Nonionic Analogs of RNA with Dimethylene Sulfone Bridges

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Abstract: Analogs of RNA have been synthesized where each of the phosphodiester linking groups is replaced by dimethylene sulfone units (sulfone-linked nucleic acid analogs of RNA, or "rSNAs"). These are the first fully nonionic analogs of RNA to be prepared as oligomers. Sequences leading to the octamer 5′-r($A_{SO_2}U_{SO_2}G_{SO_2}G_{SO_2}U_{SO_2}C_{SO_2}A_{SO_2}U$)-3′ have been prepared from 3′,5′-bishomo-β-ribonucleoside derivatives as building blocks prepared from diacetone D-glucose, and their chemistry has been explored. Coupling was performed in solution via S_N2 reactions between a thiol from one fragment and a bromide from the other, oxidation of the resulting thioether to the sulfone, and deprotection of a terminal primary hydroxyl group and regioselective conversion of it—in the presence of secondary hydroxyl groups—to an active group (thiol or bromide) to yield another fragment for coupling. Base-labile protecting groups were used for the nucleobases, and one-step full deprotection was achieved using 1 M NaOH. The target octamer and each isolated intermediate were characterized by NMR, UV spectroscopy, and mass spectrometry. While chemical reactions involving longer rSNAs were in several cases retarded relative to analogous reactions with monomers, some rates were enhanced. In water, the rSNA octamer displayed a thermal transition in the UV spectrum above 65 °C with a large hyperchromicity. The behaviors of rSNAs suggest roles for the polyanionic backbone in DNA and RNA beyond its role in conferring aqueous solubility. The repeating anionic charges in natural oligonucleotides evidently also control the potent molecular recognition properties of these richly functionalized molecules, direct strand−strand interactions to the part of the biopolymer distant from the backbone (the Watson−Crick edge of the nucleobases), cause the polymer to favor an extended conformation, and ensure that the physical properties of the oligonucleotide are largely independent of its sequence. This suggests structural features that must be built into nonionic oligonucleotide analogs generally.

Introduction

DNA and RNA are composed of a backbone consisting of repeating phosphate diester units that join sugars bearing a nonrepeating nucleobase. In the standard model of duplex structure proposed by Watson and Crick four decades ago,[1] the nucleobases play the central role in molecular recognition, while the sugar and phosphate linkers play secondary roles as scaffolding for the nucleobases, perhaps with some preorganization. Accordingly, it is widely believed that the scaffolding can be modified to improve the physical properties of oligonucleotides while retaining their molecular recognition properties, provided that the modification continues to meet preorganizational requirements. This model has underlain efforts by many groups, academic and industrial, to seek oligonucleotide analogs with improved biological properties by modification of the backbone.[2]

One class of backbone-modified oligonucleotide analogs replaces the anionic phosphate groups with neutral linkers. Among the most prominent examples of these are the methylphosphonate,[3] sulfonate,[4] sulfonamide,[5] amide,[6] and formacetal and thioformacetal[7] analogs of DNA,[8] as well as the more distantly related peptide-linked nucleic acid analogs (PNAs).[9] Some time ago, we noted that the bridging phosphate groups of natural oligonucleotides might also be replaced by a dimethylene sulfone unit to yield a hydrolytically stable oligonucleotide analog lacking the diastereoisomerism associated with the linkage in several other analogs, referred to here as sulfone-linked nucleic acid analogs (SNAs).[10,11] Because the $-SO_2-$ and $-PO_2^-$ units are largely isosteric and isoelectronic, the structural perturbation introduced by the sulfone-for-phosphate substitution should be relatively small. Further,

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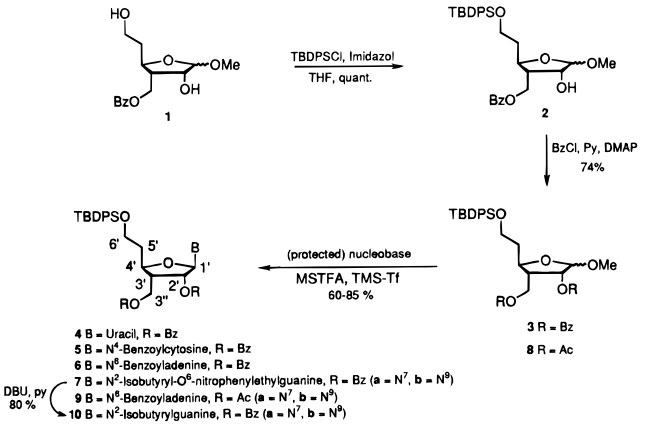
Scheme 1



because the S═O bond of sulfones retains part of the dipolar character of the phosphorus−oxygen bond in the phosphate monoanion, SNAs might retain some of the aqueous solubility of natural DNA, but be soluble in organic solvents as well, and retain some of the interstrand recognition properties of natural oligonucleotides.

This is the case with a dimeric SNA comprised of ribose-type bishomo sugars (where rSNA denotes the exclusive inclusion of 2′-hydroxyl or 2′-*O*-acyl groups), as shown by X-ray crystallography.[12] In crystals grown at room temperature, the self-complementary r($G_{SO_2}C$) sequence exhibited a duplex structure with Watson−Crick base pairing characteristics similar to those found in RNA. The r($A_{SO_2}U$) dimer, however, crystallized at high temperature and was found to be single stranded.[13] We report here the synthesis of rSNAs up to eight bases in length (the ribose-type "r" descriptor is hereafter assumed for all specific species in this context, e.g., $G_{SO_2}C$). These were prepared in mixed base sequences including all four natural nucleobases and characterized.

Synthesis

Monomers. The nucleoside analog building blocks were constructed from diacetone D-glucose. Homoallose derivative **1**[10a] (Scheme 1) was obtained in nine steps and 20% overall yield following optimization[14] of a route developed in these laboratories.[10] Silylation of the 6-position of **1** yielded **2**. Benzoylation of the remaining 2-hydroxyl group gave glycosyl donor **3**.

The glycosyl donor was reacted with (protected) nucleobases under modified Vorbrüggen conditions[15] to yield bishomonucleosides **4**−**7**[16] (Scheme 1). Pyrimidines were efficiently introduced with a stoichiometric excess of Lewis acid in CH_3CN at 40−60 °C. Under similar conditions, N^6-benzoyladenine gave mixtures of N^7 and N^9 isomers. Therefore, reaction conditions were optimized with respect to the catalyst, solvent, temperature, and reagent stoichiometry to allow isomerization during the reaction of the N^7 derivative to the favored N^9 isomer. Incubation for two days at 80 °C with 0.5 equiv of TMSTf in dichloroethane yielded the N^9 derivative of adenosine (**6**) in 54% yield as the sole product.[17] The yield was 60% when 2′,3′-diacetate **8**[18] was used as the glycosyl donor to furnish **9b**.

Table 1. ^{13}C NMR Chemical Shifts of Nucleobase Carbon Atoms for Selected Purine 3′,5′-Bishomonucleosides ($CDCl_3$)

	chemical shift (ppm)				
compd	C2	C4	C5	C6	C8
11 (N^9)	147.9/148.1[a]		120.3	155.7	137.5
7b (N^9)	151.2/152.2[a]		118.6	160.7	140.7
10b (N^9)	147.3/147.4[a]		122.2	155.5	137.8
6 (N^9)	152.8	149.7	123.8	151.3	141.8
13b (N^9)[b]	152.1	149.2	123.1	150.8	141.5
7a (N^7)	152.8/155.7[a]		108.2	163.8	144.0
10a (N^7)	147.2/152.6[a]		110.9	157.5	140.6
13a (N^7)[b]	156.9	141.8	114.2	148.5	142.6

[a] C2 and C4 resonances of guanosine residues were not unequivocally assigned. [b] The N^7 and N^9 isomers **13a** and **13b**, formed by hydrolysis of **9a** and **9b**, were resolved by HPLC chromatography.

Analogous Vorbrüggen-type glycosylation with N^2-isobutyrylguanine gave ca. 1:1 mixtures of the N^7 (**10a**) and N^9 (**10b**) regioisomers. Protection of O^6 as the 2-(*p*-nitrophenyl)ethyl ether,[19] and the use of 1.2 equiv of TMSTf in CH_3CN at 50 °C for 38 h, gave a 1:1.8 ratio of N^7 (**7a**) to N^9 (**7b**) isomers. The isomers were separated by chromatography and isolated in good combined yield (82%). The N^7 isomer was then rearranged in 80% yield to the N^9 isomer using MSTFA and 5 mol % TMSTf at 50 °C over 23 h. Recently, glycosylation involving presilylation with MSTFA and TMSCl, followed by removal of excess reagents under vacuum, and the use of 0.5 equiv of TMSTf at 60 °C for 95 h gave a more favorable 1:7 ratio of N^7 to N^9 isomers in 86% chemical yield.[20] Thus, all four bishomonucleosides (**4**, **5**, **7b**, **9b**) are accessible as pure β,N^1 or N^9 isomers in at least 60% yield. An X-ray analysis established the structure of the partially deprotected guanosine analog **11** (from **10b**; see Scheme 2), and underlay a correlation between ^{13}C NMR chemical shifts and the position of attachment of the purine bases for these molecules.[21] Table 1 gives ^{13}C resonances for **11**, its protected precursors, and selected adenosine analogs.

The octameric heterosequence 6′-$A_{SO_2}U_{SO_2}G_{SO_2}G_{SO_2}U_{SO_2}C_{SO_2}$-$A_{SO_2}U$-3″ was then prepared to provide a test of the oligomerization methodology. This octamer was prepared convergently in solution by coupling four dimers ($A_{SO_2}U$, $G_{SO_2}G$, $U_{SO_2}C$, and $A_{SO_2}U$) to yield two tetramers ($A_{SO_2}U_{SO_2}G_{SO_2}G$ and $U_{SO_2}C_{SO_2}A_{SO_2}U$), and coupling of the tetramers to yield the octamer (see Schemes 3−5).

To prepare monomers functionalized at the 3″-end as protected thiols, the 2′- and 3″-benzoyl groups in the adenosine, uridine, and guanosine analogs were removed with 0.1 M LiOH in methanol/THF/water. Compounds **4**, **9**, and **7b** were converted to diols **12**, **13b**, and **14** in 80−93% yield (Scheme 2). Reaction times were optimized to minimize a slow N^6-debenzoylation of adenine. Conversion of diols **12**, **13b**, and **14** to the corresponding 3″-thioesters **15**−**17** by a Mitsunobu

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(15) Methylene groups attached to the 3′ and the 5′ carbon atoms of natural nucleosides were designated as the 3″ and 6′ positions in 3,5-bishomonucleosides; see Scheme 1.

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(17) Obtained from **2** by hydrolysis with aqueous NaOH and subsequent acetylation with acetic anhydride in 70% yield. Huang, Z. Dissertation No. 10429, ETH Zürich, 1993.

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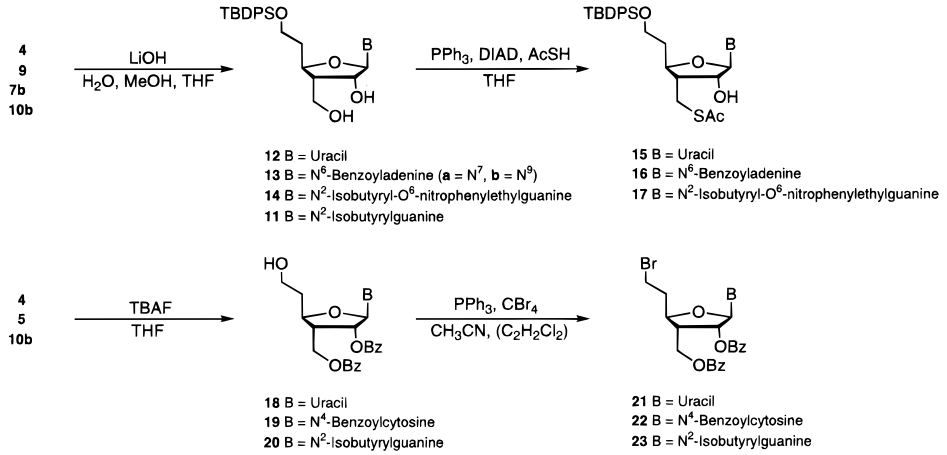
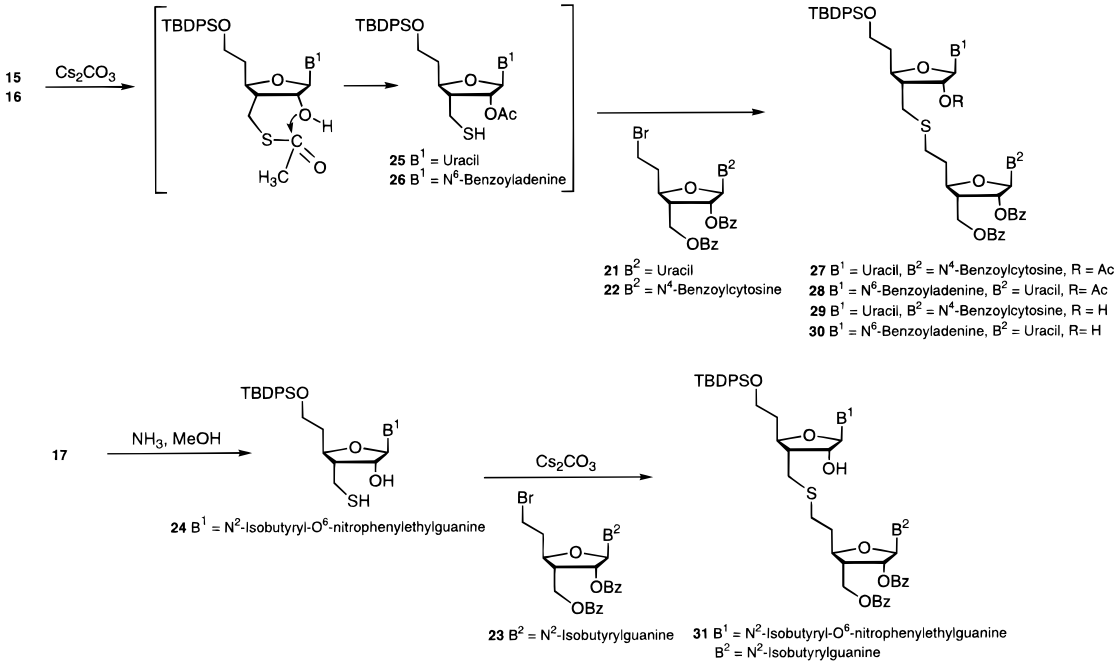
Scheme 2



Scheme 3



reaction[22] employing thioacetic acid in THF or acetonitrile proceeded quickly at room temperature, and was fully selective for the primary hydroxyl group.

Monomers carrying an electrophilic 6′-carbon were prepared by desilylation of **4**, **5**, and **7b** with TBAF in THF (Scheme 2). Partial removal of the O^6-[2-(*p*-nitrophenyl)ethyl] protection from the guanine in **7b** was always observed, however. Hence, O^6 of **7b** was first liberated by treatment with DBU in pyridine[19a] (see Scheme 1), and the resulting **10b** desilylated to give **20**. Treatment of the alcohols **18**−**20** with CBr_4/PPh_3[23,24] gave the bromides **21**−**23**. Generally, CH_3CN was used as the solvent. The low solubility of **19** necessitated the use of a mixture of CH_3CN and dichloroethane and slightly elevated temperatures. Bromination was regioselective for primary hydroxyl groups. Intramolecular displacement of the bromides by O^2 in pyrimidines, a reaction seen in natural nucleosides bearing a leaving group at the 5′-position,[25] was never observed with these molecules. While a mesylate was an alternative to the bromide as a leaving group,[8,26] it was not used here because yields were lower and side products were observed when mesylating monomers and oligomers containing uracil.

