Articles

Synthesis and Biodistribution of a Short Nonionic Oligonucleotide Analogue in Mouse with a Potential to Mimic Peptides

Bernd Eschgfäller,[†,‡] Marcel König,[†] Franziska Boess,[§] Urs A. Boelsterli,[§] and Steven A. Benner*[,‡]

Department of Chemistry, Swiss Federal Institute of Technology, CH-8092 Zurich, Switzerland, Department of Chemistry, University of Florida, Gainesville, Florida 32611, and Institute of Toxicology, Swiss Federal Institute of Technology and University of Zurich, CH-8603 Schwerzenbach, Switzerland

Received August 11, 1997

A nonionic RNA analogue of the sequence r($U_{SO_2}G_{SO_2}A_{SO_2}C$) has been synthesized where each bridging phosphate diester is replaced by a dimethylene sulfone unit (rSNA). The rSNA was synthesized in solution from 3′,5′-bishomo-β-ribonucleoside derivatives as building blocks. Full experimental procedures are provided, and the product and all synthetic intermediates are fully characterized. The tetramer is nonionic but highly dipolar due to multiple hydrogen bonding opportunities. It is freely soluble in water only at higher pH's, permitting it to be radiolabeled by exchange of the acidic protons α to the sulfones with tritiated water. The tritiated molecule was administered intravenously into the tail vein (2.6 mg/kg) of mice, and its distribution was monitored over 48 h. The rSNA was widely distributed in the biological tissues, including the brain, and excreted in both the feces and the urine. The accumulation of radioactivity was significantly higher in liver and kidney than in other tissues. Radiolabel was recovered from the urine, analyzed by HPLC, and shown to be intact oligonucleotide sulfone. This is the first bioavailability study on a short nonionic oligonucleotide analogue, a class of molecules with potential biomedical applications.

Introduction

The potential for analogues of oligonucleotides to exert biological activity has become increasingly appreciated in the past decade, complementing a much longer history where pharmaceutical research focused on nucleosides and their analogues.[1,2] Much of the drive to develop oligonucleotide analogues has come from the desire to obtain "antisense" compounds, long oligonucleotides (16−30-mers) that bind to complementary messenger RNA molecules following Watson−Crick base pairing rules (for reviews see refs 3−7). Alterations in the structure of natural oligonucleotides have been sought to improve stability, bioavailability, and hybridization potential.

Often overlooked in this work is the potential value of short (4−8 nucleotides) oligonucleotide analogues as ligands for transcription factors, restriction enzymes, or other biologically important proteins that recognize short oligonucleotide sequences. Here, replacement of anionic phosphates in the oligonucleotide backbone by nonionic linkers is known to permit the analogue to fold,[8] often to give a conformation preferentially bound by the protein.

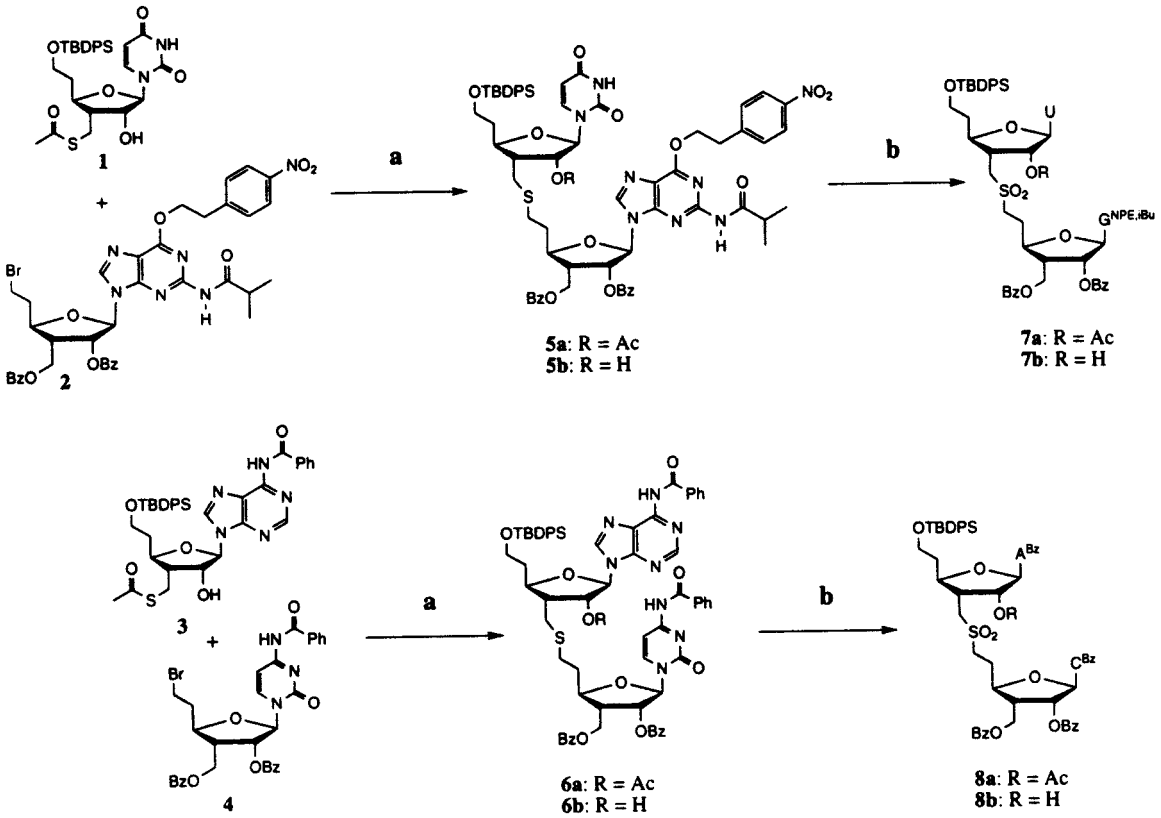
For example, Blättler et al. recently found that substitution of a dimethylene sulfone unit for a phosphate in the hexanucleotide recognition sequence of the EcoRV restriction endonuclease yielded an inhibitor (an "SNA") with $K_i \approx 20$ pM, 100-fold lower than the K_m for the corresponding DNA substrate.[9] The nonstandard sulfone linkage is known to bend the DNA duplex in the same way that the recognition site is bent prior to cleavage,[9] making the compound an analogue of the high-energy intermediate in the enzymatic reaction. These and other results suggest that the dimethylene sulfone linker might be useful in creating tight binding, low molecular weight (1000−1500 Da) oligonucleotide analogues that serve as inhibitors for restriction enzymes, transcription factors, and other proteins that bend and unwind specific DNA sequences. As the dimethylene sulfone linkage is uncharged, nuclease resistant, and extremely stable to chemical degradation, it meets many other criteria desired for a biologically active agent as well.

SNAs will have biological value *in vivo* only if they are bioavailable. While bioavailability in a number of longer oligonucleotide analogues has been studied by a variety of workers,[10−15] we were surprised to find that virtually no work had been done to examine the bioavailability of short (4−8 nucleotide) oligonucleotide analogues that might have value in applications unrelated to antisense. As noticed elsewhere, nonionic oligonucleotide analogues are curious variants of natural oligonucleotides.[8] As with DNA and RNA, they collect a large number of hydrogen bond donor and acceptor groups, which have the propensity to form "structure" (bends, folds, turns, aggregates, and precipitates). Unlike with DNA and RNA, however, the tendency to form structure is unopposed by the Cou-

[†] Swiss Federal Institute of Technology.
[‡] University of Florida.
[§] Swiss Federal Institute of Technology and University of Zurich.

S0022-2623(97)00538-4 CCC: $15.00
Published on Web 01/08/1998

Scheme 1[a]



[a] (a) Cs_2CO_3/THF; (b) Oxone/NaOAc/THF/MeOH/H_2O.

lombic repulsion. These differences make the bioavailability of SNAs especially interesting. Therefore, we describe the synthesis, biodistribution, and clearance of a tetramer sulfone oligoribonucleotide with the sequence $U_{SO_2}G_{SO_2}A_{SO_2}C$, an SNA containing one of each of the four standard nucleobases and unable to form a self-duplex by Watson−Crick rules, as a model for a short oligonucleotide analogue.

