probability ellipsoids are shown.

Fig. 3. View of the magnesium Orange G asymmetric unit, showing atom numbering. For non-H atoms, 50% probability ellipsoids are shown. Water molecules marked by an asterisk are symmetry-related to unmarked water molecules through inversion centers occupied by Mg(1) and Mg(2).
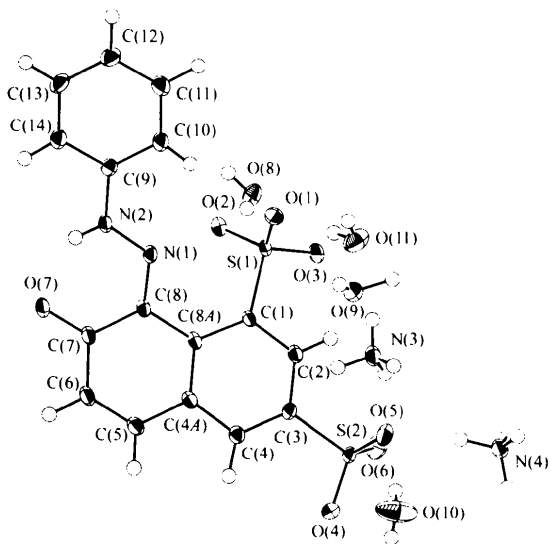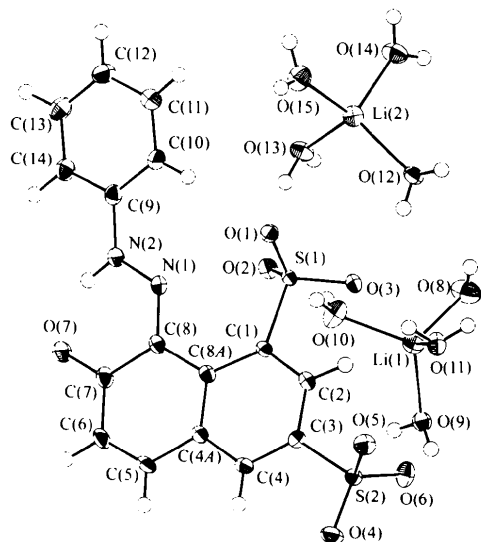

Fig. 2. View of the dilithium Orange G asymmetric unit, showing atom numbering. For non-H atoms, 50% probability ellipsoids are shown.

Fig. 4. View of the calcium Orange G asymmetric unit, showing atom numbering. For non-H atoms, 50% probability ellipsoids are shown.
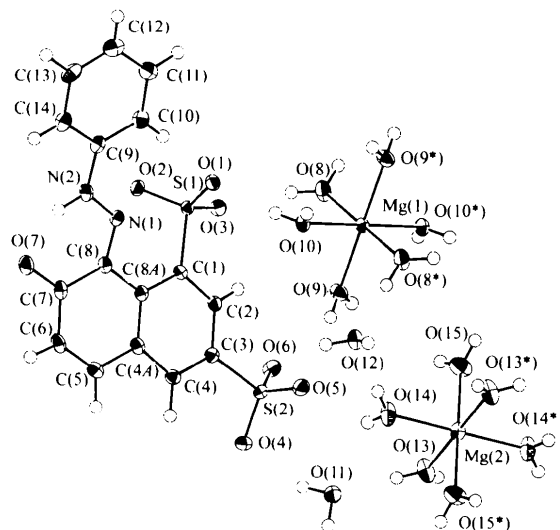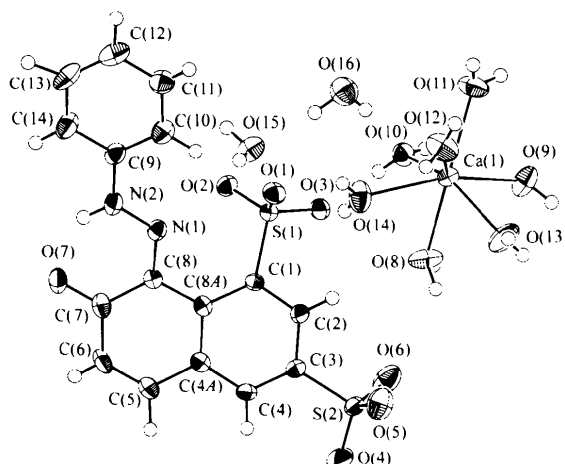
Table 1. *Experimental details for* (1)-(4)

	(1)	(2)	(3)	(4)
Crystal data				
Cation	2 NH$_4^+$	2 Li$^+$	Mg^{2+}	Ca^{2+}
No. of H$_2$O molecules	4	8	8	9
Crystal system	Triclinic	Triclinic	Triclinic	Monoclinic
Space group	$P\bar{1}$	$P\bar{1}$	$P\bar{1}$	$P2_1/n$
a (Å)	9.165 (1)	9.589 (1)	8.844 (1)	9.395 (4)
b (Å)	10.149 (4)	10.495 (1)	10.179 (1)	27.492 (9)
c (Å)	12.623 (1)	14.208 (2)	13.901 (1)	10.368 (2)
α (°)	87.43 (2)	99.61 (1)	79.18 (1)	
β (°)	88.07 (1)	100.58 (1)	79.49 (1)	111.62 (3)
γ (°)	71.00 (2)	115.81 (1)	79.49 (1)	
V (Å^3)	1108.8 (8)	1215.2 (8)	1194.2 (5)	2489 (3)
Z	2	2	2	4
M_r	514.52	564.39	574.81	608.60
D_x (g cm^{-3})	1.541	1.542	1.598	1.624
Dimensions (mm)	0.44 × 0.20 × 0.08	0.40 × 0.10 × 0.10	0.56 × 0.32 × 0.16	0.50 × 0.25 × 0.20
$F(000)$	540	588	600	1272
μ (cm^{-1})	27.24	26.25	29.46	4.82
Data collection				
Radiation	Cu $K\alpha$	Cu $K\alpha$	Cu $K\alpha$	Mo $K\alpha$
Wavelength (Å)	1.54178	1.54178	1.54178	0.71073
Number of cell reflections; 2θ range (°)	25; 40–50	25: 40–50	25: 40–52	25: 32–40
$2\theta_{max}$ (°)	139.9	140.0	140.7	51.9
Scan type	ω-2θ	ω-2θ	ω-2θ	ω-2θ
h	$-11 \rightarrow +11$	$-11 \rightarrow +11$	$-10 \rightarrow +10$	$-11 \rightarrow +11$
k	$-12 \rightarrow +12$	$-12 \rightarrow +12$	$-12 \rightarrow +12$	$0 \rightarrow +33$
l	$-12 \rightarrow +15$	$-14 \rightarrow +17$	$-13 \rightarrow +16$	$-13 \rightarrow +13$
No. of reflections measured	6514	7010	6876	10 160
No. of unique reflections	4180	4443	4377	5099
No. of observed reflections	3471	3862	4148	3083
R_{int}	0.072	0.020	0.025	0.049
Absorption correction	Psi scans	Psi scans	DIFABS (Walker & Stuart, 1983)	Psi scans
Trans. factor	0.77–1.00	0.90 1.00	0.87–1.40	0.94–1.00
Structure refinement				
No. of parameters	376	412	407	418
R	0.048	0.030	0.041	0.039
wR	0.053	0.040	0.059	0.043
$(\Delta/\sigma)_{max}$	0.04	0.00	0.15	0.06
$\Delta\rho$ (e Å^{-3})	-0.82–0.92	-0.46–0.31	-0.53–0.66	-0.27 0.84
S	1.85	1.76	2.87	1.35
Extinction coefficient	—	--	0.11015 × 10^{-4}	—
Decay correction	None	None	4.80% decl.	8.40% decl.

Definitions: $R = \Sigma(|F_{obs}| - |F_{calc}|)/\Sigma|F_{obs}|$; $wR = [\Sigma w(|F_{obs}| - |F_{calc}|)^2/\Sigma w|F_{obs}|^2]^{1/2}$; $R_{int} = \Sigma\Sigma \langle F_i^2 \rangle - F_{ij}^2|/\Sigma m\langle F_i^2 \rangle$; $S = [\Sigma(|F_{obs}| - |F_{calc}|)/\sigma](n - m)^{-1}$.

structure an absorption correction was made using the program *DIFABS* (Walker & Stuart, 1983). Absorption corrections for the other salts were made using the empirical psi-scan technique (North, Phillips & Mathews, 1968). Refinement of a secondary extinction coefficient was considered warranted only in the case of the magnesium salt (Zachariasen, 1963). Drawings were made using *ORTEP*II (Johnson, 1976) and *PLUTO* (Motherwell & Clegg, 1978).

