Org. Biomol. Chem.

Supplementary Data

The influence of protonation on molecular structure and physico-chemical properties of gossypol Schiff bases

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1. Elemental analysis data of **S1** and **S2** as well as their protonated species.

S1 - $C_{36}H_{40}N_2O_6$ Calculated: C=72.46%, H=6.76%, N=4.69%;
                                        Found: C=72.44%, H=6.72%, N=4.68%.

S1+HClO$_4$ - $C_{36}H_{41}ClN_2O_{10}$ Calculated: C=62.02%, H=5.93%, N=4.02%;
                                                     Found: C=62.00%, H=5.90%, N=3.99%.

S1+2HClO$_4$ - $C_{36}H_{42}Cl_2N_2O_{14}$ Calculated: C=54.21%, H=5.31%, N=3.51%;
                                                     Found: C=54.17%, H=5.30%, N=3.48%.

S2 - $C_{44}H_{58}N_4O_8$ Calculated: C=68.55%, H=7.58%, N=7.27%;
                                        Found: C=68.51%, H=7.53%, N=7.25%.

S2+HClO$_4$ - $C_{44}H_{59}ClN_4O_{12}$ Calculated: C=60.65%, H=6.82% N=6.43%;
                                                     Found: C=60.63%, H=6.79% N=6.44%.

S2+2HClO$_4$ - $C_{44}H_{60}Cl_2N_4O_{16}$ Calculated: C=54.38%, H=6.22%, N=5.76%;
                                                     Found: C=54.33%, H=6.18%, N=5.75%.

S2+3HClO$_4$ - $C_{44}H_{61}Cl_3N_4O_{20}$ Calculated: C=49.28%, H=5.73%, N=5.22%;
                                                     Found: C=49.22%, H=5.70%, N=5.21%.

S2+4HClO$_4$ - $C_{44}H_{62}Cl_4N_4O_{24}$ Calculated: C=45.06%, H=5.33%, N=4.78%;
                                                     Found: C=45.03%, H=5.31%, N=4.73%.

Analysis of **S1** and **S2** as well as their protonated species was carried out on Vario ELIII

(Elementar, Germany).

2. Structure of **S2** in crystal

The conformations of propylene linkers in **S2** are also different from those observed in an analogous derivative with the pyrrolidin-2-one fragment replacing the morpholine unit [24]. As indicated in the Experimental, one of the morpholine groups is disordered over two positions with equal occupancies and this disorder is strictly related to the crystal packing. The two disordered groups from two different molecules are in close contact and the pattern of disorder requires that whenever one group assumes one of the disordered orientations, the other has to assume the alternative one.

In the **S2** molecule in crystal there are four intramolecular hydrogen bonds, two strong ones involving the amine H-atoms and two much weaker ones involving hydroxyls O_4─H and O_6─H (**Fig. 1, Table 3S**). In the FT-IR spectrum of **S2** the band of ν(O-H) vibrations of the weaker hydrogen-bonded groups O_4─H$\cdots O_3$ and O_6─H$\cdots O_7$ (**Fig. 1S**) is present at 3319 cm^{-1}. The absorption of the ν(N-H) vibrations in the respective spectrum of **S2** should be observed below 3000 cm^{-1}. The fact that this absorption is not well visible in the spectrum of **S2** can be explained by the coupling of changes in the π-electron distribution of naphthalene ring with the NH proton vibration in the hydrogen bridge. The band assigned to non-bonded intramolecularly O1─H and O5─H groups is visible at about 3500 cm^{-1} in the spectrum shown in **Fig. 1S**. A comparison of the absorption of these bands in solid (**Fig. 1S**) and in solution (**Fig. 3a**) reveals that some of these hydroxyl groups in the structure of **S2** in the crystal are involved in stronger intermolecular interactions, whereas in the structure of **S2** in the solution both O1─H and O5─H groups are weakly hydrogen-bonded with the solvent molecules.

There are two types of intermolecular hydrogen-bond motifs generally formed in crystals of gossypol Schiff bases: the centrosymmetric dimer through a pair of O_5-H$\cdots O_3$ hydrogen bonds and the dimer made through a cyclic $R_2^2(10)$ motif [20, 22-31]. In **S2** only the former one is realized (**Fig. 6Sa**), leaving the carbonyl oxygen atom O_7 and the hydroxyl group O_6-H not involved in intermolecular hydrogen bonding, a quite exceptional case among gossypol Schiff bases[20, 22-31]. These two groups $C_{17}=O_7$ and O_6-H are in a close proximity of the disordered morpholine fragment and of the C_{15}-C_{20} ring, with the C_{15}-C_{20} centroid and O_7 carbonyl atom at the distance of 3.31 Å. The same naphthalene unit, but its second half, forms another short O$\cdots\pi$ contact of 3.04 Å with the O_2 atom from the ordered morpholine group. This last short contact emerges, most probably, as a result of sterical requirements of the O1-

H$\cdots$O$_2$ hydrogen bond joining hydroxyl group of one **S2** molecule with the morpholine O atom of another one. This hydrogen bond requires an acceptor atom approaching the donor group to be located directly above the naphthalene π system.

In crystal the O$_1$-H$\cdots$O$_2$ interaction bridging the neighbouring **S2** dimers furnish a two-dimensional network (**Fig. 6Sb**) parallel to (100).

