Dynamics of Alkene Radical Cation/Phosphate Anion Pair Formation from Nucleotide C4′ Radicals. The DNA/RNA Paradox Revisited

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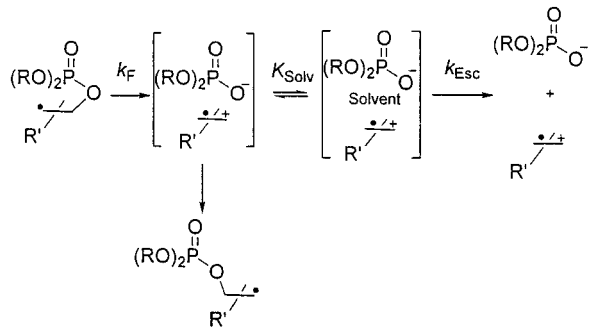
Abstract: The fragmentation of nucleotide C4′ radicals generated by thiyl radical addition to C4′C5′ exocyclic glycals has been re-examined and found to be a function of the thiol and, probably, the initiating system employed. It has been demonstrated that C4′ radicals of DNA and RNA models fragment even in the very nonpolar benzene solution if the correct (aliphatic) thiol is employed. ^{17}O-Labeling experiments are used to demonstrate that the fragmentation of nucleotide C4′ radicals (2-deoxyribo- and ribo-) to contact ion pairs is either irreversible or so rapidly reversible as to preclude prior reorganization of the contact ion pair. Formation of the solvent-separated ion pair is an irreversible step, with all such ion pairs proceeding to product formation.

Introduction

In collaboration with Newcomb and co-workers, we have recently put forward a model for the chemistry of *β*-(phosphatoxy)alkyl radicals in which rate-determining fragmentation leads to the formation of a contact ion pair consisting of an alkene radical cation and a phosphate anion. Collapse of the ion pair leads to the rearranged products. Equilibration of the contact ion pair with solvent provides a solvent-separated ion pair and eventually observable free ions (Scheme 1). The outcome of a particular reaction, rearrangement or fragmentation, is then seen to be the result of a complex interplay between collapse of the contact ion pair and its equilibration with solvent-separated and free ions. This interplay, in turn, is a function of the ability of substituents and solvents to stabilize the various charged species.[1,2] Support for this model derives from the closely related pre-exponential factors in the Arrhenius functions for either the rearrangement or fragmentation of a broad series of *β*-(phosphatoxy)alkyl and *β*-(acyloxy)alkyl radicals in a range of solvents of widely differing polarity, which suggests a common rate-determining step, namely that of fragmentation to the contact ion pair. Further support comes from the high linear correlation of rate constants, rearrangement or fragmentation, with the $E_T(30)$ solvent polarity scale in a range of solvents spanning benzene and aqueous acetonitrile.[1,2] Earlier trapping experiments with stereochemically labeled probes[3] are now best interpreted as involving nucleophilic attack on the contact ion pair.

Due to the need for strongly UV-absorbing radicals and radical cations in the laser flash photolytic method used to establish the above model, all of our studies were carried out with systems leading to benzyl radicals and styrene-type radical cations. However, it seems very reasonable to extrapolate the model to any system capable of fragmenting to give a radical cation that is at least as stable as that derived by oxidation of styrene. As ethyl vinyl ether and styrene have the same oxidation potentials in acetonitrile,[4] the model should apply to nucleotide C4′ radicals; indeed, chemically induced dynamic nuclear polarization experiments with 3-phosphatoxy-2-tetrahydrofuranyl radicals provided very strong support for the formation of enol ether radical cations.[5] More recently, using a method in which enol ether radical cations oxidize triarylamines to the highly chromophoric triarylaminium radical cations, we have established that simple α-alkoxy-*β*-(phosphatoxy)alkyl radicals (**1** and **2**) do, indeed, undergo fragmentation to diffusively free alkene radical cations (**3**) in acetonitrile solution.[6,7] From the studies conducted so far, there appears to be no reason to suspect that the reactions of α-alkoxy-*β*-(phosphatoxy)alkyl radicals do not conform to the general mechanistic picture of Scheme 1, involving equilibria between a series of ion pairs and free ions. Indeed, the approximately 100-fold rate difference for the fragmentation of radicals **2** and **4**, giving **3** and **5**, respectively, noted by two different groups in solvents of similar polarity is readily explained by this model and the different kinetic methods used (Scheme 2). Thus, the classical competition kinetic method employed for **4** by the Giese group[5] probably functions at the

