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X-ray crystal structure of a dimethylene sulfone-bridged ribonucleotide dimer in a single-stranded state

Birgitte Hyrup[+], Clemens Richert[§], Thomas Schulte-Herbrüggen[1], Steven A. Benner and Martin Egli*

Department of Chemistry, Swiss Federal Institute of Technology, CH-8092 Zürich, Switzerland and [1]Laboratory of Physical Chemistry, Swiss Federal Institute of Technology, CH-8092 Zürich, Switzerland

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ABSTRACT

A crystal structure has been solved for an analog of the r(ApU) ribodinucleotide, r(A_{so_2}U), where a bridging non-ionic dimethylene sulfone linker replaces the phosphodiester linking group found in natural RNA. Crystals of the single-stranded state of r(A_{so_2}U) were obtained from water at 50°C. In these crystals, one hydrogen bond is formed between bases from different strands and base stacking occurs in intermolecular 'homo-A' and 'homo-U' stacks. Similar to typical oligoribonucleotides, the ribose rings adopt N-type conformations and dihedral angles χ are in the *anti* range. The all-*trans* rotamer of the CH_2-SO_2-CH_2-CH_2 bridge was found, which leads to a large adenine–uracil distance. Qualitative analysis of a NOESY spectrum of the A_{so_2}U part in r($U_{so_2}C_{so_2}A_{so_2}$U) dissolved in a dimethylsulfoxide–D_2O mixture indicates that the conformation observed in the crystal is also populated in solution. Comparison with the structure of r(G_{so_2}C), which has been crystallized in the Watson–Crick paired state, shows that a rotation around ζ by +112° leads from the observed, single-stranded state to a conformation that is compatible with formation of a duplex. A concerted *trans/gauche* flip of α and γ then yields the standard conformer of A-type RNA helices. From the observed structure of r(G_{so_2}C) and other oligonucleotides it is anticipated that this flip will also revert the ribose pucker from C2′-*exo* to C3′-*endo*.

INTRODUCTION

Analogs of oligonucleotides have attracted considerable attention in recent years, in part because they have potential use as antisense agents directed at DNA and RNA targets (1–3). A large number of derivatives have been prepared where backbone atoms of oligonucleotides (mostly DNA) have been replaced to yield uncharged, non-hydrolizable analogs (4). Structural information on non-ionic oligonucleotides is rare, however, despite the fact that a few of them (5,6) have been reported to bind more strongly to complementary strands than natural oligonucleotides.

We have been exploring the dimethylene sulfone unit as a replacement for the natural phosphodiester linker in deoxyoligonucleotides (7). This linkage is dipolar, yet non-ionic, consistent with solubility both in aqueous and lipid phases. It contains no stereogenic atoms, permitting oligonucleotide analogs containing the linker to be prepared without concern for diastereoisomerism, and is stable to degradation under acidic, basic and enzymatic conditions. Most importantly, removing the charges from oligonucleotides through a largely isosteric replacement offers new insight into the molecular recognition properties of natural nucleic acids.

Our recently developed streamlined synthesis of oligoribonucleotide analogs with bridging dimethylenesulfone groups (8) provided us with an entré to the structural chemistry of close analogs of RNA. The exploration of this chemistry appeared especially interesting to us, first, because RNA is known to be less conformationally flexible than DNA and, second, because RNA analogs have recently attracted increasing attention as antisense agents (9a,b), in part due to the increased stability of RNA–RNA duplexes compared with RNA–DNA duplexes (9c). In the course of our studies the self-complementary r(G_{so_2}C) dimer crystallized at room temperature was found to form a Watson–Crick duplex (10). We have now succeeded in trapping a single-stranded state of the analogous r(A_{so_2}U) dimer **1** by crystallization at elevated temperature. Further information about single-stranded conformations of sulfone-bridged RNA was gained from NMR data of r($U_{so_2}C_{so_2}A_{so_2}$U) tetramer **3** dissolved in an aqueous solution with a high percentage of dimethylsulfoxide. The investigation of the conformational differences between these structures offers insight into molecular movements that most probably occur when these non-ionic oligonucleotides hybridize to complementary strands.