As follows from the X-ray data there are also short intramolecular H$\cdots$H and C-H$\cdots$O contacts between the substituents on the naphthalene ring (H$_4\cdots$H$_{23}$ 1.88 Å, H$_{14}\cdots$H$_{28}$ 1.94 Å, H$_{27}\cdots$O$_5$ 2.02 Å, H$_{22}\cdots$O$_1$ 1.99 Å). The naphthalene rings are almost planar with the root mean square deviation of the atoms from their best planes being 0.0073 Å for the C$_1$-C$_{10}$ unit and 0.032 Å for the C$_{11}$-C$_{20}$ unit. Furthermore, the dihedral angle (ω) between the least-square planes of the naphthalene moieties is 89.09(1)° so they are almost perpendicular to each other.
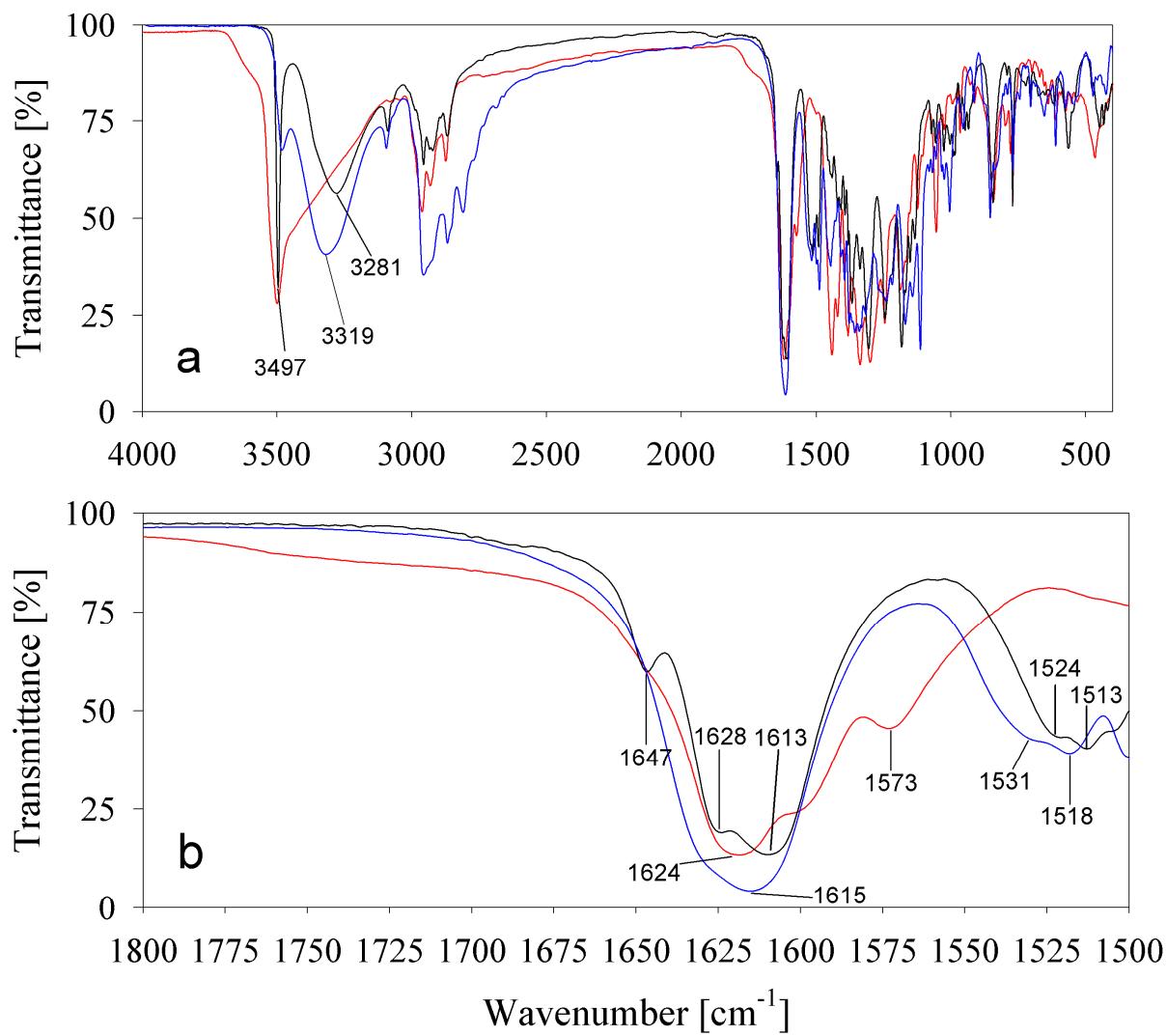