Scheme 1



(1) Newcomb, M.; Horner, J. H.; Whitted, P. O.; Crich, D.; Huang, X.; Yao, Q.; Zipse, H. *J. Am. Chem. Soc.* **1999**, *121*, 10685−10694.
(2) Whitted, P. O.; Horner, J. H.; Newcomb, M.; Huang, X.; Crich, D. *Org. Lett.* **1999**, *1*, 153−156.
(3) Crich, D.; Gastaldi, S. *Tetrahedron Lett.* **1998**, *39*, 9377−9380.
(4) Katz, M.; Riemenschneider, P.; Wendt, H. *Electrochim. Acta* **1972**, *17*, 1595−1607.
(5) Gugger, A.; Batra, R.; Rzadek, P.; Rist, G.; Giese, B. *J. Am. Chem. Soc.* **1997**, *119*, 8740−8741.
(6) Newcomb, M.; Miranda, N.; Huang, X.; Crich, D. *J. Am. Chem. Soc.* **2000**, *122*, 6128−6129.
(7) Bales, B. C.; Horner, J. H.; Huang, X.; Newcomb, M.; Crich, D.; Greenberg, M. M. *J. Am. Chem. Soc.* **2001**, *123*, 3623−3629.

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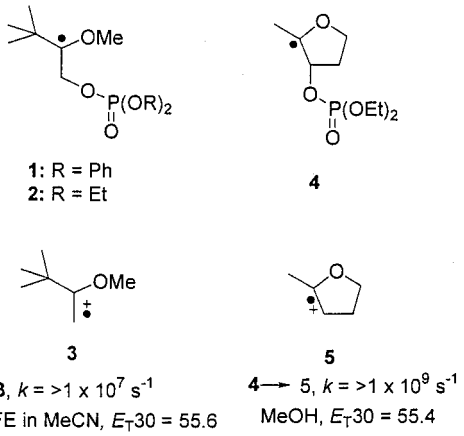
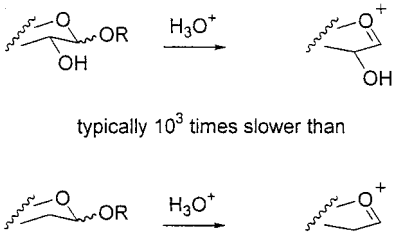
Scheme 2



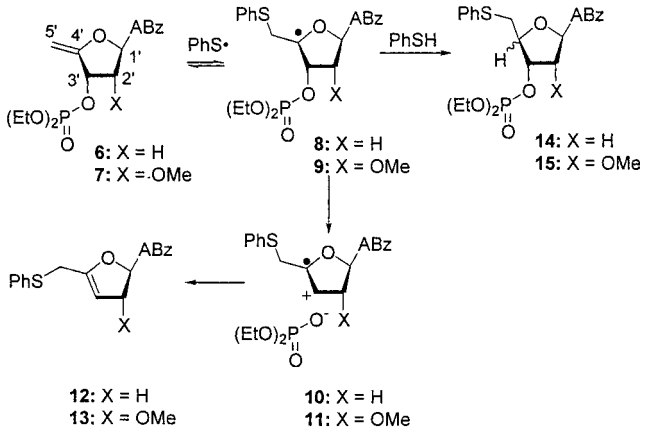
Scheme 3



level of the contact ion pair, whereas only free radical cations are reported by oxidation of triarylamines in the laser flash photolytic method developed with **2**.[6] The smaller rate constant determined by the latter method for the fragmentation of **2** is therefore a global one for fragmentation and cage escape and necessarily factors in the equilibrium constants for distribution between the various ion pairs.[6]

A number of interesting questions are raised by the general model, one of which pertains to the dynamics of the contact ion pair: does it always proceed in the forward direction to the rearranged radical or the solvent-separated ion pair, or does it partition with the initial radical, i.e., is the initial fragmentation reversible? A second point of interest to us revolves around the so-called DNA/RNA paradox,[8] in which the antitumor antibiotic iron−bleomycin (BLM) degrades "tDNA^{His}" more rapidly than the corresponding "tRNA^{His}",[9−11] through the formation and cleavage of C4′ radicals,[12−14] yet binds the RNA more tightly. By comparison with carbohydrate chemistry, wherein it is widely appreciated that 2-deoxy glycosides are solvolyzed much more rapidly than the analogous glycosides (Scheme 3),[15] a phenomenon attributed to destabilization of the anomeric cation by the adjacent C−O bond, we proposed that fragmentation of the ribonucleotide C4′ radical is retarded by the 2′-C−O bond, which destabilizes the 3′4′ radical cation.[8] Thorp and co-workers have described a very similar concept in which hydride abstraction from the 1′-site of nucleotides by an oxoruthenium(IV) complex is retarded by the presence of electron-withdrawing substituents at the 2′-position, and which is attributed to destabilization of the nascent 1′-carbocation.[16−18]