Chemistry

Synthesis of Oligonucleotide Analogues. The functionalized monomers **1**−**4** were synthesized from diacetone D-glucose as previously described (Scheme 1).[8,16,17] Hydrolysis of the thioacetates **1** and **3** with ammonia at 0 °C yielded the corresponding thiols, but generated especially with **1** large amounts of disulfide. Therefore **1** and **3** were deprotected *in situ* to thiolate *via* acetyl migration and were coupled with Cs_2CO_3 in THF with bromides **2** and **4** to yield thioethers U_SG (**5a**, 70%) and A_SC (**6a**, 74%).[8,18,19] The acetate group migrated hereby to the neighboring 2′-OH group. Approximately 10% of the thioethers were isolated with a 2′-OH group, presumably because of an intermolecular acetyl migration (**5b**, 8% and **6b**, 8%). These byproducts were used together with the acetylated thioethers in the further reaction steps.

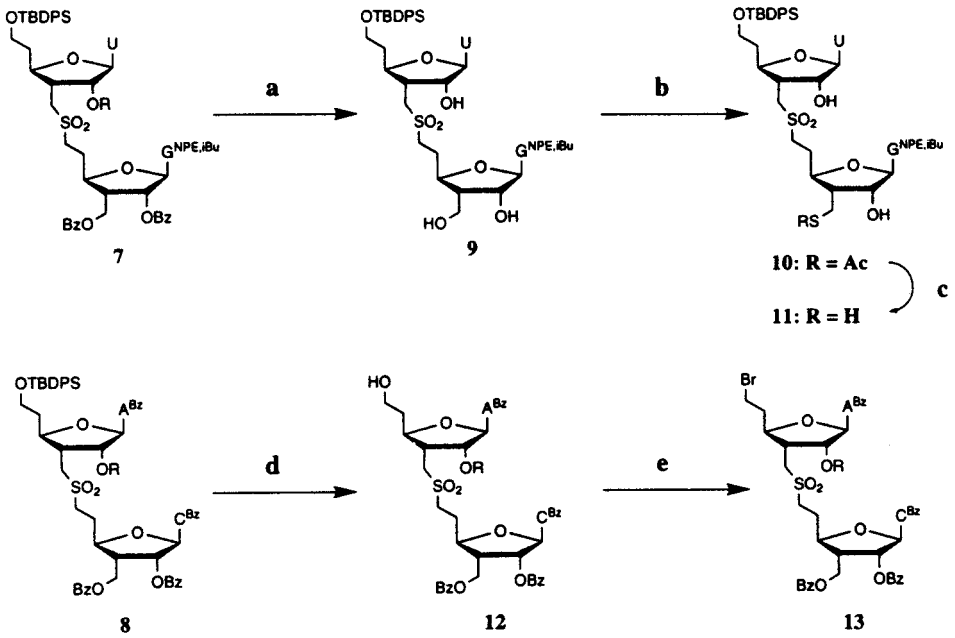
Thioethers were immediately oxidized with excess Oxone in methanol/water to give the sulfones **7** in quantitative and **8** in 85% yield (Scheme 1).[20] Earlier experiments in our laboratory showed that the oxidation at a later stage of the synthesis resulted in lower yields.[21] TLC showed the fast reaction to the more polar sulfoxide intermediates followed by a slower oxidation to the sulfones.

Dimer **7** had to be deprotected and functionalized at the 3″-end to generate a thiol group and **8** at the 6′-end to give a bromide (Scheme 2). The benzoyl and acetyl groups of **7** were hydrolyzed with 0.2 M LiOH in THF/MeOH (1:1) to triol **9** (82%). Compound **9** was converted by a Mitsunobu reaction with thioacetic acid in THF to the thioacetate **10** in 76% yield.[22] Deprotection of **10** with ammonia in degassed MeOH generated the thiol **11** in quantitative yield. Dimer **8** was desilylated with HF in pyridine to give **12** (92%). Reaction with CBr_4/PPh_3 in acetonitrile yielded bromide **13** (70%).[23,24]

Coupling of the functionalized dimers **11** and **13** with Cs_2CO_3 in THF resulted in tetramer **14** in 82% yield (Scheme 3). Only traces of disulfide were detected. Thioether **14** was oxidized with Oxone to sulfone **15** in 84% yield. **15** was fully deprotected in one step with 2 M NaOH/MeOH/THF to give **16** in 87% yield (Scheme 3).

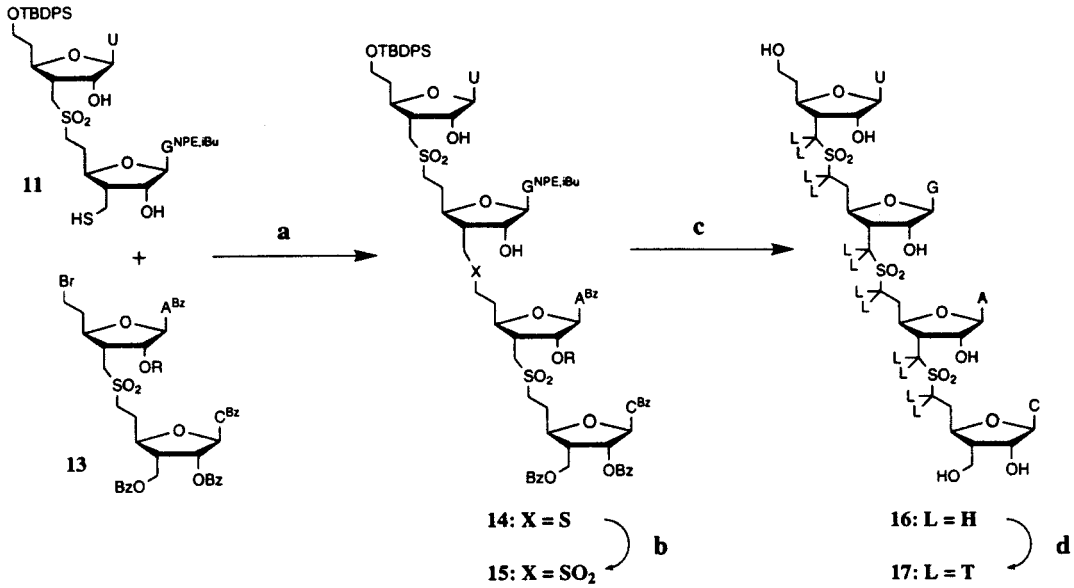
Labeling of Oligonucleotide Analogues. The protons on the CH_2 groups bonded to sulfur were exchanged with tritium derived from tritiated water (Scheme 3). Following a procedure developed using deuterium as a label, purified oligomer (2.0 mg) was suspended in tritiated water (1 mL, specific activity, 25 mCi/g = 925 MBq/g, DuPont-NEN) and treated with triethylamine (1 M). The oligomer dissolved immediately after the addition of the base. The solution was shaken for 48 h at room temperature. The sample was lyophilized in a SpeedVac, resuspended in deionized water (1 mL), and incubated with shaking at room temperature (30 min) to remove rapidly exchangeable protons followed by lyophilization. This cycle was repeated two more times. The rate of loss of the label was estimated by incubating a deuterated GC sulfone-

Scheme 2[a]



[a] (a) 0.2 M LiOH/THF/MeOH; (b) DIAD/PPh_3/AcSH/THF; (c) NH_3/MeOH; (d) HF/pyridine; (e) $PPh_3/CBr_4/CH_3CN$.

Scheme 3[a]



[a] (a) Cs_2CO_3/THF; (b) Oxone/THF/MeOH/H_2O; (c) 2 N NaOH/THF/MeOH; (d) 1 M Et_3N/T_2O. Note that tritium T is present as a tracer.

dimer dissolved in PBS buffer at pH 7.4 at 37 °C for 48 h. As analyzed by ^1H NMR, a loss of 5−10% of the label was detected. This should not interfere with the results of the bioavailability study, because the exchanged tritium should predominately appear in the form of tritiated water which is easily removed by lyophilization.