Figure 1S. The FT-IR spectra of the crystals of gossypol (red line) in KBr pellet and crystals of **S1** (black line) and **S2** (blue line) in the nujol-fluorolube mull (compilation) in the ranges: a) 4000-400 cm^{-1}, b) 1800-1500 cm^{-1}.
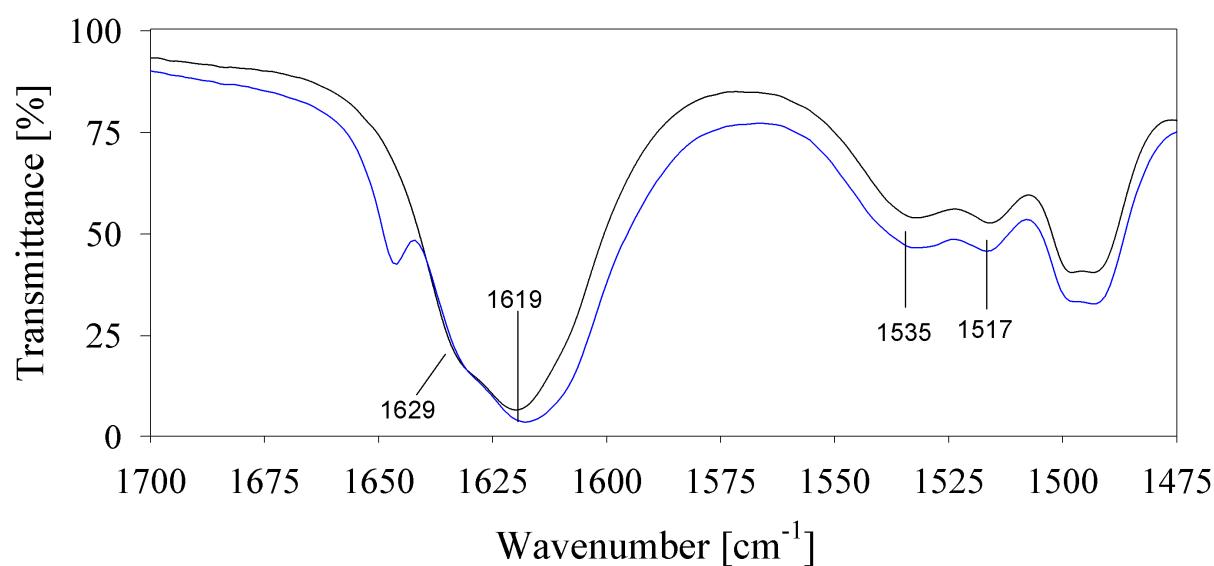
Figure 2S. Comparison of the FT-IR spectra of **S1** and **S2** in acetonitrile in the range 1700-1475 cm^{-1}.
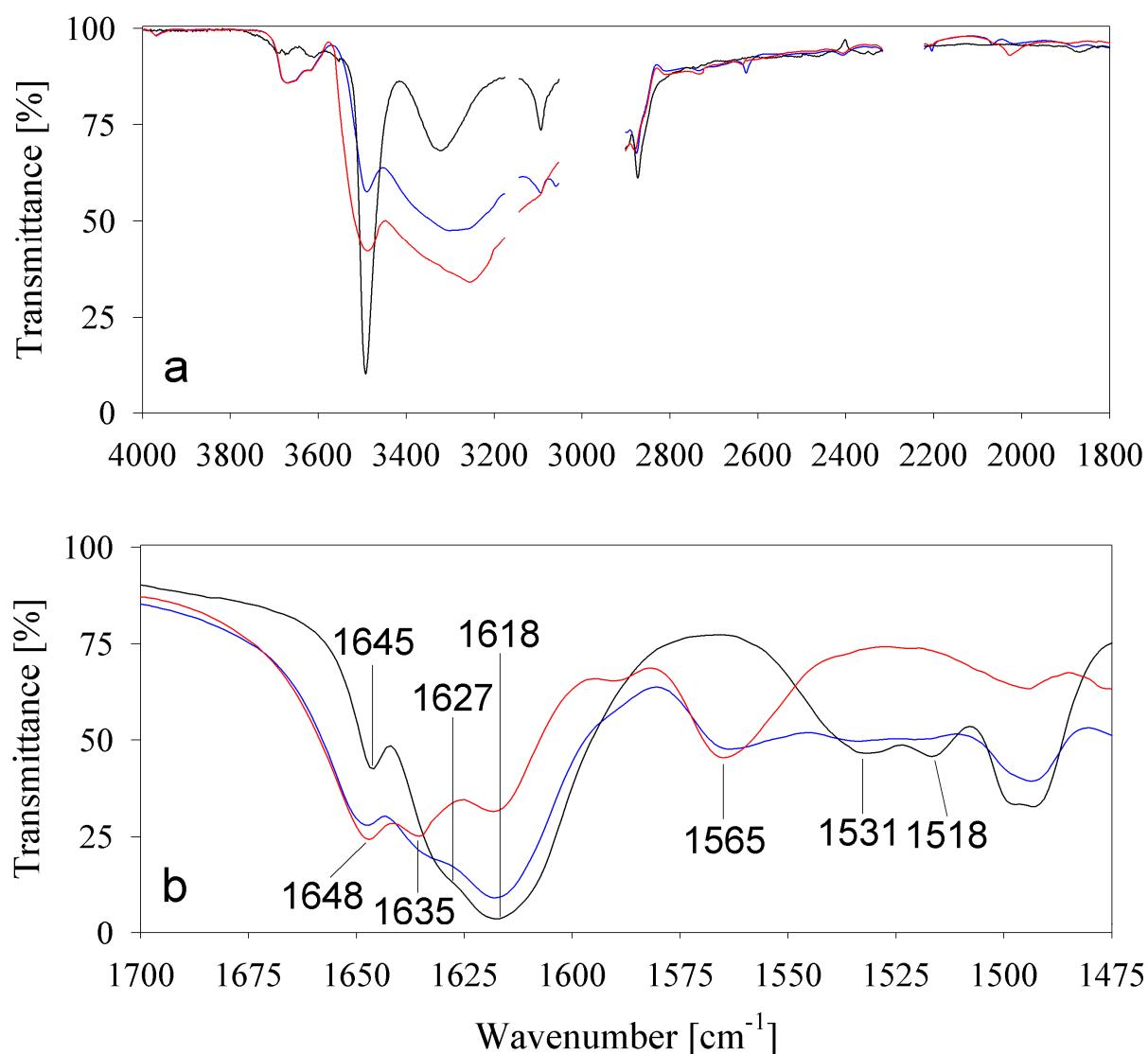
Figure 3S. The FT-IR spectra of **S1** (black line), **S1**:H$^+$ (blue line) and **S1**:2H$^+$ (red line) recorded in acetonitrile in the ranges: a) 4000-1800 cm^{-1}, b) 1700-1475 cm^{-1}.