Scheme 4



In support of our hypothesis, we found with simple model systems that the 2-deoxyribo system fragments significantly faster than the ribo. More precisely, the 2-deoxynucleotide **6** was found to undergo fragmentation, in CD_3OD/D_2O (10:1) at 40 °C in the presence of excess thiophenol and di-*tert*-butyl peroxalate, to the glycal **12** in 87% yield after only 10 min, whereas the ribo analogue **7** was recovered unchanged after 24 h under the same conditions, despite repeated addition of initiator (Scheme 4). Given that γ-C−O bonds have only a very minimal effect on the stability of free radicals,[19−24] we interpreted this difference of reactivity in terms of a reversible addition of PhS•, with comparable forward and reverse rate constants in both systems, but with the fragmentation of the ribo radical **9** being very significantly retarded over that of its deoxy analogue **8** due to the presence of the inductively withdrawing 2-C−O bond. After our initial report, Giese and co-workers described a series of experiments in which an RNA C4′ radical in an undecamer was found to cleave more slowly than the comparable single-stranded DNA C4′ radical, but only by a factor of 3.[25]

Here we present the results of experiments designed to probe the reversibility of initial fragmentation to the contact ion pair and the effect of a 2′-C−O bond on the fragmentation and ion pair equilibria. The reasons underlying the very different results previously reported by Giese and ourselves on the effect of the 2′-C−O bond on nucleotide C4′ radical fragmentation have also been determined.

Results and Discussion

Nucleotide C4′ radical generation by the addition of phenylthiyl radicals to exocyclic glycals, as employed in our initial

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(9) Holmes, C. E.; Hecht, S. M. *J. Biol. Chem.* **1993**, *268*, 25909−25913.
(10) Hecht, S. M. *Bioconjugate Chem.* **1994**, *5*, 513−526.
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(12) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107−1136.
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(14) Hecht, S. M. *Acc. Chem. Res.* **1986**, *19*, 383−391.
(15) Capon, B. *Chem. Rev.* **1969**, *69*, 407−498. Additionally, 2-azido-1,2-dimethoxypropane is solvolyzed 600 times more slowly than 2-azido-2-methoxypropane in aqueous media: Amyes, T. L.; Jencks, W. P. *J. Am. Chem. Soc.* **1989**, *111*, 7888−7900.
(16) Farrer, B. T.; Pickett, J. S.; Thorp, H. H. *J. Am. Chem. Soc.* **2000**, *122*, 549−553.
(17) Neyhart, G. A.; Cheng, C.-C.; Thorp, H. H. *J. Am. Chem. Soc.* **1995**, *117*, 1463−1471.
(18) Thorp, H. H. *Chem. Biol.* **2000**, *7*, R33−R36.
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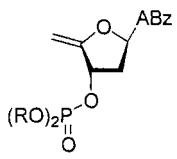
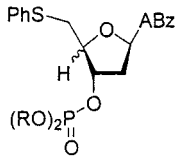
study (Scheme 4), was first described by the Giese group,[26,27] who found inter alia that cleavage of the dialkyl phosphates **6** and **16**, leading to the fragmentation product **12**, was in competition with reduction to **14** and **17** on irradiation at 30 °C with 1.0−2.4 M thiophenol in both toluene and ethanol/water (4/1). The very high differences in conversion of **6** and **7**



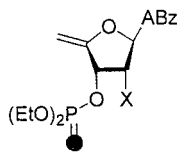
16: R = Me, *n*Pr, *i*Pr



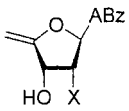
17: R = Me, *n*Pr, *i*Pr

observed earlier in this laboratory, using 1.5 M thiophenol in CD_3OD/D_2O 10/1 at 40 °C, with initiation by di-*tert*-butyl peroxalate (DBPO), coupled with the very minor influence of γ-C−O bonds on radical stability,[19−24] prompted us to modify the original Giese mechanism to include the reversibility of the initial addition of the phenylthiyl radical.[8] Were the initial addition of PhS$^\bullet$ not reversible, the slower fragmentation of **9** would have been expected to manifest itself in terms of the formation of a greater amount of reduction product **15**, rather than an apparent lack of reactivity, which was not the case.

