Articles

Growth of High-Quality Crystals Containing Peptides: Arenesulfonate Salts of L-Alanyl-L-Alanine, Glycylglycine, and L-Leucyl-L-Alanine

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X-ray quality crystals of peptides are often difficult to grow, especially for some small peptides. Common crystal growth methods such as slow evaporation or liquid diffusion are not always adequate for the growth of many peptide crystals. We have found that in some cases X-ray quality crystals containing peptides can be obtained by salt formation with arenesulfonic acids. We present here single crystal X-ray diffraction studies of arenesulfonate salts of four dipeptides: (1) 2:1 L-alanyl-L-alanine 1,5-naphthalenedisulfonate; (2) 1:1 glycylglycine 2,4-dinitrobenzenesulfonate hydrate; (3) 2:1 glycylglycine 2,6-naphthalenedisulfonate hydrate; (4) 2:1 L-leucyl-L-alanine 1,5-naphthalenedisulfonate. Molecular packing patterns and hydrogen-bonding interactions of each complex are discussed. The shortest hydrogen bonds observed in these complexes include the following interactions: acid proton/sulfonate oxygen, acid proton/water, amino proton/acid carbonyl, and amino proton/sulfonate oxygen. Weak interactions also form between amide protons and sulfonate oxygen atoms. The two glycylglycine hydrate structures show strong hydrogen bonds between the acid protons and water which in turn forms hydrogen bonds with sulfonate oxygens. The acid protons in the nonhydrated structures hydrogen-bond directly to the sulfonate oxygens. One glycylglycine salt (2) shows an unusual antiperiplanar carboxylic acid conformation, probably due to strong hydrogen-bonding between the acid proton and water.

Introduction

Interactions of biologically important molecules such as peptides and proteins are strongly influenced by factors such as molecular conformation and hydrogen-bonding ability. The analysis of such hydrogen bonding interactions is assuming an increasingly important role in the study of structure–function relationships in biochemical processes. X-ray crystallography provides a powerful means for the analysis of peptides and proteins at the atomic level of resolution and provides models for theoretical calculation of minimum energy conformations.

A significant obstacle to the determination of molecular structure by X-ray methods is the frequent difficulty in growing high-quality single crystals. Various crystal growth methods such as liquid diffusion, vapor diffusion, temperature gradient, and hanging drop methods have been used to facilitate the growth of X-ray quality crystals. Other strategies include the formation of cocrystals or salts. Each method has its associated problems. For example, cocrystals of amino acids and peptides containing neutral molecules are difficult to grow due to the strong self-association between zwitterionic molecules. As a result, few examples of amino acid cocrystals can be found in the literature.[1] The formation of amino acid salts is more common than cocrystal formation. Fischer and Bergell[2] first suggested the formation of amino acid sulfonate salts in 1902 as a method for preparing salts that are slightly soluble in water. Salt formation was subsequently used for the separation of amino acids from mixtures. Crosby and Kirk[3] characterized several amino acid sulfonates by optical microscopy. The appearance and properties of each salt were used to identify amino acid salts separated from mixtures. Stein, Moore, and

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(1) Four cocrystals of unprotected zwitterionic amino acids were found in the Cambridge Structural Database (Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, England, Version 3.4 [Vax]). An additional Speakman salt of phenylalanine has recently been published. These five amino acid cocrystals are listed as follows: (a) L-glutamic acid L-pyroglutamic acid hydrate (Taira, Z.; Watson, W. H. *Acta Crystallogr.* **1977**, *B33*, 3823); (b) glycine trimesic acid hydrate (Herbstein, F. H.; Kapon, M.; Maoir, I.; Reisner, G. M. *Acta Crystallogr.* **1981**, *B37*, 136); (c) L-serine L-ascorbic acid (Sudhakar, V.; Bhat, T. N.; Vijayan, M. *Acta Crystallogr.* **1980**, *B36*, 125); (d) L-threonine L-allothreonine (Swaminathan, P.; Srinivasan, R. *J. Cryst. Mol. Struct.* **1975**, *5*, 101); (e) L-phenylalanine L-phenylalaninium formate (Görbitz, C. H.; Etter, M. C. *Acta Crystallogr.* **1992**, *C48*, 1317).

(2) Fischer, E.; Bergell, P. *Ber. Dtsch. Chem. Ges.* **1902**, *35*, 3779.

(3) Crosby, B. L.; Kirk, P. L. *Mikrochemie* **1935**, *18*, 137.

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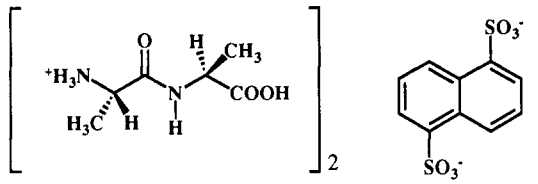
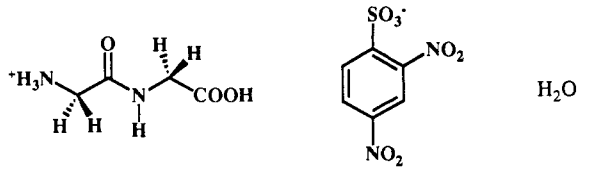
Bergman[4] used sulfonate salt formation to separate and identify amino acids isolated from protein hydrosylates and reported the solubility constants of several salts. The X-ray crystal structures of two dipeptide sulfonates were reported in the early 1970s as part of a study to compare conformations of zwitterionic peptides to those of salts.[5] In more recent years the formation of sulfonate salts of amino acids and dipeptides has been used to study conglomerate crystal formation from racemic mixtures[6] as well as to determine conformations of linear peptides.[7] Sulfonate dyes have been used as cocrystallizing agents to purify amino acids and peptides and to form brightly-colored X-ray quality salt crystals.[8] These studies suggest that the formation of sulfonate salts of peptides is a promising method for the reliable crystallization of some peptides which are otherwise difficult to grow. We are interested in studying peptide/sulfonate interactions further, as a crystal growth method in itself and also because such interactions are of interest in molecular recognition studies of biomolecules.

Our objective is to systematically analyze conformations, molecular packing patterns, and hydrogen-bond interactions in peptide sulfonate salts. We present here the crystal structures of four dipeptide arenesulfonate salts: 2:1 L-alanyl-L-alanine 1,5-napthalenedisulfonate (**1**), 1:1 glycylglycine 2,4-dinitrobenzenesulfonate hydrate (**2**), 2:1 glycylglycine 2,6-naphthalenedisulfonate hydrate (**3**), and 2:1 L-leucyl-L-alanine 1,5-naphthalenedisulfonate (**4**). X-ray crystal structures of these complexes will be discussed with emphasis on hydrogen bond interactions.