MATERIALS AND METHODS

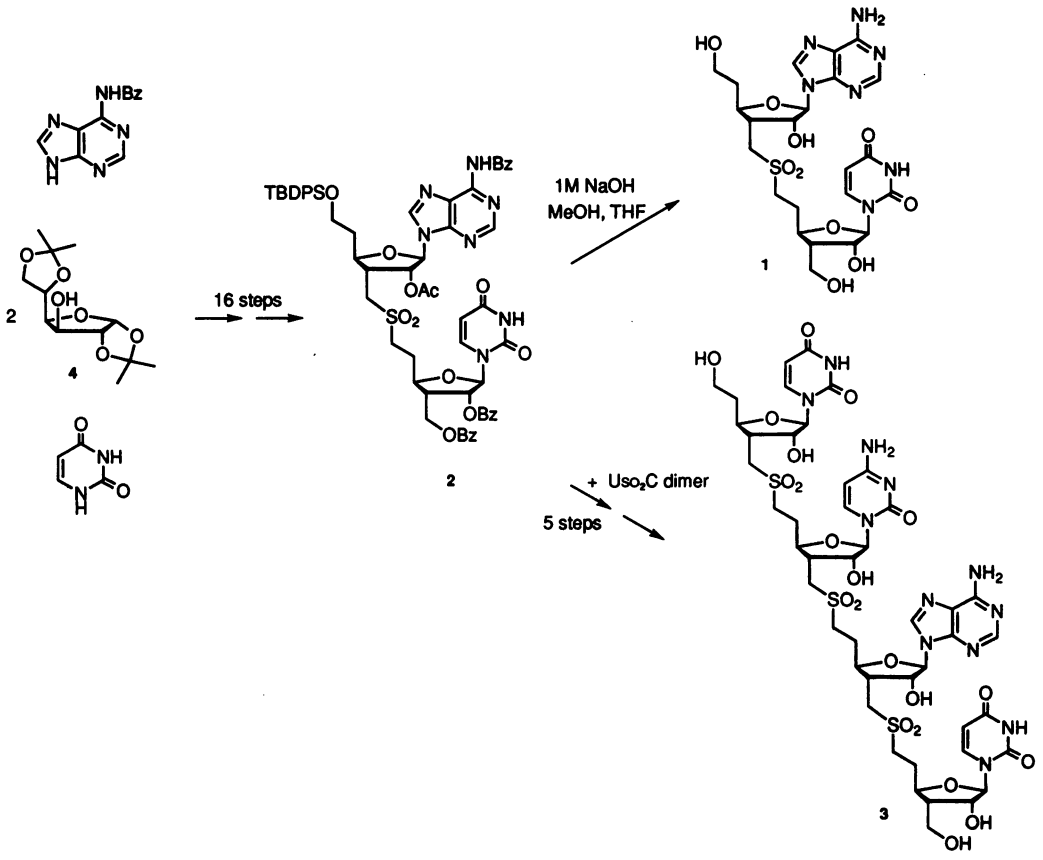
General

NMR spectra were recorded on a Varian, Gemini 300 spectrometer (300 MHz) and on a Bruker AMX 600 spectrometer (600 MHz). The residual proton signal from the deuterated solvent was used as an internal standard. Assignments of the

* To whom correspondence should be addressed

[+]Permanent address: University of Copenhagen, The H.C.Ørsted Institute, Universitetsparken 5, 2100 Copenhagen Ø, Denmark

[§]Present address: Department of Chemistry, Tufts University, Medford, MA 02155, USA



Scheme 1.

dimer spectrum in CD_3CN/D_2O were based on a ^1H–^1H COSY experiment. The fast-atom bombardment (FAB) mass spectrum was obtained on a VG ZAB2-SEQ mass spectrometer. The UV spectrum was recorded on a Shimadzu UV/VIS 240 spectrophotometer. Tetrahydrofuran was distilled from sodium; acetonitrile for HPLC was obtained from Merck.

Synthesis of r(ASO$_2$U) (1)

Protected dimer **2** (58.1 mg, 48.8 μmol) (8) was dissolved in a mixture of methanol (6.8 ml) and tetrahydrofuran (4.6 ml) and a solution of sodium hydroxide (1 M, 6.8 ml) was added (Scheme 1). The solution turned yellow upon addition of base but became colorless a few minutes later. The mixture was heated to 40°C in an oil bath; the reaction was followed by analytical HPLC (Nucleosil 100-5 RP-18, 125 × 4 mm, flow rate 0.8 ml/min, λ_{det} 260 nm, gradient 8% acetonitrile in water, isocratic for 10 min, then to 35% acetonitrile over 15 min and to 60% acetonitrile over 15 min; the retention time of the product was 18 min). After 7 h, acetate buffer (3.4 ml, 3 M acetic acid, 1 M sodium acetate) was added and the methanol and tetrahydrofuran removed *in vacuo*. The resulting suspension was diluted with water (20 ml) and extracted with methylene chloride (5 × 15 ml). Throughout the extraction, a small amount of undissolved material remained. The combined organic phases were extracted with water (50 ml) and the aqueous phases were evaporated to dryness. The resulting solid was dissolved in 7% aqueous acetonitrile (50 ml; the mixture was heated to ~60°C for a short period, then cooled to room temperature; undissolved material was removed by filtration) and purified by preparative HPLC (LiChrospher 100 RP-18, 7 μm, 250 × 50 mm, flow rate 80 ml/min, λ_{det} 260 nm, 9 ml injected per run), eluting with 7% acetonitrile in water (isocratic for 15 min) and then increasing the acetonitrile fraction to 34% over 15 min. The product had a retention time of 25.5 min. The only other detectable product eluted after 6.5 min; this material was identified as benzoic acid by NMR. Dimer **1** (17.7 mg, 61%) was isolated as a white solid.