We now suggest that the rapid reversibility of addition of RS$^\bullet$ to exocyclic glycals coupled with the use of a regiospecifically ^{17}O-labeled phosphate provides a method for probing the contact ion pair formed on expulsion of the phosphate group. Specifically, if a monolabeled phosphate **18** or **19** is allowed to react with RSH and a radical initiator and the reaction is stopped after significant conversion to the fragmentation products **12** and **13**, respectively, then scrambling of the label between the P═O and P−O in the recovered nucleoside must indicate reversibility of phosphate fragmentation. Alternatively, if it can be demonstrated that phosphate cleavage is taking place but scrambling of the label is not observed in recovered **18** and/or **19**, then the inference must be that the contact ion pair recombines before reorganization or not at all. The latter scenario implies that once the contact ion pair becomes solvent-separated, recombination cannot compete with other modes of decomposition. Toward this end, **20** and **21** were allowed to react with



18: X = H
19: X = OMe



20: X = H
21: X = OMe

diethyl chlorophosphite in THF and pyridine, followed by oxidation with a mixture of iodine and 10% ^{17}O-labeled water. In this manner, **18** and **19** were prepared in 37 and 57% isolated yields, respectively, and the label was shown to reside only in the P═O oxygen by the presence of a unique peak in the ^{17}O NMR spectra at δ 76.4 and 76.1, respectively. Determination of the exact extent of ^{17}O incorporation by mass spectrometry was complicated by the further 57% enrichment of the water employed in ^{18}O. Inspection of the ^{31}P NMR spectra of **18** and **19**, however, revealed that ^{18}O had been incorporated with little isotopic dilution. It can therefore be concluded that the same was true for ^{17}O and that the extent of enrichment of **18** and **19** in ^{17}O approached the 10% in the original source water.[28,29] Direct determination of the ^{17}O incorporation by integration of the ^{31}P NMR spectra is precluded by the quadrupolar nature of ^{17}O ($I = {}^5/_2$), which results in the ^{17}O═P resonance being dispersed in a complex multiplet.[28,29]

Exocyclic glycal **18** was allowed to react with 2.1 M thiophenol, initiated by DBPO at 40 °C in 10/1 CD_3OD/D_2O for 15 min, after which preparative TLC enabled the isolation of **12** and recovered **18** in 33 and 35% yields, respectively. Inspection of the recovered substrate (**18**) by ^{17}O NMR spectroscopy revealed the presence of a single peak at δ 76.3 which, as the P═O and P−O resonances are expected to be very readily resolved,[30−32] indicates no scrambling of the label. Under the same conditions **19** was recovered quantitatively, also with no scrambling of the label. The very considerable differences in reactivity of **18** and **19** mimic those in our original experiments with the label-free substrates **6** and **7**. To observe fragmentation with **19**, it was necessary to operate in 2/1 CD_3OD/D_2O when **13** was isolated in 26% yield after 1 h, with 18% of **19** being recovered, again without detectable scrambling of the label.[33] Although we have principally relied on ^{17}O NMR spectroscopy as a means of detecting scrambling of stereochemistry at phosphorus, the results were always fully corroborated by analysis of the ^{31}P NMR spectra.[34] In effect, the use of water enriched in both ^{17}O and ^{18}O for the synthesis of **18** and **19** enables the isotope shifts due to the presence of ^{18}O to be employed as a probe of scrambling. The ^{31}P NMR spectra of **18** and **19** as initially prepared showed two singlets resulting from the ^{16}O and ^{18}O isotopomers. Scrambling of the ^{18}O between the P═O and P−O positions would be expected to lead to ^{31}P NMR spectra containing three resonances: one each for the unlabeled, the original P═^{18}O labeled, and the inverted P−^{18}O substances.

We next turned to the use of intramolecular nucleophiles with the expectation that cyclization might be competitive with collapse of the contact ion pair. As illustrated in Scheme 5, experiments of this type were conducted with 2-mercaptoethanol, which served the dual function of thiol and nucleophile.

When **6** was exposed to 0.15 M 2-mercaptoethanol, with initiation by DBPO, in C_6D_6 at 40 °C, ^{1}H NMR spectroscopy after 45 min revealed consumption of the substrate and the formation of a rather complex reaction mixture. One nucleoside predominated in this reaction mixture to the extent of approximately 50% as judged from the intensity of the "anomeric" signals. Isolation of this product from the complex reaction mixture was not possible owing to the small scale of the experiment and the instability of the product. The experiment was therefore repeated on a somewhat larger scale in benzene, when ^{1}H NMR spectroscopy of the crude reaction mixture again

(26) Giese, B.; Beyrich-Graf, X.; Burger, J.; Kesselheim, C.; Senn, M.; Schafer, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1742−1743.