Discussion

Cell data and information relevant to structure solution and refinement are presented in Table 1. Views of the asymmetric units of the structures are given in Figs. 1–4. Fractional atomic coordinates for the four structures are listed in Tables 2–5. Selected bond lengths and angles (including torsional angles) are listed in Tables 6 and 7, respectively. Inspection of the geometrical parameters shows that from crystal structure to crystal structure, corresponding bond lengths and angles in the dye molecules are virtually identical, regardless of the chemical identity of the cation and its degree of hydration. A pairwise geometrical comparison of the dye molecule in the diammonium salt to the dye molecules of the dilithium, magnesium and calcium salts in turn using the program *MacMoMo*II (Dobler, 1993) showed r.m.s. deviations between the two molecules of only 0.087, 0.121 and 0.093 Å, respectively. The deviation between dye molecules in the magnesium and calcium structures (comparing the two structures having divalent cations) was only 0.085 Å. Clearly the varied

Table 2. *Fractional atomic coordinates and equivalent isotropic thermal parameters* (Å^2) *for diammonium Orange G* (1)

$$B_{eq} = (8\pi^2/3)\sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i . \mathbf{a}_j.$$

	x	y	z	B_{eq}
S(1)	0.38722 (6)	0.76055 (6)	0.28882 (4)	1.21 (3)
S(2)	0.95406 (6)	0.38658 (6)	0.31091 (4)	1.31 (3)
O(1)	0.2731 (2)	0.6902 (2)	0.3107 (1)	1.76 (8)
O(2)	0.3274 (2)	0.8913 (2)	0.2260 (1)	1.61 (8)
O(3)	0.4594 (2)	0.7805 (2)	0.3854 (1)	1.92 (9)
O(4)	1.0794 (2)	0.3117 (2)	0.2414 (1)	2.3 (1)
O(5)	0.8916 (2)	0.2916 (2)	0.3751 (1)	1.90 (9)
O(6)	0.9931 (2)	0.4822 (2)	0.3780 (2)	2.4 (1)
O(7)	0.3559 (2)	0.7683 (2)	−0.1530 (1)	1.81 (9)
O(8)	0.3525 (2)	1.1223 (2)	0.3292 (1)	2.1 (1)
O(9)	0.6639 (2)	0.9162 (2)	0.4537 (1)	2.1 (1)
O(10)	1.0263 (3)	0.0022 (3)	0.3278 (3)	4.6 (2)
O(11)	0.3281 (2)	0.4324 (2)	0.4233 (2)	2.9 (1)
N(1)	0.2841 (2)	0.7352 (2)	0.0661 (1)	1.3 (1)
N(2)	0.1702 (2)	0.7912 (2)	0.0022 (1)	1.4 (1)
N(3)	0.6116 (2)	0.2143 (3)	0.3783 (2)	1.9 (1)
N(4)	1.0248 (3)	0.2388 (3)	0.5782 (2)	1.9 (1)
C(1)	0.5422 (2)	0.6454 (2)	0.2130 (2)	1.1 (1)
C(2)	0.6646 (3)	0.5678 (2)	0.2754 (2)	1.4 (1)
C(3)	0.8021 (2)	0.4863 (2)	0.2289 (2)	1.3 (1)
C(4)	0.8214 (3)	0.4872 (2)	0.1201 (2)	1.4 (1)
C(4A)	0.6996 (3)	0.5632 (2)	0.0558 (2)	1.3 (1)
C(5)	0.7258 (3)	0.5648 (3)	−0.0573 (2)	1.5 (1)
C(6)	0.6159 (3)	0.6347 (3)	−0.1254 (2)	1.6 (1)
C(7)	0.4605 (3)	0.7076 (2)	−0.0877 (2)	1.4 (1)
C(8)	0.4284 (3)	0.7016 (2)	0.0260 (2)	1.3 (1)
C(8A)	0.5530 (3)	0.6378 (2)	0.1002 (2)	1.2 (1)
C(9)	0.0155 (3)	0.8206 (2)	0.0376 (2)	1.4 (1)
C(10)	−0.0225 (3)	0.7946 (3)	0.1418 (2)	2.0 (1)
C(11)	−0.1777 (3)	0.8315 (3)	0.1717 (2)	2.3 (1)
C(12)	−0.2924 (3)	0.8928 (3)	0.0982 (2)	1.9 (1)
C(13)	−0.2520 (3)	0.9137 (3)	−0.0061 (2)	2.0 (1)
C(14)	−0.0971 (3)	0.8781 (3)	−0.0370 (2)	1.8 (1)
H(2)	0.653 (3)	0.572 (3)	0.349 (3)	1.6
H(2N)	0.188 (4)	0.803 (3)	−0.058 (3)	1.6
H(3A)	0.525 (4)	0.277 (4)	0.401 (3)	2.1
H(3B)	0.629 (4)	0.128 (4)	0.402 (3)	2.1
H(3C)	0.689 (4)	0.246 (4)	0.397 (3)	2.1
H(3D)	0.612 (4)	0.212 (4)	0.306 (3)	2.1
H(4)	0.919 (4)	0.433 (3)	0.081 (2)	1.6
H(4A)	0.972 (4)	0.278 (4)	0.513 (3)	3.3
H(4B)	0.983 (5)	0.182 (5)	0.610 (3)	3.3
H(4C)	1.132 (5)	0.188 (4)	0.569 (3)	3.3
H(4D)	1.019 (5)	0.320 (5)	0.611 (3)	4.0
H(5)	0.831 (4)	0.507 (3)	−0.086 (2)	1.8
H(6)	0.637 (4)	0.637 (4)	−0.204 (3)	2.0
H(8W)	0.359 (4)	1.048 (4)	0.305 (3)	2.3
H(8A)	0.281 (4)	1.173 (4)	0.301 (3)	2.3
H(9W)	0.618 (4)	0.887 (4)	0.427 (3)	2.5
H(9A)	0.660 (4)	0.906 (4)	0.532 (3)	2.5
H(10)	0.058 (4)	0.754 (4)	0.183 (3)	2.4
H(10W)	1.094 (6)	−0.022 (6)	0.303 (4)	5.2
H(10A)	0.996 (5)	0.091 (5)	0.346 (3)	4.0
H(11W)	0.318 (4)	0.525 (5)	0.396 (3)	3.4
H(11)	−0.209 (4)	0.817 (4)	0.249 (3)	2.8
H(11A)	0.261 (5)	0.431 (5)	0.416 (4)	3.4
H(12)	−0.405 (4)	0.915 (4)	0.121 (3)	2.3
H(13)	−0.332 (4)	0.962 (4)	−0.053 (3)	2.3
H(14)	−0.068 (4)	0.886 (4)	−0.107 (3)	2.1