Oligomers. Liberation of the thiol from **15**−**17** and generation of the thiolate for the S_N2 coupling depended on the nature of the nucleobase and its protecting group. For example, the acetate was removed smoothly from **17** by ammonolysis in methanol at 0 °C, and the resulting **24** used in Cs_2CO_3-mediated[27] coupling either as a crude substance or as a chromatographically isolated substance (Scheme 3). With **15**, however, substantial amounts of (presumed) disulfide were obtained as an undesired byproduct. The benzoyl group in **16** was relatively labile under these conditions. Thioacetates **15** and **16** were therefore deprotected *in situ*, by migration of the acetyl group from sulfur to the neighboring 2′-hydroxyl group (yielding **25** and **26**) in the coupling reaction. This *in situ* deprotection reaction simultaneously protected the 2′-hydroxyl group, and yielded coupled products without the formation of

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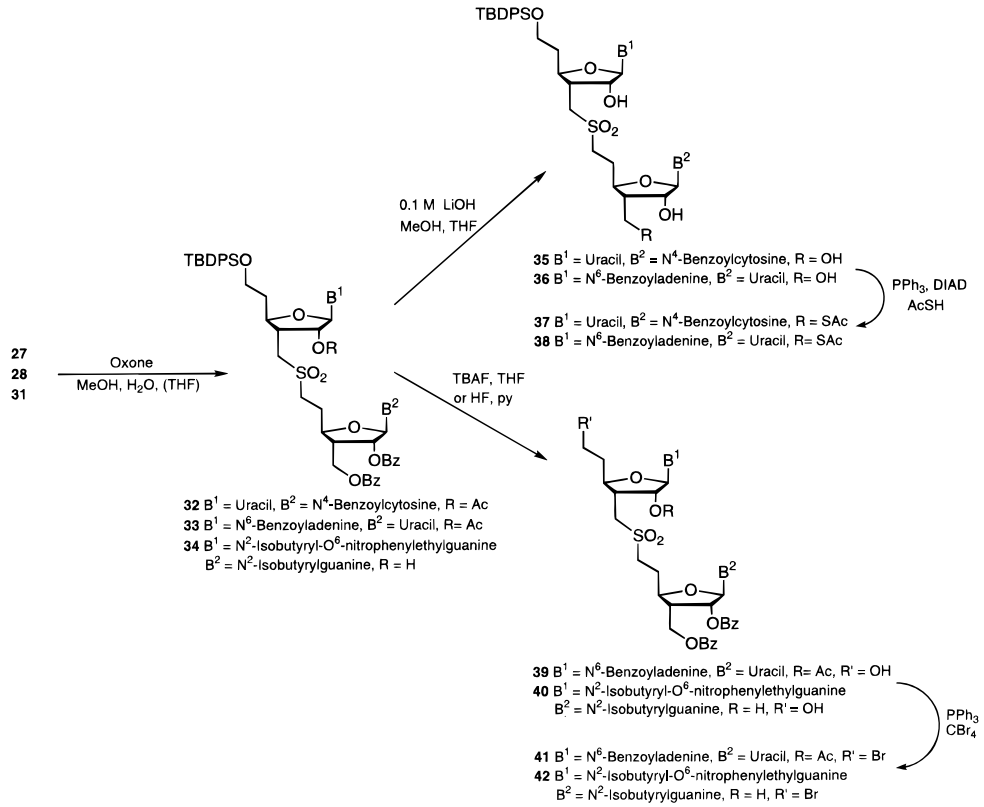
Scheme 4



detectable amounts of disulfide. The *in situ* acetyl migration and coupling of guanine-containing compound **17** were less efficient.

Migration of the acetyl groups to generate 3′′-thiol(ate)s **25** and **26** was ca. 10-fold faster than the actual coupling with **21** and **22** (both 30 mM) to give U_SC (**27**, 54%) and A_SU (**28**, 57%) dimers. Approximately 20% of the dimers isolated from these reactions lacked the 2′-*O*-acetyl group [products **29** (23%) and **30** (23%)], presumably because of an intermolecular migration of the acetyl group from the 2′-O-position. Since this acetyl group was removed two steps later (see below), no effort was made to reduce the formation of this useful byproduct. The combined yields of isolated dimers were 77% (U_SC) and 80% (A_SU) with Cs_2CO_3 in a homogeneous CH_3CN/H_2O mixture over 16−24 h at 50 °C. Yields calculated on the basis of recovered bromide starting material (≤10%) were correspondingly higher. The coupling of guanine-containing compounds **23** and **24** in a suspension of Cs_2CO_3 in THF over 2.5 h at 35 °C gave G_SG dimer **31**, isolated in 92% yield, as well as traces of bromide starting material and (presumed) disulfide. DBU as the base in the coupling reaction[10,28] led to considerable amounts of symmetrical disulfide and accordingly lower yields with thiols generated by hydrolytic deacetylation of **15** and **16**.

Thioethers were oxidized to sulfones after each coupling step, a precaution taken because full oxidation of a DNA-type thioether octamer had required forced conditions and was frequently low yielding.[28] Further, sulfones were shown on the basis of UV hyperchromicity studies (see below) to aggregate less than their parent thioethers. Thus, oxidation proved to be an important element in the strategy to manage the solubility properties of synthetic intermediates.

Treatment of dimer thioethers **27**, **28**, and **31** with Oxone in methanol/water[29] gave sulfones **32**−**34** (Scheme 4) in almost quantitative yield. Sulfoxide intermediates, formed almost immediately, could be recovered by early termination of a reaction. These were characterized by proton NMR ("doubling" of signals resulting from the diastereomeric mixture of sulfoxides) and mass spectrometry. The oxidation was followed by thin layer chromatography for the dimers, as the sulfoxides exhibited higher polarity than the sulfones, to which they were slowly but completely transformed over 1−2 h. No other products were detectable, even with longer oligomers. To improve solubility of the educts during the reaction, THF was added as required. Using $CHCl_3$ as an additive, a homogeneous reaction mixture could be obtained, although oxidations under these conditions were considerably slower.

The synthetic cycle of selective deprotection, functionalization, coupling, and oxidation was repeated starting from dimeric sulfones **32**−**34**. Deprotection of the 2′- and 3′′-alcohol groups with LiOH was lower yielding with $U_{SO_2}C$ (**32** to **35**, 54%) than with $A_{SO_2}U$ (**33** to **36**, 78%). Loss of the protection on the base was shown to account for the lower yields. Some chemical shifts of the proton signals of the parent dimer **32** exhibited a concentration dependence that may point to molecular associates, which may in turn influence the rate with which the *N*-benzoate or *O*-benzoate groups are cleaved. Further, signals in the ^1H NMR spectrum of **35** suggested that the $U_{SO_2}C$ dimer, notably in contrast to the $A_{SO_2}U$ dimer **36**, is involved in intermolecular association in solution (see the Discussion).

Mitsunobu thioacetylation (**35** to **37** and **36** to **38**) proceeded in 82% and 92% yield for the $U_{SO_2}C$ and $A_{SO_2}U$ dimers. Desilylation of the $A_{SO_2}U$ and $G_{SO_2}G$ dimers **33** and **34** used TBAF/THF and HF/pyridine, respectively. The latter was a mild alternative to TBAF compatible with the NPE protecting group. The 6′-alcohols were converted to bromides with CBr_4/PPh_3 in CH_3CN (**39**) and in 1,2-dichloroethane (**40**) to give **41** and **42** in high yields.

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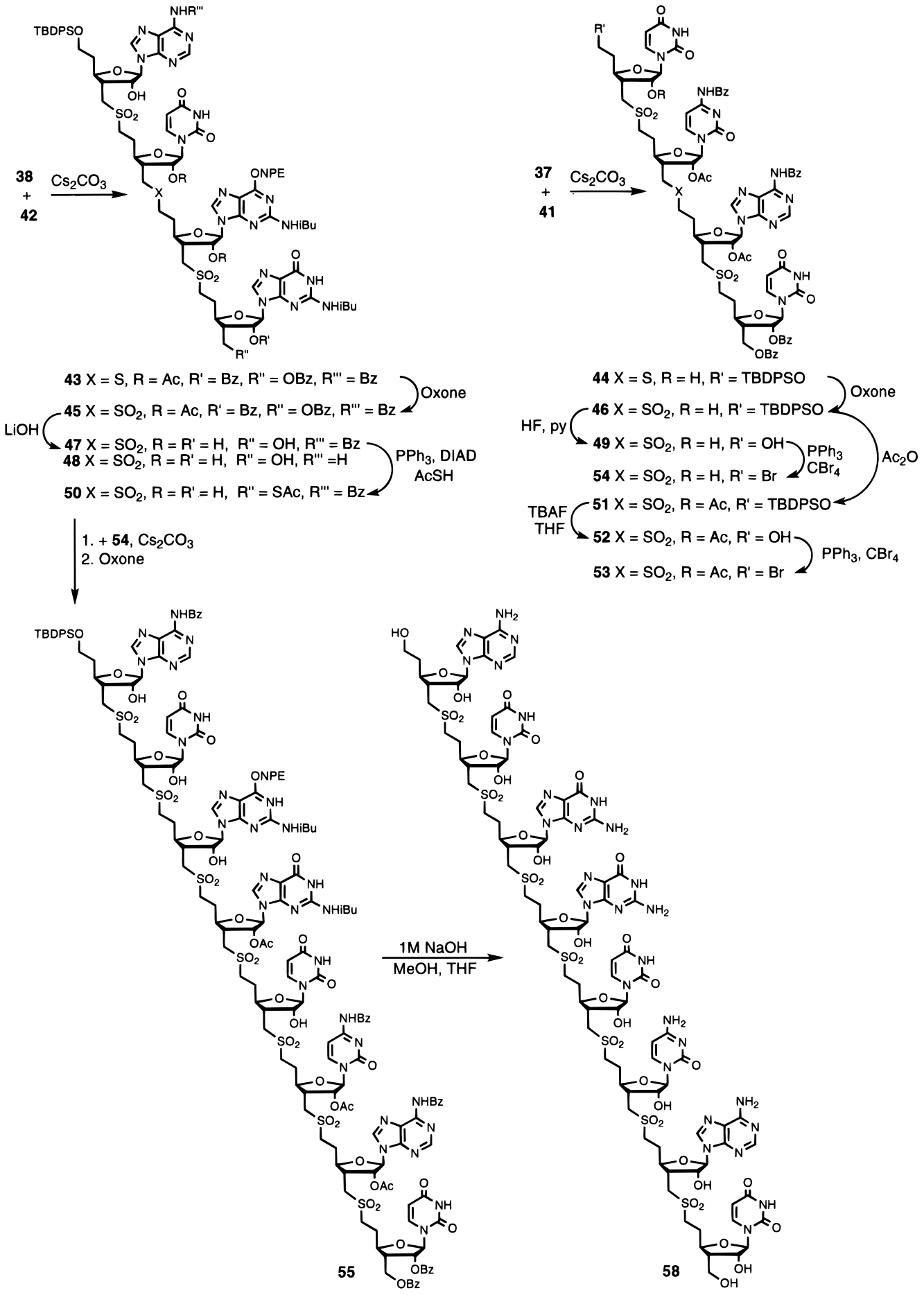
Scheme 5


38 + 42
Cs2CO3
37 + 41
43 X = S, R = Ac, R' = Bz, R'' = OBz, R''' = Bz
Oxone
45 X = SO2, R = Ac, R' = Bz, R'' = OBz, R''' = Bz
LiOH
47 X = SO2, R = R' = H, R'' = OH, R''' = Bz
48 X = SO2, R = R' = H, R'' = OH, R''' =H
PPh3, DIAD
AcSH
50 X = SO2, R = R' = H, R'' = SAc, R''' = Bz
1. + 54, Cs2CO3
2. Oxone
44 X = S, R = H, R' = TBDPSO
46 X = SO2, R = H, R' = TBDPSO
HF, py
49 X = SO2, R = H, R' = OH
54 X = SO2, R = H, R' = Br
PPh3
CBr4
Ac2O
51 X = SO2, R = Ac, R' = TBDPSO
TBAF
THF
52 X = SO2, R = Ac, R' = OH
53 X = SO2, R = Ac, R' = Br
PPh3, CBr4
1M NaOH
MeOH, THF
55
58

Coupling of the two sets of dimers to yield tetramers **43** and **44** proceeded in ca. 70% yield with Cs_2CO_3 in THF (Scheme 5). Again, ca. 25% of the products were isolated as tetramers lacking the acetyl groups on the nucleoside unit bearing the 3″-thioether, but no symmetrical disulfides were isolated. More unreacted dimer was isolated after these couplings compared to that recovered in the synthesis of the dimers from monomer building blocks. This suggested that the coupling reaction was slower with dimers. The thioethers were oxidized to yield **45** and **46**.

For the third synthetic cycle, the 2′- and 3″-hydroxyl groups of tetramer **45** were deprotected to yield **47** in 67% yield, with ca. 10% of the A-N^6-debenzoylated product **48**. Interestingly, when dissolved in a CH_2Cl_2/CH_3OH mixture (4 μM), **47** was found after one week at room temperature to have *spontaneously* transformed itself into **48** (see below). Desilylation of $U_{SO_2}C_{SO_2}A_{SO_2}U$ tetramer **46** with HF/pyridine furnished **49** in 77% yield.

Activation of the tetramers was most successful when the intermolecular association of the synthetic intermediates was managed. For example, synthesis of **50** from **47** was best in a mixed solvent system (dioxane/1,2-dichloroethane) at slightly elevated temperature, providing product in 60% yield (72% based on recovered starting material). Synthesis of a bromide from **49** was problematic under the conditions used for the dimers. Thus, **46** was 2′-O-acetylated to give **51**, desilylated (TBAF, THF) to **52**, and converted to bromide **53** in CH_3CN/dichloroethane in 52% yield. Alternatively, **49** was mixed with monomer alcohol **18**, which improved its solubility, and both alcohols were brominated together in CH_3CN/dichloroethane to give **54** in 33% yield (83% based on recovered starting material). Tetramer **45** could also be desilylated (HF, pyridine) and converted to the 6′-bromide (CBr_4/PPh_3/pyridine) in 36% yield over two steps.

To understand better the lower reactivity of the longer rSNA oligomers, partially protected oligomers **47** and **49** were examined by UV spectroscopy in mixtures of CH_2Cl_2 and CH_3OH (see the supporting information). At micromolar concentrations, strong hyperchromicity at 260 nm was observed upon addition of CH_3OH. The amount of CH_3OH necessary for maximum hyperchromicity was higher for tetramers containing primary alcohols than for the protected parent compounds, and lower for sulfones than for their parent thioethers. Proton NMR signals of the tetramers were broad in $CDCl_3$ (at millimolar concentrations) and sharpened with CD_3OD. The addition of D_2O further sharpened the signals. These observations are consistent with intermolecular aggregation via hydrogen bonding.

Coupling of the tetramers **50** and **54** (Scheme 5) was slower than with shorter fragments, yielding for the first time symmetrical disulfide as a side product of the intramolecular acetyl transfer method. Similar influence of the length of the synthetic fragment on the success of a coupling is not uniformly observed in the synthesis of RNA itself,[30] but may have been encountered in the solution phase synthesis of other nonionic oligonucleotides.[4a] Low yields are reported in esterification reactions with largely unprotected carbamate-linked thymidine trimers and tetramers.[31]

Directly treating the crude product mixture containing desired octamer sulfide and the octamer disulfide side product with Oxone converted the latter to the corresponding sulfonic acid and the former to protected sulfone **55** (Scheme 5). The sulfonic acid and residual bromide were removed easily by HPLC, yielding **55** in 53% yield for the two steps (70% based on recovered bromide **54**).