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(32) Tsai, M.-D.; Bruzik, K. In *Biological Magnetic Resonance*; Berliner, L. J., Reuben, J., Eds.; Plenum Press: New York, 1983; Vol. 5, pp 129−181.

(33) The low mass balances in most of the experiments described here are explained in large part, as verified by control experiments, by the instability of most of the substrates and products under the reaction conditions, with diethylphosphoric acid being a necessary byproduct.

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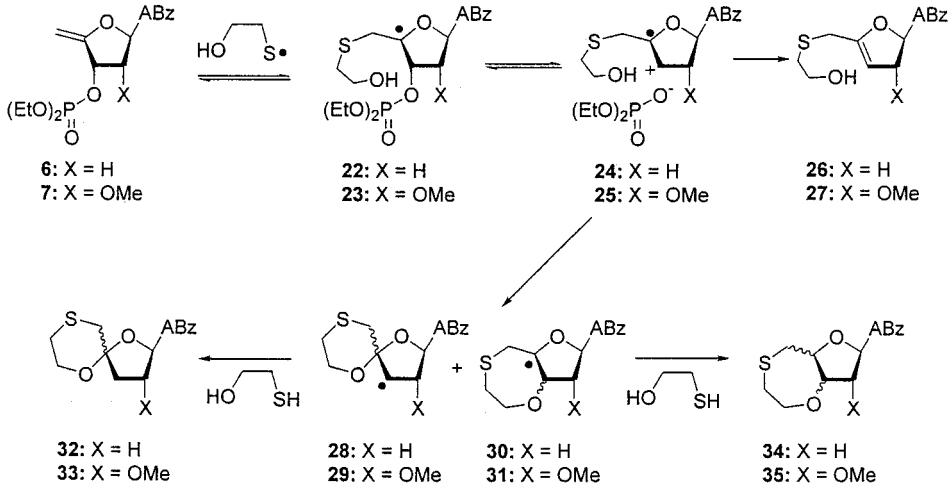
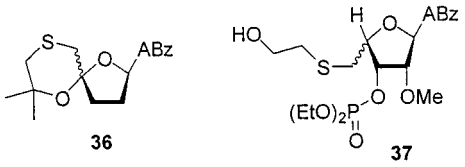
Scheme 5



indicated formation of the same major product. Repeated chromatography on silica gel eventually enabled isolation of this product in ~5% yield, which reflects the extensive decomposition in the course of the isolation. The new product decomposed rapidly in $CDCl_3$ solution but was stable for extended periods of time in C_6D_6 and CD_3OD, undergoing only a slow isomerization to a second diastereoisomer. The general instability toward chromatography and $CDCl_3$, coupled with the slow isomerization, point to a spiroacetal **32**. The spiroacetal structure was supported by the high-resolution mass spectrum, which was consistent with a molecular formula of $C_{19}H_{20}N_5O_3S$ for the protonated species. In the ^{13}C spectrum of a mixture of the two equilibrating diastereomers, weak signals at δ 104.2 and 105.1 are attributable to the acetal carbon. None of the resonances assigned to the H5′ hydrogens in the ^1H spectrum had any significant ^3J couplings which, along with the absence of a signal attributable to H4,′ further confirmed the spiroacetal structure.

In an attempt to stabilize the spiroacetal structure, the reaction was repeated using 2-mercapto-1,1-dimethylethanol but, unfortunately, with a similar result, namely a high conversion to the anticipated product (**36**) but a low isolated yield. Again, the spectroscopic data for **36** fully support the structure assigned. In particular, the ^1H NMR spectrum is a simplified version of that of **32** with the absence of resonances between δ 3.1 and 6.0 signaling the lack of a C3′-O bond and of a C4′ H, thereby excluding any structure arising from ring closure at C3′ rather than C4′.