Results and Discussion

The tetrasulfone $U_{SO_2}G_{SO_2}A_{SO_2}C$ **16** is poorly soluble in water at neutral pH (10 μM), but moderately soluble in DMSO. In water containing 1 M triethylamine, the compound was freely soluble. These results contrast markedly with the behavior of natural DNA, whose repeating anionic character renders it freely soluble in water under all conditions.[8] The enhanced solubility of **16** in water under alkaline conditions is presumed to arise from the deprotonation of the U and G bases, which have pK_as of ca. 9.2. It appears to be easier to manipulate nonionic oligonucleotide analogues under alkaline conditions, and this may provide a general strategy for nonionic oligonucleotide analogues as long as the linker is stable under alkaline conditions. This may also be valuable in synthesis and solution-phase manipulation of peptide nucleic acids (PNAs).[25]

The improved solubility of **16** in water under alkaline conditions was valuable in preparing its tritiated derivative **17**. The dimethylene sulfone unit appears to have nearly optimal acid−base properties. It is sufficiently acidic to permit incorporation of tritium from water under relatively mild conditions. At pH 7.4, however, the SO_2-C−H bond is sufficiently stable to allow properly controlled bioavailability studies to be done.

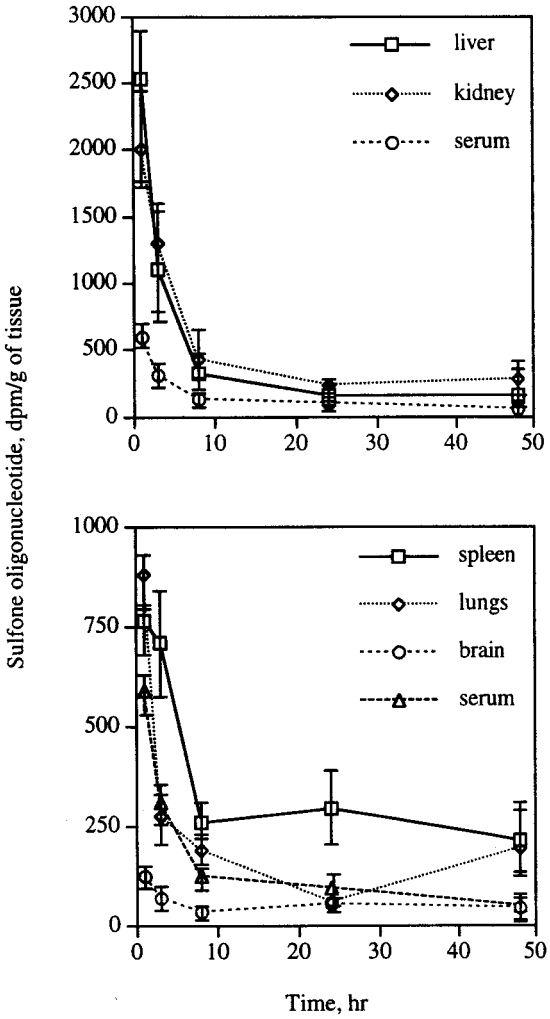


Figure 1. Measured radioactivity in the different tissues after a single iv injection of 2700 dpm/g of tissue. Three mice were sacrificed per time interval and the radioactivity was determined *via* liquid scintillation counting.

Nevertheless, in the bioavailability studies, a small but significant amount of the label was found in lyophilizable fractions, presumably as water. The slow exchange of tritium to water was expected in light of control experiments that showed that a small amount of label was lost *in vitro* under physiological conditions. The amount of released label, never more than 7% of the total label introduced in the bioavailability studies, uniformly distributed from all tissues, was determined by lyophilization. The data reported in Figure 1 reflect correction for the tritiated water.

The tritiated sulfone was widely distributed in all tissues examined (Figure 1). The concentration of label was significantly higher in liver and kidney than in all other tissues. Substantial amount of oligosulfone was found in the brain. The concentration of SNA in the liver was similar to that in the kidney, 3 times that in spleen and lungs, 4 times that in serum, and 20 times that in brain. After 8 h, the oligosulfone concentration in all tissues was higher than in the serum with the exception of brain tissue (Figure 1). The uptake of the orally administered tritiated sulfone was also tested. Low radioactivity was found in all examined tissues, and up to 95% of the administered dose was excreted within 24 h, suggesting poor gastrointestinal absorption of the oligonucleotide (data not shown).

Table 1. Total Fecal and Urinary Excretion of Dimethylene Sulfone-Bridged Oligonucleotide after Single iv Injection (% Recovered Dose)

time interval (h)	mouse	urinary excretion (%)	fecal excretion (%)
0−3	1	2.7	0.1
	2	17.3	
0−8	3	27.8	7.1
	4	20.3	18.6
	5	33.4	2.5
0−24	6	23.0	49.9
	7	20.6	19.6

The radioactivity was recovered both in the urine and in the feces, indicating that the oligosulfone was excreted *via* the kidney and also cleared *via* biliary excretion. Over the entire time course, the main pathway of excretion of the dimethylene sulfone oligonucleotide was *via* the feces. Approximately 9% of the administered dose was found in the feces 8 h after the injection, while 26% was found in the urine. After 24 h, however, approximately 35% of the label was found in the feces, while further secretion into the urine was not observed (Table 1). The large variance in radioactivity found in excreted samples is accounted for by the irregularity of the excretion phenomenon.

To establish the chemical identity of the species carrying tritium in the urine, the urine samples were filtered through SepPak C_{18} cartridges to remove salt, concentrated by lyophilization, and analyzed by HPLC. The label was present predominantly in one peak that eluted between 24 and 26 min. Authentic tritiated sulfone injected on the same column showed the same retention time, suggesting that the sulfone excreted in the urine had not undergone chemical degradation.

Studies from other laboratories with longer oligonucleotides and their analogues report that these molecules achieve widespread biodistribution in several animal systems.[9,10,12−14] The biodistribution of the $rU_{SO_2}G_{SO_2}A_{SO_2}C$ oligosulfone differs from that of other oligonucleotide analogues in several respects. First, excretion was slower with the oligosulfone than observed in a test methylphosphonate 12 nucleobases in length. The methylphosphonates are also nonionic oligonucleotide analogues. In contrast, excretion of the oligosulfone was more rapid than several phosphorothioates 20−40 nucleotides in length. These results show, therefore, no simple dependence of pharmacodynamics either on size or polarity.

The fact that the label excreted in the urine was found predominantly in the form of the oligosulfone shows that relatively little of the compound was degraded in the body. This is consistent with the expected stability of the sulfone linker.

Interestingly, the amount of oligosulfone found in the brain is much higher than that found in studies using other oligonucleotide analogues. Pardridge et al. reported that the uptake of an 18-mer PNA by the brain was negligible following intravenous administration; uptake was increased by at least 28-fold when the PNA was bound to the OX26-SA vector. The brain uptake with the vector-mediated PNA was 0.1% of the injected dose per gram of brain at 60 min after an iv injection.[26] In contrast, 0.15% of the injected dose of oligosulfone was found per gram of brain 1 h after injection.

Conclusion

The sulfone linkage, mimicking the natural phosphodiester linkage, was originally examined because of the remarkable pharmacodynamic properties displayed by simple sulfoxides and sulfones, which distribute rapidly in tissues.[27,28] However, rSNAs have the propensity to aggregate in solution, which may hinder such distribution. These studies show that an rSNA with a mass of 1200 Da is distributed rapidly and widely in mice, including the brain.[29,30] Oligonucleotide analogues of this size have the potential to be a new class of biologically active substances due to their ability to interact with and inhibit proteins that bind and catalyze transformation on DNA.[9]

Experimental Section

General Procedure. All reactions were run under a positive pressure of dry argon. Tetrahydrofuran was distilled from sodium; dimethylformamide, pyridine, acetonitrile, and methanol were purchased from Fluka (puriss, stored over molecular sieves). Reagents were purchased from Fluka with the highest commercial quality and used without further purification. Acetate buffer was made from 3 M AcOH and 1 M NaOAc in deionized water. E. Merck silica gel (60, 0.040−0.063 mm) was used for flash chromatography. NMR spectra were obtained from Bruker AMX 500, Varian EM-390, or Varian XL-300. All spectra were recorded using tetramethylsilane as an internal standard. Mass spectra (MS) were obtained from a VG ZAB2-SEQ (FAB, 3-nitrobenzyl alcohol (NOBA) matrix) or a Bruker Reflex (MALDI-TOF, 2-[(4-hydroxyphenyl)azo]benzoic acid (HABA) matrix) spectrometer.