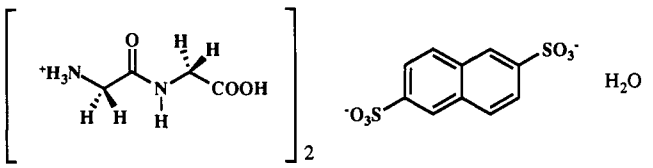
L-alanyl-L-alanine 1,5-napthalenedisulfonate

(1)



glycylglycine 2,4-dinitrobenzenesulfonate hydrate

(2)

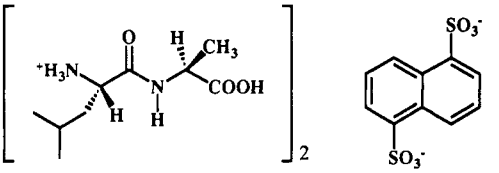


glycylglycine 2,6-naphthalenedisulfonate hydrate

(3)

Experimental Section

Several different alkane- and arenesulfonic acids were used in crystallization experiments with various dipeptides. Most



L-leucyl-L-alanine 1,5-naphthalenedisulfonate

(4)

experiments yielded only starting material or low quality crystals inappropriate for X-ray data collection. A common factor in successful crystal growth experiments was the presence of arenesulfonates. A specific arenesulfonate, 1,5-naphthalenedisulfonate, appeared to form salts with dipeptides slightly more often than other arenesulfonates, although powder was sometimes obtained rather than X-ray quality crystals (as was the case with glycylglycine 1,5-naphthalenedisulfonate, for example). Crystals of **1**, **2**, and **4** were grown at room temperature by slow evaporation from aqueous solutions containing stoichiometric amounts of peptide and sulfonic acid. Crystals of **3** were obtained by combining the peptide and the sodium sulfonate salt in 1 M HCl solution. Melting points for each complex were obtained using a Fisher-Johns melting point apparatus and are listed as follows: **1**, 225 °C dec; **2**, 170–179 °C; **3**, 243 °C dec; **4**, 170 °C dec. X-ray single crystal diffraction data were collected using an Enraf-Nonius CAD4 diffractometer (Mo Kα radiation, λ = 0.710 73 Å, graphite monochromator). Crystallographic data for each salt are listed in Table 1. Data were collected by ω–2θ scan mode up to 60° in 2θ and Lorentz–polarization corrections were applied to all data. The structures were solved by direct methods using *MITHRIL*[9] and *DIRDIF*.[10] All atomic scattering factors were from ref 11. All non-hydrogen atoms were refined anisotropically. Atom positions and isotropic thermal parameters for hydrogens involved in hydrogen bonding (amino, amide, acid, and water protons) were refined (except for one acid proton and one amine proton in **1** and the amine protons in **4**, which could not be located in the difference map), and other hydrogen atoms were included in the structure factor calculations at idealized positions (d_{C-H} or d_{N-H} = 0.95 Å). Empirical absorption correction (using *DIFABS*[12]) was applied to data for **1**, **3**, and **4** before final refinement. Refinement of the structures using reflections having $I > 2\sigma(I)$ converged at R values listed in Table 1. Fractional coordinates and isotropic displacement parameters (B(eq)) are listed for **1**–**4** in Tables 2–5.

Solid-state infrared spectra of each complex and starting materials were obtained using a Nicolet 510M FTIR. Salt formation was indicated in the IR spectrum by the appearance of a new carbonyl peak at approximately 1730 cm^{-1} due to proton transfer from the sulfonic acid to the carboxylate group of the zwitterionic peptide. For example, formation of salt **3** is indicated in Figure 1b with the characteristic appearance of a peak at 1737 cm^{-1} in the carbonyl region. ^{1}H NMR spectra of starting materials and salts were taken in DMSO-d_6 using an IBM/Bruker NR200AF spectrometer. Stoichiometric ratios of the two components of each salt were calculated from the NMR spectra and were in agreement with expected values, indicating salt formation rather than a random conglomeration of starting materials.

Results and Discussion

(1) 2:1 L-Alanyl-L-alanine 1,5-Naphthalenedisulfonate. Colorless thick needles of **1** were grown from

(4) (a) Bergmann, M.; Stein, W. H. *J. Biol. Chem.* **1939**, *129*, 609. (b) Stein, W. H.; Moore, S.; Bergman, M. *J. Biol. Chem.* **1944**, *154*, 191.
(5) van der Veen, J. M.; Low, B. W. *Acta Crystallogr.* **1972**, *B28*, 3548.
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(8) Conroy, M. J.; Lovrien, R. E. *J. Cryst. Growth* **1992**, *122*, 213.
(9) Gilmore, C. J. *J. Appl. Crystallogr.* **1984**, *17*, 42.
(10) Beurskens, P. T. *DIRDIF: Direct Methods for Difference Structures—An Automatic Procedure for Phase Extension and Refinement of Difference Structure Factors*; Technical Report 1984/1; Crystallography Laboratory: Toernooiveld, Nijmegen, The Netherlands, 1984.
(11) Cromer, D. T.; Waber, J. T. *International Tables for X-Ray Crystallography*; The Kynoch Press: Birmingham, England, 1974; Vol. IV, Table 2.2A.
(12) Walker, N.; Stuart, D. *Acta Crystallogr.* **1983**, *A39*, 158.

Table 1. Crystallographic Data for Salts 1–4

	1	2	3	4
cryst appearance	colorless needles	lt yellow prisms	colorless prisms	tan prisms
cryst size (mm^3)	0.60 × 0.30 × 0.20	0.47 × 0.35 × 0.23	0.60 × 0.40 × 0.27	0.55 × 0.46 × 0.20
empirical formula	$C_{22}H_{31}N_4O_{12}S_2$	$C_{10}H_{14}N_4O_{11}S$	$C_{18}H_{28}N_4O_{14}S_2$	$C_{28}H_{44}N_4O_{12}S_2$
radiation type (λ = 0.710 73 Å)	Mo Kα	Mo Kα	Mo Kα	Mo Kα
temp (°C)	24	24	24	24
a (Å)	5.664(6)	7.379(2)	10.348(4)	7.510(2)
b (Å)	10.376(3)	8.028(4)	9.368(2)	21.267(6)
c (Å)	11.970(3)	15.508(2)	12.92(1)	21.328(6)
α (deg)	91.36(2)	76.62(2)	90	90
β (deg)	92.92(5)	76.40(2)	99.56(5)	90
γ (deg)	99.27(5)	63.47(3)	90	90
space group	$P1$	$P\bar{1}$	$P2_1/n$	$C222_1$
Z	1	2	2	4
vol (Å^3)	693(1)	790(1)	1235(2)	3406(3)
d_{calc} (g/cm^3)	1.456	1.673	1.583	1.351
no. of measd reflns	8034	5869	6949	5135
no. of unique reflns	4017	4612	3800	4633
no. of reflns used [$I > 2\sigma(I)$]	6244	3424	2618	1561
R	0.053	0.059	0.042	0.048
R_w	0.059	0.069	0.051	0.044
goodness of fit	1.27	1.63	1.24	1.30
θ_{max} (deg)	30	30	30	28
range of h, k, l	$-7 \le h \le 7$	$-10 \le h \le 2$	$-14 \le h \le 14$	$-9 \le h \le 0$
	$-14 \le k \le 14$	$-11 \le k \le 11$	$0 \le k \le 13$	$0 \le k \le 28$
	$-16 \le l \le 16$	$-21 \le l \le 21$	$-18 \le l \le 18$	$0 \le l \le 28$
final Δ electron density (e$^-$/Å^3)	0.4	0.6	0.3	0.3
μ (cm^{-1})	2.48	2.62	2.81	2.10
(shift/error)$_{max}$	0.04	0.02	0.01	0.03