^1H-NMR (*s*, singlet; *d*, doublet; *t*, triplet; *m*, multiplet; *J*, coupling constant in Hz). (CD_3CN/D_2O, 7:1, 300 MHz, 297 K) δ (p.p.m.) 1.78–1.89 (*m*, 1H, H-5′-A); 1.96–2.10 (*m*, 3H, H-5′-A, H-5′-U, H-3′-U); 2.21–2.34 (*m*, 1H, H-5′-U); 2.69–2.79 (*m*, 1H, H-3′-A); 3.18 (*dd*, 1H, *J* = 3.2, 15.1 Hz, H-3″-A); 3.21–3.42 (*m*, 2H, 2H-6′-U); 3.53–3.79 (*m*, 5H, H-3″-A, 2H-6′-A, 2H-3″-U); 4.10 (*dt*, 1H, *J* = 2.4, 9.3 Hz, H-4′-U); 4.15 (*dt*, 1H, *J* = 2.2, 9.5 Hz, H-4′-A); 4.35 (*dd*, 1H, *J* = 1.6, 5.7 Hz, H-2′-U); 4.71 (*d*, 1H, *J* = 5.0 Hz, H-2′-A); 5.59 (*d*, 1H, *J* = 1.6 Hz, H-1′-U); 5.66 (*d*, 1H, *J* = 7.7 Hz, H-5-U); 5.97 (*s*, 1H, H-1′-A); 7.45 (*d*, 1H, *J* = 8.1 Hz, H-6-U); 8.09, 8.16 (2*s*, 2H, H-8-A, H-2-A).

(D_2O, 300 MHz, 310 K, main set of resonances; a second much smaller set was detected) δ (p.p.m.) 1.96–2.08 (*m*, 1H); 2.16–2.36 (*m*, 3H); 2.43–2.57 (*m*, 1H); 2.94–3.05 (*m*, 1H); 3.52 (*dd*, 1H, *J* ≈ 3, 15 Hz); 3.57–4.02 (*m*, 7H); 4.32 (*dt*, 1H, *J* = 2.0, 10.0 Hz); 4.41 (*dt*, 1H, *J* = 2.3, 9.9 Hz); 4.60 (*d*, 1H, *J* = 6.0 Hz); 5.12 (*d*, 1H, *J* = 5.3 Hz); 5.76 (*d*, 1H, *J* = 7.8 Hz); 5.81 (*s*, 1H); 6.21 (*s*, 1H); 7.51 (*d*, 1H, *J* = 8.1 Hz); 8.24, 8.26 (2*s*, 2H).

FAB MS. (glycerol matrix), $C_{23}H_{31}N_7O_{10}S$, *m/z* 598 ($M + H^+$).

UV. (H_2O, qualitative) λ_{max} (nm) 260; 205 (shoulder).

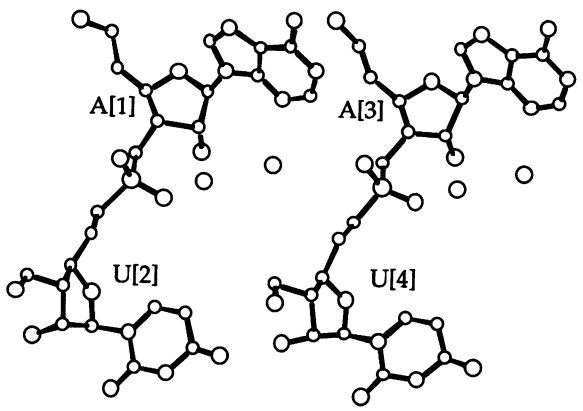


Figure 1. Crystal structure of **1**. Overall view of the two molecules forming the crystallographic asymmetric unit. Water molecules are isolated spheres and nucleosides are numbered.

Crystallization and X-ray analysis

A saturated solution of dimer **1** in water was prepared at 66°C and placed in a small sealed tube. The temperature was lowered in 1.5°/day steps over 12 days. Crystal growth was first observed between 50 and 48°C.