The experiment was repeated, using 2-mercaptoethanol and the ribonucleotide **7** in benzene. Unlike the above reactions with **6**, no evidence for the formation of a spiroacetal (**33**) was found. The two major isolated products were the fragmentation product **27** (24%) and the addition product **37** (44%),[35] arising from quenching of radical **23** (Scheme 5). Several conclusions can be drawn from this experiment. First, the isolation in high yield of the reduction product indicates that the fragmentation of the ribo radical **23** is significantly slower than that of its 2-deoxy counterpart **22**. Second, the formation of **27** rather than the spiroacetal **33** suggests that the ribo radical ion pair **25** is more reactive than its deoxy counterpart **24** and is reduced to **27** in competition with nucleophilic trapping. The actual mechanism of this reduction probably involves a competing deprotonation within the contact ion pair to give an allyl radical, followed by hydrogen transfer from the thiol. The 2′-C−O bond in the ribo series both destabilizes the radical cation and stabilizes the allyl radical, which accounts for the higher rate of deprotonation as compared to that in the deoxy ribo series.[36] Third and most important, the very formation of **27** indicates that ion pair **25** can be formed, even in benzene. This is in contrast to the earlier experiments (Scheme 4) when **7** was recovered unchanged even after 24 h in the far more polar methanol/water mixtures, leading to the original suggestion that fragmentation of radical **9** to ion pair **10** was severely retarded by the 2-C−O bond.

The obvious difference between Schemes 4 and 5 is the choice of thiol and its effect on the initial equilibrium between the exocyclic glycals and the adduct C4′ radicals. It is well known that analysis of the kinetics of addition of thiyl radicals to alkenes is complicated by the reversibility of the reaction. Nevertheless, following earlier work by Davies and Roberts,[37] Griller and co-workers established an approximate value for *n*-BuS$^\bullet$ addition to 1-octene of 1.9×10^6 M^{-1} s^{-1} in isooctane at 25 °C,[38] whereas Ito and Matsuda determined a value of 1.8×10^5 M^{-1} s^{-1} for the addition of *p*-ClPhS$^\bullet$, which is more reactive than simple PhS$^\bullet$, to the more reactive isobutyl vinyl ether.[39] Wagner and co-workers, on the other hand, determined the relative rate constants for elimination of *n*-BuS$^\bullet$ and PhS$^\bullet$ from secondary β-mercaptoalkyl radicals to be 1 and 687, respectively.[40,41] Although these numbers are only approximate

(35) The stereochemistry of **37**, formed as a single diastereomer, was not assigned rigorously. On the basis that quenching of the C4′ radical by the thiol will take place preferentially from the less hindered face, it is likely that C5′ is cis to the phosphate.

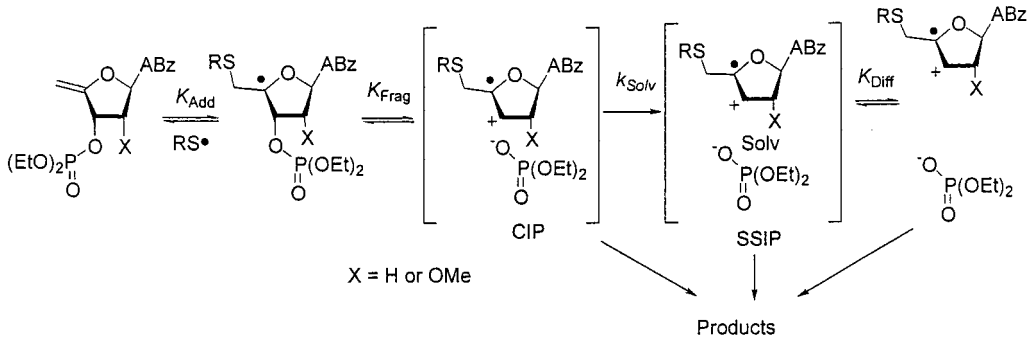
(36) It was originally suggested by Giese that the endocyclic glycal products derive from thiol reduction of the radical cation.[26] However, it has now been demonstrated that deprotonation to give allyl radicals can take place within the contact ion pair for styrene radical cations,[1,7] and likely also does so for enol ether radical cations: Horner, J. H.; Newcomb, M. *J. Am. Chem. Soc.* **2001**, *123*, 4364−4365. It is therefore highly likely, at least in nonpolar solvents such as benzene, that the products arise from deprotonation in the contact ion pair followed by subsequent reduction. A reviewer has pointed out, correctly, that this deprotonation/reduction mechanism cannot be operative in the experiments conducted in CD_3OD, as there is no incorporation from deuterium in the product from the exchanged thiol.

(37) Davies, A. G.; Roberts, B. P. *J. Chem. Soc., B* **1971**, 1830−1837.

(38) McPhee, D. J.; Campredon, M.; Lesage, M.; Griller, D. *J. Am. Chem. Soc.* **1989**, *111*, 7563−7567.