Biological Methods. All experiments were approved by the institutional supervisory board and performed according to Swiss Federal regulations. Male Zur:ICR mice (average weight, 30 g) obtained from the Institute of Laboratory Animal Sciences, University of Zurich, were used for all experiments. The animals were kept in Macrolone cages with wood shavings as bedding under controlled environmental conditions. They were fed with Nafag 890 rodent pellets (Nafag, Gossau, Switzerland) and water *ad libitum* for 1 week prior to the study. The oligosulfone was dissolved in a H_2O/DMSO solution (1:1, vol/vol). Doses were injected intravenously into the tail vein during anesthesia with Ketamine (Ketaminol, Veterinaria, Zurich, Switzerland, 150 mg/kg, ip) or administered by oral gavage in 50 μL of a solution to 2.6 mg/kg of body weight (2700 dpm/g). In toxicity tests, DMSO displayed an LD_{50} for mice of 3.8−11.0 mg/kg,[31,32] significantly above the approximately 0.9 mg/kg injected in these studies. Accordingly, no mice were lost due to toxic response, nor was toxicity detected in inspection of organs from sacrificed mice. After 1, 3, 8, 24, and 48 h, three test animals were sacrificed with carbon dioxide. Due to the limited amount of rSNA, we were only able to choose five time intervals. Vehicle control mice receiving no oligosulfone injection were also sacrificed. Blood and tissue samples (liver, kidney, spleen, lungs, and brain) of each animal were collected. Each tissue was trimmed of extraneous fat or connective tissue and weighed. Samples of blood were centrifuged to separate plasma, and the organs were homogenized (in deionized water, 3 mL/g wet weight). The urine and feces of the animals were collected in metabolism cages up to 24 h. Samples were stored at −20 °C until analysis.

All samples were lyophilized twice to remove tritiated water. The homogenized tissue was digested with Soluene 350 (1 mL/g, Canberra Packard, Meriden, CT) in a glass scintillation vial. The mixture was gently swirled and heated to 50 °C in a water bath overnight. Hionic-Fluor scintillation cocktail (13 mL, Canberra Packard, Meriden, CT) was added and swirled until a clear solution appeared. The dried feces samples were pulverized, 20 mg rehydrated with deionized water (0.1 mL) in a scintillation vial, and digested with Soluene 350 (1 mL) at 50 °C for 2 h. 2-Propanol (0.5 mL) and 30% H_2O_2 (0.2 mL) were added to decolorize the samples, the mixture was heated to 50 °C (1 h) to destroy excess peroxide, and the mixture was then suspended in Hionic-Fluor (13 mL) scintillation cocktail. The plasma and urine samples were suspended directly in Hionic-Fluor scintillation cocktail (13 mL/g of sample).

The radioactivity was measured with low level counting in a Liquid Scintillation Analyzer Tri-Cab 2250CA (Low Level) (Canberra Packard, Meriden, CT). The counts per minute were converted to disintegrations per minute by standard quench corrections. The concentration of the sulfone oligonucleotide in the sample was calculated from the specific activity of the tetramer. The background activity of the control mice were subtracted from the data reported in Figure 1.

For HPLC-analysis (Waters, Milford, MA) the urine samples were prepurified with SepPak C_{18} cartridges (Waters, Milford, MA).

U_SG Dimers 5a and 5b. Uridine thioester **1** (126.0 mg, 0.222 mmol) and guanidine bromide **2** (176.9 mg, 0.221 mmol) were dried on HV at room temperature for 24 h, Cs_2CO_3 (287.6 mg, 0.883 mmol) was added, and the mixture was dried on HV at room temperature for 15 h. THF (7.2 mL) was added, and the fine white suspension was stirred for 8 h at room temperature. The solvent was rotary evaporated and the residue chromatographed on silica gel (33 g) with CH_2Cl_2 and MeOH (1%, 100 mL; 2%, 500 mL; 10%, 200 mL) as eluent to give the 2′-OAc dimer **5a** (200.6 mg, 70%) and the 2′-OH dimer **5b** (21.5 mg, 8%). The dimers were contaminated with small amounts of side products resulting from the thioester compound **1**. The side products formed during this type of reaction are discussed elsewhere.[8]

Compound **5a**: FAB MS (3-NOBA) *m/z* 1289.4 ($M + H^+$, 35); 1231.4 (6); 919.3 ($(M\text{-}G^{NPE,\,i-Bu})^+$, 13); 371.1 ($G^{NPE,\,i-Bu}+H^+$, 17); 197.1 (21); 105.0 (Bz^+, 100). Compound **5b**: FAB MS (3-NOBA) *m/z* 1247.4 ($M + H^+$, 33); 877.3 ($(M - G^{NPE,i-Bu})^+$, 21); 371.1 ($G^{NPE,i-Bu}+H^+$, 35); 197.1 (21); 105.0 (Bz^+, 100).

A_SC Dimers 6a and 6b. **3** (232.1 mg, 0.334 mmol), **4** (215.5 mg, 0.333 mmol), and Cs_2CO_3 (433.2 mg, 1.33 mmol) were dried as described for the preparation of **5a** and **5b** above. THF (10 mL) was added, and the resulting fine white suspension was heated to 50 °C and vigorously stirred for 2 h at 50 °C. The suspension was cooled to room temperature, acetate buffer (0.83 mL), CH_2Cl_2, and 9/10 saturated brine were added, the organic layer was separated, and the aqueous layer was three times reextracted with CH_2Cl_2. The combined organic layers were concentrated *in vacuo*, and the residue was chromatographed on silica gel (40 g) with CH_2Cl_2 and MeOH (1%, 700 mL; 2%, 400 mL; 10%, 200 mL) as eluent. The dimers **6a** (311.7 mg, 74%) and **6b** (33.6 mg, 8%) were obtained slightly contaminated with side products resulting from **3** (see above).

Compound **6a**: FAB MS (3-NOBA) *m/z* 1289.4 ($M + H^+$, 10); 240.1 ($A^{Bz} + H^+$, 23); 197.1 (13); 136.0 (59); 105.0 (Bz^+, 100). Compound **6b**: FAB MS (3-NOBA) *m/z* 1219.6 ($M + H^+$, 11); 240.1 ($A^{Bz} + H^+$, 36); 197.1 (13); 136.0 (58); 105.0 (Bz^+, 100).

$U_{SO_2}G$ Dimers 7a and 7b. Thioether **5** (130.2 mg, 0.101 mmol) was dissolved in a mixture of THF (2.8 mL) and MeOH (17.8 mL). A freshly prepared solution of Oxone ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) (250.9 mg, 0.408 mmol) and NaOAc (110.3 mg, 1.34 mmol) in water (3.6 mL) was added under vigorous stirring. The resulting white suspension was stirred for 2 h at room temperature. Saturated aqueous $Na_2S_2O_3$ and CH_2Cl_2 were added resulting in a slightly cloudy solution. The organic layer was separated, and the aqueous layer was reextracted three times with CH_2Cl_2. The residue was filtered through silica gel (40 g, CH_2Cl_2/MeOH, 92:8) to give **7** in quantitative yield. An analytical sample was prepared by chromatography on silica gel with CH_2Cl_2/MeOH (98:2) as eluent. The following are data for **7a**. The ^1H NMR signals were assigned by $^1H/^1H$-COSY. ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.03 (s, 9H, CH_3-tBu); 1.14 (d, $J = 6.9$ Hz, 3H, CH_3-^{i}Bu); 1.16 (d, $J = 6.8$ Hz, 3H, CH_3-^{i}Bu); 1.73 (m, 1H, H-5′-U); 1.88 (m, 1H, H-5′-U); 2.07 (s, 3H, $COCH_3$); 2.52 (m, 2H, H-5′-G); 2.63 (m, 1H, CH-^{i}Bu); 2.84 (dd, $J = 3.3$ Hz, $J = 14.0$ Hz, 1H, H-3″-U); 2.92 (m, 1H, H-3′-U); 3.20 (m, 1H, H-6′-G); 3.24 (dd, $J = 3.5$ Hz, $J =$