A crystal (0.6 × 0.2 × 0.2 mm) was sealed in a glass capillary with a droplet of mother liquor and mounted on a four-circle diffractometer (Enraf-Nonius CAD4, CuK_α). The space group is monoclinic $P2_1$ with unit cell dimensions a = 5.437(1) Å, b = 28.253(3) Å, c = 18.623(2) Å, β = 92.32(1)°, V = 2858.6(7) Å^3 and Z = 4. The intensity pattern pointed to a higher symmetry and was compatible with space group orthorhombic $C222_1$. However, the corresponding cell transformation led to a unit cell with two angles clearly deviating from 90°. Thus the crystal lattice displays strong pseudosymmetry and the conformations of the two independent molecules per asymmetric unit are consequently almost identical. A total of 4236 unique reflections were measured to a resolution of 0.9 Å by the ω scan method and 2118 reflections were observed above the 4σ(F_o) level. No decay was observed during the measurement and data were corrected for Lorentz and polarization effects.

The structure was solved by direct methods using SHELXS-86 (11) and refined with SHELXL-93 (12). The conjugate gradient option was used during the initial refinement cycles and bases were restrained to planarity. The positions of water molecules were determined from difference electron density maps. All atoms were refined anisotropically, and the positions of hydrogen atoms were calculated and refined with the riding option. Refinement was terminated at an R factor of 3.4% (unit weights) for all data with $F_o \geq 4\sigma(F_o)$. The final asymmetric unit contains 86 heavy atoms including four solvent molecules (Fig. 1). The coordinates will be deposited in the Cambridge Structural Database.

NMR of r(USO$_2$CSO$_2$ASO$_2$U)

The synthesis of UCAU tetramer **3** (Scheme 1) is described elsewhere (8). HPLC purification was performed on a Macherey-Nagel (Düren, Germany) Nucleosil 10-CN column (250 × 22.5 mm) with a gradient of acetonitrile (85%) in water of 10–35% over 30 min. Retention time of the product **3** was 19 min.

For NMR spectroscopy a 7 mM solution (0.5 ml) of **3** in DMSO-d_6–D_2O (3.5:1) was prepared and COSY, NOESY, ROESY and HMQC spectra were recorded (600 MHz) at 300 K. The NOESY spectrum (Fig. 2) was acquired with a 100 ms mixing time. The residual solvent peak (HDO) was suppressed by 1.5 s low power (70 dB) presaturation; 1024 FIDs (à 32 scans) consisting of 4000 data points were recorded and zero-filled to 2000 (t_1) and 4000 (t_2). Peak assignment was achieved from analysis of the COSY spectrum and NOESY cross-peaks from H-6 of pyrimidines to H-5′ and H-3′ backbone resonances. A full description of the assignment and structure analysis will be published separately.

RESULTS

The dimethylene sulfone-linked r(ASO$_2$U) dimer **1** was obtained from its protected precursor **2** by alkaline hydrolysis at 40°C in 61% yield (Scheme 1). The convergent synthesis of **2**, which was obtained in 16 steps from diacetone glucose **4**, *N*6-benzoyl-adenine (13) and uracil, is described elsewhere (8). HPLC purification was performed at high dilution (~110 µM average concentration in collected solutions) to avoid association.

Crystallization occurred at 50–48°C while slowly cooling an aqueous solution of **1** saturated at 66°C. Several crystals grew rapidly and to sufficient size under these conditions, whereas at room temperature or 4°C only very small or bent crystals were obtained. These markedly changed crystallization properties at lower temperatures may be due to the transition to the duplex state.

The asymmetric unit of the crystal examined was found to harbor two r(ASO$_2$U) units with almost identical conformations, along with four water molecules (Fig. 1). Since the differences in the conformations of the two molecules are only very minor, their structural features will be discussed together. The backbone of the dimethylene sulfone-bridged dimer is in an all-*trans* conformation with the three angles ζ, α and γ differing significantly from those of standard A-RNA (Table 1, see explanatory figure for definition of angles). This leads to an extension of the intra-strand base distance to ~10 Å, compared with the 3.4 Å stacking distance in A-RNA. The conformation of the sugar rings in **1** is of the C2′-*exo* (N) type (Table 1). The dihedral angle defined by the planes of the two bases in the dimer is ~130°. However, the orientation about the glycosyl bond is in the *anti* range with both nucleosides.

The bond distances in the bases and in the furanose rings are all in the expected ranges compared with natural oligonucleotides. The backbone C–S bonds (1.70–1.81 Å) are longer than the corresponding phosphodiester P–O bonds in the natural dinucleotides (~1.6 Å). Further, the C3′–C3″ and the C5′–C6′ bonds are slightly longer (~0.1 Å) than the corresponding C3′–O3′ and C5′–O5′ bonds in RNA. These differences in bond lengths might influence the helical parameters of a potential duplex, but do not prevent hybridization, as shown by the r(GSO$_2$C) duplex (10).