Conditions for deprotecting rSNAs varied according to the protecting groups. $U_{SO_2}C_{SO_2}A_{SO_2}U$ tetramer **49** was deprotected in CH_3OH/THF treated with saturated aqueous NH_3, smoothly yielding fully deprotected tetramer $U_{SO_2}C_{SO_2}A_{SO_2}U$ **56** (structural formula not shown) at room temperature in 20 h. With tetramer **45**, which contains each type of protecting group present in **55**, the TBDPS and the NPE groups were removed only slowly (reaction times $\geq$24 h) under the conditions normally used for deprotecting synthetic DNA and RNA oligomers (NH_4OH, $\leq$60°C). They were quantitatively removed, however, together with all acyl groups, by treatment with 1 M NaOH in a mixture containing CH_3OH and THF. The reaction was complete within a few hours at 40 °C, or after one day at room temperature. No side products were detected, demonstrating the high stability of the sulfones to strongly basic conditions. Further, all of the rSNAs studied here were soluble in aqueous media at high pH, which facilitated their handling.

Precipitation/extraction yielded an oligomer pure by NMR, MALDI-TOF, and reversed phase HPLC. With this protocol, **45** and **46** were converted to the $A_{SO_2}U_{SO_2}G_{SO_2}G$ tetramer **57** (63%) and the $U_{SO_2}C_{SO_2}A_{SO_2}U$ tetramer **56** (60%), respectively (structural formulas not shown). Under the same conditions, octamer **55** was converted to **58** (Scheme 5), which after precipitation and a series of extraction and washing steps was shown to be pure by spectroscopic techniques.

Characterization

Oligomeric rSNAs were characterized by ^1H and ^{13}C NMR and UV spectroscopy and mass spectrometry, and occasionally by combustion analysis and X-ray structure determination. Mass spectrometric analysis is invaluable to identify longer deprotected oligonucleotide analogs.[32] Unsatisfactory signal to noise ratios were obtained for longer sulfone oligonucleotides in MALDI-TOF spectra recorded under conditions recommended for natural oligonucleotides.[33] The matrix 2-[(4-hydroxyphenyl)azo]benzoic acid,[34] often used for proteins, yielded sufficient sensitivity and good resolution for sulfone oligomers with data accumulation in the positive mode with repeated low power laser shots (see the supporting information). About 10 pmol of oligomer per matrix preparation was used. Monosodium adducts produced the most prominent MALDI MS peaks, as was shown independently by electrospray ionization mass spectrometry of octamer **58** (see the supporting information).

The conformations of rSNAs were explored by proton NMR; ^1H and ^{13}C assignments are reported in the supporting information. The ring pucker of the bishomo ribose rings, estimated from the $J_{1',2'}$ couplings using an approximation [2′-endo (%) = $J_{1',2'}/(J_{1',2'} + J_{3',4'}) \times 100$],[35] was predominantly 3′-endo ("north") for most protected and all unprotected rSNAs, similar to that observed in natural RNA. A north conformation of the ribose ring in solution might be expected on the basis of the conformations observed in the single crystal X-ray structure

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analyses of the guanosine analog **11** (see the supporting information) and the $G_{SO_2}C$ and $A_{SO_2}U$ dimers.[12,13]

Remarkably, (*p*-nitrophenyl)ethyl-protected guanosine analogs having a free 2′-hydroxyl group had significantly increased values for $^3J_{1',2'}$ and reciprocal changes in the multiplicities of H3′ and H4′ signals, suggesting an increased contribution of 2′-endo-type ("south") states. The $G^{iBu}{}_SG^{iBu}$ dimer, where G1 (residues take their letter plus numerical position from the 5′- to 3′-end) has a free 2′-hydroxyl group but no longer bears a NPE group at O^6, also displayed an increased contribution of south states in G1 ($J_{1',2'} = 5.3$ Hz). These results indicate that the conformational equilibrium of the tetraydrofuran ring is sensitive to small changes in structure elsewhere in the molecule. While this may be the result of a "through bond" effect, it is more likely due to an intermolecular association of rSNAs containing G.

In pure water, fully deprotected sulfone oligomers show moderate solubility. The solubility of octamer **58** was found to be $\geq 5\ \mu$M in water and >1 mM in DMSO and DMF; higher solubilities were often observed, but not reproducibly. Dissolution in organic solvents and water is slow, and requires gentle heating. The rSNAs without guanine dissolve considerably faster than rSNAs containing G. Those rSNAs containing guanine could, however, be efficiently solubilized with 0.1 M NaOH.

Octamer **58** displayed a transition in UV absorbance (258 nm) as a function of temperature. Remarkably, this began at ca. 65 °C, and was not complete at 95 °C. The first derivative of the curve, extracted from plots obtained at three different concentrations (ca. 1, 0.5, and 0.33 μM), yield in each case a "melting point" of 83 °C.[36] The hyperchromicity was large compared to that observed in a model RNA with the same sequence. To rule out the possibility that the hyperchromicity reflects simple, reversible solution/dissolution of the octamer, the melting experiment was repeated at pH 12.5, where **58** dissolves well in water. The hyperchromicity was lower at this higher pH, but the transition did not disappear. The transition did nearly completely disappear, however, when 20% DMF was added to the neutral aqueous solution.

Discussion

Our synthetic strategy makes available rSNAs having any sequence, and therefore allows examination of the physical and biological properties of these nonionic oligonucleotide analogs to begin. Preliminary results show that rSNAs have many characteristics that deserve detailed exploration. These characteristics offer insights into why natural oligoribonucleotides have phosphate bridging groups, and how nonionic oligonucleotides might be designed to exhibit altered physical properties while retaining Watson−Crick recognition.

Physical and Catalytic Properties of rSNAs. Some interesting reactivities were observed at the outset in the partially protected rSNAs used as synthetic intermediates. For example, the rates and yields of the coupling reactions were sensitive to the sequences being coupled. Since a key step in the coupling is the rate of migration of the acetate from the 3″-thiol to the 2′-hydroxyl group (e.g., **15** to **25**, Scheme 3), this reaction should be influenced by the conformation of the sugar ring. When the sugar is in a 3′-endo-type conformation, the transfer can proceed via a six-membered ring transition state in a chair-type conformation that is free from significant steric clashes. In contrast, if the sugar ring adopts the 2′-endo-type conformation, acetyl transfer should be slower. Thus, lower yields in migration/coupling reactions observed with $G^{NPE,iBu}$-3″-SAc/2′-OH monomer **17**, where NMR spectra suggest that more of the south conformation is present, can be rationalized. Similarly, the thioacetylation of 3″-alcohol **14**, also showing a significant south contribution, gave almost exclusively the 3″-SAc/2′-OH product **17**. However, the G^{iBu} diol **11**, with more north conformation, gave a 1.9:1 ratio of 3″-SAc/2′-OH to 3″-SH/2′-OAc under the same conditions.

Detailed analysis of the NMR spectra of several intermediates suggested that intermolecular association could also be an important factor determining reactivity. For example, in contrast to other monomeric and dimeric rSNAs bearing two or more free hydroxyl groups, all signals of the more easily N-debenzoylated $U_{SO_2}C^{Bz}$ triol **35** were sharp in neat $CDCl_3$, consistent with the molecule forming a single defined structure. Signals for N^3-H of uracil and both 2′-OH protons of the dimer are ca. 1−1.5 ppm downfield of the corresponding signals in other compounds, suggesting that one of the base NHs and at least two of the ribose OHs are involved in hydrogen bonding. Further, each of the base hydrogens gives at least one NOESY cross peak to a proton of the other pyrimidine, indicating base stacking. Strong interresidue base-to-ribose NOE cross peaks (e.g., NH(U) to H-2′(C)) indicate that **35** forms at least a bimolecular associate. Formation of this associate is sensitive to small structural changes. For example, functionalization of the 3″-hydroxyl group as a thioester (**35** to **37**) seems to block association.

The yields of coupling reactions correlated with increasing chain length. This effect could be explained by an increase in intermolecular association through collection of a critical mass of hydrogen bonding groups in the intermediates. Evidence for this comes from the solvent effects on yield in the coupling. Yields of monomer couplings to produce dimers (e.g., **15** and **22** to give **27**) were higher in THF and lower in DMF; the opposite was observed in the tetramer to octamer coupling (**50** and **54** to give the precursor of **55**), where disrupting the hydrogen bonding with the polar solvent may have been essential.

From a synthetic point of view, these effects are simply technological annoyances. As such, they were managed by adjusting the polarity of the solvents, manipulating the sulfide−sulfone oxidation states of intermediates, and changing purification strategies. With optimized conditions, coupling, oxidation, and either 6′- or 3″-deprotection without chromatography proceed in approximately 70% overall yield for the three steps at the dimer and trimer levels.[37]

At a scientific level, however, these effects are interesting statements about the conformation and reactivity of nonionic oligonucleotide analogs. For example, not all effects observed in rSNAs with higher molecular weight involved slower chemical reactions. In some cases, longer oligomers had *increased* rates of reactions. For example, as a solution (4 μM) in CH_2Cl_2/CH_3OH, **47** was observed to transform itself spontaneously into **48** after one week at room temperature. In more detailed studies at 20 μM concentration, **47** showed a half-life for the debenzoylation of ca. 30 h at 25 °C. The analogous reaction was also observed in dimer **36** ($t_{1/2} \approx 12$ h), but was much slower in the 2′,3″-deprotected adenosine monomer **13b** ($t_{1/2} \geq 10$ days).[38] Comparison of the debenzoylation rates of the quickest self-debenzoylating SNA **36** with that of N^6-

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benzoyl-2′-deoxyadenosine as a reference showed a rate enhancement of ≥30-fold.

Because **36** and **47** are not recovered unchanged from the reaction mixture, it is incorrect to state that they catalyze their own debenzoylations. However, if the reaction occurs *via* an aggregate, then the rSNA molecules might be acting as a true catalyst in the debenzoylation reaction. The structural origin of the "catalytic" activity of these rSNAs is unknown. The phenomenon deserves further exploration, especially as it is reminiscent of the self-transformations catalyzed by natural RNA.[39]

The rich molecular association properties extended to the fully deprotected rSNAs. First, rSNAs can participate in Watson−Crick pairing. For example, the $G_{SO_2}C$ dimer forms in the crystal a duplex quite similar in overall structure to the analogous RNA (G_PC) dimer.[12] Non-Watson−Crick pairing presumably is involved in the molecular aggregation of the $G_{SO_2}G$ dimer, however, as it is noticeably less soluble in water than the $G_{SO_2}C$ dimer (data not shown). Self-association in G-rich RNA and DNA is well known.[40]

However, it is clear that non-Watson−Crick interactions play a far more important role in rSNAs than in RNAs. The molecular association of the octameric rSNA **58** in water proved to be especially interesting. This sequence is nearly self-complementary. If Watson−Crick rules are followed, two of these molecules might form an antiparallel duplex joined by six Watson−Crick base pairs and two G−U wobble base pairs. An RNA molecule with the identical sequence indeed displayed a melting transition consistent with this structure. With this natural RNA strand at 4 and 1.4 μM concentrations, the melting transitions were at ca. 16 and 12 °C, respectively.

In contrast, the "melting temperature" of octameric rSNA **58** displayed a transition at much higher temperature. The "melting temperature" was difficult to obtain precisely, as the end point was >95 °C. The transition temperature did not change notably with a 3-fold change in concentration of the octamer, suggesting that the transition reflects a unimolecular change in the conformation rather than a disassociation of two octamer strands. Further, the relatively weak dependence of the transition on pH suggests that the structure being melted is formed by interaction between hydrogen bonding groups that are not easily ionizable (the 2′-OH group of the backbone sugars and the exocyclic NH_2 groups of the bases, for example). One conformation consistent with these data is a tight "hairpin". These suggestions must be qualified, however, in light of the extremely high transition temperature, making it impossible to establish a high-temperature baseline, to determine accurately the midpoint of the transition, and thereby to assess accurately the stoichiometry of the transition. The self-association of octameric **58** appears to preclude intermolecular association of **58** with complementary RNA and DNA. The melting transition of **58** was the same in the presence of both complementary RNA and DNA as in their absence.

Comparison with Phosphate-Linked Oligonucleotides. The "standard model" of nucleic acid structure, proposed by Watson and Crick in 1953,[1] assigns to the nucleobases the predominant role for the strand−strand recognition displayed by oligonucleotides. Accordingly, the backbone is often viewed as being largely irrelevant to their molecular recognition properties, even as it is recognized as being a key to the solubility properties of oligonucleotides in aqueous and nonaqueous environments. In particular, the polyanionic character of the backbone has long been believed to account for the difficulty with which oligonucleotides cross the lipid phase of membranes to enter cells.

This model has underlain many efforts to generate backbone-modified oligonucleotides with altered solubility properties. Best known are efforts to develop nuclease-resistant and membrane-permeable DNA analogs for "antisense" applications[41] by replacing the repeating phosphate polyanion.[42−44] In a very early study, nonionic but randomly polymeric oligonucleotide analogs were prepared.[45]

The behaviors of rSNAs documented here underscore the incompleteness of the standard model in describing the role of the backbone. Much of the current disenchantment[46] with oligonucleotide analogs as therapeutic agents may be due to the failure of the standard model in this respect. These results also suggest at least three mechanisms by which the phosphate groups define the molecular recognition properties of oligonucleotides in ways incidental to their effect on solubility in water.

First, the phosphate groups evidently force the preferred interaction surface between oligonucleotide strands to the parts of the molecule that are as distant from the polyanionic backbone as possible. This is, of course, the Watson−Crick "edge" of the nucleobases. In the absence of interstrand phosphate−phosphate repulsion, sugar−sugar interstrand interactions, sugar−backbone interstrand interactions, interactions between the sugar and backbone groups of one strand and the Hoogsteen edge of the nucleobases on the other, Hoogsteen−Hoogsteen interstrand interactions, and Watson−Crick−Hoogsteen interstrand interactions all become more important. In rSNAs, the absence of the repeating negative charges in the backbone allows the interactions between the two strands to "move in" from the Watson−Crick edge of the nucleobases to involve other parts of the strand.

Second, the repeating polyanionic backbone in natural DNA and RNA makes the properties of the oligonucleotide largely independent of its sequence. Interactions between monopoles (charges) dominate dipolar interactions, which in turn dominate quadrapolar interactions (and so on). The presence of a repeating monopole in oligonucleotides implies that dipolar interactions (hydrogen bonding, for example) are secondary in defining the properties of an oligonucleotide. This means that the behavior of a natural oligonucleotide will, to a first approximation, be independent of its sequence. This property is important for an encoding molecule. With rSNAs, virtually every behavior is strongly dependent on sequence and length, including solubility, conformation, and reactivity, especially when compared with natural oligonucleotides. In this respect,

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rSNAs behave more like peptides than oligonucleotides,[47] which also self-associate, form conformations strongly dependent on sequence, and display low levels of catalytic activity.[48,49] By amplifying interactions that are only second-order in RNA, sRNAs should reveal second-order factors influencing behavior in natural oligonucleotides, factors that may have biological significance.[50]

Third, the statistical mechanical theory of polymers suggests that the polyanionic backbone will cause natural oligonucleotides to adopt an extended structure.[51] This deters the formation of hairpins and other folded unimolecular structures that bring the phosphate groups together. An extended structure can, of course, be viewed as "preorganization" to prepare a single strand for binding to a complementary strand. Conversely, it can be viewed as an effect disfavoring unimolecular conformations in an oligonucleotide that might compete with its binding to complementary oligonucleotides. Thus, the polyanionic RNA sequence $A_PU_PG_PG_PU_PC_PA_PU$ forms duplexes. The self-structure of the corresponding rSNA **58** precludes association with complementary oligonucleotides.