a)
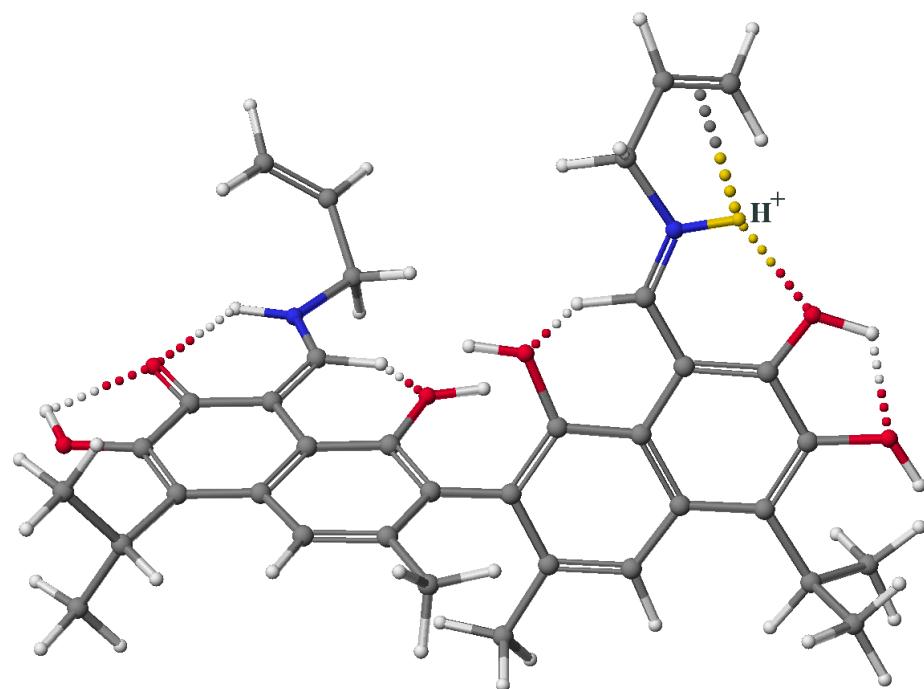
b)
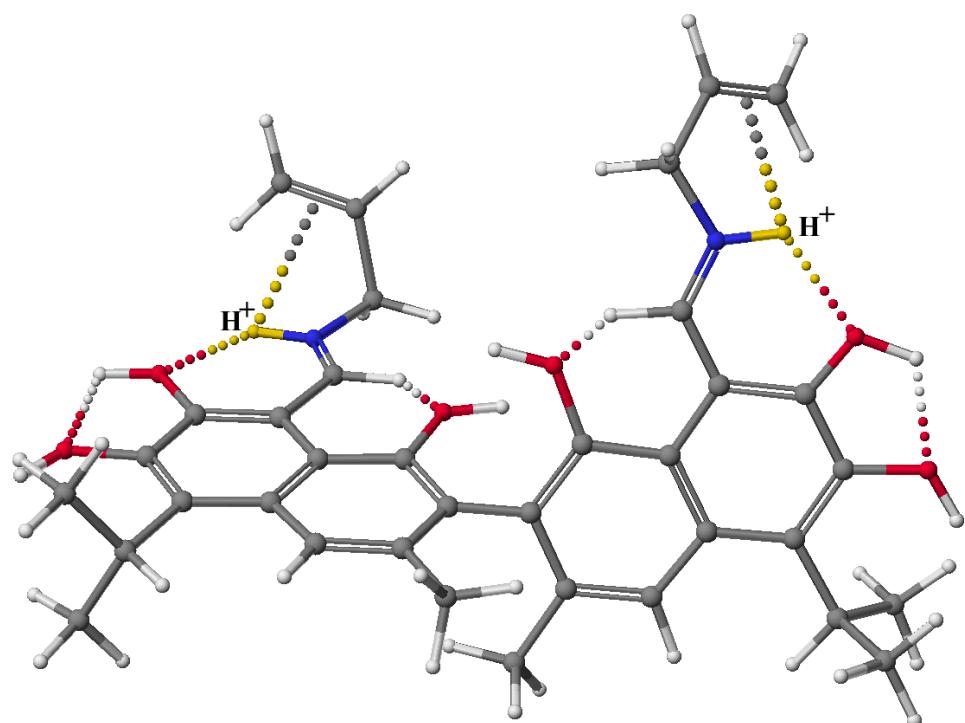
Figure 4S. The lowest-energy structures of the protonated **S1** Schiff base: a) **S2**:H$^+$, b) **S2**:2H$^+$, calculated by the PM5 method at semi-empirical level of theory; the excess protons are marked by yellow.
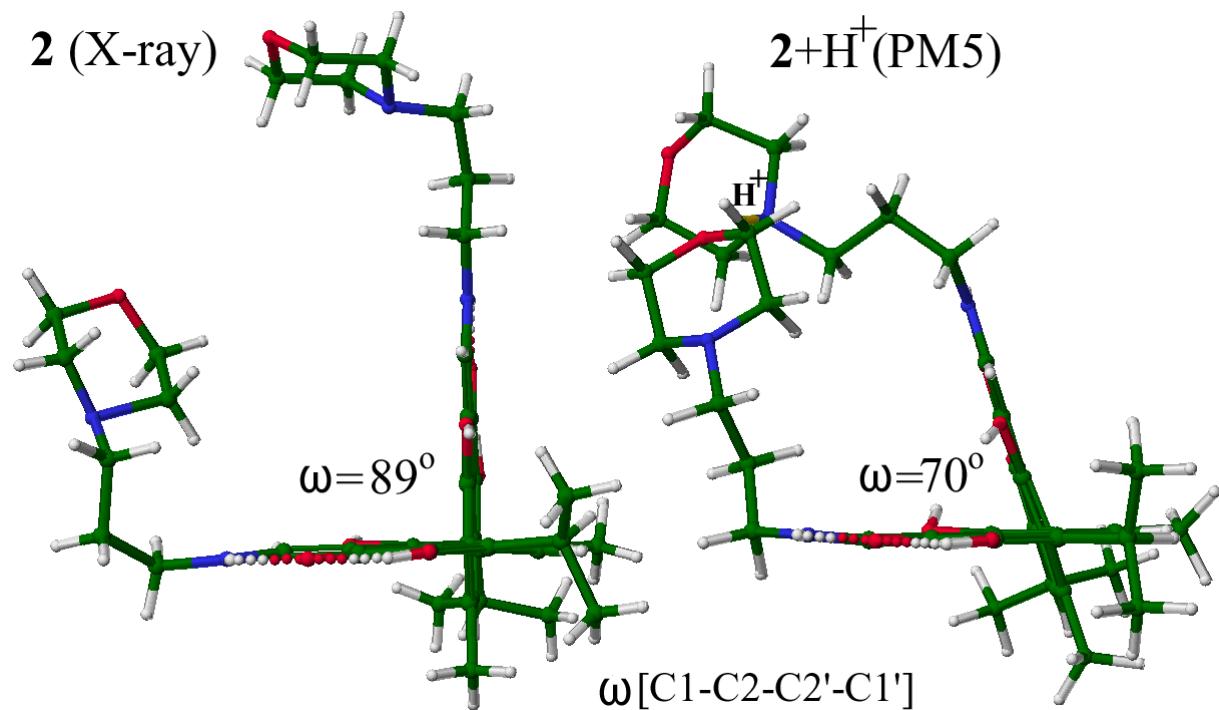
Figure 5S. Comparison of the dihedral angles (ω) found in the structures of unprotonated **S2** (X-ray) and monoprotonated **S2** (PM5).