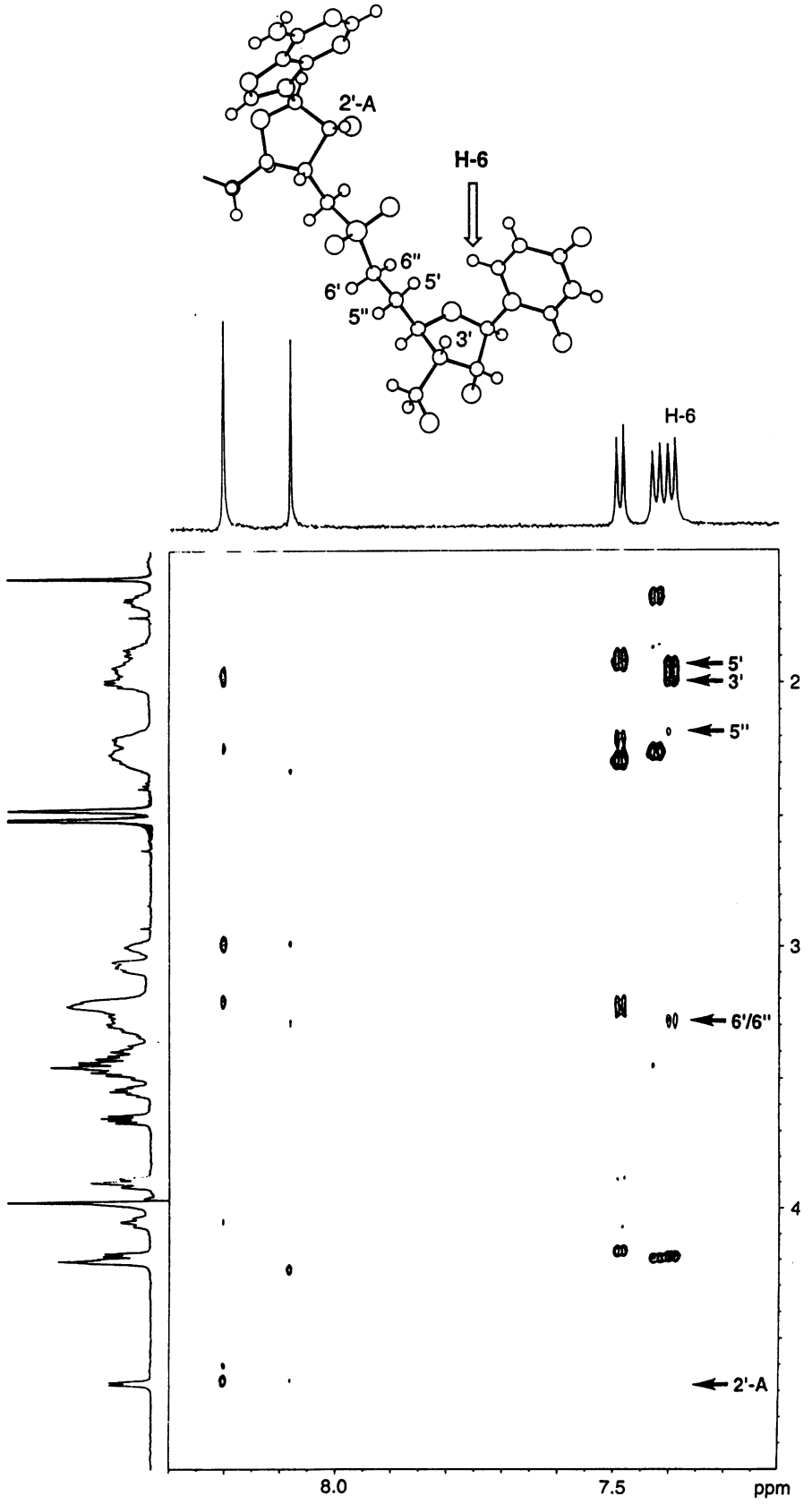


Figure 2. Expansion of a region of nucleobase resonances of the NOESY spectrum of r($Uso_2Cso_2Aso_2U$) tetramer **3** in DMSO–D_2O (3.5:1, 300 K, 600 MHz), mixing time 100 ms. Cross-peaks (or their absence) between U-H-6 of the terminal Aso_2U fragment and selected protons are indicated by arrows. The structure drawing in the top part was taken from the X-ray structure of **1**.