12.4 Hz, 1H, H-3″-U); 3.29 (t, J = 6 Hz, 2H, CH_2-NPE); 3.47 (m, 1H, H-6′ G); 3.79 (m, 2H, H-6′-U); 3.93 (dt, J = 2.2 Hz, J = 9.6 Hz, 1H, H-4′-U); 4.18 (m, 1H, H-3′-G); 4.55 (m, 1H, H-4′-G); 4.64 (dd, J = 6.5 Hz, J = 11.6 Hz, 1H, H-3″-G); 4.76 (d, J = 6.2 Hz, 1H, H-3″-G); 4.78 (t, J = 6.6 Hz, 2H, CH_2-NPE); 5.21 (d, J = 1.7 Hz, 1H, H-1′-U); 5.40 (dd, J = 1.7 Hz, J = 6.5 Hz, 1H, H-2′-U); 5.62 (dd, J = 2.3 Hz, J = 8.0 Hz, 1H, H-5-U); 5.99 (d, J = 1.6 Hz, 1H, H-1′-G); 6.01 (dd, J = 1.6 Hz, J = 6.2 Hz, 1H, H-2′-G); 7.01 (d, J = 8.2 Hz, 1H, H-6-U); 7.34−7.55 (m, 14H, *p*-Ph, *p*-Bz, *o*-NPE); 7.58−7.63 (m, 4H, *o*-Ph); 7.91 (s, 1H, H-8-G); 7.99−8.06 (m, 4H, *o*-Bz); 8.13 (d, J = 8.8 Hz, 2H, *m*-NPE); 8.89 (br, 1H, NH). FAB MS (3-NOBA): *m/z* 1321.2 (M + H^+, 13); 1263.5 ((M − ^{i}Bu)$^+$, 3); 951.1 ((M − $G^{NPE,i-Bu}$)$^+$, 3); 371.8 ($G^{NPE,i-Bu}$ + H^+, 8); 307. 0 (32); 105.0 (Bz^+, 91); 76.9 (Ph^+, 54).

$A_{SO_2}C$ Dimers 8a and 8b. Following the procedure used for the preparation of **7** above, AC-thioether **6** (310.4 mg, 0.246 mmol) was dissolved in THF (27.2 mL)/MeOH (42.7 mL), and a mixture of Oxone (604.8 mg, 0.984 mmol) and NaOAc (266.5 mg, 3.25 mmol) in water (3.6 mL) was added. After 2 h, saturated aqueous $Na_2S_2O_3$ and CH_2Cl_2 were added, and the product was extracted as above. Chromatography on silica gel (40 g, CH_2Cl_2/MeOH, 98.5:1.5; 98:2; 95:5) gave **8** (269.0 mg, 85%) as a white foam. The following are data for **8a**. The ^{1}H NMR signals were assigned by ^{1}H/^{1}H-COSY. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.05 (s, 9H, CH_3-tBu); 1.97 (m, 1H, H-5′-A); 2.04 (m, 1H, H-5′-A); 2.18 (s, 3H, $COCH_3$); 2.46 (m, 1H, H-5′-C); 2.57 (m, 1H, H-5′-C); 3.09 (d, J = 12.4 Hz, 1H, H-3″-A); 3.46 (m, 4H, 1 × H-3″-A, 2 × H-6′-C, H-3′-C); 3.62 (m, 1H, H-3′-A); 3.86 (m, 2H, H-6′-A); 4.25 (t, J = 8.8 Hz, 1H, H-4′-A); 4.47 (t, J = 6.7 Hz, 1H, H-4′-C); 4.53 (dd, J = 7.0 Hz, J = 11.4 Hz, 1H, H-3″-C); 4.72 (dd, J = 6.4 Hz, J = 11.5 Hz, 1H, H-3″-C); 5.45 (d, J = 1.7 Hz, 1H, H-1′-C); 5.88 (d, J = 5.6 Hz, 1H, H-2′-A); 5.96 (d, J = 1.6 Hz, 1H, H-1′-A); 6.97 (dd, J = 1.6 Hz, J = 6.2 Hz, 1H, H-2′-C); 7.33−7.60 (m, 20H, *p*-Ph, *p*-Bz, H-5-C, H-6-C); 7.62−7.68 (m, 4H, *o*-Ph); 7.77 (d, J = 7.6 Hz, 2H, *o*-NBz-A); 7.91 (d, J = 7.4 Hz, 2H, *o*-NBz-C); 7.96−8.00 (m, 4H, *o*-OBz); 8.23 (s, 1H, H-8-A); 8.71 (s, 1H, H-2-A); 8.92 (br, 1H, NH); 9.32 (br, 1H, NH). FAB MS (3-NOBA): *m/z* 1293.5 (M + H^+, 18); 240.0 (A^{Bz} + H^+, 9); 105.0 (Bz^+, 100); 76.9 (Ph^+, 28).

$U_{SO_2}G$ Triol 9. Dimer **7** (136.3 mg, 103.1 μmol) was dissolved in a mixture of THF (1.1 mL) and MeOH (1.2 mL) and was cooled to 0 °C. A solution of 0.2 M LiOH (1.70 mL, 340.0 μmol) was added dropwise. The solution was stirred for 2 h at 0 °C before acetate buffer (163 μL) was added slowly. Then, CH_2Cl_2 was added and the organic layer was separated. The aqueous layer was reextracted five times with CH_2Cl_2. The combined organic layers were concentrated in vacuo. Chromatography on silica gel (12 g, CH_2Cl_2/MeOH, 96:4; 95:5; 90:10) afforded triol **9** (90.5 mg, 82%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.03 (s, 9H, CH_3-^{t}Bu); 1.24 (2d, J = 6.9 Hz, J = 6.9 Hz, 6 H, 2 × CH_3-^{i}Bu); 1.83 (m, 1H, H-5′-U); 1.99 (m, 1H, H-5′-U); 2.12, 2.28 (m, 2H, H-3′-U/G); 2.42 (m, 2H, H-5′-G); 2.64 (m, 1H, CH-^{i}Bu); 2.90 (d, J = 12.5 Hz, 1H, H-3″-U); 3.21 (m, 1H, H-3″-U); 3.26 (t, J = 6.8 Hz, 2H, CH_2-NPE); 3.34 (m, 1H, H-6′ G); 3.55 (br, 1H, OH-3″-G); 3.64 (dt, J = 3.4 Hz, J = 12.6 Hz, 1H, H-6′-G); 3.75 (m, 1H, H-3″-G); 3.88 (t, J = 6.1 Hz, 2H, H-6′-U); 3.97 (m, 1H, H-3″-G); 4.25 (m, 2H, H-4′-U, H-2′-U); 4.56 (br, 1H, H-2′-G); 4.72 (m, 1H, H-4′-G); 4.72 (t, J = 6.7 Hz, 2H, CH_2-NPE); 5.56, 5.57 (s, 1H, H-1′-U/G); 5.62 (d, J = 8.1 Hz, 1H, H-5-U); 5.85 (br, 1H, OH-2′); 6.37 (br, 1H, OH-2′); 7.31 (d, J = 8.2 Hz, 1H, H-6-U); 7.34−7.44 (m, 6H, *p*-Ph); 7.49 (d, J = 8.7 Hz, 2H, *o*-NPE); 7.63−7.65 (m, 4H, *o*-Ph); 8.06 (s, 1H, H-8-G); 8.13 (d, J = 8.7 Hz, 2H, *m*-NPE); 8.24 (br, 1H, NH). FAB MS (3-NOBA): *m/z* 1093.3 (M + Na^+, 8); 1071.1 (M + H^+, 28); 370.9 ($G^{NPE,i-Bu}$ + H^+, 100); 76.9 (Ph^+, 36).