Table 2. Fractional Coordinates and Isotropic Displacement Parameters [B(eq)] for L-Alanyl-L-alanine 1,5-Naphthalenedisulfonate (1) at 24 °C

atom	x	y	z	B(eq) (Å^2)	atom	x	y	z	B(eq) (Å^2)
S1	0.3958	0.5072	0.2151	2.40(3)	H1O	0.713(8)	−0.249(4)	1.267(4)	4(1)
S2	0.6042(2)	0.49276(9)	0.78496(7)	2.34(3)	H2N	1.118(8)	0.319(4)	1.061(4)	4(1)
O1	0.3747(5)	0.3671(3)	0.1970(2)	3.4(1)	H3N	1.0533	0.2259	0.9708	3.4
O2	0.6285(5)	0.5767(3)	0.1900(2)	3.3(1)	H4N	0.550(6)	0.168(3)	1.224(3)	1.8(7)
O3	0.2025(5)	0.5570(3)	0.1553(2)	3.6(1)	H20	0.4523	0.1532	1.0225	4.8
O4	0.6119(5)	0.6330(3)	0.8040(2)	3.0(1)	H21	0.5600	0.2385	0.9271	4.8
O5	0.3731(5)	0.4198(3)	0.8089(2)	3.3(1)	H22	0.6507	0.1086	0.9533	4.8
O6	0.8027(5)	0.4453(3)	0.8412(2)	3.4(1)	H23	0.7262	0.3272	1.0988	3.3
C1	0.6315(6)	0.4663(4)	0.6394(3)	2.2(1)	H24	0.7996	0.0451	1.3567	3.5
C2	0.7954(7)	0.3903(4)	0.6101(3)	2.9(2)	H26	0.2986	0.0104	1.3287	5.3
C3	0.8222(8)	0.3615(4)	0.4955(4)	3.3(2)	H27	0.4453	0.1041	1.4191	5.3
C4	0.6871(7)	0.4095(4)	0.4148(3)	2.8(1)	H28	0.4360	−0.0456	1.4268	5.3
C5	0.5143(6)	0.4881(4)	0.4419(3)	2.0(1)	O33	0.4100(5)	0.8998(3)	−0.1739(2)	3.4(1)
C6	0.3676(7)	0.5390(4)	0.3598(3)	2.3(1)	O36	0.2388	1.1920	−0.3085	4.3(1)
C7	0.2083(7)	0.6196(4)	0.3892(3)	2.9(2)	O37	0.0708(5)	1.1094(3)	−0.1554(2)	3.8(1)
C8	0.1901(8)	0.6525(4)	0.5029(3)	3.2(2)	N30	0.3510(6)	0.7139(3)	−0.0232(2)	2.7(1)
C9	0.3203(7)	0.6034(4)	0.5837(3)	3.0(2)	N33	0.0298(6)	0.8597(3)	−0.2443(2)	3.0(1)
C10	0.4876(6)	0.5202(4)	0.5571(3)	2.3(1)	C30	−0.0095(8)	0.8095(5)	0.0057(3)	4.1(2)
H2	0.8911	0.3567	0.6663	3.5	C31	0.1325(6)	0.7486(4)	−0.0787(3)	2.5(1)
H3	0.9355	0.3081	0.4752	4.0	C32	0.2072(7)	0.8423(4)	−0.1712(3)	2.4(1)
H4	0.7091	0.3901	0.3385	3.3	C34	0.0603(7)	0.9676(3)	−0.3185(3)	2.9(1)
H7	0.1116	0.6527	0.3332	3.5	C35	0.1248(7)	1.0962(3)	−0.2497(3)	2.9(1)
H8	0.0838	0.7102	0.5229	3.8	C36	−0.164(1)	0.9702(4)	−0.3908(4)	4.8(2)
H9	0.3005	0.6247	0.6598	3.6	H5N	0.447(6)	0.784(3)	0.025(3)	1.7(7)
O23	1.0113(5)	0.1078(3)	1.1606(2)	3.6(1)	H6N	0.305(9)	0.660(5)	0.041(4)	5(1)
O26	0.7415(6)	−0.1813(3)	1.2960(3)	4.0(1)	H7N	0.445(8)	0.688(4)	−0.080(4)	4(1)
O27	0.5504(6)	−0.1110(3)	1.1488(2)	4.1(1)	H8N	−0.102(8)	0.816(4)	−0.239(3)	4(1)
N20	1.0078(6)	0.2980(3)	1.0098(3)	2.8(1)	H30	−0.1504	0.8310	−0.0305	4.9
N23	0.6685(7)	0.1416(3)	1.2335(3)	3.2(1)	H31	0.0852	0.8865	0.0382	4.9
C20	0.5941(8)	0.1821(5)	0.9849(3)	4.0(2)	H32	−0.0524	0.7493	0.0627	4.9
C21	0.7821(7)	0.2540(4)	1.0666(3)	2.7(1)	H33	0.0370	0.6716	−0.1109	2.9
C22	0.8376(7)	0.1622(4)	1.1577(3)	2.7(1)	H34	0.1878	0.9587	−0.3652	3.5
C24	0.6593(7)	0.0329(3)	1.3081(3)	3.0(1)	H36	−0.2018	0.8910	−0.4343	5.8
C25	0.6441(6)	−0.0941(3)	1.2409(3)	2.8(1)	H37	−0.1384	1.0418	−0.4392	5.8
C26	0.4391(9)	0.0247(5)	1.3772(3)	4.5(2)	H38	−0.2921	0.9793	−0.3448	5.8
H1N	0.972(7)	0.350(4)	0.960(4)	3.5(9)					

water. The unit cell consists of two crystallographically independent peptide molecules and one sulfonate ion, as shown in Figure 2. A stereoview of the molecular packing pattern of 1 is shown in Figure 3. The hydrogen bonds in the structures were determined from an analysis of hydrogen bond donor-to-acceptor distances ($d_{D\cdots A} \le 3.3$ Å) and donor-hydrogen···acceptor angles (D–H···A angle ≥ 115°)[13] and are listed in Table 6. The strongest hydrogen bonds for this complex, based on hydrogen bond donor-to-acceptor distances[14] include acid proton/sulfonate oxygen, amino proton/acid carbonyl, and amino proton/sulfonate oxygen. The amide/sulfonate interaction shown below is

Table 3. Fractional Coordinates and Isotropic Displacement Parameters [*B*(eq)] for 1:1 Glycylglycine 2,4-Dinitrobenzenesulfonate Hydrate (2) at 24 °C

atom	*x*	*y*	*z*	*B*(eq) (Å²)	atom	*x*	*y*	*z*	*B*(eq) (Å²)
O8	0.7687(3)	0.972(12)	0.5096(1)	2.2(1)	H2W	0.678(7)	0.688(6)	0.263(3)	5(1)
O10	0.8138(3)	0.3538(2)	0.5612(1)	2.7(1)	S1	0.8646(1)	1.32149(8)	0.80198(4)	1.77(4)
O11	0.7256(3)	0.4188(3)	0.4274(1)	2.4(1)	O1	0.9525(3)	1.1280(3)	0.7867(1)	2.6(1)
N3	0.9458(4)	0.9742(3)	0.6402(2)	2.1(2)	O2	0.6828(3)	1.4395(3)	0.7620(1)	3.2(2)
N4	0.7332(3)	0.7052(3)	0.5753(1)	2.0(2)	O3	1.0100(3)	1.4031(3)	0.7837(1)	3.6(2)
C7	0.8163(4)	0.8690(3)	0.6624(2)	2.0(2)	O4	0.5714(4)	1.1146(4)	0.8774(1)	4.0(2)
C8	0.7701(4)	0.8531(3)	0.5754(2)	1.5(2)	O5	0.7704(4)	0.8746(4)	0.9581(2)	4.2(2)
C9	0.6915(4)	0.6742(3)	0.4951(2)	1.9(2)	O6	0.6087(3)	1.1395(3)	1.2460(1)	3.6(2)
C10	0.7492(4)	0.4695(3)	0.4976(2)	1.7(2)	O7	0.7720(4)	1.3093(3)	1.2384(1)	3.6(2)
H1N	1.081(7)	0.899(7)	0.617(3)	7(1)	N1	0.6902(4)	1.0422(5)	0.9320(1)	2.2(2)
H2N	0.883(5)	1.093(5)	0.599(2)	3.8(8)	N2	0.6962(3)	1.2381(3)	1.2042(1)	2.3(2)
H3N	0.942(5)	1.014(5)	0.691(2)	3.9(8)	C1	0.7861(3)	1.3088(3)	0.9212(1)	1.4(2)
H4N	0.741(6)	0.615(6)	0.624(3)	5(1)	C2	0.7287(3)	1.1691(3)	0.9731(2)	1.5(2)
H7	0.6922	0.9346	0.6991	2.3	C3	0.6965(4)	1.1448(3)	1.0657(2)	1.7(2)
H8	0.8868	0.7472	0.6932	2.3	C4	0.7176(4)	1.2697(3)	1.1060(2)	1.7(2)
H9	0.7687	0.7165	0.4441	2.3	C5	0.7637(4)	1.4161(4)	1.0581(2)	2.2(2)
H10	0.5495	0.7429	0.4914	2.3	C6	0.7994(4)	1.4344(3)	0.9653(2)	2.0(2)
H11	0.666(6)	0.536(6)	0.389(3)	5(1)	H3	0.6611	1.0462	1.1000	2.1
O1W	0.5739(4)	0.6926(3)	0.3029(1)	2.9(2)	H5	0.7707	1.5033	1.0880	2.6
H1W	0.524(6)	0.659(5)	0.279(3)	4(1)	H6	0.8334	1.5341	0.9314	2.4