Table 1. Backbone and glycosidic torsion angles (in degrees) in the r(ASo$_2$U) dimer **1**, the r(GSo$_2$C) duplex structure (10) and in r(ApU) (14)

Dimer	Residue	α	β	γ	δ	ε	ξ	χ	*P*[a]
r(ASo$_2$U) (**1**)	A1			171	87	−147	169	−172	−3
	U2	−177	−166	−177	91			−175	−16
	A3			175	85	−158	−175	−165	−10
	U4	−158	−164	174	86			−167	−19
r(GSo$_2$C)	G1			176	93	−140	−66	−178	12
	C2	−58	−179	44	86			−170	29
r(ApU)	Strand 1	−72	177	57	79[b]	−147	−67	−175[b]	12[b]
	Strand 2	−65	168	58	78[b]	−139	−76	−151[b]	22[b]

[a]Pseudorotation phase angle (18,19).
[b]Mean value for the two ribose units.

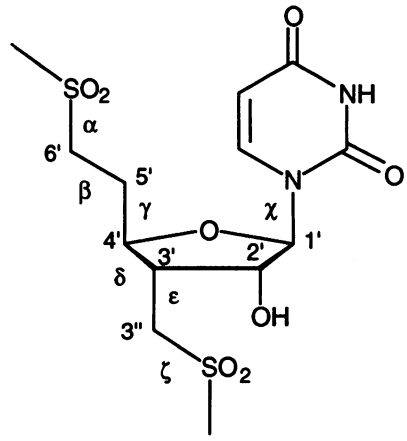


Table 2. Observed hydrogen bonds in the crystal structure of **1**

Residue	Hydrogen bond[a]	Distance (Å)
Adenosine [1]	N1–O5′[c] A[3][b]	2.72
	N3–H_2O W[1]	2.95
	N6–O4 U[4][b]	2.84
	N6–O2′ U[2][b]	3.18
	N7–O2′ U[2][b]	2.67
	O5′–N1 A[3][b]	2.82
	O5′–N3 U[2][b]	2.79
	O2′–H_2O W[1]	2.84
	O2′–H_2O W[3]	2.92
Uridine [2]	O2S[d]–H_2O W[3][b]	3.31
	O3′–H_2O W[4][b]	2.86
	O3′–O4 U[4][b]	2.83
	O2–C8 A[1][b]	3.08
	O4–N6 A[3][b]	2.90
	O4–O3′ U[4]	2.91
Adenosine [3]	N3–H_2O W[2]	2.91
	N6–O2′ U[4][b]	3.22
	N7–O2′ U[4][b]	2.66
	O5′–N3 U[4][b]	2.89
	O2′–H_2O W[2]	2.84
	O2′–H_2O W[4]	2.67
Uridine [4]	O2S–H_2O W[4][b]	3.43
	O3′–H_2O W[3][b]	2.66
	O2–C8 A[3][b]	3.02

[a]Residue numbers in brackets.
[b]A symmetry related nucleoside.
[c]terminal hydroxyl oxygens are termed 05′ and 03′.
[d]the SO_2 oxygens are termed 01S and 02S.

In the crystal no pairing between bases involving more than one hydrogen bond is observed. Adenine and uracil rings are integrated in 'homo-A' and 'homo-U' stacks situated next to each other, with direct interactions between the adenine and uracil bases from adjacent stacks (Table 2). N6 of adenine is hydrogen bonded to O4 of uracil (2.84 Å and 2.90 Å). In addition, a non-classical hydrogen bond (3.08 Å and 3.02 Å) between C8 of the same adenine base and O2 of a second uracil is suggested. The water molecules form hydrogen bonds to backbone and base atoms (Table 2) and the interaction modes are similar for the two intedpendent r(ASo$_2$U) molecules. One water molecule is situated between the 2′-hydroxyl group and nitrogen N3 of adenosine, whereas the second water molecule forms a weak hydrogen bond to one of the two SO_2 oxygens of the bridge. The second oxygen of the sulfone moiety is surrounded by hydrophobic groups only. A full list of observed hydrogen bonds in the crystal lattice (upper distance limit 3.5 Å) is given in Table 2.

The analysis of selected distance information gained from the NOESY spectrum of the r(USo$_2$CSo$_2$ASo$_2$U) tetramer **3** dissolved in a DMSO-D_2O mixture at 27°C (Fig. 2) shows conformational characteristics in the terminal ASo$_2$U part that are similar to the high temperature crystal structure of dimer **1** (Table 3). The absence of an H-6-U/H-2′-A NOE indicates an extended backbone. Much stronger intranucleoside NOESY cross-peaks between uracil H-6 and H-5′ compared with uracil H-6 and H-6′/H-6″ point to a *trans* conformation for γ. Furthermore, strong cross-peaks of H-6 (pyrimidines), H-8 (adenine) and H-3′ of riboses show that χ is preferentially *anti* for all nucleoside units in the tetramer. Similarities in the chemical shift and signal multiplicities of the DMSO–D_2O spectrum of **3** and the ^{1}H-NMR spectrum of **1** in D_2O at 37°C suggest a strong contribution of extended structures to the conformational equilibrium of **1** under physiological conditions.