(a)

(b)
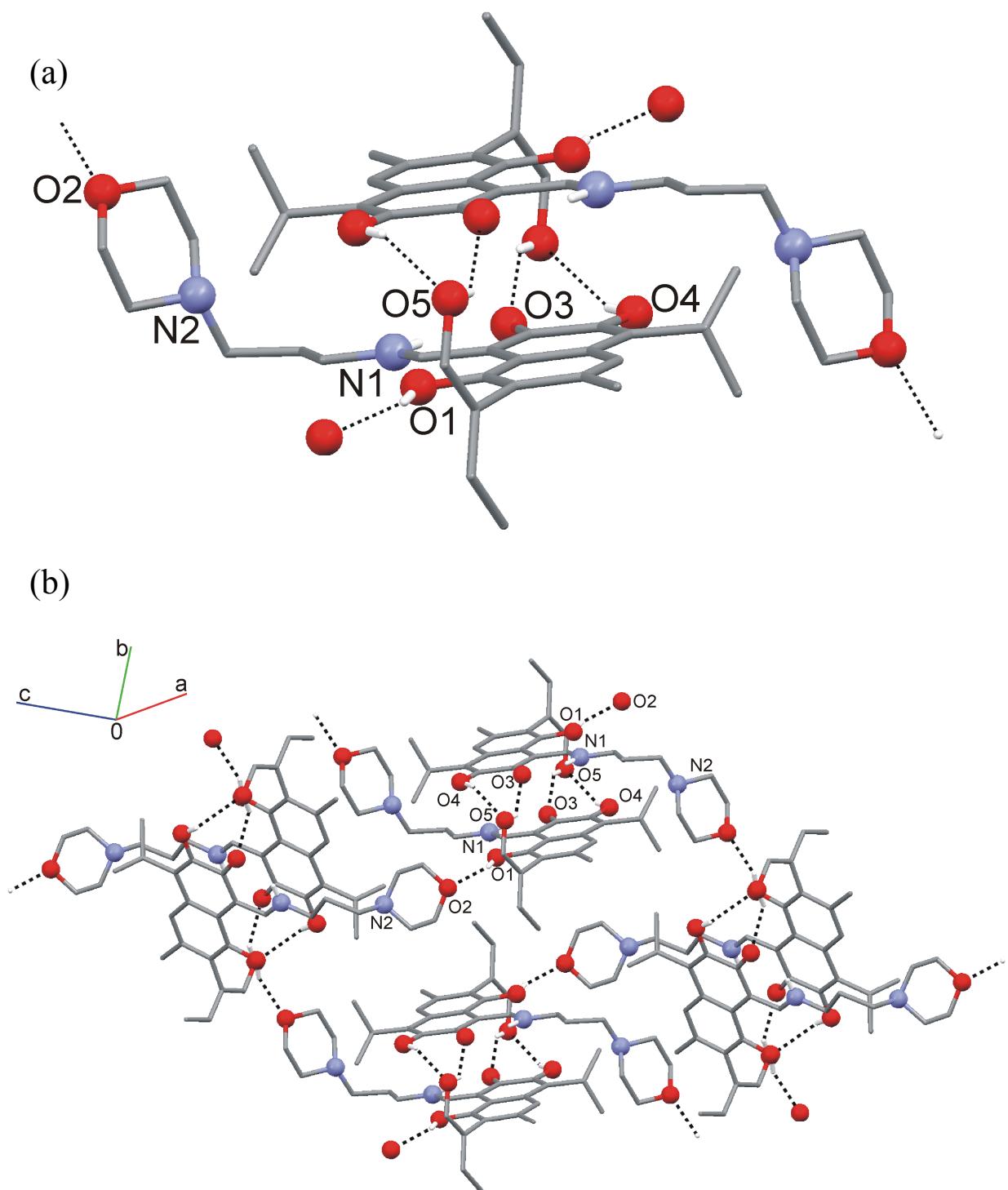
Figure 6S.   (a) Two-dimensional assembly of **S2** molecules *via* hydrogen bonds.