Table 4. Fractional Coordinates and Isotropic Displacement Parameters [*B*(eq)] for 2:1 Glycylglycine 2,6-Naphthalenedisulfonate Hydrate (3) at 24 °C

atom	*x*	*y*	*z*	*B*(eq) (Å²)
S1	0.83428(4)	0.14325(5)	0.82197(4)	2.01(2)
O1	0.7173(1)	0.0997(2)	0.8615(1)	2.87(6)
O2	0.9301(1)	0.0288(2)	0.8279(1)	3.31(6)
O3	0.8037(2)	0.2072(2)	0.7195(1)	3.42(6)
C1	0.9256(2)	0.4121(2)	0.8744(1)	2.08(6)
C2	0.9078(2)	0.2767(2)	0.9090(1)	1.99(6)
C3	0.9440(2)	0.2407(2)	1.0159(2)	2.48(7)
C4	0.9982(2)	0.3410(2)	1.0866(2)	2.48(7)
C9	0.9814(2)	0.5181(2)	0.9463(1)	1.92(6)
H1	0.9005	0.4350	0.8023	2.5
H3	0.9307	0.1462	1.0388	3.0
H4	1.0223	0.3146	1.1585	2.9
O12	0.9241(1)	0.4441(2)	0.3715(1)	3.38(6)
O13	0.6746(2)	0.5092(2)	0.0254(1)	3.32(7)
O14	0.6199(2)	0.3250(2)	0.1187(1)	4.21(8)
N11	0.8872(2)	0.3366(2)	0.5551(2)	3.16(8)
N12	0.7099(2)	0.4621(2)	0.3050(1)	2.94(7)
C11	0.7714(2)	0.3550(2)	0.4743(2)	2.40(7)
C12	0.8092(2)	0.4257(2)	0.3787(1)	2.17(7)
C13	0.7276(2)	0.5279(2)	0.2077(2)	2.97(8)
C14	0.6679(2)	0.4411(2)	0.1136(2)	2.67(8)
H1N	0.862(3)	0.287(3)	0.613(2)	4.8(6)
H2N	0.946(2)	0.290(3)	0.535(2)	3.0(5)
H3N	0.930(3)	0.421(4)	0.577(2)	6.0(8)
H5	0.7339	0.2643	0.4553	2.9
H6	0.7093	0.4130	0.5012	2.9
H7	0.6874	0.6194	0.2032	3.6
H8	0.8187	0.5381	0.2070	3.6
H4N	0.637(3)	0.454(3)	0.316(2)	3.5(6)
H13	0.643(3)	0.448(4)	−0.027(3)	6.6(9)
O1W	0.5710(2)	0.3626(2)	−0.1406(1)	3.21(7)
H1W	0.585(3)	0.401(4)	−0.202(3)	7.0(9)
H2W	0.603(3)	0.282(3)	−0.145(2)	5.7(8)

Table 5. Fractional Coordinates and Isotropic Displacement Parameters [*B*(eq)] for 2:1 L-Leucyl-L-alanine 1,5-Naphthalenedisulfonate (4) at 24 °C

atom	*x*	*y*	*z*	*B*(eq) (Å²)
S1	−0.2517(2)	0.15670(4)	0.46126(5)	2.56(4)
O1	−0.2527(6)	0.1822(1)	0.3979(1)	3.5(1)
O2	−0.0896(4)	0.1723(2)	0.4940(2)	3.5(2)
O3	−0.4102(4)	0.1739(2)	0.4966(2)	3.2(2)
O11	−0.2164(4)	0.1698(1)	0.6614(1)	3.3(2)
O12	0.3745(5)	0.2021(1)	0.7065(1)	3.2(2)
O13	0.3538(5)	0.0987(1)	0.7189(2)	4.1(2)
N11	−0.3803(5)	0.2569(2)	0.5976(2)	2.9(2)
N14	0.0599(5)	0.2051(2)	0.6400(2)	2.9(2)
C1	−0.2553(8)	0.0736(2)	0.4519(2)	2.4(2)
C2	−0.2553(9)	0.0496(2)	0.3929(2)	2.9(2)
C3	−0.2571(9)	−0.0150(2)	0.3829(2)	3.4(2)
C4	−0.2581(9)	−0.0553(2)	0.4323(2)	2.9(2)
C10	−0.2581(7)	−0.0330(2)	0.4946(2)	2.2(2)
C12	−0.1943(6)	0.2723(2)	0.6176(2)	2.2(2)
C13	−0.1173(6)	0.2104(2)	0.6418(2)	2.2(2)
C15	0.1469(7)	0.1453(2)	0.6513(2)	2.9(2)
C16	0.3010(6)	0.1497(2)	0.6960(2)	2.8(2)
C17	0.2163(8)	0.1162(2)	0.5903(2)	4.7(3)
C18	−0.1925(7)	0.3226(2)	0.6680(2)	3.4(2)
C19	−0.2995(6)	0.3823(2)	0.6542(2)	3.6(2)
C20	−0.249(1)	0.4134(2)	0.5933(3)	4.8(3)
C21	−0.275(1)	0.4286(3)	0.7085(3)	7.0(4)
H1N	−0.3776	0.2256	0.5658	3.4
H1	−0.2539	0.0774	0.3579	3.4
H2	−0.2586	−0.0310	0.3413	4.1
H2N	−0.4461	0.2419	0.6325	3.4
H3	−0.2587	−0.0993	0.4247	3.4
H3N	−0.4359	0.2938	0.5815	3.4
H4N	0.119(6)	0.233(2)	0.626(2)	2(1)
H4	−0.1273	0.2862	0.5824	2.7
H5	0.0613	0.1173	0.6688	3.4
H6	0.2686	0.0765	0.5989	5.6
H7	0.1205	0.1110	0.5617	5.6
H8	0.3033	0.1432	0.5723	5.6
H9	−0.2396	0.3044	0.7052	4.0
H10	−0.0726	0.3346	0.6748	4.0
H11	−0.4221	0.3713	0.6522	4.3
H12	−0.1274	0.4255	0.5948	5.8
H13	−0.2670	0.3848	0.5597	5.8
H14	−0.3212	0.4497	0.5871	5.8
H15	−0.1527	0.4394	0.7122	8.4
H16	−0.3427	0.4655	0.7007	8.4
H17	−0.3144	0.4097	0.7465	8.4

a recurring pattern in all four of these peptide salts. This hydrogen bond interaction is particularly interesting because it is analogous to the molecular recognition of sulfate groups by sulfate binding proteins. For example, Quiocho and co-workers reported that in the structure of a sulfate-binding protein NH···O hydrogen bonds between the peptide backbone and sulfate ions were mainly responsible for the binding.[15]

Figure 4 illustrates a two-dimensional representation of the complex three-dimensional hydrogen bonding network surrounding the asymmetric unit of **1**. Distances and angles of all hydrogen bonds shown in the figure are listed in Table 6. Shown in Figure 4 are two peptide

(13) Görbitz, C. H.; Etter, M. C. *Int. J. Peptide Protein Res.* **1992**, *39*, 93.
(14) Jeffrey, G. A.; Saenger, W. *Hydrogen Bonding in Biological Structures*; Springer-Verlag: New York, 1991; Chapter 2.
(15) Quiocho, F. A.; Sack, J. S.; Vyas, N. K. *Nature* **1987**, *329*, 561.