As nonionic oligonucleotide backbones can no longer direct the interaction between strands toward the extremities of the nucleobases (the "Watson−Crick edges"), or force the molecule to adopt an extended structure, nonionic oligonucleotide analogs must incorporate other features if they are to consistently achieve binding to complementary oligonucleotides following simple rules. For example, functionality not directly involved in Watson−Crick pairing (nitrogens 3 and 7 of the purines, the furanose ring oxygen, the 2′-hydroxyl group) might be removed. Further, because the properties of nonionic oligonucleotides depend strongly on their sequence, the sequence of a nonionic analog to be used in a biological system (for example, as an antisense agent) cannot be selected simply to make it complementary to a target sequence, but must also be mindful of the physicochemical properties of the antisense sequence itself.

Experimental Section

General Techniques. Reactions were carried out under an argon atmosphere with freshly distilled (THF and dioxane from Na) or commercially dried (DMF, pyridine, CH_3CN, and $Cl(CH_2)_2Cl$; puriss quality, absolute, stored over molecular sieves) solvents (Fluka) under anhydrous conditions, unless otherwise noted. Reagents: highest commercial quality (Fluka), used without further purification. Acetate buffer: HOAc (3 M), NaOAc (1 M), and deionized water. Analytical thin layer chromatography: E. Merck 60 F_{254} precoated silica gel plates, compounds visualized by UV light or staining with cerium(IV) sulfate/phosphomolybdic acid/H_2SO_4 (concentrated) and subsequent heating. Flash chromatography: E. Merck silica gel (60, 0.040−0.063 mm mesh), solvents distilled from anhydrous $CaSO_4$ (Sikkon, Fluka), stepwise (3−5 steps) eluant gradients unless otherwise noted. NMR spectra: Bruker AMX 500, AMX 600, or Varian Gemini 300, 200 spectrometer. Ultraviolet (UV) spectra: Shimadzu UV/VIS 240 or Perkin-Elmer Lambda 2 spectrophotometer, the latter being equipped with a thermoelectric cuvette holder, thermoelement, and Perkin-Elmer Digital Controller 570-071 for the collection of UV data as a function of temperature. Infrared spectra: Perkin-Elmer 781 spectrophotometer. Low-resolution mass spectra (MS): VG Tribrid (EI), VG ZAB2-SEQ (FAB, 3-nitrobenzyl alcohol (NOBA) matrix), or Bruker Reflex (MALDI-TOF, 2-[(4-hydroxyphenyl)azo]benzoic acid (HABA) matrix) spectrometer. Electrospray ionization spectra: VG Biotech BIO Q spectrometer, acquired by MScan Corp., Geneva. Optical rotations: Perkin-Elmer 241 or Jasco DIP-370 polarimeter. Microanalyses: Microanalytical Laboratory, ETH, Zürich. The following experimental part gives representative procedures and data only; additional procedures and assigned (for oligonucleotides, residues take their letter, or letter plus numerical position from the 5′- to 3′-end, e.g., A or G2) spectroscopic data for *all* compounds are reported in the supporting information.

1-[2′-Benzoyl-3′-[(benzoyloxy)methyl]-6′-(*tert*-butyldiphenylsilyl)-3′,5′-dideoxy-*β*-allofuranosyl]uracil (4). Uracil (1.3 g, 11 mmol) was dried under high vacuum at 60 °C and suspended in CH_3CN (20 mL). *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) (8.8 g, 8.1 mL, 44 mmol) was added under stirring, resulting in a clear solution after 15 min. Glycosyl donor **3** (3.5 g, 5.5 mmol), which had been coevaporated with toluene and dried under high vacuum at 50 °C, was dissolved in CH_3CN (40 mL) and added to the silylated base. The resulting solution was cooled (0 °C), and trimethylsilyl triflate (TMSTf) (1.8 g, 1.5 mL, 8.3 mmol) was injected. The solution was warmed to 40 °C over 30 min and then stirred for 6 h before being cooled to 0 °C and poured into a mixture of saturated Na_2CO_3 (200 mL), ice (100 g), and CH_2Cl_2 (300 mL). The mixture was filtered through Celite, the organic layer separated, and the aqueous layer reextracted 2× with CH_2Cl_2. The combined organic phases were washed with brine, which was reextracted 2× with CH_2Cl_2. The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*, and the residue chromatographed on silica (120 g) with CH_2Cl_2/EtOAc (4:1) and a MeOH gradient (1.5% to 6%). Removal of residual MSTFA under high vacuum yielded bishomouridine **4** (3.4 g, 4.7 mmol, 85%) as a colorless glass. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.07 (s, 9H, CH_3); 1.98, 2.16 (2m, 2H, H-5′); 2.95 (m, 1H, H-3′); 3.93 (m, 2H, H-6′); 4.39 (m, 1H, H-4′); 4.54 (m, 2H, H-3′′); 5.74 (d, 1H, J = 8.1 Hz, H-5); 5.81 (m, 2H, H-1′, H-2′); 7.24 (d, 1H, J = 8.1 Hz, H-6); 7.40 (m, 10H, *m*-Bz, *m*,*p*-Ph); 7.55 (2 AA′BB′C systems, 2 C parts, 2H, *p*-Bz); 7.68 (m, 4H, *o*-Ph); 7.99 (2 AA′BB′C systems, 2 AA′ parts, 4H, *o*-Bz); 9.38 (br s, 1H, NH). FABMS: *m/z* 741 ($M + Na^+$). Anal. Calcd for $C_{41}H_{42}N_2O_8Si$: C, 68.50; H, 5.89; N, 3.90. Found: C, 68.57; H, 5.74; N, 3.99.

***N*^{4}-Benzoyl-1-[2′-benzoyl-3′-[(benzoyloxy)methyl]-6′-(*tert*-butyldiphenylsilyl)-3′,5′-dideoxy-*β*-allofuranosyl]cytosine (5).** Following the procedure for preparing **4**, 2 equiv of TMSTf was stirred with *N*^{4}-benzoylcytosine[52] (1.23 g, 5.73 mmol) and glycosyl donor **3** (2.92 g, 4.58 mmol) (1 h, 60 °C). The residue was chromatographed using EtOAc/petroleum ether/chloroform/EtOH (100:100:60:1) to yield **5** (2.29 g, 2.80 mmol, 61%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.09 (s, 9H, CH_3); 2.00, 2.20 (2m, 2H, H-5′); 2.79 (m, 1H, H-3′); 3.97 (m, 2H, H-6′); 4.19 (d, 2H, J = 6.9 Hz, H-3′′); 4.54 (dt, 1H, J = 10, 2.7 Hz, H-4′); 5.93 (d, 1H, J = 1.4 Hz, H-1′); 6.06 (d, 1H, J = 4.5 Hz, H-2′); 7.32−7.69 (m, 16H, H-5, *m*,*p*-Bz, *m*,*p*-Ph); 7.75 (m, 4H, *o*-Ph); 7.77 (d, 1H, J = 6.7 Hz, H-6); 7.98 (3 AA′BB′C systems, 3 AA′ parts, 6H, *o*-Bz); 9.4 (br s, 1H, NH). FABMS: *m/z* 822 ($M + H^+$). Anal. Calcd for $C_{48}H_{47}N_3O_8Si$: C, 70.14; H, 5.76; N, 5.11. Found: C, 69.70; H, 5.83; N, 5.23.

***N*^{2}-Isobutyryl-*O*^{6}-[2-(4-nitrophenyl)ethyl]-9-[2′-benzoyl-3′-[(benzoyloxy)methyl]-6′-(*tert*-butyldiphenylsilyl)-3′,5′-dideoxy-*β*-allofuranosyl]guanine (7b).** *N*^{2}-Isobutyryl-*O*^{6}-[2-(4-nitrophenyl)ethyl]guanine[19] (3.54 g, 9.55 mmol, dried under HV, 2 h) was suspended in CH_3CN (60 mL) at room temperature (RT). MSTFA (3.53 mL, 19.09 mmol) was added to the thick white paste and stirring continued at RT for ca. 2 h, giving a faintly yellow solution containing a small amount of fine, suspended white material. A solution of **3** (4.10 g, 6.36 mmol) in CH_3CN (30 mL) was added at RT, followed by TMSTf (1.39 mL, 7.64 mmol). The mixture was heated to 50 °C for 38 h and then cooled to 0 °C and quenched with saturated aqueous $NaHCO_3$ (30 mL) under vigorous stirring. The suspension was filtered through cotton/sand, and the pellet was washed with CH_2Cl_2 (150 mL). The filtrate was shaken with $NaHCO_3$ (50 mL), the aqueous phase was extracted with CH_2Cl_2 (2 × 30 mL), and the combined organic phases were washed

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with saturated aqueous NaCl (150 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to a yellowish foam. Chromatography on silica gel (325 g, petroleum ether/EtOAc (2:3)) followed by removal of *N*-methyltrifluoroacetamide under high vacuum yielded regioisomers **7a** (N^7; 1.79 g, 29%) and **7b** (N^9; 3.28 g, 53%), isolated as white foams. For the data of **7a**, see the supporting information. **7b** ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.045 (s, 9H, $C(CH_3)_3$); 1.182 (d, J = 5.7 Hz, 3H, $CH(CH_3)_2$); 1.199 (d, J = 5.7 Hz, 3H, $CH(CH_3)_2$); 1.952−2.042 (m, 1H, H-5′); 2.153−2.232 (m, 1H, H-5′); 2.92−3.05 (m, 1H, $CH(CH_3)_2$); 3.311 (t, J = 6.7 Hz, 2H, OCH_2CH_2Ar); 3.62−3.73 (m, 1H, H-3′); 3.872 (d, J = 5.4 Hz, 1H, H-6′); 3.894 (d, J = 4.9 Hz, 1H, H-6′); 4.547−4.688 (m, 3H, H-4′, 2 × H-3″); 4.811 (t, J = 6.7 Hz, 2H, OCH_2CH_2Ar); 5.963 (d, J = 1.6 Hz, 1H, H-1′); 6.108 (dd, J = 1.6, 6.0 Hz, 1H, H-2′); 7.296−7.452 (m, 10H, 4 × *m*-Bz, 2 × *p*-Ph, 4 × *m*-Ph); 7.488−7.522 (AA′BB′ system, AA′ part, 2H, 2 × *o*-$PhNO_2$); 7.530−7.661 (m, 6H, 2 × *p*-Bz, 4 × *o*-Ph); 7.861 (s, 1H, N^2-H); 7.867 (s, 1H, H-8); 7.988−8.022 (2 AA′BB′C systems, 2 AA′ parts, 4H, 4 × *o*-Bz); 8.145−8.179 (AA′BB′ system, BB′ part, 2H, 2 × *m*-$PhNO_2$). FABMS: *m/z* 978 ($M + H^+$). Anal. Calcd for $C_{54}H_{56}N_6O_{10}Si$: C, 66.38; H, 5.78; N, 8.60. Found: C, 65.76; H, 5.86; N, 8.50.

***N*^{6}-Benzoyl-9-[2′-acetyl-3′-[(acetyloxy)methyl]-6′-(*tert*-butyldiphenylsilyl)-3′,5′-dideoxy-*β*-allofuranosyl]adenine (9b).** *N*^{6}-Benzoyladenine[53] (770 mg, 3.2 mmol, recrystallized from EtOH, dried over P_4O_{10}) was suspended in $Cl(CH_2)_2Cl$ and silylated with MSTFA (1.5 g, 1.4 mL, 7.6 mmol) over 30 min at RT. Glycosyl donor **8**[18] (1.0 g, 2.0 mmol) in $Cl(CH_2)_2Cl$ (5 mL) was added to the clear solution, and TMSTf (220 mg, 180 μL, 1 mmol) injected. The mixture was stirred (80 °C, 50 h), cooled (0 °C), and diluted with CH_2Cl_2 (25 mL). Under slow stirring, saturated Na_2CO_3 solution was added dropwise and the resulting mixture filtered through sand. The organic layer was separated and the aqueous layer reextracted 3× with CH_2Cl_2. The sand was washed (CH_2Cl_2/MeOH (99:1), 300 mL), and the washings were combined with the organic extracts. These were washed with brine, dried ($MgSO_4$), filtered, and dried *in vacuo*. The residue was chromatographed on silica (120 g, EtOAc/CH_2Cl_2/MeOH (60:40:1)) to yield **9b** (870 mg, 1.2 mmol, 60%) as a colorless glass. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.06 (s, 9H, CH_3-*t*Bu); 1.96, 2.03 (2m, 2H, H-5′); 2.06, 2.15 (2s, 2 × 3H, $COCH_3$); 3.16 (m, 1H, H-3′); 3.84 (m, 2H, H-6′); 4.18 (AA′X system = q, 1H, J = 5.8, 11.4 Hz, H-3″); 4.33 (m, 2H, H-3″, H-4′); 5.84 (dd, 1H, J = 1.7, 6.2 Hz; H-2′); 5.96 (d, 1H, J = 1.8 Hz, H-1′); 7.38 (m, 8H, *m*-Bz, *m*,*p*-Ph); 7.55 (AA′BB′C system, C part, 1H, *p*-Bz); 7.64 (AA′BB′C system, AA′ part, 4H, *o*-Ph); 8.01 (AA′BB′C system, AA′ part = d, 2H, J = 7 Hz, *o*-Bz); 8.03 (s, 1H, H-8); 8.75 (s, 1H, H-2); 9.31 (br s, 1H, NH). FABMS: *m/z* 722 ($M + H^+$). Anal. Calcd for $C_{39}H_{43}N_5O_7Si$: C, 64.89; H, 6.00; N, 9.70. Found: C, 64.46; H, 6.11; N, 9.68.

***N*^{2}-Isobutyryl-9-[2′-benzoyl-3′-[(benzoyloxy)methyl]-6′-(*tert*-butyldiphenylsilyl)-3′,5′-dideoxy-*β*-allofuranosyl]guanine (10b).** A solution of N^9 isomer **7b** (2.0 g, 2.1 mmol) in absolute pyridine (21 mL) was cooled (0 °C) and treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 1.53 mL, 10.3 mmol). The ice bath was removed and the solution stirred for 2.75 h before recooling the solution to 0 °C, adding glacial HOAc (586 μL, 10.3 mmol), rotoevaporating the solvents to near dryness, and coevaporating the residue with toluene (3 × 30 mL). The oil was chromatographed (silica gel, 60 g, petroleum ether/EtOAc (2:3)). This afforded a mixture of *p*-vinylnitrobenzene and **10b** in a ca. 4:1 ratio, as determined by ^{1}H NMR (1.69 g, ca. 80% yield of **10b**). A pure analytical sample (data below) was obtained after a second column under similar conditions. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.055 (s, 9H, $C(CH_3)_3$); 1.235 (d, J = 6.9 Hz, 3H, $CH(CH_3)_2$); 1.241 (d, J = 6.9 Hz, 3H, $CH(CH_3)_2$); 1.845−1.946 (m, 1H, H-5′); 2.096−2.175 (m, 1H, H-5′); 2.604 (hept, J = 6.9 Hz, 1H, $CH(CH_3)_2$); 3.476−3.549 (m, 1H, H-3′); 3.880 (d, J = 5.1 Hz, 1H, H-6′); 3.898 (d, J = 5.0 Hz, 1H, H-6′); 4.480−4.592 (m, 3H, H-4′, 2 × H-3″); 5.858 (d, J = 1.2 Hz, 1H, H-1′); 6.117 (dd, J = 1.1, 5.5 Hz, 1H, H-2′); 7.317−7.462 (m, 10H, 4 × *m*-Bz, 2 × *p*-Ph, 4 × *m*-Ph); 7.531−7.664 (m, 6H, 2 × *p*-Bz, 4 × *o*-Ph); 7.689 (s, 1H, H-8); 7.906−7.930 (AA′BB′C system, AA′ part, 2H, 2 × *o*-Bz); 8.002−8.025 (AA′BB′C system, AA′ part, 2H, 2 × *o*-Bz); 8.700 (s, 1H, N^2-H); 12.00 (br s, 1H, N^1-H). FABMS: *m/z* 829 ($M + H^+$). Anal. Calcd for $C_{46}H_{49}N_5O_8Si$: C, 66.73; H, 5.96; N, 8.46. Found: C, 66.46; H, 5.93; N, 8.31.