Table 3. *Fractional atomic coordinates and equivalent isotropic thermal parameters* (Å^2) *for dilithium Orange G* (2)

$$B_{eq} = (8\pi^2/3)\sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i . \mathbf{a}_j.$$

	x	y	z	B_{eq}
S(1)	1.00315 (5)	0.63309 (4)	0.22359 (3)	1.13 (1)
S(2)	0.38441 (5)	0.29603 (4)	0.20603 (3)	1.22 (1)
O(1)	1.0861 (1)	0.5469 (1)	0.21063 (9)	1.56 (3)
O(2)	1.1112 (2)	0.7896 (1)	0.27382 (9)	1.54 (3)
O(3)	0.8952 (2)	0.6128 (1)	0.12786 (9)	1.59 (3)
O(4)	0.2836 (2)	0.2601 (2)	0.2719 (1)	2.09 (4)
O(5)	0.3851 (2)	0.1673 (1)	0.1474 (1)	1.83 (4)
O(6)	0.3500 (2)	0.3782 (1)	0.1403 (1)	2.07 (4)
O(7)	1.2360 (2)	0.8151 (2)	0.63808 (9)	2.06 (4)
O(8)	0.8934 (2)	1.0517 (2)	0.0345 (1)	3.02 (6)
O(9)	0.6761 (2)	1.0054 (2)	0.1782 (1)	1.82 (4)
O(10)	1.0055 (2)	0.9902 (2)	0.2351 (1)	2.45 (4)
O(11)	0.7061 (2)	0.7344 (1)	0.0629 (1)	1.92 (4)
O(12)	1.0459 (2)	0.6631 (1)	−0.0236 (1)	1.66 (4)
O(13)	1.3370 (2)	0.8905 (1)	0.1622 (1)	1.85 (4)
O(14)	1.3810 (2)	0.8275 (2)	−0.0511 (1)	2.22 (4)
O(15)	1.3848 (2)	0.6275 (2)	0.0766 (1)	2.38 (4)
N(1)	1.1949 (2)	0.7032 (2)	0.4335 (1)	1.29 (4)
N(2)	1.3405 (2)	0.7835 (2)	0.4919 (1)	1.54 (4)
C(1)	0.8675 (2)	0.5608 (2)	0.2964 (1)	1.15 (4)
C(2)	0.7092 (2)	0.4700 (2)	0.2393 (1)	1.29 (4)
C(3)	0.5849 (2)	0.4127 (2)	0.2834 (1)	1.26 (4)
C(4)	0.6186 (2)	0.4518 (2)	0.3857 (1)	1.36 (4)
C(4A)	0.7783 (2)	0.5448 (2)	0.4453 (1)	1.30 (5)
C(5)	0.8078 (2)	0.5887 (2)	0.5520 (1)	1.54 (5)
C(6)	0.9561 (2)	0.6797 (2)	0.6150 (1)	1.70 (5)
C(7)	1.0948 (2)	0.7322 (2)	0.5786 (1)	1.58 (5)
C(8)	1.0715 (2)	0.6832 (2)	0.4717 (1)	1.29 (4)
C(8A)	0.9090 (2)	0.5966 (2)	0.4032 (1)	1.20 (4)
C(9)	1.4757 (2)	0.8037 (2)	0.4584 (1)	1.49 (5)
C(10)	1.4612 (2)	0.7376 (2)	0.3609 (1)	1.76 (5)
C(11)	1.6003 (2)	0.7652 (2)	0.3334 (1)	2.04 (5)
C(12)	1.7518 (2)	0.8567 (2)	0.4013 (2)	2.21 (6)
C(13)	1.7648 (2)	0.9203 (2)	0.4984 (2)	2.51 (6)
C(14)	1.6268 (2)	0.8945 (2)	0.5274 (1)	2.07 (5)
Li(1)	0.8208 (4)	0.9469 (3)	0.1307 (2)	1.87 (9)
Li(2)	1.2767 (4)	0.7350 (3)	0.0380 (2)	1.83 (8)
H(2N)	1.349 (3)	0.826 (2)	0.554 (2)	1.9
H(2)	0.679 (3)	0.446 (2)	0.168 (2)	1.5
H(4)	0.536 (3)	0.420 (2)	0.415 (2)	1.6
H(5)	0.716 (3)	0.555 (2)	0.578 (2)	1.9
H(6)	0.973 (3)	0.706 (3)	0.687 (2)	2.0
H(8W)	0.926 (4)	1.024 (4)	0.009 (2)	3.5
H(8A)	0.917 (3)	1.140 (3)	0.036 (2)	3.5
H(9W)	0.589 (3)	0.957 (3)	0.175 (2)	2.2
H(9A)	0.718 (3)	1.061 (3)	0.234 (2)	2.2
H(10W)	1.075 (3)	1.062 (3)	0.249 (2)	3.0
H(10)	1.362 (3)	0.681 (3)	0.317 (2)	2.1
H(10A)	1.034 (3)	0.926 (3)	0.243 (2)	3.0
H(11W)	0.763 (3)	0.697 (3)	0.088 (2)	2.3
H(11)	1.592 (3)	0.715 (3)	0.268 (2)	2.5
H(11A)	0.690 (3)	0.706 (3)	0.000 (2)	2.3
H(12)	1.843 (3)	0.869 (3)	0.380 (2)	2.6
H(12W)	0.985 (3)	0.635 (3)	0.008 (2)	2.0
H(12A)	0.993 (3)	0.596 (3)	−0.080 (2)	2.0
H(13)	1.869 (3)	0.979 (3)	0.547 (2)	3.0
H(13W)	1.288 (3)	0.867 (3)	0.204 (2)	2.3
H(13A)	1.333 (3)	0.966 (3)	0.163 (2)	2.3
H(14)	1.636 (3)	0.940 (3)	0.596 (2)	2.5
H(14W)	1.350 (3)	0.872 (3)	−0.084 (2)	2.7
H(14A)	1.453 (3)	0.822 (3)	−0.074 (2)	2.7
H(15W)	1.365 (3)	0.560 (3)	0.100 (2)	2.9
H(15A)	1.481 (3)	0.652 (3)	0.071 (2)	2.9

crystalline environments found in these four salts have little effect on the geometry of the dye molecule itself.

Naphtholic azo compounds with the hydroxyl group adjacent to the azo linkage have been shown to be capable of existing as the azo compound, the hydrazo compound or as an equilibrium mixture of the two in the solid state as well as in solution (Olivieri, Wilson, Paul & Curtin, 1989). Evidence for this tautomerism can be found in the published crystal structure of Arsenazo I mentioned previously,

Table 4. *Fractional atomic coordinates and equivalent isotropic thermal parameters (Å^2) for magnesium Orange G (3)*

$$B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij}a_i^* a_j^* \mathbf{a}_i.\mathbf{a}_j.$$