$U_{SO_2}G$ Thioacetate 10. A solution of PPh_3 (24.7 mg, 94.2 μmol) in THF (1.0 mL) was cooled to 0 °C. Diisopropyl azodicarboxylate (DIAD) (13.6 μL, 70.4 μmol) was added, and the mixture was stirred for 35 min. **9** (50.4 mg, 47.0 μmol) was dried (48 h, HV at room temperature), dissolved in THF (0.78 mL), and added to the mixture. Immediately thereafter, thioacetic acid (6.7 μL, 70.4 μmol) was added. The slightly yellow solution was stirred for 1.5 h at 0 °C, the solvents were rotary evaporated, and the residue was chromatographed on silica gel (11.2 g, CH_2Cl_2/MeOH, 97:3; 95:5; 90:10) to yield thioester **10** (40.1 mg, 76%) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.03 (s, 9H, CH_3-tBu); 1.26 (d, J = 6.9 Hz, 6 H, CH_3-^{i}Bu); 1.86 (m, 1H, H-5′-U); 2.02 (m, 1H, H-5′-U); 2.14 (m, 1H, H-3′-U); 2.30 (s, 3H, SAc); 2.35 (m, 2H, H-5′-G); 2.49 (m, 1H, H-3′-G); 2.73 (m, 1H, CH-^{i}Bu); 2.97 (m, 2H, 1 × H-3″-U, 1 × H-3″-G); 3.28 (m, 2H, H-6′-U); 3.40 (dt, J = 4.9 Hz, J = 12.8 Hz, 1H, H-6′-G); 3.68 (dt, J = 3.4 Hz, J = 12.7 Hz, 1H, H-6′-G); 3.90 (t, J = 6.1 Hz, 2H, CH_2-NPE); 3.92 (m, 1H, H-4′-G); 4.29 (t, J = 9.9 Hz, 1H, H-4′-G); 4.42 (d, J = 3.5 Hz, 1H, H-2′-U); 4.53 (d, J = 4.9 Hz, 1H, H-2′-G); 4.75 (m, 2H, CH_2-NPE); 5.52 (d, J = 1.8 Hz, 1H, H-1′-U); 5.59 (d, J = 8.1 Hz, 1H, H-5-U); 5.62 (s, 1H, H-1′-G); 6.35 (br, 1H, OH-2′); 7.32 (d, J = 8.2 Hz, 1H, H-6-U); 7.35−7.44 (m, 6H, *p*-Ph); 7.53 (d, J = 8.7 Hz, 2H, *o*-NPE); 7.64−7.66 (m, 4H, *o*-Ph); 8.02 (s, 1H, H-8-G); 8.14 (d, J = 8.7 Hz, 2H, *m*-NPE); 8.29 (br, 1H, NH). FAB MS (3-NOBA): *m/z* 1129.3 (M + H^+, 25); 743.2 (9); 371.1 ($G^{NPE,i-Bu}$ + H^+, 100); 221.1 (28); 76.9 (Ph^+, 36).

$U_{SO_2}G$ Thiol 11. Thioacetate **10** (40.1 mg, 35.5 μmol) was dissolved in Ar-saturated MeOH (3.6 mL) at 0 °C. Ammonia was bubbled through the solution for 15 min. The lines were removed, and the solution was stirred a further 105 min at 0 °C before carefully removing the solvent and NH_3 by evaporation at ca. 1 °C with the exclusion of air contact. The white solid was dried on HV for 12 h.

$A_{SO_2}C$ Alcohol 12. Dimer **8** (253.3 mg, 0.196 mmol) was dissolved in a solution of HF (1.25 mmol) in pyridine. The reaction mixture was vigorously stirred for 3.5 h, methoxytrimethylsilane (0.34 mL, 2.48 mmol) was added, and the mixture was stirred for 10 min. The solvent was removed, and the residue was chromatographed on silica gel (40 g, CH_2Cl_2/MeOH, 95:5) to yield **12** (190.7 mg, 92%). ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 2.04 (m, 2H, H-5′-A); 2.16 (s, 3H, $COCH_3$); 2.57 (m, 2H, H-5′-C); 3.36 (dd, J = 11.7 Hz, J = 14.4 Hz, 1H, H-3″-A); 3.94 (m, 1H, H-6′-C); 3.54 (dd, J = 2.2 Hz, J = 14.6 Hz, 1H, H-3″-A); 3.68 (m, 2H, H-3′-A/C); 3.85 (m, 2H, H-6′-A); 4.04 (br, 1H, OH); 4.23 (m, 1H, H-4′-A); 4.47 (m, 1H, H-4′-C); 4.53 (dd J = 7.2 Hz, J = 11.5 Hz, 1H, H-3″-C); 4.75 (dd, J = 6.4 Hz, J = 11.5 Hz, 1H, H-3″-C); 5.38 (d, J = 1.7 Hz, 1H, H-1′-C); 5.93 (dd, J = 1.9 Hz, J = 6.4 Hz, 1H, H-2′-A); 6.00 (s, 1H, H-1′-A); 6.06 (d, J = 5.5 Hz, 1H, H-2′-C); 7.37 (m, 2H, m-NBz-A); 7.43 (m, 6H, m-Bz); 7.50 (d, J = 7.5 Hz, 1H, H-5-C); 7.52−7.61 (m, 4H, *p*-Bz); 7.71 (d, J = 7.4 Hz, 1H, H-6-C); 7.77 (d, J = 7.7 Hz, 2H, *o*-NBz-A); 7.89 (d, J = 7.8 Hz, 2H, *o*-NBz-C); 7.99 (m, 4H, *p*-OBz); 8.18 (s, 1H, H-8-A); 8.61 (s, 1H, H-2-A); 8.95 (br, 1H, NH); 9.11 (br, 1H, NH). FAB MS (3-NOBA): *m/z* 1055.3 (M + H^+, 11); 601.2 ((M − A^{Bz} − C^{Bz})$^+$, 22); 240.1 (A^{Bz} + H^+, 22); 105.0 (Bz^+, 100).

$A_{SO_2}C$ Bromide 13. Alcohol **12** (86.7 mg, 82.2 μmol) and PPh_3 (43.4 mg, 165.1 μmol) were dissolved in CH_3CN (3.0 mL). CBr_4 (49.2 mg, 148.3 μmol), dissolved in CH_3CN (1.0 mL), was added at room temperature. After 1 h, the solution was poured into a mixture of saturated sodium bicarbonate, ice, and CH_2Cl_2. The organic layer was separated, and the aqueous layer was reextracted four times with CH_2Cl_2. The organic layers were combined, the solvent was removed, and the residue was chromatographed on silica gel (20 g, CH_2Cl_2/MeOH, 98:1.5; 98:2; 96:4; 90:10) to yield **13** (64.5 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.18 (s, 3H, $COCH_3$); 2.34 (m, 1H, H-5′-A); 2.43 (m, 1H, H-5′-A); 2.46 (m, 1H, H-5′-C); 2.58 (m, 1H, H-5′-C); 3.17 (dd, J = 3.2 Hz, J = 14.3 Hz, 1H, H-3″-A); 3.37−3.52, 3.58 (m, 6H, H-3′-A, 4 × H-6′-A/C, 1 × H-3″-A); 3.82 (m, 1H, H-3′-C); 4.30 (dt, J = 2.1 Hz, J = 9.2 Hz, 1H, H-4′-A); 4.46 (m, 1H, H-4′-C); 4.54 (dd, J = 7.0 Hz, J = 11.7 Hz, 1H, H-3″-C); 4.72 (dd, J = 6.4 Hz, J = 11.5 Hz, 1H, H-3″-C); 5.44 (d, J = 2.0 Hz, 1H, H-1′-C); 5.93 (d, J = 5.7 Hz, 1H, H-2′-A); 5.99 (dd, J = 1.8 Hz, J = 6.2 Hz, 1H, H-2′-C); 6.06 (s, 1H, H-1′-A); 7.33−7.49 (m, 8H, *m*-Bz); 7.51−7.61 (m, 5H, *p*-Bz, H-5-C); 7.64 (d, J = 7.4 Hz, 1H, H-6-C); 7.86 (d, J = 7.7 Hz, 2H, *o*-NBz-A); 7.91 (d, J = 7.3 Hz, 2H, *o*-NBz-C); 7.98 (m, 4H, *p*-OBz); 8.29 (s, 1H, H-8-A); 8.72 (s, 1H, H-2-A); 8.95 (br, 1H, NH); 9.20 (br,

1H, NH). FAB MS (3-NOBA): *m/z* 1117.1, 1119.1 ($M + H^+$, 7/8); 663.0, 665.0 ($(M - A^{Bz} - C^{Bz})^+$, 4/4); 240.0 ($A^{Bz} + H^+$, 12); 104.9 (Bz^+, 68).