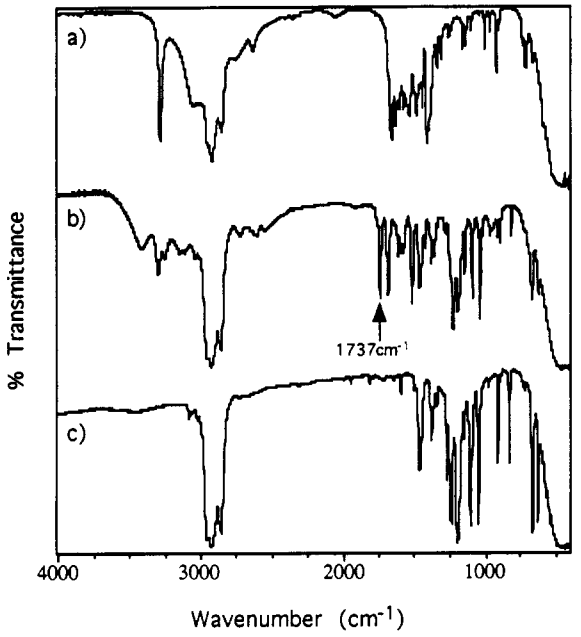


Figure 1. Infrared spectra (Nujol mull) of (a) Gly-Gly, (b) 2:1 Gly-Gly–2,6-naphthalenedisulfonate hydrate (3), and (c) 2,6-naphthalenedisulfonic acid, disodium salt. Peptide salt formation is indicated in part b by the appearance of a carbonyl peak at 1737cm^{-1}.

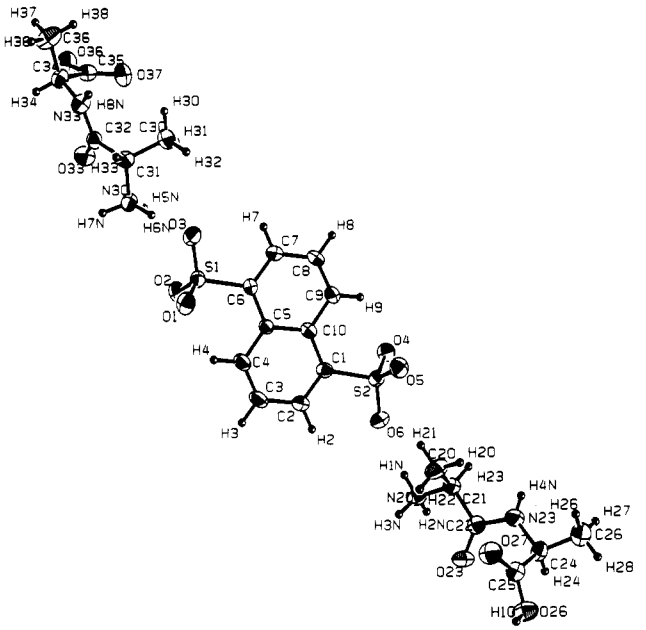


Figure 2. ORTEP picture (50% probability ellipsoids) of the asymmetric unit for 2:1 L-Ala-L-Ala–1,5-naphthalenedisulfonate (1).

molecules stacked together on the right-hand side of the figure. One of these peptides forms a hydrogen bond bridge between the sulfonate ion and the other peptide within the asymmetric unit.

(2) Glycylglycine 2,4-Dinitrobenzenesulfonate Hydrate. Light yellow rectangular prisms of **2** were obtained from water. Figure 5 shows the asymmetric unit consisting of one peptide molecule, one sulfonate ion, and one water molecule. A stereoview of the molecular packing pattern of **2** is shown in Figure 6. The acid group of the peptide adopts an unusual antiperiplanar conformation with respect to the carbonyl instead of the more common synperiplanar orientation. Few such crystal structures showing this *anti* conformation have been reported, and

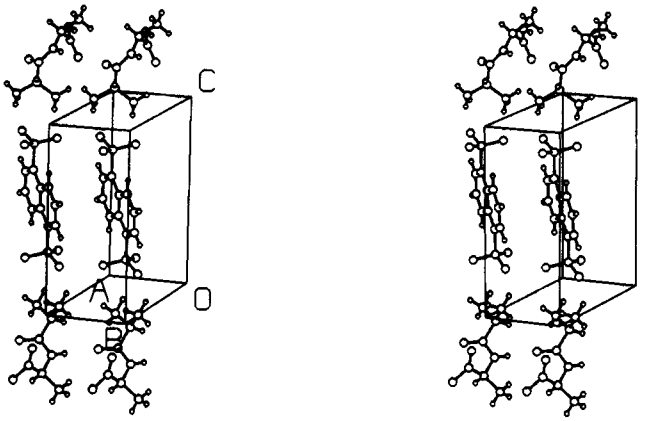


Figure 3. Stereoview of the molecular packing pattern of 2:1 L-Ala-L-Ala 1,5-naphthalenedisulfonate (1).

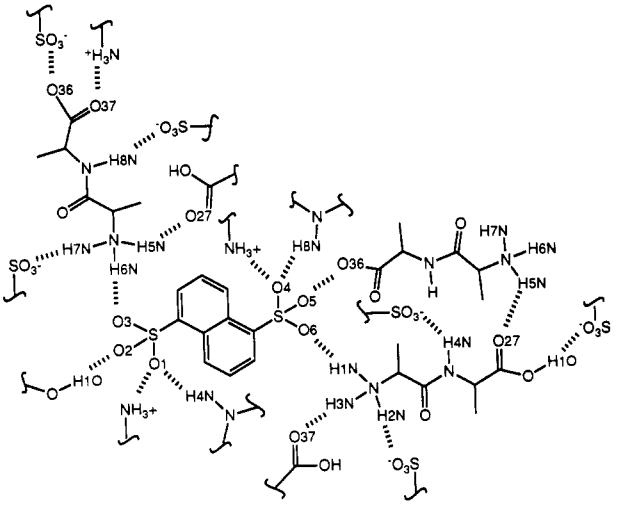
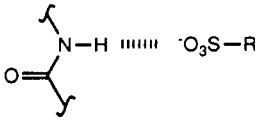


Figure 4. Two-dimensional representation of the three-dimensional hydrogen bonding network surrounding the asymmetric unit of 2:1 L-Ala-L-Ala 1,5-naphthalenedisulfonate (1). Hydrogen bonds are indicated as dashed lines.