|	x	y	z	B_{eq}
S(1)	0.53042 (5)	0.19071 (4)	0.27538 (3)	1.10 (3)
S(2)	0.00603 (5)	0.54971 (4)	0.30859 (3)	1.24 (3)
Mg(1)	½	½	½	1.27 (5)
Mg(2)	0	1.0000	½	1.44 (5)
O(1)	0.6651 (2)	0.2515 (1)	0.2805 (1)	1.46 (7)
O(2)	0.5687 (2)	0.0730 (1)	0.2249 (1)	1.46 (7)
O(3)	0.4418 (2)	0.1604 (1)	0.3743 (1)	1.53 (7)
O(4)	−0.1286 (2)	0.6030 (1)	0.2578 (1)	1.65 (8)
O(5)	0.0763 (2)	0.6569 (1)	0.3299 (1)	1.82 (8)
O(6)	−0.0280 (2)	0.4481 (1)	0.3954 (1)	1.75 (8)
O(7)	0.6270 (2)	0.2535 (2)	−0.1344 (1)	2.00 (8)
O(8)	0.4874 (2)	0.2959 (1)	0.5281 (1)	2.10 (9)
O(9)	0.2846 (2)	0.5335 (2)	0.4575 (1)	1.92 (8)
O(10)	0.6042 (2)	0.4925 (1)	0.3555 (1)	1.54 (8)
O(11)	−0.1977 (2)	0.8798 (2)	0.3109 (1)	2.6 (1)
O(12)	0.4077 (2)	0.8482 (1)	0.2996 (1)	1.87 (8)
O(13)	−0.1973 (2)	0.9293 (2)	0.4944 (1)	2.4 (1)
O(14)	0.0996 (2)	0.9144 (2)	0.3777 (1)	2.5 (1)
O(15)	0.0768 (2)	0.8233 (2)	0.5960 (1)	2.7 (1)
N(1)	0.6776 (2)	0.2547 (2)	0.0617 (1)	1.27 (8)
N(2)	0.7979 (2)	0.2144 (2)	−0.0011 (1)	1.45 (9)
C(1)	0.3991 (2)	0.3143 (2)	0.2076 (1)	1.2 (1)
C(2)	0.2731 (2)	0.3754 (2)	0.2675 (1)	1.3 (1)
C(3)	0.1520 (2)	0.4639 (2)	0.2274 (1)	1.3 (1)
C(4)	0.1515 (2)	0.4874 (2)	0.1268 (1)	1.4 (1)
C(4A)	0.2775 (2)	0.4257 (2)	0.0647 (1)	1.4 (1)
C(5)	0.2689 (3)	0.4466 (2)	−0.0402 (1)	1.6 (1)
C(6)	0.3809 (3)	0.3864 (2)	−0.1044 (1)	1.7 (1)
C(7)	0.5202 (3)	0.3072 (2)	−0.0730 (1)	1.5 (1)
C(8)	0.5396 (2)	0.2956 (2)	0.0305 (1)	1.3 (1)
C(8A)	0.4082 (2)	0.3437 (2)	0.1022 (1)	1.2 (1)
C(9)	0.9479 (2)	0.1777 (2)	0.0250 (1)	1.5 (1)
C(10)	0.9751 (3)	0.1704 (2)	0.1212 (2)	1.8 (1)
C(11)	1.1249 (3)	0.1252 (2)	0.1428 (2)	2.1 (1)
C(12)	1.2470 (3)	0.0895 (2)	0.0687 (2)	2.1 (1)
C(13)	1.2189 (3)	0.1001 (2)	−0.0274 (2)	2.1 (1)
C(14)	1.0695 (3)	0.1446 (2)	−0.0504 (2)	1.8 (1)
H(2)	0.273 (3)	0.355 (3)	0.331 (2)	1.6
H(2N)	0.770 (3)	0.213 (3)	−0.059 (2)	1.8
H(4)	0.060 (3)	0.553 (3)	0.096 (2)	1.6
H(5)	0.179 (3)	0.509 (3)	−0.063 (2)	1.9
H(6)	0.379 (3)	0.394 (3)	−0.171 (2)	2.1
H(8W)	0.518 (3)	0.248 (3)	0.586 (2)	2.5
H(8A)	0.480 (4)	0.246 (3)	0.486 (2)	2.5
H(9W)	0.208 (4)	0.528 (3)	0.501 (2)	2.3
H(9A)	0.251 (3)	0.585 (3)	0.413 (2)	2.3
H(10W)	0.615 (3)	0.420 (3)	0.333 (2)	1.9
H(10)	0.885 (3)	0.190 (3)	0.173 (2)	2.2
H(10A)	0.685 (3)	0.531 (3)	0.331 (2)	1.9
H(11)	1.143 (3)	0.115 (3)	0.212 (2)	2.5
H(11W)	−0.202 (4)	0.801 (3)	0.289 (2)	3.1
H(11A)	−0.266 (4)	0.932 (3)	0.280 (2)	3.1
H(12)	1.348 (4)	0.060 (3)	0.080 (2)	2.6
H(12W)	0.454 (3)	0.921 (3)	0.281 (2)	2.3
H(12A)	0.401 (3)	0.816 (3)	0.251 (2)	2.3
H(13W)	−0.268 (4)	0.911 (3)	0.541 (2)	2.9
H(13)	1.307 (3)	0.070 (3)	−0.080 (2)	2.5
H(13A)	−0.206 (4)	0.905 (3)	0.443 (2)	2.9
H(14)	1.048 (3)	0.147 (3)	−0.114 (2)	2.2
H(14W)	0.059 (4)	0.880 (3)	0.356 (2)	3.0
H(14A)	0.194 (4)	0.892 (3)	0.354 (2)	3.0
H(15W)	0.153 (4)	0.811 (3)	0.626 (2)	3.3
H(15A)	0.050 (4)	0.748 (4)	0.597 (2)	3.3

Table 5. *Fractional atomic coordinates and equivalent isotropic thermal parameters (Å^2) for calcium Orange G (4)*

$$B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij}a_i^* a_j^* \mathbf{a}_i.\mathbf{a}_j.$$

	x	y	z	B_{eq}
Ca(1)	0.32869 (9)	0.28694 (3)	0.02138 (8)	2.16 (3)
S(1)	0.3643 (1)	0.12993 (3)	−0.2521 (1)	1.90 (3)
S(2)	0.9477 (1)	0.13876 (3)	0.0918 (1)	2.06 (3)
O(1)	0.2647 (3)	0.1369 (1)	−0.1762 (3)	2.5 (1)
O(2)	0.2911 (3)	0.1052 (1)	−0.3816 (3)	2.6 (1)
O(3)	0.4318 (3)	0.1760 (1)	−0.2707 (3)	2.5 (1)
O(4)	1.0835 (4)	0.1097 (1)	0.1361 (4)	5.2 (2)
O(5)	0.9126 (4)	0.1577 (1)	0.2038 (3)	4.2 (1)
O(6)	0.9523 (4)	0.1777 (1)	0.0002 (3)	4.4 (1)
O(7)	0.3297 (3)	−0.0722 (1)	−0.2715 (3)	3.3 (1)
O(8)	0.5594 (4)	0.2435 (1)	0.0536 (3)	3.9 (1)
O(9)	0.4701 (4)	0.3539 (1)	−0.0243 (3)	3.8 (1)
O(10)	0.3186 (3)	0.2621 (1)	−0.2028 (3)	2.7 (1)
O(11)	0.1302 (4)	0.3389 (1)	−0.1145 (4)	4.2 (1)
O(12)	0.1540 (4)	0.2857 (1)	0.1410 (4)	4.0 (1)
O(13)	0.4838 (4)	0.3108 (1)	0.2447 (3)	3.8 (1)
O(14)	0.2696 (4)	0.2037 (1)	0.0408 (4)	3.7 (1)
O(15)	0.3284 (4)	0.1442 (1)	−0.6146 (3)	3.6 (1)
O(16)	0.2478 (4)	0.2415 (1)	−0.6021 (4)	4.3 (2)
N(1)	0.2667 (3)	0.0267 (1)	−0.2349 (3)	2.1 (1)
N(2)	0.1514 (4)	−0.0019 (1)	−0.2911 (3)	2.5 (1)
C(1)	0.5256 (4)	0.0942 (1)	−0.1478 (3)	1.7 (1)
C(2)	0.6515 (4)	0.1224 (1)	−0.0745 (4)	2.0 (1)
C(3)	0.7918 (4)	0.1012 (1)	−0.0005 (3)	2.0 (1)
C(4)	0.8086 (4)	0.0515 (1)	−0.0033 (4)	2.0 (1)
C(4A)	0.6832 (4)	0.0223 (1)	−0.0732 (4)	2.0 (1)
C(5)	0.7057 (5)	−0.0296 (1)	−0.0768 (4)	2.6 (1)
C(6)	0.5917 (5)	−0.0600 (1)	−0.1444 (5)	2.8 (1)
C(7)	0.4379 (5)	−0.0432 (1)	−0.2111 (4)	2.4 (1)
C(8)	0.4094 (4)	0.0087 (1)	−0.2022 (4)	2.0 (1)
C(8A)	0.5348 (4)	0.0424 (1)	−0.1426 (3)	1.8 (1)
C(9)	0.0003 (4)	0.0143 (1)	−0.3209 (4)	2.4 (1)
C(10)	−0.0309 (5)	0.0604 (2)	−0.2846 (4)	2.9 (2)
C(11)	−0.1818 (5)	0.0745 (2)	−0.3225 (5)	3.2 (2)
C(12)	−0.3006 (5)	0.0431 (2)	−0.3931 (5)	3.7 (2)
C(13)	−0.2668 (5)	−0.0028 (2)	−0.4265 (5)	4.1 (2)
C(14)	−0.1171 (5)	−0.0173 (2)	−0.3914 (5)	3.4 (2)
H(2)	0.644 (4)	0.155 (2)	−0.077 (4)	2.5
H(2N)	0.168 (5)	−0.031 (2)	−0.309 (4)	3.2
H(4)	0.906 (5)	0.040 (1)	0.048 (4)	2.5
H(5)	0.818 (5)	−0.042 (2)	−0.019 (4)	3.2
H(6)	0.605 (5)	−0.089 (2)	−0.142 (5)	3.3
H(8W)	0.635 (6)	0.241 (2)	0.130 (6)	4.7
H(8A)	0.619 (6)	0.250 (2)	0.005 (5)	4.7
H(9W)	0.552 (6)	0.370 (2)	0.022 (5)	4.4
H(9A)	0.492 (6)	0.354 (2)	−0.088 (6)	4.6
H(10)	0.051 (5)	0.082 (2)	−0.238 (4)	3.6
H(10W)	0.353 (5)	0.236 (2)	−0.221 (5)	3.2
H(10A)	0.347 (5)	0.284 (2)	−0.238 (5)	3.2
H(11W)	0.053 (6)	0.344 (2)	−0.112 (6)	5.0
H(11)	−0.202 (5)	0.101 (2)	−0.302 (5)	3.8
H(11A)	0.144 (6)	0.367 (2)	−0.148 (5)	4.8
H(12W)	0.164 (6)	0.264 (2)	0.203 (5)	4.7
H(12)	−0.405 (6)	0.055 (2)	−0.420 (5)	4.5
H(12A)	0.093 (6)	0.303 (2)	0.136 (5)	4.7
H(13)	−0.342 (6)	−0.031 (2)	−0.470 (5)	5.1
H(13W)	0.460 (6)	0.312 (2)	0.304 (5)	4.4
H(13A)	0.558 (6)	0.322 (2)	0.262 (5)	4.5
H(14)	−0.090 (6)	−0.048 (2)	−0.413 (5)	4.3
H(14W)	0.196 (6)	0.192 (2)	0.050 (5)	4.3
H(14A)	0.309 (6)	0.187 (2)	0.020 (6)	4.3
H(15W)	0.312 (6)	0.129 (2)	−0.548 (5)	4.3
H(15A)	0.264 (6)	0.132 (2)	−0.688 (5)	4.3
H(16W)	0.321 (7)	0.254 (2)	−0.567 (6)	5.1
H(16A)	0.280 (6)	0.210 (2)	−0.598 (5)	5.1