***N*^{6}-Benzoyl-9-[6′-(*tert*-butyldiphenylsilyl)-2′,3′-dideoxy-3′-(hydroxymethyl)-*β*-allofuranosyl]adenine (13b).** Diacetate **9b** (3.6 g, 5 mmol) in a mixture of MeOH (60 mL) and THF (50 mL) was treated with 0.2 N LiOH (62.5 mL, 12.5 mmol) with stirring at RT. The hydrolysis was stopped after 12 min by addition of acetate buffer (6.25 mL). CH_2Cl_2 (150 mL) was added, the organic layer was separated, and the aqueous layer was reextracted four times with CH_2Cl_2. The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and dried *in vacuo*. The residue was chromatographed on silica (200 g, EtOAc/CH_2Cl_2/MeOH (6:4:1)) to yield **13b** (2.55 g, 4 mmol, 80%) as a colorless foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.06 (s, 9H, CH_3); 1.91 (m, 2H, H-5′); 2.34 (m, 1H, H-3′); 3.83 (m, 3H, H-6′, H-3″); 3.94 (m, 1H, H-3″); 4.1 (br s, 1H, OH); 4.38 (dt, 1H, J = 3.8, 8.2 Hz, H-4′); 4.90 (dd, 1H, J = 2.4, 6.2 Hz, H-2′); 5.95 (d, 1H, J = 2.7 Hz, H-1′); 6.0 (br s, 1H, OH); 7.39 (m, 8H, *m*-Bz, *m*,*p*-Ph); 7.49 (AA′BB′C system, C part, 1H, *m*-Bz); 7.67 (AA′BB′C system, AA′ part, 4H, *o*-Ph); 7.95 (AA′BB′C system, AA′ part = d, 2H, J = 7.5 Hz, *o*-Bz); 8.02 (s, 1H, H-8); 8.52 (s, 1H, H-2); 9.47 (br s, 1H, NH). FABMS: *m/z* 660 ($M + Na^+$). Anal. Calcd for $C_{35}H_{39}N_5O_5Si$: C, 65.91; H, 6.16; N, 10.98. Found: C, 65.87; H, 6.49; N, 10.90. For the related conversions of **4**, **7b**, and **10b**, see the supporting information.

1-[3′-[(Acetylthio)methyl]-6′-(*tert*-butyldiphenylsilyl)-3′,5′-dideoxy-*β*-allofuranosyl]uracil (15). Diol **12** (3.4 g, 6.67 mmol) was coevaporated from toluene, dried under high vacuum, and dissolved in THF (60 mL). PPh_3 (2.62 g, 10 mmol) in THF (60 mL) was cooled to 0 °C and treated with diisopropyl azodicarboxylate (DIAD; 2.02 g, 1.95 mL, 10 mmol). Within 5 min, a white precipitate formed. After 30 min, the solution of **12** and thioacetic acid (0.76 g, 0.71 mL, 10 mmol) were added concurrently. The precipitate dissolved, and the reaction was allowed to warm to RT. After overnight stirring, MeOH (0.5 mL) was added and the solvents were removed by rotary evaporation. The residue was chromatographed twice on silica (EtOAc/CH_2Cl_2, first run 3:1, second run 1:1) to yield thioester **15** (2.43 g, 4.3 mmol, 64%). The acetyl group had migrated to the 2′-position in ca. 20% of this product. A reaction with 70 mg of **12** gave a 75% yield of pure 2′-OH product after one chromatographic separation. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.06 (s, 9H, CH_3-*t*Bu); 1.82 (m, 2H, H-5′); 2.17 (m, 1H, H-3′); 2.33 (s, 3H, $SCOCH_3$); 3.05 (m, 2H, H-3″); 3.93 (m, 2H, H-6′); 4.29 (m, 2H, H-4′, OH); 5.04 (d, 1H, J = 3 Hz, H-2′); 5.67 (s, 1H, H-1′); 5.69 (d, 1H, J = 8.1 Hz, H-5); 7.38 (d, 1H, J = 8.1 Hz, H-6); 7.43 (m, 6H, *m*,*p*-Ph); 7.70 (AA′BB′C system, AA′ part, 4H, *o*-Ph); 10.54 (br s, 1H, NH). FABMS: *m/z* 591 ($M + Na^+$). Anal. Calcd for $C_{29}H_{36}N_2O_6SSi$: C, 61.24; H, 6.38; N, 4.93. Found: C, 61.05; H, 6.42; N, 4.90. For the related conversions of **13** and **14**, see the supporting information.

***N*^{4}-Benzoyl-1-[2′-benzoyl-3′-[(benzoyloxy)methyl]-3′,5′-dideoxy-*β*-allofuranosyl]cytosine (19).** Compound **5** (361 mg, 0.44 mmol) was dissolved in THF (10 mL) under stirring and treated with tetrabutylammonium fluoride (TBAF; 153 mg, 0.484 mmol) in THF (10 mL) at RT. After 4 h, additional TBAF (25 mg, 0.08 mmol) was added. 30 min later, the solvent was removed by rotary evaporation at 30 °C and the residue was directly applied to a silica column (20 g). The product was eluted using CH_2Cl_2/EtOAc (1:4, 100 mL) followed by pure EtOAc (50 mL) and CH_2Cl_2/MeOH (9:1, 100 mL) to yield pure **19** (240 mg, 0.41 mmol, 93%) as a colorless foam. ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD (4:1)): δ 1.93, 2.06 (2m, 2H, H-5′); 2.78 (m, 1H, H-3′); 3.75 (m, 2H, H-6′), 4.39 (m, 3H, H-4′, H-3″); 5.81 (s, 1H, H-1′); 5.82 (d, 1H, J = 4.2 Hz, H-2′); 7.22−7.58 (m, 10H, H-5, *m*,*p*-Bz); 7.78−7.92 (m, 7H, H-6, *o*-Bz). FABMS: *m/z* 606 ($M + Na^+$). Anal. Calcd for $C_{32}H_{29}N_3O_8$: C, 65.86; H, 5.01; N, 7.20. Found: C, 65.79; H, 5.31; N, 6.99. For the related conversions of **4** and **10b**, see the supporting information.

***N*^{2}-Isobutyryl-9-[2′-benzoyl-3′-[(benzoyloxy)methyl]-6′-bromo-3′,5′,6′-trideoxy-*β*-allofuranosyl]guanine (23).** Compound **20** (765 mg, 1.30 mmol) and PPh_3 (511 mg, 1.95 mmol) were dried under high vacuum and then dissolved in CH_3CN (12 mL). A solution of CBr_4 (535 mg, 1.61 mmol) in CH_3CN (3 mL) was added and stirring continued for 45 min. The crude foam resulting from concentration of the solution *in vacuo* was first chromatographed on silica gel (120

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g, EtOAc/CH_2Cl_2/MeOH (80:20:1)). Two fractions were collected, one of pure bromide and one containing bromide/P(O)Ph_3. The latter yielded pure bromide after a second column (120 g silica) using EtOAc/CH_2Cl_2 (3:1) and a MeOH gradient (1% to 2% to 4%). The combined pure fractions of bromide **23** were concentrated *in vacuo* to a white foam (635 mg, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.227 (d, J = 6.9 Hz, 3H, CH(CH_3)$_2$); 1.254 (d, J = 6.9 Hz, 3H, CH(CH_3)$_2$); 2.291−2.427 (m, 2H, 2 × H-5′); 2.698 (hept, J = 6.9 Hz, 1H, C*H*(CH_3)$_2$); 3.475−3.553 (m, 1H, H-6′); 3.592−3.645 (m, 1H, H-6′); 3.699−3.772 (m, 1H, H-3′); 4.471(dd, J = 11.5, 6.7 Hz, 1H, H-3″); 4.486−4.530 (m, 1H, H-4′); 4.574 (dd, J = 11.5, 7.1 Hz, 1H, H-3″); 5.933 (d, J = 1.5 Hz, 1H, H-1′); 6.147 (dd, J = 1.4, 5.9 Hz, 1H, H-2′); 7.373−7.455 (m, 4H, 4 × *m*-Bz); 7.522−7.618 (m, 2H, 2 × *p*-Bz); 7.750 (s, 1H, H-8); 7.764−7.928 (AA′BB′C system, AA′ part, 2H, 2 × *o*-Bz); 7.990−8.018 (AA′BB′C system, AA′ part, 2H, 2 × *o*-Bz); 9.470 (br s, 1H, N^2-H); 12.120 (br s, 1H, N^1-H). FABMS: *m/z* 652 (M + H$^+$). Anal. Calcd for $C_{30}H_{30}N_5O_7Br$: C, 55.22; H, 4.63; N, 10.73; Br, 12.25. Found: C, 55.03; H, 4.79; N, 10.42; Br, 12.45. For the related conversions of **18** and **19**, see the supporting information.

***N*^{2}-Isobutyryl-*O*^{6}-[2-(4-nitrophenyl)ethyl]-9-[3′-(mercaptomethyl)-6′-(*tert*-butyldiphenylsilyl)-3′,5′-dideoxy-β-allofuranosyl]guanine (24).** Compound **17** (400 mg, 485 μmol) was dissolved in Ar-saturated MeOH (36 mL) at 0 °C. Gaseous NH_3 was gently bubbled into the stirred solution for 10 min. The lines were removed, and the solution was stirred for 65 min at 0 °C before the solvent and NH_3 were removed by rotary evaporation (P = 700−25 Torr, T_{bath} = ca. 10 °C). Residual NH_3 and acetamide were removed by high vacuum (14 h) to yield the glass **24**, which was used in the subsequent coupling reaction (to give **31**) without further purification. An analytically pure sample of **24** (14 mg, 87%; data below) was obtained by the ammonolysis of 17 mg of **17** in a similar manner, followed by chromatographic removal of traces of presumed disulfide and/or C2′-OAc side products (the latter resulting from migration of the acetyl group from C3″-sulfur) using silica gel (1.3 g) and petroleum ether/EtOAc (2:3) as the eluant. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.064 (s, 9H, C(CH_3)$_3$); 1.279 (d, J = 6.9 Hz, 3H, CH(C*H*$_3$)$_2$); 1.283 (d, J = 6.9 Hz, 3H, CH(C*H*$_3$)$_2$); 1.622 (t, J = 8.5 Hz, 1H, SH); 1.728−1.838 (m, 1H, H-5′); 1.853−1.920 (m, 1H, H-5′); 2.403−2.468 (m, 1H, H-3′); 2.581 (hept, J = 6.9 Hz, 1H, C*H*(CH_3)$_2$); 2.644 (ddd, J = 13.7, 8.6, ca. 8.4, 1H, H-3″); 3.115 (ddd, J = 13.8, 8.2, 5.5 Hz, 1H, H-3″); 3.309 (t, J = 6.8 Hz, 2H, OC*H*$_2$-CH_2Ar); 3.7616−3.860 (m, 2H, 2 × H-6′); 4.540−4.584 (m = pent, 1H, H-4′); 4.706 (ddd, J = 7.8, 4.8, 3.2 Hz, 1H, H-2′); 4.734−4.826 (m = dt, 2H, OCH$_2$C*H*$_2$Ar); 5.736 (d, J = 5.1 Hz, 1H, H-1′); 6.385 (br s, 1H, C-2′-OH); 7.356−7.452 (m, 6H, 4 × *m*-Ph, 2 × *p*-Ph); 7.496 (AA′BB′ system, AA′ part, 2H, 2 × *o*-PhNO_2); 7.635−7.702 (m, 4H, 4 × *o*-Ph); 7.863 (d, J = 0.2 Hz, 1H, H-8); 7.883 (s, 1H, N^2-H); 8.159−8.194 (AA′BB′ system, BB′ part, 2H, 2 × *m*-PhNO_2). FABMS: *m/z* 785 (M + H$^+$).

Dimer Thioether 27. A mixture of **22** (599 mg, 929 μmol), **15** (527 mg, 930 μmol), and Cs_2CO_3 (1.21 g, 3.72 mmol) was dissolved in CH_3CN (25 mL) and water (2.9 mL) under Ar. The solution was stirred (50 °C, 24 h). The solution was concentrated (rotary evaporation) by 75% and neutralized with acetate buffer (1.9 mL). CH_2Cl_2 (30 mL) and 9/10 saturated brine (10 mL) were added, the organic layer was separated, and the aqueous phase was extracted 2× with CH_2Cl_2. The combined organic phases were concentrated *in vacuo*, and the residue was chromatographed (120 g of silica, CH_2Cl_2/EtOAc (2:1) and a MeOH gradient (1% to 5%)) to give unreacted bromide (52.9 mg, 80 μmol), 2′,3″-diacetate of the uridine thiol (80.2 mg, 130 μmol), **27** (572 mg, 505 μmol), and **29** (228 mg, 209 μmol). The (combined) yield of dimer was 77% (84% based on recovered bromide). The following are data for **27**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05 (s, 9H, CH_3-*t*Bu); 1.80 (m, 1H, H-5′-U); 2.0−2.25 (m, 3H, 2 × H-5′-C, H-5′-U); 2.13 (s, 3H, $COCH_3$); 2.50−2.70 (m, 3H, H-6′-C, H-3′-U); 2.72, 2.83 (2m, 2H, H-3″-U); 3.06 (m, 1H, H-3′-C); 3.84 (m, 2H, H-6′-U); 4.04 (m, 1H, H-4′-U); 4.39 (m, 1H, H-4′-C); 4.57 (AA′B system, AA′ part, 2H, H-3″-C); 5.39 (d, 1H, J = 1.9 Hz, H-1′-U); 5.49 (dd = d, 1H, J = 3.6 Hz, H-2′-U); 5.67 (d, 1H, J = 8.2 Hz, H-5-U); 5.83 (d, 1H, J = 1.9 Hz, H-1′-C); 5.96 (dd, 1H, J = 1.6, 6.8 Hz, H-2′-C); 7.13 (d, 1H, J = 8.2 Hz, H-6-U); 7.33−7.70 (m, 20H, *o*,*m*,*p*-Ph, *m*,*p*-Bz, H-5-C); 7.77 (d, 1H, J = 7 Hz, H-6-C); 7.90−8.06 (m, 6H, *o*-Bz); 8.56 (br s, 1H, NH); 8.85 (br s, 1H, NH). FABMS: *m/z* 1157 (M + Na$^+$). For the related conversions of **16**/**21** and **17**/**24**, see the supporting information.