Table 6. Hydrogen-Bond Interactions for L-Alanyl-L-alanine 1,5-Naphthalenedisulfonate (1)

	type of interaction	$d_{D\cdots A}$ (Å)	$d_{H\cdots A}$ (Å)	angle$_{D-H\cdots A}$ (deg)
O26–H10···O2	acid/SO_3^-	2.752(4)	1.98(5)	177
N30–H5N···O27	amino/acid carbonyl	2.782(4)	1.83(4)	161
N30–H7N···O4	amino/SO_3^-	2.792(4)	1.84(5)	176
N30–H6N···O3	amino/SO_3^-	2.799(4)	1.81(5)	176
N20–H3N···O37	amino/acid carbonyl	2.825(4)	1.932	156
N20–H1N···O6	amino/SO_3^-	2.870(4)	2.04(4)	164
N20–H2N···O1	amino/SO_3^-	2.970(5)	2.12(5)	175
N23–H4N···O1	amide/SO_3^-	3.108(5)	2.45(3)	144
N33–H8N···O4	amide/SO_3^-	3.144(5)	2.38(4)	157



in general this occurs only when strong intramolecular hydrogen bonding ispresent[16] or when intermolecular hydrogen bonding occurs between the acid proton and a carbonyl oxygen[17] or a carboxylate oxygen[18] of a neighboring molecule. The carboxylic acid geometry in **2**,

(16) Leiserowitz, L. *Acta Crystallogr.* **1976**, *B32*, 775.
(17) Fujinaga, M.; James, M. N. G. *Acta Crystallogr.* **1980**, *B36*, 3196.
(18) Eggleston, D. S.; Hodgson, D. J. *Int. J. Peptide Protein Res.* **1982**, *20*, 66.

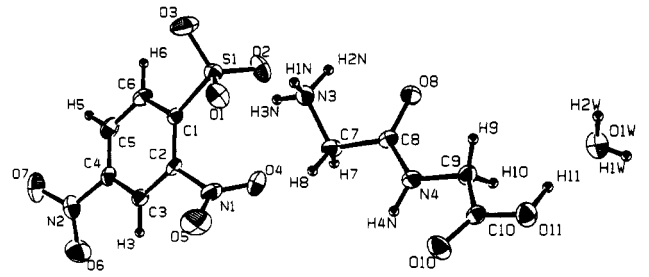


Figure 5. ORTEP picture (50% probability ellipsoids) of the asymmetric unit for 1:1 Gly-Gly 2,4-dinitrobenzenesulfonate hydrate (2).

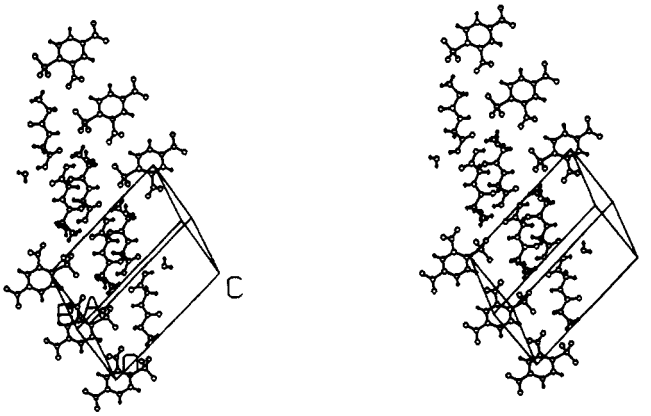


Figure 6. Stereoview of the molecular packing pattern of 1:1 Gly-Gly 2,4-dinitrobenzenesulfonate hydrate (2).

Table 7. Hydrogen-Bond Interactions for Glycylglycine 2,4-Dinitrobenzenesulfonate Hydrate (2)

	type of interaction	$d_{D\cdots A}$ (Å)	$d_{H\cdots A}$ (Å)	angle$_{D-H\cdots A}$ (deg)
O11–H11···O1W	acid/water	2.582(3)	1.65(4)	163
N3–H2N···O10	amino/acid carbonyl	2.814(3)	1.90(3)	152
N3–H1N···O8	amino/amide	2.841(3)	2.28(4)	116
N3–H3N···O1	amino/SO_3^-	2.844(3)	1.95(4)	171
O1W–H2W···O3	water/SO_3^-	2.873(4)	2.08(5)	147
O1W–H1W···O2	water/SO_3^-	2.986(3)	2.26(4)	166
N4–H4N···O2	amide/SO_3^-	3.227(3)	2.34(4)	165

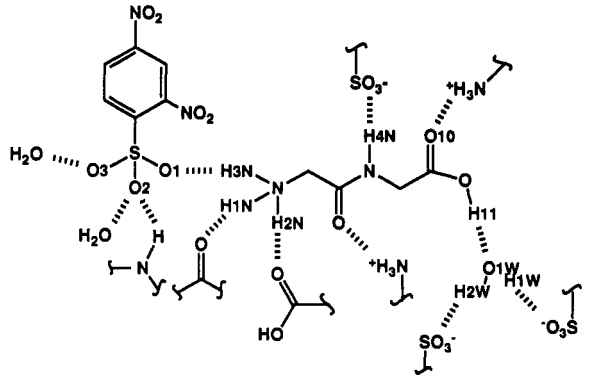


Figure 7. Two-dimensional representation of the three-dimensional hydrogen bonding network of 1:1 Gly-Gly 2,4-dinitrobenzenesulfonate hydrate (2). Hydrogen bonds are indicated as dashed lines.

however, is induced by strong intermolecular hydrogen bonding between the acid proton and a neighboring *water* molecule ($d_{donor\cdots acceptor}$ = 2.582(3) Å). Other strong hydrogen bonds for 2, based on hydrogen bond donor-to-acceptor distances, are listed in Table 7 and include the following interactions: acid proton/water, amino proton/acid carbonyl, amino proton/amide oxygen, and amino proton/sulfonate oxygen. A weak intermolecular

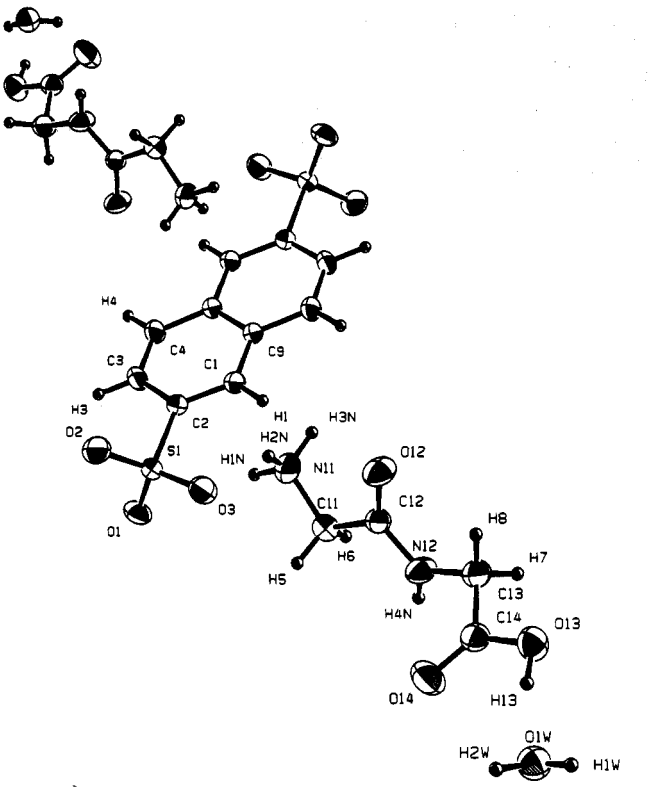


Figure 8. ORTEP picture (50% probability ellipsoids) illustrating the asymmetric unit and inversion related atoms of 2:1 Gly-Gly 2,6-naphthalenedisulfonate hydrate (3).