        (b) Centrosymmetric dimer of **S2** formed *via* O_5-H···O_3 hydrogen bonds.

Table 1S. Crystal data and some details of data collection and structure refinement for **S2**.

Empirical formula	$C_{44} H_{58} N_4 O_8$
Formula weight	770.94
Temperature (K)	105 (2)
Wavelength (Å)	1.54184
Crystal system	monoclinic
Space group	C 2/c
Unit cell dimensions	
a (Å)	37.523(3)
b (Å)	11.2668(6)
c (Å)	21.482(2)
β (°)	120.07(1)
Volume (Å^3)	7859.2(9)
Z	8
Density calculated (g/cm^3)	1.303
F(000)	3312
Crystal size (mm)	0.35 x 0.1 x 0.01
θ range for data collection (°)	2.56 – 76.06
Limiting indices	$-44 \le h \ge 45, -12 \le k \ge 13, -25 \le l \ge 22$
Reflection collected/unique	18723/7176
Data/restraints/parameters	7176/0/561
Completeness $\theta = 68.25$ °	99.4 %
Goodness-of-fit on F^2	1.031
Final R indices [I > 2σ(I)]	$R_1 = 0.0486$ w$R_2 = 0.1298$
R indices (all data)	$R_1 = 0.0844$ w$R_2 = 0.1480$
$\Delta_{\rho max}$ and $\Delta_{\rho min}$ (eÅ^{-3})	0.248 and -0.275

Table 2S. Torsion angles (°) describing conformation of the trimethylene linkers and conformation of the morpholine fragments within **S2**.

Torsion angle		Torsion angle	
C22-N1-C31-C32	-179.4 (2)	C27-N3-C38-C39	-149.2 (2)
N1-C31-C32-C33	173.8 (2)	N3-C38-C39-C40	63.8 (3)
C31-C32-C33-N2	-69.0 (3)	C38-C39-C40-N4	68.5 (3)
C34 N2 C33 C32	-85.9(2)	C41 N4 C40 C39	-100.8(3)
C37 N2 C33 C32	153.7(2)	C44 N4 C40 C39	148.0(5)
		C41' N4 C40 C39	-167.1(4)
		C44' N4 C40 C39	81.5(3)
C37 N2 C34 C35	-59.6(3)	C44 N4 C41 C42	-63.5(6)
N2 C34 C35 O2	58.9(3)	N4 C41 C42 O8	58.3(7)
C34 C35 O2 C36	-55.7(3)	C41 C42 O8 C43	-53.7(9)
C35 O2 C36 C37	55.0(3)	C42 O8 C43 C44	53.6(9)
O2 C36 C37 N2	-58.4(3)	O8 C43 C44 N4	-64.3(9)
C36 C37 N2 C34	60.4(3)	C43 C44 N4 C41	64.3(8)
		C44' N4 C41' C42'	-57.2(7)
		N4 C41' C42' O8'	63.4(9)
		C41' C42' O8' C43'	-58.7(9)
		C42' O8' C43' C44'	58.9(7)
		O8' C43' C44' N4	-59.3(6)
		C43' C44' N4 C41'	56.3(5)