$U_{SO_2}G_SA_{SO_2}C$ Tetramer 14. Cs_2CO_3 (37.4 mg, 114.8 μmol), dried on HV (2 min at 130 °C, 10 min at room temperature), was placed under Ar, and THF (2.0 mL) was added. Crude thiol **11** (ca. 35.5 μmol, based on 100% ammonolysis yield) and **13** (47 mg, 42.0 μmol) dissolved in THF (1.6 mL) were added. The suspension was vigorously stirred at 50 °C for 2 h. The reaction was cooled to room temperature and neutralized with acetate buffer (57 μL). CH_2Cl_2 and saturated aqueous NaCl/H_2O (9:1) were added, the organic layer was separated, and the aqueous layer was reextracted five times with CH_2Cl_2. The organic layers were combined, and the solvent was removed by evaporation. Chromatography on silica gel (12 g, CH_2Cl_2/MeOH, 97:3; 96:4; 95:5; 90:10) afforded tetramer **14** (62.2 mg, 82%). ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 0.94 (s, 9H, CH_3-^{t}Bu); 1.06 (d, J = 7.2 Hz, 6H, 2 × CH_3-^{i}Bu); 1.78 (m, 1H, H-5′-U); 1.92 (m, 1H, H-5′-U); 2.10 (m, 2H, H-5′); 2.12 (s, 3H, $COCH_3$); 2.21, 2.33 (m, 4H, H-5′); 2.46 (m, 1H, H-3′-U); 2.58, 2.66 (m, 2H, 1 × H-3″-G, H-3′-G); 2.80 (m, 4H, 2 × H-6′-A, 1 × H-3″-G, CH-^{i}Bu); 3.10 (dd, J = 3.5 Hz, J = 12.7 Hz, 1H, H-3″-U); 3.15 (dd, J = 6.5 Hz, J = 9.5 Hz, 1H, H-3″-A); 3.22 (m, 1H, H-3′); 3.41 (dd, J = 8.6 Hz, J = 14.6 Hz, 1H, H-3″); 3.50, 3.57, 3.74 (m, 8H, partially covered by the DMSO signal, 4 × H-6′-G/C), 2 × H-3″-A/U, 2 × H-3′-A/C); 3.74 (t, J = 6.5 Hz, 2H, H-6′-U); 3.95 (t, J = 10.7 Hz, 1H, H-4′-U); 4.03 (m, 1H, H-4′-G); 4.14 (t, J = 8.3 Hz, 1H, H-4′-A); 4.23 (m, 1H, H-2′-U); 4.51, 4.58 (m, 4H, H-4′-C, H-3″-C, H-2′-G); 4.77 (t, J = 6.8 Hz, 2H, CH_2-NPE); 5.57 (d, J = 8.1 Hz, 1H, H-5-U); 5.60 (d, J = 1.7 Hz, 1H, H-1′-C); 5.73 (d, J = 5.2 Hz, 1H, OH); 5.89 (d, 1H, OH); 5.89 (d, J = 1.8 Hz, 1H, H-1′-U); 5.93 (dd, J = 2.0 Hz, J = 6.8 Hz, 1H, H-2′-A); 5.99 (d, J = 5.0 Hz, 1H, H-2′-C); 6.01 (d, J = 2.0 Hz, 1H, H-1′-G); 6.18 (d, J = 1.2 Hz, 1H, H-1′-A); 7.37−7.67 (m, 27H, H-6-U, H-5-C, H-6-C, *p*-Ph, *p*-Bz, *o*-NPE); 7.91 (d, J = 7.2 Hz, 2H, *o*-NBz-A); 7.98−8.05 (m, 6H, *o*-NBz-C, *o*-OBz); 8.16 (d, J = 8.7 Hz, 2H, *m*-NPE); 8.25 (d, J = 7.5 Hz, 1H, NH-^{i}Bu); 8.38 (s, 1H, H-8-G); 8.67 (s, 1H, H-8-A); 8.75 (s, 1H, H-2-A); 10.30 (s, 1H, NH); 11.30 (br, 2H, NH). FAB MS (3-NOBA): *m/z* 2123.6 ($M + H^+$, 100); 1885.4 ($(M - A^{Bz})^+$, 4); 1063.3 (12); 766.4 (10); 666.8 (9); 613.1 (30).

$U_{SO_2}G_{SO_2}A_{SO_2}C$ Tetramer 15. UGAC-thioether **14** (62.2 mg, 28.2 μmol) was dissolved in THF (1.9 mL)/MeOH (5.2 mL), and a mixture of Oxone (71.1 mg, 115.7 μmol) and NaOAc (31.5 mg, 384.0 μmol) in water (0.95 mL) was added. After 1.5 h, aqueous $Na_2S_2O_3$ and CH_2Cl_2 were added, and the product was extracted as above. Chromatography on silica gel (12 g, CH_2Cl_2/MeOH, 96.5:3.5; 96:4; 95:5; 90:10) yielded **15** (53.1 mg, 84%) as a white foam. ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 0.95 (s, 9H, CH_3-^{t}Bu); 1.07 (d, J = 7.1 Hz, 6H, 2 × CH_3-^{i}Bu); 1.78 (m, 1H, H-5′-U); 2.08 (m, 1H, H-5′-U); 2.13 (s, 3H, $COCH_3$); 2.31, 2.47 (m, 5H, 4 × H-5′, H-3′-U); 2.81 (m, 1H, CH-^{i}Bu); 3.11, 3.30, 3.45, 3.65 (m, 17H, partially covered by the DMSO signal, CH_2-NPE, 6 × H-6′-G/A/C, 6 × H-3″-U/G/A, 3 × H-3′-G/A/C); 3.74 (t, J = 6.3 Hz, 2H, H-6′-U); 3.95 (t, J = 9.5 Hz, 1H, H-4′-U); 4.03 (t, J = 8.8 Hz, 1H, H-4′-G); 4.16 (t, J = 8.3 Hz, 1H, H-4′-A); 4.23 (br, 1H, H-2′-U); 4.51 (m, 2H, H-4′-A, 1 × H-3″-C); 4.59 (m, 1H, H-3″-C); 4.67 (br, 1H, H-2′-G); 4.77 (t, J = 6.6 Hz, 2H, CH_2-NPE); 5.57 (d, J = 8.1 Hz, 1H, H-5-U); 5.60 (d, J = 1.7 Hz, H-1′-C); 5.73 (d, J = 5.2 Hz, 1H, OH); 5.90 (s, 1H, H-1′-U); 5.93 (d, J = 8.5 Hz, 1H, H-2′-A); 6.00 (s, d, 2H, H-2′-C, H-1′-G); 6.15 (d, J = 4.7 Hz, 1H, OH); 6.23 (s, 1H, H-1′-A); 7.38−7.66 (m, 27H, H-6-U, H-5-C, H-6-C, *o*-, *m*-, *p*-Ph, *m*-, *p*-Bz, *o*-NPE); 7.91 (d, J = 7.2 Hz, 2H, *o*-NBz-A); 7.98−8.05 (m, 6H, *o*-NBz-C, *o*-OBz); 8.16 (d, J = 8.7 Hz, 2H, *m*-NPE); 8.25 (d, J = 7.5 Hz, 1H, NH-^{i}Bu); 8.38 (s, 1H, H-8-G); 8.67 (s, 1H, H-8-A); 8.75 (s, 1H, H-2-A); 10.30 (s, 1H, NH); 11.30 (br, 2H, NH). FAB MS (3-NOBA): *m/z* 2155.4 ($M + H^+$, 5); 371.1 ($G^{NPE,i-Bu} + H^+$, 16); 289.0 (21); 240.1 ($A^{Bz} + H^+$, 9); 216.1 ($C^{Bz} + H^+$, 5); 105.0 (Bz^+, 59); 76.9 (Ph^+, 43).

Deprotected $U_{SO_2}G_{SO_2}A_{SO_2}C$ 16. Tetramer **15** (4.0 mg, 1.86 μmol) was dissolved in MeOH (0.5 mL) and THF (0.5 mL). NaOH (2 M, 0.5 mL) was added at room temperature, and the yellow solution was stirred for 23 h at room temperature. Acetate buffer (0.37 mL) was added, yielding a white precipitate. The organic solvents were removed by rotary evaporation, and CH_2Cl_2/diethyl ether 9:1 and H_2O were added to yield a clear, slightly yellow organic layer and a milky aqueous layer. The organic layer was separated and the aqueous layer washed three times with CH_2Cl_2/diethyl ether (9:1). The white precipitate was recovered by centrifugation from the aqueous layer. The white precipitate was suspended in water in an Eppendorf tube, shaken and the product recovered by precipitation. This procedure was repeated twice. **16** (2.0 mg, 87%) was recovered as a white solid. Analytical HPLC (Waters RP-Nova-Pak C_{18} column, 3.9 × 150 mm, flow 0.9 mL/min; A = H_2O, B = CH_3CN, 0% B for 5 min, gradient 0% to 25% B in 40 min, 25% to 100% B in 15 min, 100% B for 10 min, retention time of the single peak: 24−26 min) showed a single product. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) The spectrum upfield from 3.70 ppm could not be interpreted; 3.70 (m, 2H, H-3″); 3.85−4.13 (m, 4H, H-4′); 4.19 (m, 1H, H-2′-Py); 4.27 (m, 1H, H-2′-Py); 4.57 (m, 1H, H-1′-Pu); 4.72 (m, 1H, H-2′-Pu); 5.50 (br, 1H, OH); 5.66 (m, 2H, H-1′, H-5-U); 5.71 (s, 1H, H-1′); 5.76 (s, d, 2H, H-1′, H-5-C); 5.95 (s, 1H, H-1′-A); 6.01 (br, 1H, OH); 6.13 (br, 1H, OH); 6.22 (br, 1H, OH); 6.53 (br, 2H); 7.15 (br, 2H, NH_2); 7.30 (br, 1H, NH); 7.54 (d, 1H, H-6-U); 7.58 (d, 1H, H-6-C); 7.90 (s, 1H, H-2-A); 8.17 (s, H-8-A); 8.35 (s, 1H, H-8-A); 10.95 (br, NH). MALDI-TOF MS (HABA-matrix, linear, positive mode, 7 kV): *m/z* 1284.4 ($M - H^{+2} Na^+$); 1262.6 ($M + Na^+$).