Dimer Sulfone 34. Compound **31** (563 mg, 0.42 mmol) was dissolved in MeOH (76 mL) and THF (11 mL). A freshly prepared solution of Oxone (2 $KHSO_5$·$KHSO_4$·K_2SO_4; 1.02 g, 1.66 mmol) and NaOAc (450 mg, 5.50 mmol) in water (15 mL) was added under vigorous stirring. The white slurry was stirred (2 h, RT). Approximately half of the organic solvent was evaporated, and saturated aqueous $Na_2S_2O_3$ (30 mL) and CH_2Cl_2 (120 mL) were added. The organic layer was separated and the aqueous layer extracted twice with CH_2Cl_2. The combined organic phases were washed with brine, which was again extracted twice with CH_2Cl_2. The combined organic layers were concentrated *in vacuo*, and the crude foam was chromatographed on silica gel (95 g, stepwise gradient CH_2Cl_2/MeOH/acetone (96:3:1 to 93:5:2 to 91:7:3)). Compound **34** was isolated as a white foam (555 mg, 96%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.041 (s, 9H, C(CH_3)$_3$); 1.151−1.207 (m = 4 × d, 12H, 2 × CH(C*H*$_3$)$_2$); 1.763−1.829 (m, 1H, H-5′-G1); 1.958−2.022 (m, 1H, H-5′-G1); 2.482−2.530 (m, 1H, H-5′-G2); 2.594−2.621 (m, 1H, C*H*(CH_3)$_2$-G1); 2.649−2.772 (m, 2H, C*H*(CH_3)$_2$-G2, H-5′-G2); 2.980−3.001 (m, 2H, H-3″-G1, H-3′-G1); 3.310 (t, J = 6.8 Hz, 2H, OC*H*$_2$CH$_2$Ar); 3.374−3.452 (m, 2H, H-6′-G2, H-3′-G2); 3.588−3.647 (m, 1H, H-6′-G2); 3.823−3.848 (m, 2H, 2 × H-6′-G1); 3.909 (dd, J = 16.0, 9.2, 1H, H-3″-G1); 4.385−4.423 (m, 1H, H-4′-G1); 4.509 (dd, J = 11.6, 6.3, 1H, H-3″-G2); 4.532−4.571 (m, 1H, H-4′-G2); 4.648 (dd, J = 11.6, 6.4, 1H, H-3″-G2); 4.697−4.726 (m = ddd, 1H, H-2′-G1); 4.726−4.837 (m, 2H, OCH$_2$C*H*$_2$-Ar); 5.617 (d, J = 3.4 Hz, 1H, H-1′-G1); 5.994 (s, 1H, H-1′-G2); 6.000 (dd, J = 3.7, 8.7 Hz, 1H, H-2′-G2); 6.402 (br s = d, 1H, OH); 7.287−7.419 (m, 10H, 4 × *m*-Bz, 2 × *p*-Ph, 4 × *m*-Ph); 7.450−7.482 (m, 1H, *p*-Bz); 7.482−7.510 (m, 2H, AA′BB′ system, AA′ part, 2H, 2 × *o*-PhNO_2); 7.552−7.588 (m, 1H, *p*-Bz); 7.626−7.666 (m, 4H, 4 × *o*-Ph); 7.716 (s, 1H, H-8-G2); 7.801 (s, 1H, H-8-G1); 7.872−7.892 (AA′BB′C system, AA′ part, 2H, 2 × *o*-Bz); 7.935−7.955 (AA′BB′C system, AA′ part, 2H, 2 × *o*-Bz); 8.133−8.168 (m, 3H, N^2-H-G1, AA′BB′ system, BB′ part, 2H, 2 × *m*-PhNO_2); 9.528 (br s, 1H, N^2-H-G2); 12.190 (br s, 1H, N^1-H-G2). FABMS: *m/z* 1389 (M + H$^+$). For the related conversions of **27** and **28**, see the supporting information.

Dimer Triol 35. A solution of **32** (73.7 mg, 63.3 μmol) in MeOH (20 mL) and THF (7 mL) was cooled to 0 °C, and a 0.2 M aqueous LiOH solution (1.55 mL, 309 μmol) was added. The solution was stirred (90 min, 0 °C). Acetate buffer (2 mL) and CH_2Cl_2 (120 mL) were added, and the aqueous layer was separated and extracted five times with CH_2Cl_2. The combined organic phases were concentrated *in vacuo* and the residue dried under high vacuum. Silica gel chromatography (18 g of silica, CH_2Cl_2 with an EtOH/MeOH (1:1) gradient of 4% to 15% and a water gradient of 0.1% to 1%) yielded **35** (31.3 mg, 34.2 μmol, 54%) as a glass. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.00 (s, 9H, CH_3-*t*Bu); 1.11 (m, 1H, H-5′-U); 1.83 (m, 1H, H-5′-U); 2.02 (m, 1H, H-3′-C); 2.23 (m, 2H, H-5′-C); 2.41 (m, 1H, H-3′-U); 2.93 (m, 1H, H-3″-U); 3.33 (m, 1H, H-6′-C); 3.50, 3.57 (2m, 2H, H-6′-U); 3.74 (m, 1H, H-3″-C); 3.87 (m, 1H, H-3″-C); 3.94 (m, 1H, H-6′-C); 3.99 (dd, 1H, H-3″-U); 4.20 (t, 1H, H-4′-U); 4.29 (t, 1H, H-4′-C); 4.42 (br s, 1H, H-2′-C); 4.59 (d, 1H, J = 8.6 Hz, OH 3″-C); 4.96 (t, 1H, H-2′-U); 5.22 (dd, 1H, J = 8, 2 Hz, H-5-U); 5.67, 5.69 (2s, 2H, H-1′-C and H-1′-U); 6.32 (d, 1H, J = 4.3 Hz, OH 2′-U); 6.65 (br s, 1H, OH 2′-C); 6.97 (d, 1H, J = 8.1 Hz, H-6-U); 7.16 (d, 1H, J = 7.3 Hz, H-5-C); 7.25−7.5 (m, 9H, *m*,*p*-Bz, *m*,*p*-Ph); 7.58 (AA′BB′C system, AA′ part, 4H, *o*-Ph); 7.81 (d, 1H, J = 7.5 Hz, H-6-C); 8.01 (AA′BB′C system, AA′ part, 2H, *o*-Bz); 9.18 (br s, 1H, NH); 11.72 (br s, 1H, NH). FABMS: *m/z* 938 (M + Na$^+$). For the related conversion of **33**, see the supporting information.

Dimer Thioester 38. A CH_2Cl_2 solution of **36** (446 mg, 475 μmol) was coevaporated with toluene, dried (14 h, 35 °C, high vacuum), and dissolved in CH_3CN (5 mL) and THF (3 mL). PPh_3 (249 mg, 950 μmol) was dissolved in CH_3CN (2 mL), cooled quickly to 0 °C, and treated with DIAD (178 μL, 183 mg, 902 μmol). After 15 min, the solution of **36** was added, immediately followed by thioacetic acid (67.8 μL, 72.6 mg, 950 μmol). The solution was stirred (45 min at RT), concentrated by rotary evaporation, and dried (high vacuum). The residue was chromatographed on silica (135 g; CH_2Cl_2/2-propanol (95:5) and a MeOH gradient of 2% to 12%) to yield **38** (436 mg, 437 μmol, 92%) as a foam. ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD/D_2O

(4:1:saturated)): δ 0.99 (s, 9H, CH_3-*t*Bu); 1.86, 2.01 (2m, 2 × 2H, H-5′); 2.24 (s, 3H, Ac); 2.37 (m, 1H, H-3′); 2.69 (m, 1H, H-3′); 2.91 (d, 2H, J = 7.4 Hz, H-3″); 3.18, 3.30 (2m, 2H, H-6′-U); 3.65 (m, 2H, H-3″); 3.82 (m, 2H, H-6′-A); 3.98 (dt, 1H, J = 2.0, 10.0 Hz, H-4′); 4.18 (d, 1H, J = 3.5 Hz, H-2′-U); 4.24 (dt, 1H, J = 2.2, 10.0 Hz, H-4′); 4.70 (dd, 1H, J = 5.2 Hz, H-2′-A); 5.52 (s, 1H, H-1′-U); 5.60 (d, 1H, J = 8.0 Hz, H-5-U); 6.00 (s, 1H, H-1′-A); 7.27−7.57 (m, 10H, H-6-U, *m*,*p*-Ph, *m*,*p*-Bz); 7.58 (2 AA′BB′C systems, 2 AA′ parts, 4H, *o*-Ph); 7.99 (AA′BB′C system, AA′ part, 2H, *o*-Bz); 8.04 (s, 1H, H-8-A); 8.66 (s, 1H, H-2-A). FABMS: *m*/*z* 1020 (M + Na^+). For the related conversion of **35**, see the supporting information.

Dimer Alcohol 40. Compound **34** (455 mg, 0.33 mmol) was dissolved in pyridine (46 μL) in a 10 mL polypropylene tube under Ar and treated with HF/pyridine (319 μL, 1.64 mmol of a 5.2 M solution) for 14 h at RT. Methoxytrimethylsilane (0.45 mL, 3.28 mmol) was added. The mixture was stirred (5 min), transferred (CH_2Cl_2 wash) to a glass flask, and concentrated *in vacuo*. The oily foam was chromatographed (silica gel, 32 g, gradient of CH_2Cl_2/MeOH/acetone (92:6:2 to 9:1:0)) to yield **40** as a foam (339 mg, 90%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.151−1.197 (m = 4 × d, 12H, 2 × CH(CH_3)$_2$); 1.866−1.962 (m, 2H, 2 × H-5′-G1); 2.39−2.47 (m, 1H, H-5′-G2); 2.53−2.730 (m, 3H, H-5′-G2, C*H*(CH_3)$_2$-G2, C*H*(CH_3)$_2$-G1); 3.23−3−31 (m, 5H, H-3″-G1, H-3′-G1, OCH_2CH_2Ar, C-6′-OH); 3.33−3.464 (m, 2H, H-6′-G2, H-3′-G2); 3.58−3.66 (m, 1H, H-6′-G2); 3.70−3.811 (m, 3H, 2 × H-6′-G1, H-3″-G1); 4.346−4.383 (m, 1H, H-4′-G1); 4.43−4.491 (m, 2H, H-4′-G2, H-3″-G2) 4.556−4.592 (m, 1H, H-3″-G2); 4.730−4.797 (m, 2H, OCH_2CH_2Ar); 4.80−4.85 (m, 1H, H-2′-G1); 5.788 (d, J = 2.7 Hz, 1H, H-1′-G1); 5.842 (br s, 1H, C-2′-G1-OH); 5.948 (d, J = 2.7 Hz, 1H, H-1′-G2); 5.995 (dd, J = 2.8, 6.3 Hz, 1H, H-2′-G2); 7.28−7.41 (m, 4H, 4 × *m*-Bz); 7.42−7.50 (m, 3H, *p*-Bz, AA′BB′ system, AA′ part, 2H, 2 × *o*-PhNO_2); 7.52−7.57 (m, 1H, *p*-Bz); 7.777 (s, 1H, H-8-G2); 7.84−7.94 (2 × AA′BB′C system, 2 × AA′ part, 4H, 4 × *o*-Bz); 7.963 (s, 1H, H-8-G1); 8.114−8.149 (m, 2H, AA′BB′ system, BB′ part, 2H, 2 × *m*-PhNO_2); 8.351 (s, 1H, N^2-H-G1); 9.82 (br s, 1H, N^2-H-G2); 12.21 (br s, 1H, N^1-H-G2). FABMS: *m*/*z* 1151 (M + H^+). For the related conversion of **33**, see the supporting information.

Dimer Bromide 41. A mixture of **39** (50.8 mg, 54 μmol) and PPh_3 (28.1 mg, 107 μmol) in CH_3CN (3 mL) was mixed with a solution of CBr_4 (42.6 mg, 129 μmol) in CH_3CN (0.5 mL) at RT. The solution became yellow after a few minutes. After 90 min, the solution was added to a mixture of saturated bicarbonate (15 mL), ice (10 g), and CH_2Cl_2 (100 mL). The organic layer was separated and the aqueous layer reextracted four times with CH_2Cl_2. The combined organic layers were concentrated and dried (high vacuum). The residue was applied to a silica (57 g) column. Elution with EtOAc/CH_2Cl_2/THF (75:25:10, 300 mL) and then CH_2Cl_2/EtOAc (83:17, and a MeOH gradient of 4% to 8%) yielded **41** (47.0 mg, 46.4 μmol, 86%) as a colorless foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.19 (s, 3H, Ac); 2.31 (m, 3H, 2 × H-5′-A, H-5′-U); 2.58 (m, 1H, H-5′-U); 3.10 (dd, 1H, H-3″-A); 3.12 (m, 1H, H-3′); 3.45 (m, 5H, H-3′, H-3″-A, 3 × H-6′); 3.87 (m, 1H, H-6′); 4.28 (quint, 1H, H-4′); 4.39 (m, 1H, H-4′); 4.52, 4.69 (2dd, 2H, H-3″-U); 5.41 (d, 1H, J = 1.9 Hz, H-1′-U); 5.70 (dd, 1H, J = 1.9, 7.9 Hz, H-5-U); 5.80 (dd, 1H, J = 1.9, 7.2 Hz, H-2′-U); 5.85 (d, 1H, J = 5.3 Hz, H-2′-A); 6.05 (s, 1H, H-1′-A); 7.21 (d, 1H, J = 8.1 Hz, H-6-U); 7.38−7.63 (m, 9H, *o*,*m*-Bz) 7.98 (2 AA′BB′C systems, 2 AA′ parts, 4H, *o*-OBz); 8.09 (AA′BB′C system, AA′ part = d, 2H, J = 7.2 Hz, *o*-NBz); 8.15 (s, 1H, H-8-A); 8.79 (s, 1H, H-2-A); 9.53 (br s, 1H, NH); 10.13 (br s, 1H, NH). FABMS: *m*/*z* 1038/1040 (M + Na^+). For the related conversions of **40**, see the supporting information.

Tetramer Thioether 43. Compound **38** (83.3 mg, 84 μmol), compound **42** (92 mg, 76 μmol), and Cs_2CO_3 (149 mg, 456 μmol) were dried (high vacuum, 50 °C) and pulverized with a stirring bar. THF (30 mL) was added under stirring, and after 11 h the solvent was removed from the slurry *in vacuo*. Acetate buffer (0.28 mL) and 2/3 saturated brine (9 mL) were added, and the mixture was extracted 5× with CH_2Cl_2. The organic layers were concentrated and the residue chromatographed (50 g of silica, CH_2Cl_2 and a 2-propanol gradient of 3% to 0% with a concurrent MeOH gradient of 3% to 12.5%) to yield **42** (5.5 mg, 4.5 μmol), AU-2′,3″-diacetate (7.4 mg, 7 μmol), **43** (57 mg, 27 μmol, 36%), the U2-2′-alcohol tetramer (20.8 mg, 10 μmol, 13%), and a mixture of both tetrameric species (30.5 mg, 14 μmol, 18%). The combined yield of tetramers was 67% (71% based on recovered bromide). A reaction on a 0.1-fold scale gave a 76% combined yield of tetramers. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.95 (s, 9H, CH_3-*t*Bu); 1.06 (m, 12H, CH_3-iBu); 1.93 (3H), 2.00 (1H), 2.14−2.37 (4H), 2.44 (1H) (4m, 8 × H-5′, H-3′); 2.51−2.70 (m, 4H, 2 × H-6′-G3, 2 × H-3″-U); 2.74, 2.85 (2m, 2H, CH-iBu); 2.97 (m, 2H, H-3′-A, H-3′-G4); 3.26−3.39 (partly under H_2O), 3.40−3.58 (6H) (2m, CH_2-NPE, 2 × H-6′-U, 2 × H-6′-G4, H-3′-G4, 2 × H-3″-A, 2 × H3″-G3); 3.77 (m, 2H, 2 × H-6′-A); 3.90 (dt, 1H, J = 2.2, 8.9 Hz, H-4′); 4.01 (dt, 1H, J = 2.7, 8.9 Hz, H-4′); 4.16 (dt, 1H, J = 2.1, 9.2 Hz, H-4′); 4.34 (br s, 1H, OH); 4.51 (dt, 1H, J = 2.8, 8.3 Hz, H-4′-G4); 4.57 (dd, 1H, H-3″-G4); 4.67 (m, 2H, H-3″-G4, H-2′-A); 4.78 (m, 3H, H-2′-G3, CH_2-NPE); 5.31 (dd, 1H, J = 2.2, 6.3 Hz, H-2′-U); 5.63 (d, 1H, J = 8.0 Hz, H-5-U); 5.65 (d, 1H, J = 2.4 Hz, H-1′-U); 5.88 (d, 1H, J = 2.0 Hz, H-1′-G3); 6.03 (s, 1H, H-1′-A); 6.04 (dd, partly hidden, J = 1.8 Hz, H-2′-G4); 6.14 (d, 1H, J = 2.1 Hz, H-1′-G4); 6.23 (br s, 1H, OH); 7.27−7.52 (10H), 7.54−7.62 (7H); 7.63−7.68 (5H) (3m, H-6-U, 3 × *m*,*p*-Bz, 2 × *o*,*m*,*p*-Ph, 2 × *o*-NPE); 7.91, 7.97 (2 AA′BB′C systems, 2 AA′ parts, 2 × 2H, *o*-OBz); 8.05 (AA′BB′C system, AA′ part, 2H, *o*-Bz-A); 8.17 (AA′BB′ system, BB′ part, 2H, *m*-NPE); 8.26 (s, 1H, H-8-G); 8.34 (s, 1H, H-8-G); 8.55 (s, 1H, H-8-A); 8.70 (s, 1H, H-2-A); 10.32 (br s, 1H, NH); 11.4 (br s, ca. 3H, NH); 12.4 (very br s, 1H, NH). FABMS: *m*/*z* 2152 (M + Na^+). For the related conversion of **37**/**41**, see the supporting information.