in which the C—O bond to the hydroxyl group adjacent to the azo linkage is significantly shorter [1.263 (12) *versus* 1.350 (13) Å] than the C—O bond to the more remote hydroxyl. Although the chemical structure diagram given in the paper shows the short C—O bond as a single bond to a hydroxyl O atom, these bond lengths suggest instead that this group is

actually a carbonyl and that the molecule exists as the hydrazo tautomer rather than as the azo tautomer. In the four Orange G crystal structures we describe here, the dye molecules assume predominantly the hydrazo form. Bond lengths are consistent with the hydrazo structure, as is the fact that it was possible to locate the H atom on the N atom rather than on the O atom in the difference maps of all four salts. The distance between the protonated N atom and the 'naphthoquinone' O atom is approximately 2.5 Å in all four crystal structures, a short approach consistent with the presence of an intramolecular hydrogen bond. The existence of this bond is clearly one factor that causes the dye molecules in all four structures to assume almost identical conformations at the phenylazo group.
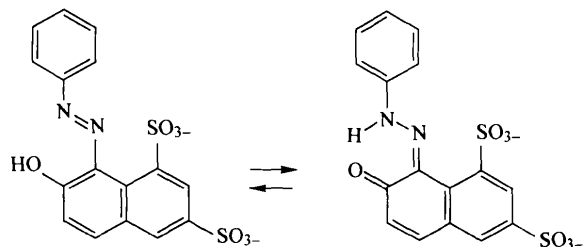
The conformational similarity shown by the dye molecules from structure to structure extends even to the orientations of their sulfonate groups. As indicated by the O(1)—S(1)—C(1)—C(2) torsional angle (Table 7), the rotational orientation of the S(1) sulfonate group varies over a range of no more than 5° from one structure to another. Judging from the O(4)—S(2)—C(3)—C(2) angle, the orientation of the S(2) sulfonate varies by no more than 18°. On the other hand, interesting differences are observed between the two sulfonate groups of individual dye molecules. In each crystal structure, the two sulfonate groups of the dye molecule assume contrasting orientations with respect to the naphthalene ring, the S(1) sulfonate showing a staggered orientation, but the S(2) sulfonate showing an eclipsed orientation. The S(1)—C(1) bond is also significantly longer than the S(2)—C(3) bond in all four structures, perhaps as a result of strain due to its location near the bulky phenylazo substituent on C(8).

The presence of the bulky phenylazo and sulfonate groups on the naphthalene ring causes it to deviate significantly from planarity. The phenylazo group and the S(1) sulfonate group are displaced to opposite sides of the least-squares plane of the naphthalene ring, as are the S(1) sulfonate and S(2) sulfonate. Displacements of C(1) and C(8), the ring atoms bearing the S(1) sulfonate and the phenylazo group, from the ring plane are among the largest (when not *the* largest) shown by the ring C atoms in the four crystal structures.

Table 6. Selected bond lengths (Å) for (1)-(4)

Dye molecule in (1)-(4)	(1)	(2)	(3)	(4)
S(1)—O(1)	1.459 (2)	1.452 (1)	1.457 (1)	1.440 (3)
S(1)—O(2)	1.466 (2)	1.461 (1)	1.457 (1)	1.434 (3)
S(1)—O(3)	1.460 (2)	1.470 (1)	1.462 (1)	1.462 (3)
S(1)—C(1)	1.796 (2)	1.798 (2)	1.800 (2)	1.795 (4)
S(2)—O(4)	1.446 (2)	1.443 (1)	1.462 (2)	1.430 (3)
S(2)—O(5)	1.473 (2)	1.468 (1)	1.449 (1)	1.417 (3)
S(2)—O(6)	1.452 (2)	1.459 (1)	1.459 (1)	1.443 (3)
S(2)—C(3)	1.766 (2)	1.769 (2)	1.770 (2)	1.759 (4)
O(7)—C(7)	1.265 (3)	1.270 (2)	1.269 (3)	1.262 (5)
N(1)—N(2)	1.301 (3)	1.290 (2)	1.303 (2)	1.291 (4)
N(1)—C(8)	1.341 (3)	1.341 (2)	1.339 (3)	1.350 (5)
N(2)—C(9)	1.412 (3)	1.405 (2)	1.405 (3)	1.409 (5)
C(1)—C(2)	1.388 (3)	1.379 (2)	1.387 (3)	1.385 (5)
C(1)—C(8A)	1.428 (3)	1.435 (2)	1.429 (2)	1.427 (5)
C(2)—C(3)	1.390 (3)	1.397 (3)	1.395 (3)	1.386 (5)
C(3)—C(4)	1.379 (3)	1.377 (2)	1.374 (3)	1.376 (5)
C(4)—C(4A)	1.394 (3)	1.401 (2)	1.407 (3)	1.390 (5)
C(4A)—C(5)	1.440 (3)	1.444 (2)	1.447 (3)	1.444 (5)
C(4A)—C(8A)	1.421 (3)	1.420 (2)	1.417 (3)	1.424 (5)
C(5)—C(6)	1.340 (3)	1.342 (3)	1.347 (3)	1.336 (6)
C(6)—C(7)	1.450 (3)	1.431 (3)	1.435 (3)	1.430 (6)
C(7)—C(8)	1.459 (3)	1.461 (2)	1.460 (3)	1.459 (5)
C(8)—C(8A)	1.461 (3)	1.455 (2)	1.462 (3)	1.447 (5)

Metal atom coordination in (2), (3) and (4)

(2)		(3)		(4)	
Li(1)—O(8)	1.935 (4)	Mg(1)—O(8)	2.061 (2)	Ca(1)—O(8)	2.388 (3)
Li(1)—O(9)	1.925 (4)	Mg(1)—O(8)*	2.061 (2)	Ca(1)—O(9)	2.418 (3)
Li(1)—O(10)	1.911 (4)	Mg(1)—O(9)	2.047 (2)	Ca(1)—O(10)	2.390 (3)
Li(1)—O(11)	1.968 (4)	Mg(1)—O(9*)	2.047 (2)	Ca(1)—O(11)	2.360 (3)
Li(2)—O(12)	1.962 (4)	Mg(1)—O(10)	2.060 (1)	Ca(1)—O(12)	2.394 (3)
Li(2)—O(13)	1.989 (3)	Mg(1)—O(10)*	2.060 (1)	Ca(1)—O(13)	2.329 (3)
Li(2)—O(14)	1.890 (4)	Mg(2)—O(13)	2.024 (2)	Ca(1)—O(14)	2.382 (4)
Li(2)—O(15)	1.915 (4)	Mg(2)—O(13)†	2.024 (2)		
		Mg(2)—O(14)	2.038 (2)		
		Mg(2)—O(14)†	2.038 (2)		
		Mg(2)—O(15)	2.112 (2)		
		Mg(2)—O(15)†	2.112 (2)		

* Located at $1 - x$, $1 - y$, $1 - z$.
† Located at $- x$, $2 - y$, $1 - z$.