Acknowledgment. We are indebted to the Swiss National Science Foundation for partial support of this work, to Dr. I. Allmann at the Institute of Toxicology for veterinary assistance, and to Klaus Girgenrath at the Institute for Organic Chemistry for help in radiochemical work.

Supporting Information Available: Spectral assignments for **7a,b**, **8a,b**, **9**, **10**, and **12**−**15** (4 pages). Ordering information is given on any current masthead page.

References

(1) Elion, G. B. The purine path to chemotherapy. *Science* **1989**, *244*, 41−47.
(2) Hitchings, G. H. Selective inhibitors of dihydrofolate reductase (Nobel Lecture). *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 879−885.
(3) Uhlmann, E.; Peyman, A. Antisense oligonucleotides: A new therapeutic principle. *Chem. Rev. (Washington, D.C.)* **1990**, *90*, 543−584.
(4) Wickstrom, E. In *Prospects for Antisense Nucleic Acid Therapy of Cancer and Aids*; Wickstrom, E., Ed.; Wiley-Liss: New York, 1991.
(5) Milligan, J. F.; Matteucci, M. D.; Martin, J. C. Current concepts in antisense drug design. *J. Med. Chem.* **1993**, ***36***, 1923−1937.
(6) Stein, C. A.; Chen, Y.-C. Antisense oligonucleotides as therapeutic agents—Is the bullet really magical? *Science* **1993**, *261*, 1004−1012.
(7) Crooke, S. T. Therapeutic applications of oligonucleotides. *Annu. Rev. Pharm. Tox.* **1992**, ***32***, 327−374.
(8) Richert, C.; Roughton, A. L.; Benner, S. A. Nonionic analogues of RNA with dimethylene sulfone bridges. *J. Am. Chem. Soc.* **1996**, *118*, 4518−4531.
(9) Blättler, M. O.; Wenz, C.; Pingoud, A.; Benner, S. A. Distorting duplex DNA by dimethylene sulfone substitutions: A new class of inhibitor for restriction enzymes, *J. Am. Chem. Soc.*, submitted 1997.
(10) Agrawal, S.; Temsamani, J.; Tang, J. Y. Pharmacokinetics, biodistribution, and stability of oligodeoxynucleotide phosphorothioates in mice. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 7595−7599.
(11) Chem, T.-L.; Miller, P. S.; Ts'o, P. O. P.; Colvin, O. M. Disposition and metabolism of oligodeoxynucleoside methylphosphonate following a single iv injection in mice. *Drug Metab. Dispos.* **1990**, *18*, 815−818.
(12) Temsamani, J.; Tang, J. Y.; Padmapriya, A.; Kubert, M.; Agrawal, S. Pharmacokinetics, biodistribution and stability of capped oligodeoxynucleotide phosphorothioates in mice. *Antisense Res. Dev.* **1993**, *3*, 277−284.

(13) Iversen, P.; Mata, J.; Tracewell, W. G.; Zon, G. Pharmacokinetics of an antisense phosphorothioate oligodeoxynucleotide against *rev* from human immunodeficiency virus type 1 in the adult male rat following single injections and continuous infusion. *Antisense Res. Dev.* **1994**, *4*, 43−52.
(14) Zhang, R.; Lu, Z.; Zhao, H.; Zhang, X.; Diasio, R. B.; Habus, I.; Jiang, Z.; Iyer, R. P.; Yu, D.; Agrawal, S. *In vivo* stability, disposition and metabolism of a "hybrid" oligonucleotide phosphorothioate in rats. *Biochem. Pharmacol.* **1995**, *50*, 545−556.
(15) Zhang, R.; Diasio, R. B.; Lu, Z.; Liu, T.; Jiang, Z.; Galbraith, W. M.; Agrawal, S. Pharmacokinetics and tissue distribution in rats of an oligodeoxynucleotide phosphorothioate (GEM 91) developed as a therapeutic agent for human immunodeficiency virus type-1. *Biochem. Pharmacol.* **1995**, *49*, 929−939.
(16) Huang, Z.; Schneider, K. C.; Benner, S. A. Building blocks for oligonucleotide analogues with dimethylene sulfide, sulfoxide, and sulfone groups replacing phosphodiester linkages. *J. Org. Chem.* **1991**, *56*, 3869−3882.
(17) Huang, Z.; Benner, S. A. Selective protection and deprotection procedures for thiol and hydroxyl groups. *SynLett* **1993**, 83−84.
(18) Buter, J.; Kellogg, R. M. Synthesis of macrocyclic and medium ring dithia compounds using cesium thiolates. *J. Chem. Soc., Chem. Commun.* **1980**, *1980*, 466−468.
(19) Buter, J.; Kellogg, R. M. Synthesis of sulfur-containing macrocycles using cesium thiolates. *J. Org. Chem.* **1981**, *46*, 4481−4485.
(20) Trost, B. M.; Curran, D. P. Chemoselective oxidation of sulfides to sulfones. *Tetrahedron Lett.* **1981**, *22*, 1287−1290.
(21) Huang, Z. Dissertation No. 10429, ETH Zurich, Switzerland, 1993.
(22) Mitsunobu, O. The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis* **1981**, *1981*, 1−28.
(23) Ramirez, F.; Desai, N. B.; McKelvie, N. A new synthesis of 1,1-dibromoolefines *via* phosphine-dibromomethylenes. The reaction of triphenylphosphine with carbon tetrabromide. *J. Am. Chem. Soc.* **1962**, *84*, 1745−1747.
(24) Verheyden, J. P. H.; Moffat, J. G. Halo sugar nucleosides. III. Reactions for the chlorination and bromination of nucleoside hydroxyl groups. *J. Org. Chem.* **1972**, *37*, 2289−2299.
(25) Farese, A.; Patino, N.; Condom, R.; Dalleu, S.; Guedj, R. Liquid-phase synthesis of a peptidic nucleic-acid dimer. *Tetrahedron Lett.* **1996**, *37*, 1413−1416.
(26) Pardridge, W. M.; Boado, R. J.; Kang, Y.-S. Vector-mediated delivery of a polyamide ("peptide") nucleic acid analogue through the blood-brain barrier *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5592−5596.
(27) Hanahan, D. Studies on transformation of *Escherichia coli* with plasmids. *J. Mol. Biol.* **1983**, *166*, 557−580.
(28) Nambiar, K. P.; Stackhouse, J.; Stauffer, D. M.; Kennedy, W. P.; Eldredge, J. K.; Benner, S. A. Total synthesis and cloning of a gene coding for the ribonuclease S protein. *Science* **1984**, *223*, 1299−1301.
(29) Cefalu, W. T.; Pardridge, W. M. Restrictive transport of a lipid-soluble peptide (cyclosporin) through the blood-brain barrier. *J. Neurochem.* **1985**, *45*, 1954−1956.
(30) Fahr, A. Cyclosporin clinical pharmacokinetics. *Clin. Pharmacokinet.* **1993**, *24*, 472−495.
(31) Caujolle, F. M. E.; Caujolle, D. H.; Cros, S. B.; Calvet, M.-M. J. Limits of toxic and teratogenic tolerance of dimethyl sulfoxide. *Ann. N.Y. Acad. Sci.* **1967**, *141*, 110−125.
(32) Rubin, L. F. Toxicity of dimethyl sulfoxide, alone and in combination. *Ann. N.Y. Acad. Sci.* **1975**, *243*, 98−103.

JM970538O