Tetramer Sulfone 46. Following the procedure and general workup used for **34**, **44** (86.0 mg, 45.5 μmol) was reacted for 3 h with Oxone (112 mg, 192 μmol) and NaOAc (49.3 mg, 600 μmol) in a mixture of MeOH (25 mL), THF (10 mL), and water (5 mL). The residue was filtered over silica, and **46** (87.2 mg, 45 μmol, 99%) was isolated as a colorless foam. ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD/D_2O (3:1:saturated)): δ 0.94 (s, 9H, CH_3-*t*Bu); 1.69 (2H); 1.90 (2H), 2.09 (3H), 2.32 (3H) (4m, 8 × H-5′, 2 × H-3′); 2.00, 2.05 (2s, 2 × 3H, 2 × $COCH_3$); 2.73 (d, 1H, J = 12.8 Hz, H-3″); 2.98−3.42 (9H), 3.43−3.68 (3H), 3.69−3.81 (3H) (3m, 2 × H-3′, 5 × H-3″, 8 × H-6′), 3.88 (m, 1H, H-4′); 4.04 (dt, 1H, J = 2.5, 8.8 Hz, H-4′); 4.13 (dt, 1H, J = 2, 9 Hz, H-4′); 4.27 (m, 1H, H-4′); 4.37 (d, 1H, J = 5.1 Hz, H-2′-U1); 4.41, 4.53 (2dd, 2H, H-3″-U2); 5.28 (s, 1H, H-1′-Py); 5.49 (m, 3H, H-1′-Py, H-2′-Py, H-5-U), 5.55 (s, 1H, H-1′-Py); 5.59 (d, 1H, J = 8.0 Hz, H-5-U); 5.73 (m, 2H, H-2′-Py, H-2′-A); 5.96 (s, 1H, H.-1′-A); 7.20 (d, 1H, J = 8.1 Hz, H-6-U); 7.23−7.48 (m, 20H, H-6-U, H-5-C, *m*,*p*-Ph, *m*,*p*-Bz); 7.53 (2 AA′BB′C systems, 2 AA′ parts, 4H, *o*-Ph); 7.62 (d, 1H, J = 7.6 Hz, H-6-C); 7.82 (m, 6H, 6 × *o*-Bz); 7.94 (AA′BB′C system, AA′ part = d, 2H, J = 7.2 Hz, *o*-Bz-A); 8.18 (s, 1H, H-8-A); 8.63 (s, 1H, H-2-A). FABMS: *m*/*z* 1963 (M + Na^+). For the related conversion of **43**, see the supporting information.

Tetramer Pentaol 47. Compound **45** (62.2 mg, 29.2 μmol) was hydrolyzed with 0.2 M LiOH (1.1 mL, 220 μmol; 65 min). The mixture was neutralized and extracted with a mixture of CH_2Cl_2 and EtOH (9:1), the solvents were removed, and the residue was chromatographed (17 g of silica; CH_2Cl_2/MeOH/EtOH/water (84:7.5:7.5:1), 200 mL, and then EtOAc/MeOH/water (76:18:3), 150 mL). Pentaol **47** (36.8 mg, 19.3 μmol, 66%) and the debenzoylated adenine side product **48** (3.3 mg, 1.8 μmol) were obtained as colorless solids. The following data are for **47**. ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD/D_2O (3:1:saturated)): δ 1.00 (s, 9H, CH_3-*t*Bu); 1.17 (m, 12H, CH_3-iBu); 1.84 (m, 1H, H-5′-A); 2.02 (4H), 2.20−2.42 (4H), (2m, 7 × H-5′, H-3′-U); 2.52, 2.60, 2.66 (3m, 3H, 2 × CH-iBu, H-3′-A); 2.78, 2.83 (2m, 2H, 2 × H-3′-G); 2.92 (d, 1H, J = 12.4 Hz, H-3″); 2.99 (m, 2H, 2 × H-6′); 3.15 (dd, 1H, J = 2.5, 14.5 H, H-3″); 3.21 (2H), 3.33 (3H), 3.54 (2H), 3.72 (2H) (4m, 5 × H-3″, 4 × H-6′); 3.21 (m, 2H, CH_2-NPE); 3.83 (m, 3H, 2 × H-6′-A, H-3″-G4); 3.97 (t, 1H, J = 8.0; H-4′); 4.16 (m, 2H, 2 × H-4′); 4.24 (dt, 1H, J = 2.3, 9.7 Hz, H-4′); 4.45 (d, 1H, J = 5.0 Hz, H-2′-U); 4.69 (m, 4H, 2 × H-2′, CH_2-NPE); 4.81 (d, 1H, J = 5.5 Hz, H-2′-G3); 5.44 (s, 1H, H-1′-U); 5.54 (d, 1H, J = 8.0 Hz, H-5-U); 5.69 (s, 1H, H-1′-G); 5.88 (s, 1H, H-1′-G); 6.01 (s, 1H, H-1′-A); 7.29−7.39 (7H), 7.45 (4H), (2m, H-6-U, *m*,*p*-Ph, *m*-Bz, *o*-NPE); 7.54 (AA′BB′C system, C part, 1H, *p*-Bz); 7.61 (2 AA′BB′C systems, 2 AA′ parts, 4H, *o*-Ph); 7.71 (br s, 1H, H-8-G4); 7.99 (s, 1H, H-8-G3); 8.01 (AA′BB′C system, AA′ part = d, 2H, J = 7.2 Hz, *o*-Bz-A); 8.06 (s, 1H, H-8-A); 8.07 (AA′BB′ system, BB′ part, 2H, *m*-NPE); 8.58 (s, 1H, H-2-A). FABMS: *m*/*z* 1956 (M + 2Na^+ − H^+). For the related conversion of **48**, see the supporting information.

Tetramer Diol 49. Compound **46** (29.0 mg, 15.0 μmol) in CH_2Cl_2/MeOH (9:1, 1.2 mL) was dried in an Eppendorf reaction vessel under Ar stream and then high vacuum. The residue, in 0.25 mL of pyridine, was treated with a solution of HF in pyridine (5 M, 250 μL, 1.25 mmol), and the mixture was stirred for 8 h at RT. Trimethylmethoxysilane (350 μL, 317 mg, 3.03 mmol) was added to quench residual HF, yielding a colorless precipitate. After 30 min at RT, the mixture was transferred to a flask and dried *in vacuo*. The residue was chromatographed on silica (14 g; CH_2Cl_2/MeOH/H_2O (93:7:0.5 (80 mL) followed by 88:11:0.65 (75 mL) and then EtOAc/MeOH/H_2O (79:18:3)) to yield dialcohol **49** (19.7 mg, 11.6 μmol, 77%) as a colorless solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.74 (m, 1H, H-5′-U1); 1.94 (1H), 2.03 (3H), 2.23 (2H), 2.37 (2H), 2.45 (2H) (5m, 7 × H-5′, 3 × H-3′); 2.07 (s, 3H, $COCH_3$); 2.13 (s, 3H, $COCH_3$); 3.10 (m, 3H, H-3′-A, 2 × H-3″); 3.23−3.66 (m, 12H, 8 × H-6′, 4 × H-3″); 3.94 (m, 2H, 2 × H-4′); 4.16 (t, 1H, J = 10 Hz, H-4′); 4.26 (m, 1H, OH-6′-U1); 4.40 (dt, 1H, J = 2.8, 8.8 Hz, H-4′); 4.52 (m, 2H, H-3″-U2, H-2′-U1); 4.61 (dd, 1H, H-3″-U2); 5.45 (d, 1H, J = 8.2 Hz, H-2′-Py); 5.62 (d, 1H, J = 8.0 Hz, H-5-U); 5.63 (d, 1H, J = 1.6 Hz, H-1′-Py); 5.68 (d, 1H, J = 1.6 Hz, H-1′-Py); 5.71 (d, 1H, J = 8.0 Hz, H-5-U); 5.74 (d, 1H, J = 7.2 Hz, H-5-C); 5.81 (dd, 1H, J = 3.0, 7.5 Hz, H-2′-Py); 5.94 (d, 1H, J = 3.0 Hz, H-1′-Py); 6.01 (d, 1H, J = 5.1 Hz, H-2′-A); 6.24 (d, 1H, J = 1.4 Hz, H-1′-A); 7.44 (m, 1H, H-6-U); 7.46−7.62 (m, 12H, 4 × *o*,*m*-Bz); 7.78 (d, 1H, J = 8.1 Hz, H-6-U); 7.90, 7.96, 8.00 (3 AA′BB′C systems, 3 AA′ parts, 3 × 2H, *o*-Bz); 8.05 (AA′BB′C system, AA′ part, 2H, *o*-Bz-A); 8.13 (d, 1H, J = 7.3 Hz, H-6-C); 8.66 (s, 1H, H-8-A); 8.75 (s, 1H, H-2-A); 11.33 (br s, 3H, 3 × NH). FABMS: *m/z* 1739 (M + K^+). For the related conversion of **51**, see the supporting information.

Tetramer Thioester 50. Pentaol **47** (19.7 mg, 10.3 μmol) was coevaporated with pyridine, dried (high vacuum), and reacted with PPh_3 (9.5 mg, 36.0 μmol), DIAD (6.1 μL, 6.3 mg, 31 μmol), and thioacetic acid (2.5 μL, 2.8 mg, 36 μmol) in a mixture of dioxane (0.45 mL) and $Cl(CH_2)_2Cl$ (0.2 mL) for 90 min. The reagent was prepared first, in a manner similar to that used for compound **38**. Chromatography on silica (10 g; CH_2Cl_2/MeOH/EtOH/H_2O (100:7.5:2.5:0.1 (100 mL) followed by 100:7.5:7.5:1 (50 mL))) furnished **50** (12.1 mg, 6.1 μmol, 60%, 72% based on recovered **47**) and unreacted **47** (3.5 mg, 1.8 μmol) as colorless solids. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.96 (s, 9H, CH_3-*t*Bu); 1.12 (m, 12H, CH_3-iBu); 1.93 (m, 1H, H-5′-A); 2.05 (2H), 2.12 (1H), 2.22 (2H), 2.32 (1H), 2.46 (1H), 2.52 (1H) (6m, 7 × H-5′, H-3′-U2); 2.32 (s, 3H, $COCH_3$); 2.77, 2.83 (2m, 2H, 2 × CH-iBu); 2.94 (m, 1H, H-3′-A); 3.02 (m, 2H, 2 × H-3″-G4); 3.11 (m, 1H, H-3′-G); 3.22 (dd, 1H, J = 2.0, 11.1 Hz, H-3″); 3.28−3.43 (m, partly under HDO), 3.51 (3H) (2m, H-3′-G, 5 H-3″, 6 × H-6′, CH_2-NPE); 3.77 (t, 2H, J = 6.8 Hz, 2 × H-6′-A); 3.91 (t, 1H, J = 10.4 Hz; H-4′); 4.03 (m, 2H, 2 × H-4′); 4.24 (dt, 1H, J = 1.6, 9.7 Hz, H-4′); 4.30 (m, 1H, H-2′-U); 4.52 (m, 1H, H-2′); 4.69 (m, 1H, H-2′); 4.76 (m, 1H, H-2′); 4.78 (t, 2H, J = 6.8 Hz, CH_2-NPE); 5.61 (d, 1H, J = 8.1 Hz, H-5-U); 5.65 (d, 1H, J = 1.9 Hz, H-1′-U); 5.78 (d, 1H, J = 2.6 Hz, H-1′-G); 5.91 (d, 1H, J = 2.1 Hz, H-1′-G); 6.01 (d, 1H, J = 5.4 Hz, OH); 6.03 (d, 1H, J = 1.6 Hz, H-1′-A); 6.17 (d, 1H, J = 4.6 Hz, OH); 6.22 (br s, 1H, OH); 7.34−7.44 (6H), 7.52−7.66 (10H) (2m, H-6-U, *o*,*m*,*p*-Ph, *m*,*p*-Bz, *o*-NPE); 8.04 (AA′BB′C system, AA′ part, 2H, *o*-Bz-A); 8.14 (s, 1H, H-8-G4); 8.17 (AA′BB′ system, BB′ part, 2H, *m*-NPE); 8.39 (s, 1H, H-8-G3); 8.5 (s, 1H, H-8-A); 8.70 (s, 1H, H-2-A), 10.31 (s, 1H, NH); 11.2 (br s, 1H, NH); 11.35 (br s, 2H, 2 × NH); 12.1 (br s, 1H, NH). FABMS: *m/z* 1993 (M + Na^+).

Tetramer Bromide 54. Monomer **18** (25.2 mg, 52.5 μmol), tetramer **49** (6.5 mg, 3.8 μmol), and PPh_3 (22 mg, 84.5 μmol) in CH_3CN (3 mL) and $Cl(CH_2)_2Cl$ (2 mL) were treated with CBr_4 (23.3 mg, 70.4 μmol) in $Cl(CH_2)_2Cl$ (0.5 mL) for 2 h. Chromatography (12 g of silica; CH_2Cl_2/EtOAc/MeOH/H_2O (50:50:3:0.5) (50 mL) followed by CH_2Cl_2/MeOH/H_2O (92:7:0.5 (50 mL) and then 88:11:0.65 (40 mL))) gave **21** (14.9 mg, 27.4 μmol, 52%), **18** (9.5 mg, 19.8 μmol), **54** (2.2 mg, 1.25 μmol, 33%, 83% based on recovered starting material), and **49** (3.9 mg, 2.3 μmol) as colorless glasses or foams. The following are data for **54**. ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD/D_2O (4:1:saturated)): δ 1.95−2.28 (5H), 2.36 (4H), 2.55 (2H) (3m, 8 × H-5′, 3 × H-3′); 2.08 (s, 3H, $COCH_3$-C); 2.13 (s, 3H, $COCH_3$-A); 2.80 (m, 2H, H-3′-A, H-3″); 3.15 (m, ca. 3H, partly under HDO), 3.21−3.80 (10H), (2m, 5 × H-3″, 8 × H-6′); 4.08 (m, 2H, 2 × H-4′); 4.19 (m, 1H, H-4′); 4.35 (m, 1H, H-4′); 4.48 (m, 2H, H-3″-U2, H-2′-U1); 4.64 (dd, 1H, H-3″-U2); 5.20 (s, 1H, H-1′-Py); 5.46 (d, 1H, J = 2.1 Hz, H-1′-Py); 5.51 (d, 1H, J = 5.3 Hz, H-2′-Py); 5.62 (d, 1H, J = 8.0 Hz, H-5-U); 5.65 (d, 1H, J = 8.1 Hz, H-5-U); 5.66 (s, 1H, H-1′-Py); 5.81 (d, 2H, J = 5.6 Hz, H-2′-Py, H-2′-A); 5.99 (s, 1H, H-1′-A); 7.35−7.62 (m, 16H, *m*,*p*-Bz, 2 × H-6-U, H-5,6-C); 7.92 (m, 6H, *o*-Bz); 8.02 (AA′BB′C system, AA′ part, 2H, *o*-Bz-A); 8.17 (s, 1H, H-8-A); 8.70 (s, 1H, H-2-A). FABMS: *m/z* 1788 (M + Na^+). For the related conversion of **52**, see the supporting information.