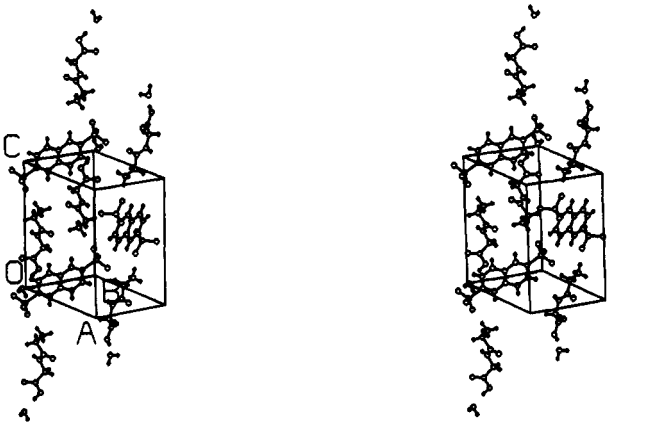


Figure 9. Molecular packing pattern for 2:1 Gly-Gly–2,6-naphthalenedisulfonate hydrate (3). Sulfonate ions, peptide molecules, and water molecules are partitioned into layers throughout the crystal.

interaction forms between amide NH and sulfonate oxygen. Water protons also hydrogen-bond to sulfonate oxygens. Unlike the acid proton in 1, the acid proton in 2 does not directly interact with sulfonate oxygens. Instead the proton forms a hydrogen bond to water which in turn interacts with the sulfonate oxygens. Figure 7 illustrates the three-dimensional hydrogen bonding network surrounding the asymmetric unit of 2.

(3) 2:1 Glycylglycine 2,6-Naphthalenedisulfonate Hydrate. Clusters of colorless prisms of 3 having well-defined faces were obtained from 1 M HCl. Figure 8 shows the unit cell, consisting of two peptide molecules, one sulfonate ion, and two water molecules. Figure 9 shows a stereoview of the molecular packing pattern viewed down the *a* axis and illustrates the partitioning of peptide molecules, water molecules, and sulfonate ions into layers

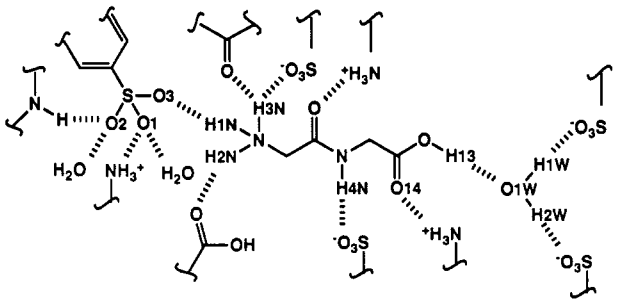


Figure 10. Two-dimensional representation of the three-dimensional hydrogen bonding network of 2:1 Gly-Gly–2,6-naphthalenedisulfonate hydrate (**3**). Hydrogen bonds are indicated as dashed lines.

Table 8. Hydrogen-Bond Interactions for Glycylglycine–2,6-Naphthalenedisulfonate Hydrate (3)

	type of interaction	$d_{D\cdots A}$ (Å)	$d_{H\cdots A}$ (Å)	angle$_{D-H\cdots A}$ (deg)
O13–H13···O1W	acid/water	2.622(3)	1.73(3)	169
N11–H1N···O3	amino/SO_3^-	2.709(3)	1.76(3)	174
N11–H2N···O14	amino/acid carbonyl	2.848(3)	2.22(2)	133
O1W–H1W···O2	water/SO_3^-	2.876(3)	2.00(4)	161
N11–H3N···O12	amino/amide	2.884(3)	2.00(3)	160
O1W–H2W···O1	water/SO_3^-	2.889(2)	2.07(3)	168
N12–H4N···O2	amide/SO_3^-	2.960(3)	2.18(3)	168
N11–H3N···O1	amino/SO_3^-	2.965(3)	2.48(3)	113

within the crystal. This partitioning is found in all four peptide sulfonate salts presented in this paper. Table 8 lists the strongest hydrogen bonds, based on hydrogen-bond donor-to-acceptor distances, which include the following interactions: acid proton/water, amino proton/sulfonate oxygen, and amino proton/acid carbonyl. The interaction between acid protons and sulfonate oxygens is also mediated by a water molecule (as in **2**). Figure 10 illustrates the three-dimensional hydrogen bonding network surrounding the asymmetric unit of **3**.

(4) 2:1 L-Leucyl-L-alanine 1,5-Naphthalenedisulfonate. Light tan prisms of **4** were obtained from water. The asymmetric unit consists of one half of the sulfonate ion and one dipeptide molecule. The similarities in both C–O bond distances of the acid group (1.265(5) Å and 1.252(5) Å) indicate a disordered acid proton. This proton disorder is due to hydrogen-bonded acid dimers formed between two peptide acid groups related by a 2-fold axis. The disorder leads to a hydrogen occupancy of one-half on each oxygen of the acid group. (Because the disordered proton did not refine well, it was not included in the final structure.) A stereoview of the molecular packing pattern of **4** is shown in Figure 12. Hydrogen bond interactions are listed in Table 9 and include the following interactions: acid dimers, amino proton/sulfonate oxygen, and amino proton/acid carbonyl. The three-dimensional hydrogen-bonding network surrounding the asymmetric unit of **4** is illustrated in Figure 13. Represented in the figure is the disordered acid proton having an occupancy of one-half.

The hydrogen bond between the amide hydrogen and sulfonate oxygen in each structure is weaker than other hydrogen bonds present (based on N···O distance criteria). This may be due to the greater steric hindrace of the amide hydrogen resulting in fewer opportunities to form stronger hydrogen bonds with available donors.

Bond lengths, angles, and torsion angles for salts **1–4** were analyzed to gauge the accuracy of the structure and to compare conformations between salts. Bond lengths

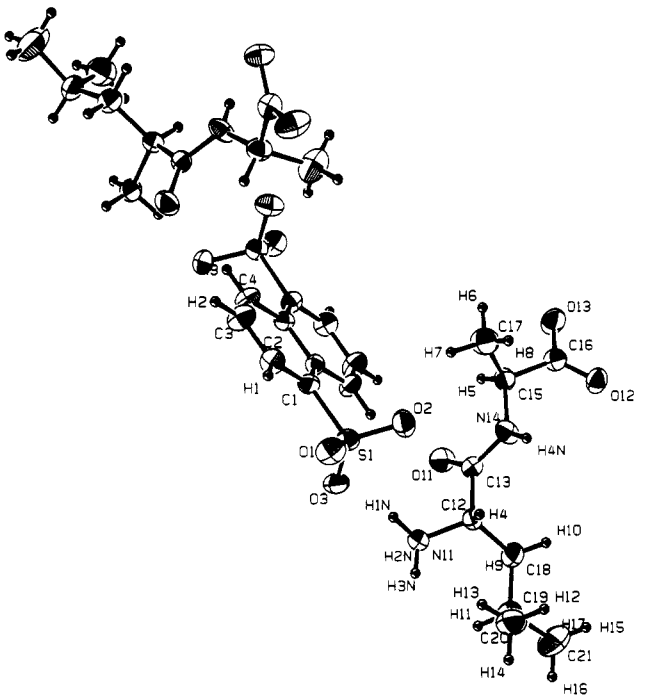
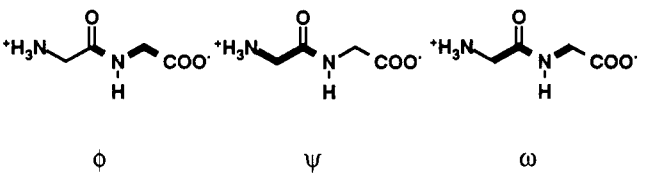


Figure 11. ORTEP picture (50% probability ellipsoids) illustrating the asymmetric unit and symmetry related atoms of 2:1 L-Leu-L-Ala 1,5-naphthalenedisulfonate (**4**). The acid proton was disordered about the 2-fold axis between acid dimers and was not included in the structure.

for salts **1–4** were generally found to be within the expected range for organic compounds. Torsion angles ϕ, ψ, and ω (as defined below[19]) are listed in Table 10 for salts **1–4**