Selected intermolecular contacts in the four crystal structures are listed in Table 8. Recent studies have found and described preferential patterns of hydrogen bonding and metal ion coordination to sulfate, sulfonate and phosphate groups (Kanyo & Christianson, 1991). In our own investigation we have attempted to identify modes of intermolecular interaction involving the sulfonate group that might resemble the interactions occurring between large sulfated biomolecules (such as heparin) and peptides or proteins capable of recognizing these biomolecules. In this light it is of interest to identify the various types of intermolecular contacts, such as any hydrogen bonds or metal coordination contacts, made by the sulfonate groups in these four salts. The occurrence of a particular type of interaction in several different crystal structures involving these comparatively small dye molecules would suggest that this same interaction could readily occur between biomolecules as well.

Inspection of the intermolecular contacts involving the sulfonate oxygens reveals some general trends.

Table 7. *Selected bond angles and torsional angles* (°) *for* (1)–(4)

Dye molecule in (1)–(4)	(1)	(2)	(3)	(4)
O(1)—S(1)—O(2)	113.5 (1)	114.05 (8)	113.78 (8)	113.0 (2)
O(1)—S(1)—O(3)	111.9 (1)	110.93 (7)	110.26 (8)	111.0 (2)
O(1)—S(1)—C(1)	107.4 (1)	109.01 (7)	108.71 (8)	108.5 (2)
O(2)—S(1)—O(3)	111.5 (1)	110.82 (7)	111.84 (8)	111.7 (2)
O(2)—S(1)—C(1)	107.3 (1)	107.76 (7)	107.11 (8)	108.1 (2)
O(3)—S(1)—C(1)	104.5 (1)	103.68 (7)	104.63 (9)	104.1 (2)
O(4)—S(2)—O(5)	111.9 (1)	113.17 (8)	111.96 (9)	113.0 (2)
O(4)—S(2)—O(6)	113.6 (1)	114.55 (9)	112.67 (9)	112.8 (2)
O(4)—S(2)—C(3)	106.9 (1)	106.32 (8)	108.41 (9)	108.2 (2)
O(5)—S(2)—O(6)	110.7 (1)	109.81 (8)	112.95 (9)	109.8 (2)
O(5)—S(2)—C(3)	105.7 (1)	106.09 (8)	105.04 (9)	105.2 (2)
O(6)—S(2)—C(3)	107.6 (1)	106.25 (8)	105.18 (8)	107.4 (2)
N(2)—N(1)—C(8)	118.2 (2)	118.4 (1)	118.9 (2)	118.7 (3)
N(1)—N(2)—C(9)	121.1 (2)	121.6 (1)	121.9 (2)	121.0 (3)
S(1)—C(1)—C(2)	112.7 (2)	113.1 (1)	113.8 (1)	112.6 (3)
S(1)—C(1)—C(8A)	126.5 (2)	126.2 (1)	126.1 (1)	126.5 (3)
C(2)—C(1)—C(8A)	120.5 (2)	120.5 (2)	119.9 (2)	120.7 (3)
C(1)—C(2)—C(3)	120.6 (2)	121.2 (1)	121.5 (2)	120.9 (3)
S(2)—C(3)—C(2)	119.3 (2)	119.0 (1)	118.2 (1)	119.0 (3)
S(2)—C(3)—C(4)	120.5 (2)	121.0 (1)	121.5 (2)	121.0 (3)
C(2)—C(3)—C(4)	120.2 (2)	119.9 (2)	120.2 (2)	120.0 (3)
C(3)—C(4)—C(4A)	120.1 (2)	120.0 (2)	119.2 (2)	120.3 (3)
C(4)—C(4A)—C(5)	118.4 (2)	118.3 (2)	117.6 (2)	118.8 (3)
C(4)—C(4A)—C(8A)	121.1 (2)	121.6 (1)	122.0 (2)	121.5 (3)
C(5)—C(4A)—C(8A)	120.5 (2)	120.1 (2)	120.3 (2)	119.7 (3)
C(4A)—C(5)—C(6)	122.7 (2)	122.6 (2)	121.6 (2)	122.4 (4)
C(5)—C(6)—C(7)	120.6 (2)	120.6 (2)	121.3 (2)	121.4 (4)
O(7)—C(7)—C(6)	120.2 (2)	120.9 (2)	121.0 (2)	121.4 (4)
O(7)—C(7)—C(8)	121.9 (2)	120.4 (2)	120.7 (2)	121.0 (4)
C(6)—C(7)—C(8)	117.8 (2)	118.6 (2)	118.2 (2)	117.6 (3)
N(1)—C(8)—C(7)	122.1 (2)	122.5 (2)	122.7 (2)	122.1 (3)
N(1)—C(8)—C(8A)	116.5 (2)	117.0 (1)	117.4 (2)	116.7 (3)
C(7)—C(8)—C(8A)	120.7 (2)	120.2 (2)	119.6 (2)	120.9 (3)
C(1)—C(8A)—C(4A)	116.8 (2)	116.5 (2)	116.8 (2)	116.2 (3)
C(1)—C(8A)—C(8)	126.3 (2)	126.1 (2)	125.7 (2)	126.5 (3)
C(4A)—C(8A)—C(8)	116.9 (2)	117.3 (1)	117.5 (2)	117.3 (3)
N(2)—C(9)—C(10)	122.0 (2)	122.4 (2)	122.2 (2)	121.8 (3)
N(2)—C(9)—C(14)	116.6 (2)	116.6 (2)	116.8 (2)	117.4 (4)
C(8)—N(1)—N(2)—C(9)	175.8 (2)	177.0 (1)	176.2 (2)	176.8 (3)
N(1)—N(2)—C(9)—C(10)	1.5 (3)	– 0.7 (3)	5.9 (3)	– 3.7 (5)
N(2)—N(1)—C(8)—C(7)	– 8.7 (3)	– 8.3 (2)	– 5.0 (3)	– 8.3 (5)
O(1)—S(1)—C(1)—C(2)	– 96.5 (2)	– 99.6 (1)	– 101.2 (1)	– 96.5 (3)
O(4)—S(2)—C(3)—C(2)	179.0 (2)	– 177.6 (1)	– 163.4 (1)	– 169.0 (3)

Metal atom coordination in (2), (3) and (4)

(2)		(3)		(4)	
O(8)—Li(1)—O(9)	108.1 (2)	O(8)—Mg(1)—O(9)	88.37 (7)	O(8)—Ca(1)—O(9)	82.7 (1)
O(8)—Li(1)—O(10)	108.8 (2)	O(8)—Mg(1)—O(9)*	91.63 (7)	O(8)—Ca(1)—O(10)	72.7 (1)
O(8)—Li(1)—O(11)	108.1 (2)	O(8)—Mg(1)—O(10)	92.60 (6)	O(8)—Ca(1)—O(11)	149.7 (1)
O(9)—Li(1)—O(10)	112.7 (2)	O(8)—Mg(1)—O(10)*	87.40 (6)	O(8)—Ca(1)—O(12)	132.9 (1)
O(9)—Li(1)—O(11)	110.2 (2)	O(9)—Mg(1)—O(10)	90.43 (6)	O(8)—Ca(1)—O(13)	78.4 (1)
O(10)—Li(1)—O(11)	108.8 (2)	O(9)—Mg(1)—O(10)*	89.57 (6)	O(8)—Ca(1)—O(14)	74.6 (1)
O(12)—Li(2)—O(13)	105.2 (2)	O(13)—Mg(2)—O(14)	87.85 (7)	O(9)—Ca(1)—O(10)	81.7 (1)
O(12)—Li(2)—O(14)	104.8 (2)	O(13)—Mg(2)—O(14)†	92.15 (7)	O(9)—Ca(1)—O(11)	78.0 (1)
O(12)—Li(2)—O(15)	129.3 (2)	O(13)—Mg(2)—O(15)	91.33 (8)	O(9)—Ca(1)—O(12)	130.1 (1)
O(13)—Li(2)—O(14)	107.9 (2)	O(13)—Mg(2)—O(15)†	88.67 (8)	O(9)—Ca(1)—O(13)	78.0 (1)
O(13)—Li(2)—O(15)	104.2 (2)	O(14)—Mg(2)—O(15)	91.89 (8)	O(9)—Ca(1)—O(14)	155.6 (1)
O(14)—Li(2)—O(15)	104.2 (2)	O(14)—Mg(2)—O(15)†	88.11 (8)	O(10)—Ca(1)—O(11)	81.5 (1)
				O(10)—Ca(1)—O(12)	135.2 (1)
				O(10)—Ca(1)—O(13)	146.4 (1)
				O(10)—Ca(1)—O(14)	83.1 (1)
				O(11)—Ca(1)—O(12)	77.1 (1)
				O(11)—Ca(1)—O(13)	119.4 (2)
				O(11)—Ca(1)—O(14)	118.4 (1)
				O(12)—Ca(1)—O(13)	77.7 (1)
				O(12)—Ca(1)—O(14)	73.5 (1)
				O(13)—Ca(1)—O(14)	105.4 (1)

* Located at $1 - x, 1 - y, 1 - z$.
† Located at $-x, 2 - y, 1 - z$.