Octamer 55. Cs_2CO_3 (4.6 mg, 14 μmol) was dried at 100 °C under high vacuum for 5 min. **50** (6.8 mg, 3.45 μmol), **54** (6.2 mg, 3.5 μmol), and anhydrous DMF (0.45 mL) were added, and the slurry was stirred (RT, 4 h, Ar). Acetate buffer (10 mL) was added, and the solvents were evaporated *in vacuo*. The residue was treated with 50% brine (2 mL) and extracted 5× with CH_2Cl_2/EtOH (4:1, 5 mL each). The combined organic phases were concentrated *in vacuo* to yield a colorless solid (18 mg). This was dissolved in MeOH (4 mL), THF (3 mL), and water (0.2 mL) under stirring and moderate heating. A solution of Oxone (18.6 mg, 30 μmol) and anhydrous NaOAc (8.1 mg, 99 μmol) in water (0.6 mL) was added at RT and the turbid suspension stirred for 9 h. Saturated $Na_2S_2O_3$ solution (0.2 mL) was added, resulting in a clear organic phase. Water (2 mL) was added and the mixture extracted five times with CH_2Cl_2/EtOH (9:1, 20 mL). The combined organic phases were evaporated to dryness, and the residue was dissolved in a mixture of CH_3CN and water (3:1, 7 mL) under slight heating. The solution was filtered and purified by HPLC (Lichrospher RP_{18} column, 10 μm, 250 × 16 mm, guard column 40 × 16 mm; flow 9 mL/min; gradient: B = 85% CH_3CN in water, A = water; 40% to 90% B in 53 min; elution of product after 48 min). Evaporation of solvents yielded **55** (7.0 mg, 1.85 μmol, 53%; 70% based on recovered bromide) as a white amorphous solid. *In vacuo* concentration of solutions containing compounds having shorter retention times yielded the G4-2′-acetyl-3″-sulfonic acid of **50** (5 min, 2.1 mg, 1.0 μmol), and **54** (24 min, 1.5 mg, 0.84 μmol). ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.96 (s, 9H, CH_3-*t*Bu); 1.07 (m, 12H, CH_3-iBu); 1.82 (2H), 2.05 (8H), 2.19 (2H), 2.28 (4H), 2.39 (2H), 2.47 (partly under DMSO-d_5) (6m, 16 × H-5′, 4 × H-3′-Py); 2.07, 2.07, 2.12 (3s, 3 × 3H, 3 × $COCH_3$); 2.86 (m, 2H, 2 × CH-iBu); 2.96 (m, 3H, 3 × H-3′-Pu); 3.02−3.68 (m, partly under H_2O, H-3′-Pu, 16 × H-3″, CH_2-NPE, 14 × H-6′); 3.77 (m, 2H, H-6′-A1); 3.92 (m, 3H, 3 × H-4′-Py); 4.02 (m, 2H, 2 × H-4′); 4.15 (m, 2H, 2 × 4′); 4.31 (d, 1H, J = 5.4 Hz, H-2′-U); 4.34 (d, 1H, J = 5.0 Hz, H-2′-U); 4.39 (dt, 1H, J = 2.3, 9.0 Hz, H-4′-A6); 4.52 (dd, 1H, J = 5.8, 11.3 Hz, H-3″-U8); 4.61 (dd, 1H, J = 6.7, 11.3 Hz, H-3″-U8); 4.64 (d, 1H, J = 4.8 Hz, H-2′-Pu); 4.77 (m, 3H, H-2′-Pu, CH_2-NPE); 5.01, 5.12 (2 br s, 2H, 2 × OH); 5.38 (d, 1H, J = 7.2 Hz, H-2′-Py); 5.55 (m, 1H, H-2′-Py); 5.60 (d, 1H, J = 8.1 Hz, H-5-U); 5.62 (d, 1H, J = 8.1 Hz, H-5-U); 5.66 (s, 4H, 4 × H-1′-Py); 5.69 (d, 1H, J = 7.9 Hz, H-5-U); 5.73 (d, 1H, J = 6.7 Hz, H-5-C); 5.81 (dd, 1H, J = 3.0, 7.1 Hz, H-2′); 5.87 (m, 2H, H-2′-A6, H-1′-G); 5.93 (d, 1H, J = 2.9 Hz, H-1′-G); 5.93 (s, 1H, H-1′-A1); 6.15 (br s, 2H, 2 × OH); 6.23 (s, 1H, H-1′-A6); 7.28 (d, 1H, J = 7.1 Hz, H-6-C); 7.34−7.66 (m, 29H, 2 × H-6-U, *o*,*m*,*p*-Ph, *m*,*p*-Bz, *o*-NPE); 7.78 (d, 1H, J = 8.0 Hz, H-6-U); 7.90 (2H); 7.94 (2H), 7.99 (2H), (3 AA′BB′C systems, 3 AA′ parts, 4 × *o*-Bz-U8, 2 × *o*-Bz-C); 7.96 (s, 1H, H-8-G); 8.06 (m, 4H, 4 × *o*-Bz-A); 8.17 (AA′BB′ system, BB′ part, 2H, *m*-NPE); 8.34 (s, 1H, H-8-G); 8.53, 8.62, 8.67, 8.71 (4s, 4H, 2 × H-8-A, 2 × H-2-A); 10.35 (br s, ca. 2H, NH); 11.4 (br s, ca. 5H, NH). For other 500 MHz ^{1}H NMR (DMSO-d_6/D_2O (4:1) and $CDCl_3$/CD_3OD/D_2O (5:2:saturated), see the supporting information. FABMS: *m/z* 3708 (M + Na^+).

Deprotected Tetramer 56. Compound **46** (16.7 mg, 8.9 μmol) in MeOH (1.2 mL) and THF (0.9 mL) was treated with 1 M NaOH (1.2 mL, 1.2 mmol) with stirring (40 °C, 4 h). The mixture was cooled to RT and diluted with acetate buffer (0.55 mL). A white precipitate formed. A mixture of CH_3CN and water (1:1, 13 mL) was added, and the resulting solution filtered and injected onto an HPLC column (Nucleosil 10-CN, 250 × 22.5 mm; flow 11 mL/min; B = CH_3CN (85%) in water, A = water; gradient 10% to 35% B in 30 min; retention time of the product 19 min, no detectable side products) to yield **56** (6.3 mg, 5.3 μmol, 60%) as a colorless solid. Injection of larger amounts of the crude product onto the HPLC column led to a peak eluting after 16 min. This was assigned as an aggregate, since it had

identical retention time upon reinjection at higher dilution. ^{1}H NMR (600 MHz, DMSO-d_6/D_2O (3.5:1), 300 K): δ 1.710 (m, 1H, H-5′-U1); 1.903 (m, 1H, H-5′-U1); 1.950 (m, 1H, H-5′-C); 1.973 (m, 1H, H-5′-U4); 2.009 (m, 1H, H-5′-A); 2.033 (m, 1H, H-3′-U4); 2.227 (m, 1H, H-5′-U4); 2.239 (m, 1H, H-5′-C); 2.287 (m, 1H, H-5′-A); 2.296 (m, 1H, H-3′-U1); 2.333 (m, 1H, H-3′-C); 3.025 (m, 1H, H-3′-A); 3.093 (dd, 1H, J = 2.6, 11.3 Hz, H-3″-U1); 3.106 (dd, 1H, J = 3.0, 8.7 Hz, H-3″-C); 3.239 (m, 1H, H-6′-A); 3.240 (m, 1H, H-3″-A); 3.247 (m, 1H, H-6′-A); 3.253 (m, 1H, H-6′-C); 3.276 (m, 1H, H-6′-C); 3.314 (m, 1H, H-6′-U4); 3.331 (m, 1H, H-6′-U4); 3.415 (m, 1H, H-3″-C); 3.439 (m, 1H, H-3″-U1); 3.460 (m, 1H, H-3″-U4); 3.489 (m, 1H, H-6′-U1); 3.504 (m, 1H, H-3″-A); 3.568 (m, 1H, H-6′-U1); 3.676 (dd, 1H, J = 6.0, 11.0 Hz, H-3″-U4); 3.922 (dt, 2H, J = 2.7, 9.6 Hz, H-4′-U1, H-4′-C); 3.996 (m, partly under HDO, H-4′-U4); 4.070 (dt, 1H, J = 2.6, 8.6 Hz, H-4′-A); 4.199 (d, 1H, J = 6.7 Hz, H-2′-C); 4.220 (dd, 1H, J = 2.5, 5.9 Hz, H-2′-U4); 4.228 (dd, 1H, J = 2.6, 4.3 Hz, H-2′-U1); 4.697 (dd, 1H, J = 1.5, 5.7 Hz, H-2′-A); 5.575 (d, 1H, J = 7.9 Hz, H-5-U); 5.581 (d, 1H, J = 8.0 Hz, H-5-U); 5.587 (m, 3H, 3 × H-1′-Py); 5.815 (d, 1H, J = 7.4 Hz, H-5-C); 5.899 (d, 1H, J = 1.6 Hz, H-1′-A); 7.422 (d, 1H, J = 8.1 Hz, H-6-U4); 7.449 (d, 1H, J = 8.0 Hz, H-6-U1); 7.517 (d, 1H, J = 7.5 Hz, H-6-C); 8.112 (s, 1H, H-2-A); 8.231 (s, 1H, H-8-A). For 500 MHz ^{1}H and 125 MHz ^{13}C NMR in DMSO-d_6, see the supporting information. MALDI-TOFMS (linear positive mode, 20 kV): m/z 1222 (M + Na^+).

Deprotected Tetramer 57. Compound **45** (23 mg, 10.6 μmol) was treated with 1 M NaOH (1.3 mL), MeOH (1.3 mL), and THF (1 mL) for 6 h at 40 °C. HPLC (gradient of CH_3CN (85%) in water from 10% to 30% in 33 min, retention time 18 min) yielded **57** (8.5 mg, 6.6 μmol, 63%) as a colorless solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.77 (m, 1H, H-5′-A); 2.01 (3H), 2.23 (1H), 2.30 (2H), 2.43 (1H), 2.50 (partly under DMSO-d_5) (5m, 8H, 7 × H-5′, H-3′-U); 2.86 (m, 2H, 2 × H-3′); 3.13−3.39 (partly under H_2O, integrated after addition of D_2O; 9H), 3.40−3.59 (7H) (2m, 8 × H-6′, 7 × H-3″, H-3′); 3.75 (m, 1H, H-3″-G4); 3.91 (dt, 1H, J = 2.0, 7.5 Hz, H-4′); 4.04 (m, 2H, 2 × H-4′); 4.09 (dt, 1H, J = 3.1, 6.0 Hz, H-4′); 4.31 (d, 1H, J = 6.2 Hz, H-2′-U); 4.57 (m, 2H, 2 × H-2′); 4.66 (d, 1H, J = 5.6 Hz, H-2′); 5.54 (d, 1H, J = 7.8 Hz, H-5-U); 5.65 (s, 1H, H-1′); 5.66 (d, 1H, J = 2.7 Hz, H-1′); 5.70 (d, 1H, J = 1.9 Hz, H-1′); 5.92 (d, 1H, J = 1.2 Hz, H-1′-A); 6.25 (br s, 2H, 2 × OH); 6.88, 6.96 (2 br s, 2 × 2H, 2 × NH_2-G); 7.28 (br s, 2H, NH_2-A); 7.51 (d, 1H, J = 8.1 Hz, H-6-U); 7.73, 7.77 (2s, 2H, 2 × H-8-G); 8.13 (s, 1H, H-2-A); 8.24 (s, 1H, H-8-A). MALDI-TOFMS (linear, positive mode, 20 kV): m/z 1304 (M + Na^+).

Deprotected Octamer 58. Compound **55** (2.0 mg, 0.54 μmol) was treated with 1 M NaOH (0.4 mL), MeOH (0.3 mL), and THF (0.2 mL) for 10 h at 40 °C. Acidification with acetate buffer (0.2 mL) and concentration to 0.8 mL under an Ar stream led to a white precipitate. After cooling to −20 °C for 5 min, the mixture was centrifuged for 5 min at 8000g and the supernatant was carefully aspired. The precipitate was washed twice with water and four times with diethyl ether/CH_2Cl_2 (9:1) and dried under high vacuum to yield deprotected octamer **58** (0.9 mg, 0.36 μmol, 72%) as an amorphous, colorless solid. This compound was characterized by HPLC using a Lichrospher RP18 column (10 μm, 250 × 16 mm, guard column 40 × 16 mm, flow 9 mL/min; B = CH_3CN, A = water, gradient 10% to 25% B in 5 min, 25% to 35% B in 15 min; elution of **58** after 13.5 min). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.78 (m, 1H, H-5′-A1); 1.93−2.10 (10H), 2.22−2.42 (8H), 2.55 (partly under DMSO-d_5) (3m, 15 × H-5′, 4 × H-3′-Py); 2.87 (3H), 3.02 (1H) (2m, 4 × H-3′-Pu); 3.14−3.43 (partly under H_2O), 3.43−3.60 (12H) (2m, 15 × H-3″, 16 × H-6′); 3.70 (dd, 1H, J = 5.4, 10.5 Hz, H-3″-U8); 3.79 (br s, 1H, OH); 3.92 (m, 3H, 3 × H-4′); 4.02 (m, 5H, 5 × H-4′); 4.22 (d, 1H, J = 5.0 Hz, H-2′-Py); 4.27 (d, 1H, J = 6.2 Hz, H-2′-Py); 4.32 (m, 2H, 2 × H-2′-Py); 4.57 (m, 2H, 2 × H-2′-Pu); 4.60 (br s, 1H, OH); 4.64 (d, 1H, J = 5.3 Hz, H-2′-Pu); 4.70 (d, 1H, J = 5.1 Hz, H-2′-Pu); 5.59−5.68 (m, 7H, 3 × H-5-U, 4 × H-1′); 5.70, 5.72 (2s, 1H, 2 × H-1′); 5.76 (d, 1H, J = 7.6 Hz, H-5-C); 5.91, 5.95 (2s, 2H, 2 × H-1′-A); 6.05 (1H), 6.18 (4H), 6.38 (1H) (3br s, 6 × OH); 6.56 (2H), 6.64 (2H) (2 br s, 2 × NH_2-G); 7.16−7.29 (m, 6H, 2 × NH_2-A, NH_2-C); 7.39 (m, H-6-U); 7.55−7.64 (m, 3H, 2 × H-6U, H-6-C); 7.84, 7.85 (2s, 2H, 2 × H-8-G); 8.13, 8.14, 8.23, 8.30 (4s, 4H, 2 × H-8-A, 2 × H-2-A). MALDI-TOFMS (linear, positive mode, 20 kV): m/z 2534.9 (M + Na^+), calcd (average mass) 2534.5. Electrospray MS: m/z 2511.4 (M^+), calcd (average mass) 2511.5.

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Supporting Information Available: Additional experimental procedures, assigned NMR and mass spectra (generally FAB) for all compounds, crystallographic data (crystal data, positional and thermal parameters, bond lengths and angles; also in CIF format) and an Ortep plot of **11**, UV spectra of **55** in CH_2Cl_2 with 0.5% and 4% methanol and methanol adjuvant-induced hyperchromicity plots for **47**, **49**, and **55**, MALDI-TOF spectra of **57** and **58**, an electrospray ionization spectrum of **58**, and UV absorbance versus temperature profile of **58** and of its natural RNA counterpart (57 pages). This material is contained in many librairies on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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