ϕ ψ ω

(two crystallographically independent peptides A and B are listed for **1** in the table). X-ray structures for zwitterionic forms of the peptides were retrieved from the Cambridge Structural Database.[20]

A comparison of the torsion angles for salts **1–4** with those found in zwitterionic forms reveals few differences. The largest difference occurs in the ϕ angle for Ala-Ala. In the neutral Ala-Ala molecule, ϕ is much larger (−113°) compared to ~56° for the Ala-Ala salt. For Gly-Gly ϕ ranges from 121° in **3** to 178° for neutral β-Gly-Gly. As expected, torsion angles for Gly-Gly are closer to 180° than for the other dipeptides due to the peptide side chains on Ala-Ala and Leu-Ala. Torsion angles ψ and ω for **1** agree closely with those for the neutral species. The same is true for Gly-Gly, except for a 20° difference in ψ for **3**. (Since the X-ray structure of neutral Leu-Ala has not been reported in the Cambridge Structural Database,[20] a comparison of conformations to the neutral peptide could not be made for **4**.) Values of ω for **1–4** deviate from planarity by as much as 16°. Such deviations from 180° for peptide bonds in linear peptides have been observed

(19) IUPAC-IUB Commission on Biochemical Nomenclature. *Biochemistry* **1970**, *9*, 3471.

(20) Cambridge Structural Database; Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, England, Oct 1993, Version 5.06 (Vax).

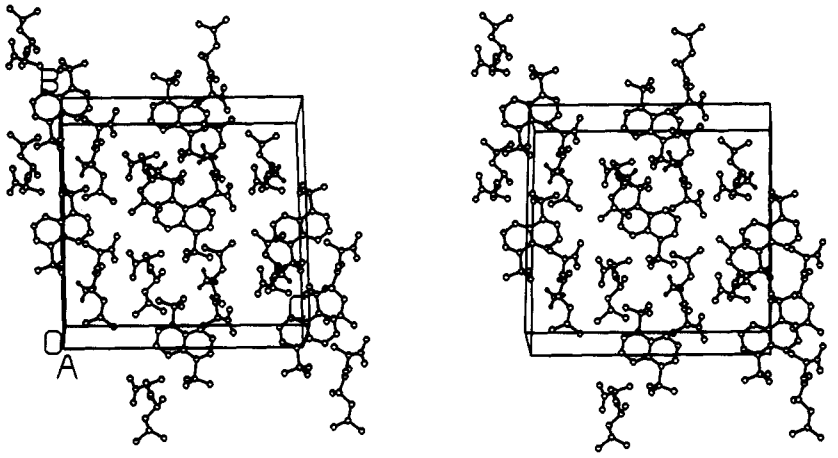


Figure 12. Stereoview of the molecular packing pattern of 2:1 L-Leu-L-Ala 1,5-naphthalenedisulfonate (4) viewed down the *a* axis.

Table 9. Hydrogen-Bond Interactions for L-Leucyl-L-alanine 1,5-Naphthalenedisulfonate (4)

	type of interaction	$d_{D\cdots A}$ (Å)	$d_{H\cdots A}$ (Å)	angle$_{D-H\cdots A}$ (deg)
O13–H···O13	acid dimer	2.566(7)		
O12–H···O12	acid dimer	2.645(6)		
N11–H1N···O3	amino/SO_3^-	2.792(5)	1.86	168
N14–H4N···O1	amide/SO_3^-	2.894(5)	2.11(4)	168
N11–H3N···O2	amino/SO_3^-	2.925(5)	2.11	143
N11–H3N···O1	amino/SO_3^-	3.084(6)	2.47	122
N11–H2N···O12	amino/acid	3.185(5)	2.24	173

Table 10. Selected Torsion Angles for the Peptides in Four Sulfonate Salts (1–4) and Their Zwitterionic Counterparts (L-alanyl-L-alanine, α-glycylglycine, and β-glycylglycine)

peptide	torsion angle		
	φ (deg)	ψ (deg)	ω (deg)
L-Ala-L-Ala peptide A in 1	−55.2(4)	163.9(3)	163.9(3)
L-Ala-L-Ala peptide B in 1	−57.2(4)	165.9(3)	164.8(3)
L-Ala-L-Ala[23]	−113.0	165.4	175.7
Gly-Gly in 2	154.2(2)	152.7(2)	−178.7(2)
Gly-Gly in 3	121.0(2)	−172.8(2)	−178.7(2)
α-Gly-Gly[24]	154.6	152.3	174.5
β-Gly-Gly[25]	177.7	156.2	178.7
L-Leu-L-Ala in 4	−133.3(5)	155.4(4)	−169.2(4)

in other structures.[21] It has been suggested that these peptide bond distortions are caused by hydrogen-bond interactions of the amide group.[21a] Deviations of 10–20° from planarity for an amide bond require only a few kilojoules per mole of energy[22] and thus are not uncommon in hydrogen-bonded structures of linear peptides.

Conclusions

We have shown that the formation of arenesulfonate salts of dipeptides is a useful technique for the growth of X-ray quality crystals and in one case have shown the utility of such a method for the crystallization and structure determination of a dipeptide for which the native zwitterionic form is currently unavailable. Structural data gained from arenesulfonate salts of peptides can provide useful information about the peptides themselves in addition to information applicable to molecular recognition of the peptide backbone in proteins. Common interactions observed in all four dipeptide sulfonate salts include the following hydrogen-bonded donor/acceptor pairs: amine/sulfonate, amide/sufonate, and amine/carboxylate. Additional hydrogen bonds observed in hydrated structures include acid/water and water/sulfonate interactions. Given their high hydration state, such interactions may also occur in protein sulfonate complexes although the positions of water molecules would be more difficult to determine.

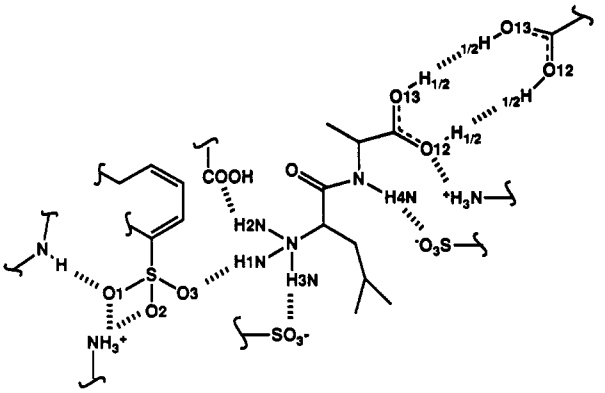


Figure 13. Two-dimensional representation of the three-dimensional hydrogen bonding network of 2:1 L-Leu-L-Ala-1,5-naphthalenedisulfonate (4). The disordered acid proton is shown with an occupancy of one-half. Hydrogen bonds are indicated as dashed lines.

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Supplementary Material Available: Text describing the structure determination and tables of crystallographic data, fractional coordinates, anisotropic displacement parameters, bond lengths, bond angles, torsion angles, and intermolecular distances for salts 1–4 (105 pages); structure factor tables for salts 1–4 (127 pages). Ordering information can be found on any current masthead page.

(21) Some observed deviations of ω from 180° are listed as follows: (a) 21° for L-leucyl-L-tyrosine (Krause, J. A.; Baures, P. W.; Eggleston, D. S. *Acta Crystallogr.* **1993**, *B49*, 123); (b) 19° for glycylaspartic acid (Eggleston, D. S.; Hodgson, D. J. *Int. J. Peptide Protein Res.* **1985**, *26*, 509); (c) 12° or more for 2 of 3 peptide bonds in the N-terminal tetrapeptide from angiotensin II (Feldman, S. H.; Eggleston, D. S., *Acta Crystallogr.* **1990**, *C46*, 678).

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