Table 3. Comparison of U-H6 distances (Å) to selected protons in the X-ray structures of the r(A_{so_2}U) dimer **1**, in the r(G_{so_2}C) duplex (10) and in the A_{so_2}U part of the NMR structure of r($U_{so_2}C_{so_2}A_{so_2}$U) tetramer **3** in DMSO–D_2O (3.5:1, 300 K)

Structure	Intranucleoside distance from pyrimidine H6					
	H-3′	H-5′	H-5″	H-6′	H-6″	H-2′–Pu[a]
r(A_{so_2}U) (**1**)	3.1	2.2	3.7	4.5	3.4	6.3
r(G_{so_2}C)	2.8	4.0	4.6	2.2	3.1	3.3
r(A_{so_2}U) in **3**	2.5–3.5	2.5–3.5	3.5–4.5	>4.5[b,c]	3.5–4.5[b]	>4.5[c]

NMR distances were estimated by classifying NOESY cross-peaks (Fig. 2) as strong (r_{ij} = 1.8–2.5 Å), medium (r_{ij} = 2.5–3.5 Å), weak (r_{ij} = 3.5–4.5 Å).
[a]Adjacent purine nucleoside.
[b]No assignment of the diastereotopic protons was attempted.
[c]No NOESY cross-peak observed.

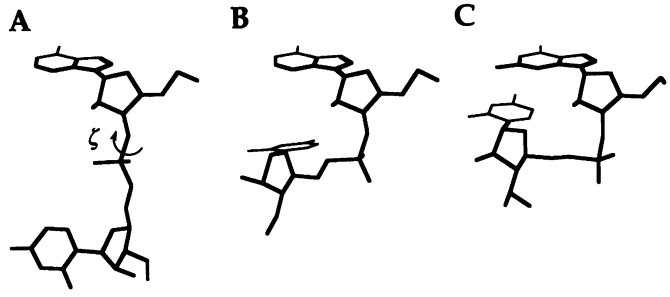


Figure 3. Comparison of the conformations of r(A_{so_2}U) **1** (**A**) (molecule A[1]–U[2] as observed in the crystal structure), modeled **1** (**B**) (crystal structure with ζ rotated by +112°) and r(G_{so_2}C) (**C**) [as observed in the duplex (10)].

DISCUSSION

The structures obtained in this work can be compared with two other structures, those of the r(G_{so_2}C) derivative crystallized at room temperature (10) and of the natural r(ApU) dimer crystallized as the sodium salt at 4°C (14). Both of these form Watson–Crick duplexes giving helix fragments that are approximately of the A type. In contrast, r(A_{so_2}U) crystallized at 50°C forms a single-stranded structure. It is intriguing to suggest that the single-stranded structure (instead of the Watson–Crick duplex) is seen in the r(A_{so_2}U) crystal because of the high temperature of crystallization. The fact that crystals with a different macroscopic appearance are formed when r(A_{so_2}U) is crystallized at lower temperatures is consistent with this hypothesis. Further evidence comes from the conformation found in solution in a denaturing solvent mixture. In this solvent the conformational characteristics of r(A_{so_2}U) incorporated in the non-complementary r($U_{so_2}C_{so_2}A_{so_2}$U) tetramer (**3**) resemble those of the high temperature X-ray crystal structure in water (Fig. 2 and Table 3). There also appears to be some conformational flexibility of the type needed to form a stacked Watson–Crick base pairing, as observed in the [r(G_{so_2}C)]$_2$ duplex. For example, moderately intense NOESY cross-peaks are observed between the base protons (H-8-A and H-6-C) and the H-6′/6″ backbone protons with the internal bases in the tetramer. This suggest that γ is also to some extent *gauche*.

The crystal structure of **1** also suggests that the sulfone substitution has had the expected effect on lipophilicity. The more lipophilic nature of the backbone is reflected in a reduced hydration [two water molecules per r(A_{so_2}U) compared with six in r(ApU); 14]. The findings that only one of the sulfone oxygens per dimer is involved in hydrogen bonding and that the other displays hydrophobic contacts are taken as an indication for both hydrophobic and hydrophilic properties of the sulfone functionality. Hence, it may have the necessary characteristics for solubility in water and membranes and therefore facilitate cellular uptake. The extensive hydration of the 2′-hydroxyl groups in the case of the r(A_{so_2}U) dimer indicates that this functional group contributes significantly to the increased solubility of the RNA sulfones (8) compared with their DNA counterparts (7).