Table 8. *Selected intermolecular contacts* (Å) *for* (1)–(4)

Diammonium salt

O(1)···O(11')	2.824 (3)	O(5)···N(3')	2.916 (3)
O(1)···N(4'')	2.914 (3)	O(6)···N(4')	2.860 (3)
O(2)···O(8')	2.809 (3)	O(7)···N(3'')	2.853 (3)
O(2)···O(10''')	2.899 (3)	O(8)···O(9''')	2.756 (3)
O(3)···O(9')	2.836 (3)	O(8)···N(3'''')	2.914 (3)
O(4)···O(8'')	2.837 (3)	O(9)···N(4')	2.799 (3)
O(5)···N(4')	2.831 (3)	O(10)···N(4'')	2.839 (4)
O(5)···O(10')	2.873 (4)	O(11)···N(3')	2.871 (3)

Dilithium salt

O(1)···O(12ˣ)	2.811 (2)	O(6)···O(11'')	2.785 (2)
O(2)···O(10')	2.793 (2)	O(6)···O(15''')	2.814 (2)
O(2)···O(13')	2.856 (2)	O(7)···O(9''')	2.691 (2)
O(3)···O(11')	2.750 (2)	O(8)···O(12ˣᵛ)	2.827 (2)
O(3)···O(12')	2.803 (2)	O(8)···O(8ᵛ)	2.939 (3)
O(4)···O(10ˣ)	2.798 (2)	O(9)···O(14ˣᵛ)	2.858 (2)
O(5)···O(13ˣ)	2.787 (2)	O(9)···O(13ˣ)	2.880 (2)
O(5)···O(14ˣ)	2.820 (2)	O(11)···O(15ˣ)	2.841 (2)

Magnesium salt

O(1)···O(10')	2.769 (2)	O(5)···O(14')	2.874 (2)
O(2)···O(11ˣ)	2.846 (2)	O(6)···O(9ᵛ')	2.768 (2)
O(2)···O(12ˣᵛ)	2.846 (2)	O(6)···O(15ˣᵛ)	2.851 (2)
O(3)···O(13ˣᵛ)	2.737 (2)	O(7)···O(12ˣ)	2.777 (2)
O(3)···O(8')	2.873 (2)	O(8)···O(12'')	2.783 (2)
O(4)···O(10ˣᵛ)	2.814 (2)	O(11)···O(13')	2.692 (2)
O(4)···O(11')	2.974 (2)	O(12)···O(14')	2.753 (2)
O(5)···O(9')	2.761 (2)		

Calcium salt

O(1)···O(13ˣᵛ'')	2.849 (4)	O(6)···O(16ˣˣ)	2.871 (5)
O(1)···O(14')	2.891 (4)	O(6)···O(14ˣ')	2.941 (5)
O(2)···O(15')	2.781 (4)	O(7)···O(11ˣ')	2.800 (4)
O(3)···O(12ˣᵛ'')	2.777 (4)	O(8)···O(10ˣˣ)	2.793 (4)
O(3)···O(10')	2.789 (4)	O(8)···O(16ˣˣ)	2.830 (5)
O(4)···O(15ˣˣ)	2.915 (5)	O(11)···O(15ˣˣ'')	2.873 (5)
O(5)···O(10ˣˣ)	2.686 (5)	O(12)···O(16ˣˣᵛ)	2.759 (5)
O(5)···O(9ˣˣ)	2.688 (5)	O(15)···O(16')	2.796 (5)
O(6)···O(13ˣᵛ'')	2.790 (5)		

Symmetry codes: (i) x, y, z; (ii) $1 - x$, $1 - y$, $1 - z$; (iii) $-1 + x$, $1 + y$, z; (iv) $1 + x$, $-1 + y$, z; (v) $2 - x$, $1 - y$, $1 - z$; (vi) $1 - x$, $1 - y$, $-z$; (vii) $1 - x$, $2 - y$, $1 - z$; (viii) x, $1 + y$, z; (ix) $2 - x$, $-y$, $1 - z$; (x) $2 - x$, $1 - y$, $-z$; (xi) $-1 + x$, $-1 + y$, z; (xii) $-1 + x$, y, z; (xiii) $2 - x$, $2 - y$, $1 - z$; (xiv) $2 - x$, $2 - y$, $-z$; (xv) x, $-1 + y$, z; (xvi) $-x$, $1 - y$, $1 - z$; (xvii) $-\frac{1}{2} + x$, $\frac{1}{2} - y$, $-\frac{1}{2} + z$; (xviii) $\frac{1}{2} + x$, $\frac{1}{2} - y$, $-\frac{1}{2} + z$; (xix) $1 + x$, y, $1 + z$; (xx) $\frac{1}{2} + x$, $\frac{1}{2} - y$, $\frac{1}{2} + z$; (xxi) $1 + x$, y, z; (xxii) $\frac{1}{2} - x$, $-\frac{1}{2} + y$, $-\frac{1}{2} - z$; (xxiii) $-\frac{1}{2} + x$, $\frac{1}{2} - y$, $\frac{1}{2} + z$; (xxiv) x, y, $1 + z$.

Most of the sulfonate oxygens in all four crystal structures participate in at least two close hydrogen bonding contacts (< 3.0 Å between the O atom and the hydrogen-bond donor atom). The sulfonate O(4) atom, perhaps rendered less accessible by its location in the eclipsed position in all four crystal structures, is relatively isolated in the sense that in each of the diammonium, dilithium and calcium structures it participates in only one close hydrogen-bonding contact. In the diammonium salt the close intermolecular contacts involving sulfonate oxygens are to water molecules and also to ammonium ions; in the three metal salts (lithium, magnesium and calcium), the contacts are exclusively to water molecules. It is striking that in none of these structures is a sulfonate

O atom coordinated directly to a metal ion. In other words, all contacts between the sulfonate groups and the metal atoms are water-mediated.

A noteworthy intermolecular interaction found in all three of the metal salt structures is one in which two water molecules coordinated to the same metal ion are held in an orientation such that each water O atom comes into close contact with a different sulfonate O atom on a given sulfonate group. We consider this sulfonate-bridging interaction to be particularly interesting because a similar interaction could be expected to occur at molecular binding sites where large biomolecules recognize each other; for example, the guanidinium side-chain terminus of an arginine residue in a peptide might well bridge two O atoms of a sulfate group in heparin in this fashion. The plentiful examples of this bridging interaction we have found in the crystal structures of these Orange G metal salts indicate that this interaction is significant for sulfonate groups and, therefore, probably also significant for the sulfate groups of biomolecules.