Table 3S. Geometrical parameters (Å,°) of inter- or intramolecular hydrogen bonds in the crystal of **S2.**

D-H...A	d(D-H)	d(H…A)	d(D-A)	<(D-H…A)
O_4-H⋯O_3	0.82	2.09	2.575(2)	118
O_6-H⋯O_7	0.82	2.05	2.546(2)	118
N_1-H⋯O_3	0.86	1.89	2.559(2)	133
N_3-H⋯O_7	0.86	1.88	2.550(3)	134
O_1-H⋯$O_2{}^i$	0.82	2.02	2.734(2)	145
O_5-H⋯$O_3{}^{ii}$	0.82	2.06	2.720(2)	137

Symmetry code: (i) -x+1/2, y+1/2, -z+1/2; (ii) -x+1/2, -y+1/2, -z+1.

Table 4S. The statistical parameters of models 1°-4° tested for **S1** and **S2** protonated species.

	Compound **S1**		Compound **S2**			
	model 1°	model 2°	model 1°	model 2°	model 3°	model 4°
Standard deviation in EMF	27.19	9.91	77.26	77.60	28.29	14.48
ΔBIC	0.48	0.4	0.93	0.93	0.5	0.2

EMF – electromotive force. Difference between potential measured and calculated,

ΔBIC- Bayesian Information Criteria. $BIC = n \cdot \log \dfrac{RSS}{n} + k \cdot \log(n)$ and

$\Delta BIC_i = BIC_i - BIC^{\min}$

Table 5S. $\Delta H_f°$ values (kcal/mol) determined for the structures of **S1** and **S2** protonated molecules at different sites considered.

Structure calculated	Stoichiometry	$\Delta H_f°$ (kcal/mol)
S1$_{en-en}$-H$^+$ (A)	**S1**-H$^+$	-1.12
S1$_{im-en}$-H$^+$ (A) _{Fig 4Sa}		-7.89
S1$_{en-en}$-H$^+$ (B)		-4.12
S1$_{im-en}$ -H$^+$ (B)		-5.11
S1$_{en-en}$-H$^+$ (C)		+7.14
S1$_{im-en}$-H$^+$ (C)		+6.52
S1$_{en-en}$-H$^+$ (D)		+5.74
S1$_{im-im}$-H$^+$ (D)		+4.11
S1$_{en-en}$-H$^+$ (G)		-0.17
S1$_{im-en}$-H$^+$ (G)		-0.22
S1$_{en-en}$-2H$^+$ (A, A')	**S1**-2H$^+$	-3.85
S1$_{im-im}$-2H$^+$ (A, A') _{Fig 4Sb}		-12.73
S1$_{en-en}$-2H$^+$ (B, B')		-7.53
S1$_{im-im}$-2H$^+$ (B, B')		-9.11
S1$_{en-en}$-2H$^+$ (C, C')		+10.11
S1$_{im-im}$-2H$^+$ (C, C')		+8.29
S1$_{en-en}$-2H$^+$ (D, D')		+6.73
S1$_{im-im}$-2H$^+$ (D, D')		+6.18
S1$_{en-en}$-2H$^+$ (G, G')		-2.32
S1$_{im-im}$-2H$^+$ (G, G')		-2.54
S2$_{en-en}$-H$^+$ (A)	**S2**-H$^+$	-1.26
S2$_{im-en}$-H$^+$ (A)		-5.73
S2$_{en-en}$-H$^+$ (B)		-3.59
S2$_{im-en}$ -H$^+$ (B)		-4.02
S2$_{en-en}$-H$^+$ (C)		+8.34
S2$_{im-en}$-H$^+$ (C)		+7.92
S2$_{en-en}$-H$^+$ (D)		+5.92
S2$_{im-im}$-H$^+$ (D)		+5.19
S2$_{en-en}$-H$^+$ (E) _{Fig 4a}		-12.42
S2$_{im-en}$-H$^+$ (E)		-10.71
S2$_{en-en}$-H$^+$ (F)		-4.11
S2$_{im-en}$-H$^+$ (F)		-4.86
S2$_{en-en}$-2H$^+$ (A, A')	**S2**-2H$^+$	-2.08
S2$_{im-im}$-2H$^+$ (A, A')		-11.74
S2$_{en-en}$-2H$^+$ (B, B')		-7.35
S2$_{im-im}$-2H$^+$ (B, B')		-9.17
S2$_{en-en}$-2H$^+$ (C, C')		+15.98
S2$_{im-im}$-2H$^+$ (C, C')		+13.84
S2$_{en-en}$-2H$^+$ (D, D')		+13.26
S2$_{im-im}$-2H$^+$ (D, D')		+13.03
S2$_{en-en}$-2H$^+$ (E, E') _{Fig 4b}		-20.03
S2$_{im-im}$-2H$^+$ (E, E')		-16.45
S2$_{en-en}$-2H$^+$ (F, F')		-7.42
S2$_{im-im}$-2H$^+$ (F, F')		-8.06