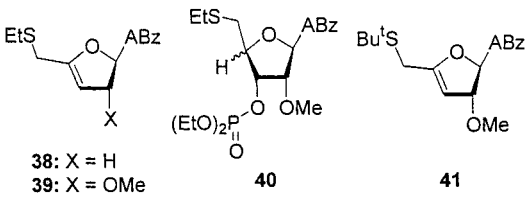
(39) Ito, O.; Matsuda, M. *J. Am. Chem. Soc.* **1979**, *101*, 1815−1819.

Scheme 6



and are not measured for vinyl ethers, it is evident that the reversible addition of thiyl radicals to alkenes favors the adduct radical to a much greater extent for alkylthiyl radicals than for arylthiyl radicals. This leads directly to the conclusion that radical **23** (Scheme 5) has a longer lifetime than radical **9** (Scheme 4) and that the increase in lifetime of **23** is sufficient to permit fragmentation to **25** to compete with the back reaction. A revised general reaction scheme for the fragmentation should therefore be as presented in Scheme 6.

The fragmentation of **6** and **7** in benzene with 2-mercaptoethanol but not with thiophenol is not a function of the hydroxy group on 2-mercaptoethanol. The reaction of **6** with ethanethiol in benzene at 40 °C on initiation with DBPO resulted in extensive degradation, and it was not possible to isolate the anticipated product **38**. However, reaction of **7** with ethanethiol under the same conditions resulted in the formation of the fragmentation product **39** and the reduction product **40**, in 21 and 34% yields respectively, together with recovered **7**, demonstrating again that fragmentation of the C4′ radical is a function of thiol. Reaction of **6** with ethanethiol and of **7** with *tert*-butanethiol in CD_3OD resulted in the formation and isolation of the fragmentation products **38** and **41** in 79 and 24% yields, respectively.



The reaction of ethanethiol, DBPO, and **7** in benzene was repeated using the ^{17}O-labeled isotopomer **19** and C_6D_6 as solvent. The fragmentation product **39** was isolated in 19% yield, the reduced product ^{17}O-**40** in 38% yield, and the substrate **19** in 33% yield. Examination of recovered **19** and ^{17}O-**40** by ^{17}O NMR spectroscopy revealed that no scrambling of the label had taken place in either case; i.e., each substance exhibited a single peak in the ^{17}O NMR spectrum corresponding to the P═O labeled substance. It is therefore established beyond reasonable doubt that C4′ radicals in both the 2-deoxy and ribo series do fragment to give contact ion pairs, even in benzene solution. It is likewise established that if the formation of the contact ion pair is reversible, the reverse reaction is more rapid than reorganization of the contact ion pair. After equilibration with the solvent, collapse back to the initial radical is not observed, and it is therefore concluded that all solvent-separated ion pairs proceed in the forward direction toward products. This last result is in full agreement with earlier failed crossover experiments.[42,43]

A further conundrum concerns the discrepancy between the original work of the Giese group,[26] who observed fragmentation product **12** and addition products **17** on addition of PhS• radicals to exocyclic glycals **6** and **16** in toluene at 30 °C, and our own experiments with PhS• and **6** in C_6D_6, which resulted in very little reaction at 40 °C. The answer, we believe, is again to be found in the initial equilibrium between the thiyl radical and its adduct with the glycal. In effect, the position of this equilibrium is affected not only by the nature of the thiol but also by the concentration of thiyl radicals in the reaction mixture. This, in turn, is a function of the radical initiation method. In the work described here, as in our previous work, we have preferred chemical initiation with DBPO, as this allows us to constantly monitor experiments in the probe of the NMR spectrometer. Under these conditions, the flux of PhS• radicals is determined by the half-life for the decomposition of the initiator at the temperature employed ($t_{1/2}$(DBPO) at 40 °C ≈ 2 h).[44] Giese, on the other hand, employed photolytic initiation which, presumably, provided a higher flux of radicals and so shifted the equilibrium in the forward direction.

Conclusion

It has been demonstrated that the overall rate of fragmentation of nucleotide C4′ radicals, when generated by addition of thiyl radicals to exocyclic glycals, is a function of the position of the equilibrium between the thiyl radical and alkene and their adduct. This, in turn, means that the observed rate of fragmentation will be a function of the thiol used and of the initiating system. These complications suffice to explain the discrepancies between our earlier work[8] and that of the Giese group[25] on the differing rates of fragmentation between nucleotide C4′ radicals and their 2-deoxy analogues. While the results reported earlier were correct and were shown to be reproducible, the results from the Giese group, using a more straightforward method of radical generation, probably reflect more accurately the true extent of the phenomenon. ^{17}O-Labeling experiments unambiguously demonstrate that the fragmentation of nucleotide C4′ radicals is either not reversible or so rapid as to preclude reorganization of the contact ion pair. All solvent-separated ion pairs proceed to product formation.