In the lithium structure, O(2) and O(3) of the S(1) sulfonate group are bridged by the O(10)—Li(1)—O(11) group [O(2)—O(10) distance = 2.793 (2), O(3)—O(11) distance = 2.750 (2) Å]. The approach to the sulfonate by these coordinated water molecules is 'edge-on' in the sense that the water O atoms lie approximately in the plane of the three sulfonate O atoms. The displacements of O(10) and O(11) from the O(1)/O(2)/O(3) least-squares plane are only 0.31 and −0.06 Å, respectively, and the angle between the O(1)/O(2)/O(3) and O(10)/Li(1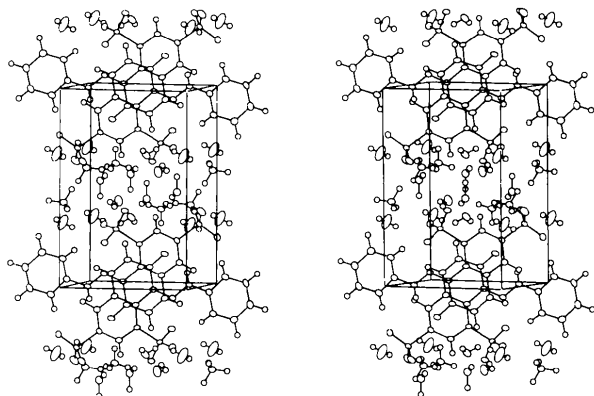)/O(11) least-squares planes is 167.5°. A second bridging interaction is found at this same pair of sulfonate oxygens, where O(2) and O(3) are also bridged by the O(12)/Li(2)/O(13) group [O(2)—O(13) distance = 2.856 (2), O(3)—O(12) distance = 2.803 (2) Å]. In this case there is considerable deviation from an edge-on approach. O(12) and O(13) lie 2.73 and 2.83 Å from the O(1)/O(2)/O(3) least-squares plane, and the angle between the O(1)/O(2)/O(3) and O(12)/Li(2)/O(13) planes is 141.0°. Both these bridging interactions can be seen in Fig. 2.

Two bridging interactions are also found in the magnesium structure. One (visible in Fig. 3) occurs between the O(8)—Mg(1)—O(10) group and two O atoms, O(1) and O(3), from the S(1) sulfonate group [O(1)—O(10) distance = 2.769 (2), O(3)—O(8) distance = 2.873 (2) Å]. This bridging contact deviates from a purely edge-on approach, with O(8) and O(10) lying 0.68 and −0.98 Å away from the O(1)/O(2)/O(3) least-squares plane and the O(1)/O(2)/O(3) and O(8)/Mg(1)/O(10) planes intersecting at an angle of 142.6°, an angle close to that found for the nonedgewise contact in the lithium structure. Perhaps in the magnesium structure the deviation from

a purely edge-on approach facilitates tridentate binding of the hydrated cation to the dye molecule; a third O atom, O(5) from the dye molecule's other sulfonate group, also makes contact with the Mg atom's hydration shell [O(5)—O(9) distance = 2.761 (2) Å]. The two-point bridging contact from the first sulfonate plus the single-point contact from the second sulfonate thus amounts to a three-point contact to the hydrated cation. The second bridging interaction (not shown in Fig. 3) is found at the S(2) sulfonate group, where O(4) and O(6) are bridged by the O(9)—Mg(1)—O(10) group of a hydrated magnesium cation related by symmetry to that bridging the S(1) sulfonate [O(4)—O(10^i) distance = 2.814 (2), O(6)—O(9^{ii}) distance = 2.768 (2) Å; symmetry codes: (i) $-1 + x$, y, z; (ii) $-x$, $1 - y$, $1 - z$]. This bridging interaction is approximately 'face-on', with the water molecules approaching the sulfonate group from a direction roughly perpendicular to the plane of the sulfonate O atoms. The deviations of the O(9) and O(10) atoms (of the symmetry-related hydrated cation) from the O(4)/O(5)/O(6) least-squares plane are 2.49 and 1.48 Å, respectively, and the angle between the O(4)/O(5)/O(6) and O(9)/Mg(1)/O(10) least-squares planes is 113.0°.

A bridging interaction is also found in the calcium structure and can be seen in Fig. 4. It is an approximately edge-on approach by the O(10)—Ca(1)—O(14) group to O(1) and O(3) of the S(1) sulfonate group [O(1)—O(14) distance = 2.891 (4), O(3)—O(10) distance = 2.789 (4) Å]. The deviations of O(10) and O(14) from the O(1)/O(2)/O(3) plane are 1.78 and 0.35 Å, respectively, and the angle between the O(1)/O(2)/O(3) and O(10)/Ca(1)/O(14) least-squares planes is 29.6°.

Molecular packing in the four crystal structures is shown in Figs. 5–8. In all four salts the inversion-related stacking of dye molecules defines hydrophobic layers in the crystal structure. Surrounding and connecting the hydrophobic dye-containing regions are hydrophilic regions composed of water molecules and cations. The charged sulfonate groups

Fig. 7. Stereoview of the magnesium Orange G molecular packing. H atoms have been omitted for clarity.

Fig. 5. Stereoview of the diammonium Orange G molecular packing.
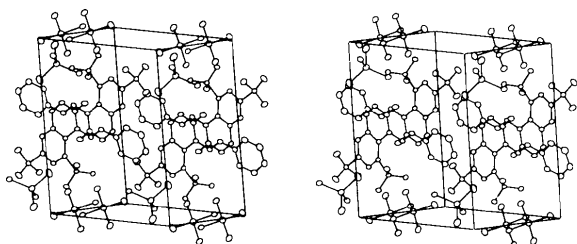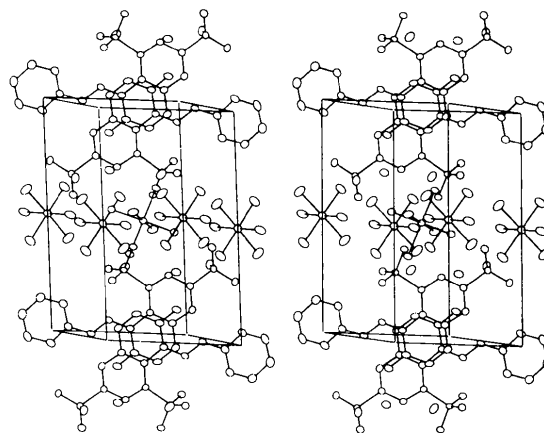

Fig. 6. Stereoview of the dilithium Orange G molecular packing. H atoms have been omitted for clarity.
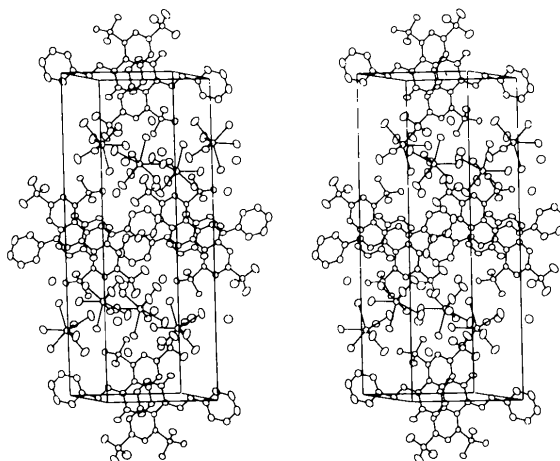

Fig. 8. Stereoview of the calcium Orange G molecular packing. H atoms have been omitted for clarity.

of the dye molecules project into these regions. While similar in these general aspects, the crystal structures assumed by the four salts are nonetheless clearly different. It is likely that the structural change brought about by the exchange of one otherwise rather similar cation for another (for example, Ca^{2+} for Mg^{2+}) is greatly amplified by the dramatic differences between these cations in their degree and geometry of hydration. If it is primarily the different hydration patterns assumed by the lithium, magnesium and calcium cations in these salts that cause the crystal structures to differ, it is reasonable to conclude that these varied hydration patterns also facilitate the recognition and differentiation of these cations by biomolecules. The number of previously described organic crystal structures containing these differently hydrated cations is not large. A search of the Cambridge Structural Database (CSD, 1993) located five structures containing the tetra-aqualithium cation, 39 structures containing the hexaaquamagnesium cation and only one structure containing the heptaaquacalcium cation. It is interesting that this lone compound containing the hepta-hydrated calcium cation is, like Orange G, a sulfonated naphthalene derivative.

We are continuing to investigate the structures and packing arrangements of sulfonated azo dyes, crystallized independently and also cocrystallized with biomolecules such as amino acids and nucleotide bases. We expect the results to be useful to protein crystallographers, cell biologists and medicinal chemists working in the area of biomolecular recognition, especially that involving the sulfonate and sulfate groups.

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