The sites of protonation considered:
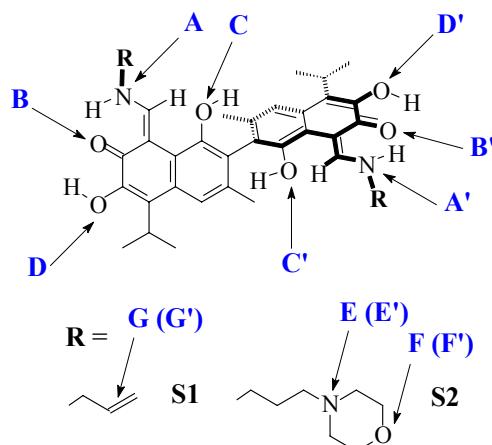
S1$_{en-en}$ - Schiff base in the enamine-enamine tautomeric form, **S1**$_{en}$-$_{im}$ -the Schiff base in the enamine-imine tautomeric form, **S1**$_{im}$-$_{im}$ - Schiff base in the imine-imine tautomeric form; $H_f°$ = standard enthalpy of formation

$\Delta H_f°$= $H_f°_{bonded}$-$H_f°_{non-bonded}$, where $H_f°_{bonded}$ = proton and the Schiff base molecule are bonded to each other; $H_f°_{non-bonded}$ = proton and the Schiff base are distanced to each other above 30Å.

Structure calculated	Stoichiometry	$\Delta H_f°$ (kcal/mol)
$\mathbf{S2}_{en-en}$-3H$^+$ (A, A', D)	$\mathbf{S2}$-3H$^+$	-0.73
$\mathbf{S2}_{im-im}$-3H$^+$ (A, A', D)		-6.59
$\mathbf{S2}_{en-en}$-3H$^+$ (B, B', C)		+3.58
$\mathbf{S2}_{im-im}$-3H$^+$ (B, B', C)		+1.94
$\mathbf{S2}_{en-im}$-3H$^+$ (B, B', C)		+2.44
$\mathbf{S2}_{en-en}$-3H$^+$ (C, C', A)		+11.93
$\mathbf{S2}_{im-im}$-3H$^+$ (C, C', A)		+10.31
$\mathbf{S2}_{en-im}$-3H$^+$ (C, C', A)		+9.98
$\mathbf{S2}_{en-en}$-3H$^+$ (D, D', A)		+10.27
$\mathbf{S2}_{im-im}$-3H$^+$ (D, D', A)		+9.25
$\mathbf{S2}_{en-im}$-3H$^+$ (D, D', A)		+7.14
$\mathbf{S2}_{en-en}$-3H$^+$ (E, E', A)		-24.81
$\mathbf{S2}_{im-im}$-3H$^+$ (E, E', A)		-25.15
$\mathbf{S2}_{im-en}$-3H$^+$ (E, E', A) Fig 4c		-29.82
$\mathbf{S2}_{en-en}$-3H$^+$ (F, F', A)		-10.73
$\mathbf{S2}_{im-im}$-3H$^+$ (F, F', A)		-10.21
$\mathbf{S2}_{im-en}$-3H$^+$ (F, F', A)		-11.59
$\mathbf{S2}_{en-en}$-4H$^+$ (A, A', D, D')	$\mathbf{S2}$-4H$^+$	+6.98
$\mathbf{S2}_{im-en}$-4H$^+$ (A, A', D, D')		+11.42
$\mathbf{S2}_{im-im}$-4H$^+$ (A, A', D, D')		+2.16
$\mathbf{S2}_{en-en}$-4H$^+$ (B, B', C, C')		+13.72
$\mathbf{S2}_{im-en}$-4H$^+$ (B, B', C, C')		+8.21
$\mathbf{S2}_{im-im}$-4H$^+$ (B, B', C, C')		+7.91
$\mathbf{S2}_{en-en}$-4H$^+$ (B, B', D, D')		+7.02
$\mathbf{S2}_{im-en}$-4H$^+$ (B, B', D, D')		+4.41
$\mathbf{S2}_{im-im}$-4H$^+$ (B, B', D, D')		+2.52
$\mathbf{S2}_{en-en}$-4H$^+$(E, E',D, D')		-7.12
$\mathbf{S2}_{im-en}$-4H$^+$(E, E',D, D')		-8.34
$\mathbf{S2}_{im-im}$-4H$^+$(E, E',D, D')		-9.51
$\mathbf{S2}_{en-en}$-4H$^+$ (C, C', A, A')		+9.42
$\mathbf{S2}_{im-en}$-4H$^+$ (C, C', A, A')		+4.21
$\mathbf{S2}_{im-im}$-4H$^+$ (C, C', A, A')		-1.67
$\mathbf{S2}_{en-en}$-4H$^+$ (D, D', A, A')		+7.14
$\mathbf{S2}_{im-en}$-4H$^+$ (D, D', A, A')		+2.43
$\mathbf{S2}_{im-im}$-4H$^+$ (D, D', A, A')		-3.86
$\mathbf{S2}_{en-en}$-4H$^+$(E, E',A, A')		-26.74
$\mathbf{S2}_{im-en}$-4H$^+$(E, E',A, A')		-33.32
$\mathbf{S2}_{im-im}$-4H$^+$(E, E',A, A')Fig 4d		-38.15
$\mathbf{S2}_{en-en}$-4H$^+$ (F, F', A, A')		-14.73
$\mathbf{S2}_{im-en}$-4H$^+$ (F, F', A, A')		-16.23
$\mathbf{S2}_{im-en}$-4H$^+$ (F, F', A, A')		-19.98