The availability of crystal structures of a sulfone analog of RNA crystallized at high temperature as an unpaired single strand r(A_{so_2}U) and at room temperature as a Watson–Crick duplex [r(G_{so_2}C)]$_2$ (10) allows one to infer the effects of duplex formation on the conformation of the extended backbone. The most striking difference between the single strand formed by r(A_{so_2}U) and the Watson–Crick duplex formed by r(G_{so_2}C) is the backbone torsion angles, which are rotated to *trans* conformations in the single strand (Table 1), as expected from a model that minimizes steric interactions. Thus only β and ε adopt their typical A-RNA conformations in the single-stranded arrangement, but α, γ and ζ are changed compared with the r(G_{so_2}C) and natural r(ApU) duplex structures.

The second significant difference between r(A_{so_2}U) and [r(G_{so_2}C)]$_2$ lies in the sugar puckers of the uncomplexed and complexed sulfones. In the single-stranded r(A_{so_2}U) dimer the average pseudorotation angle is changed by ~30° towards the 2′-*exo* conformation when compared with the [r(G_{so_2}C)]$_2$ duplex. Interestingly, several nucleotides in oligonucleotide structures display this 2′-*exo* pucker mode when γ is *trans* (Table 4) (15–17). In close analogs or different crystal forms, however, where γ adopts a different torsion angle, the sugar rings show the expected 3′-*endo* pucker (Table 4). A comparatively short H-3′ to H-5′ distance in the γ *trans* structures (2.35 Å for U2 in **1**) leads us to the assumption that a steric interaction between H-3′ and H-5′ is responsible for this phenomenon.

Table 4. Comparison of torsion angle γ and furanose ring pucker of selected nucleosides in X-ray structures of modified and natural oligonucleotides

Structure	Sugar type	γ (degrees)	Sugar pucker (*P*)[a]	Reference
U(2) in r(A_{so_2}U) (1)	Ribo	–177	C2′-*exo* (–16)	This work
C(2) in [r(G_{so_2}C)]$_2$	Ribo	44	C3′-*endo* (29)	(10)
G(13) in [r(C_4G_4)]$_2$ rhombohedral	Ribo	178	C2′-*exo* (–16)	(15)
G(13) in [r(C_4G_4)]$_2$ hexagonal	Ribo	22	C3′-*endo* (34)	(15)
G(5) in [d(GCCCG*p*GGC)]$_2$[b]	2′-Deoxy	–179	C2′-*exo* (–8)	(16)
G(5) in [d(GCCCGGGC)]$_2$	2′-Deoxy	143	C3′-*endo* (16)	(17)

[a]Pseudorotation phase angle (18,19).
[b]The letter *p* indicates a 3′-methylene phosphonate linkage.

Considering the large differences in α, γ and ζ in the single-stranded sulfone compared with the duplex, it is interesting how simple it is to transform one conformation into the other. A single rotation around ζ by +112° in the r(ASO_2U) dimer yields a conformation where the bases could pair in a Watson–Crick sense, which is seen in the duplex-forming r(GSO_2C) (Fig. 3). Only minor adjustments in α and γ are needed to get to a base-stacked 'ready for binding' conformation. A concerted α, γ *trans* to *gauche* flip that leads to the A-RNA 'standard' binding conformation is conceivable. The rotation about ζ does not seem to be restricted by any detectable barrier greater than the CH–SO eclipsed maximum. Thus in solution these conformations should be easily accessible at room temperature.

Our results allay concerns that the atomic substitutions in the sulfone backbone may prevent the formation of a Watson–Crick-type double helix with a complementary strand. Further, conclusions can be drawn that may be valuable for the design of similar RNA analogs. First, replacement of the 3′ and 5′ oxygen atoms by CH_2 groups, which do not exert a stereoelectronic effect, does not abolish the preference for N-type conformers in the ribose ring. Second, the transition from the unpaired to the duplex state seems to be critically influenced by the conformational state of ζ and its fixation may be an entropic advantage for hybridization. The latter conclusion is confirmed by the reported single-strand (20) and duplex structures (21) of self-complementary, methylphosphonate-linked deoxynucleotide dimers, where ζ adopted the unusual value of 117.8° in the unpaired state (20). The two dimethylene sulfone-bridged dimer structures are better amenable to this type of analysis, however, because the sulfones exist as the same single stereochemical isomer.

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