Experimental Section

General. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions unless otherwise stated. ^{17}O NMR spectra were recorded in $CDCl_3$

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Table 1. Reaction of Exocyclic Glycals **6**, **7**, **18**, and **19** with Thiols

	substrate	thiol	solvent (v/v)	DBPO (mol %)	time (min)	fragmentation product (isolated yield)	recovered substrate (isolated yield)	^{31}P (ppm) of recovered substrate	^{17}O (ppm) of recovered substrate
1	**18**	PhSH	CD_3OD/D_2O (10/1)	40	15	**12** (33)	(35)	−3.40, −3.36	76.29
2	**18**	PhSH	CD_3CN	246	195		(28)	−3.37, −3.40	76.13
3	**18**	PhSH	CD_3CN/CD_3OD (10/1)	465	75		(71)	−3.37, −3.41	75.99
4	**18**	PhSH	CD_3OD	100	45	**12** (62)	(24)	−3.36, −3.40	76.42
5	**19**	PhSH	CD_3OD/D_2O (10/1)	40	45		(100)	−3.25, −3.21	75.12
6	**19**	PhSH	CD_3OD/D_2O (2/1)	158	105	**13** (26)	(18)	−3.26, −3.22	75.12
7	**7**	PhSH	CD_3OD/D_2O (2/1)	120	105	**13** (41)	nd		
8	**6**	EtSH	CD_3OD	100	30	**38** (79)	nd		
9	**19**	EtSH	C_6D_6	50	30	**39** (19)	(33)	−3.25, −3.22	75.78
10	**7**	EtSH	CD_3OD	150	45	**39** (26)	nd		
11	**7**	Bu^{t}SH	CD_3OD	150	45	**41** (24)	nd		

solution on a Bruker Avance spectrometer at 68 MHz. ^{17}O chemical shifts are given with respect to external H_2O. High-resolution FAB and ESI mass spectra were recorded by the University of Minnesota Mass Spec Laboratory and the UIC Research Resources Center, respectively. Deuterated solvents and *O*-labeled water (10% ^{17}O, 55% ^{18}O) were purchased from Cambridge Isotope Laboratories, Inc., and used directly. Other solvents was dried and distilled by standard procedures before use. All reactions were performed under argon. Thiophenol, ethanethiol, and 2-methyl-2-propanethiol were purchased from ACROS and used directly. 2-Mercaptoethanol was distilled before use. 2-Hydroxy-2-methylpropanethiol,[45] di-*tert*-butylperoxyoxalate,[46] and substrates[8] **6** and **7** were prepared according to literature procedures.

^{17}O-Labeled 6-*N*-Benzoyl-9-(2,5-dideoxy-3-*O*-diethylphosphoryl-*β*-D-*glycero*-pent-4-enofuranosyl)adenine (18). Diethyl chlorophosphite (0.34 mL, 2.36 mmol) was added dropwise to a solution of 6-*N*-benzoyl-9-(2,5-dideoxy-*β*-D-*glycero*-pent-4-enofuranosyl)adenine[8] (200 mg, 0.59 mmol) and pyridine (1.0 mL) in THF (10.0 mL) at 0 °C. After 30 min, a solution of iodine (1.27 g, 5.0 mmol) in a mixture of THF, pyridine, and O-labeled water (5.0:1.0:0.2 mL) was added dropwise until the red color of iodine persisted. The reaction mixture was then treated with 10% aqueous $Na_2S_2O_3$ (10.0 mL) and phosphate buffer solution (10.0 mL, pH = 7.0). The aqueous layer was extracted with EtOAc (2 × 50 mL), and the combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. Preparative TLC on silica gel (eluent: 20/1 $CHCl_3$/MeOH) yielded the regioselectively labeled nucleotide **18** (103 mg, 37%) as a white solid. ^{1}H NMR δ: 1.38−1.41 (6 H, m), 1.81−1.87 (1 H, m), 2.12−2.20 (1 H, m), 4.16−4.20 (4 H, m), 4.60 (1 H, d, J = 2.6 Hz), 4.09 (1 H, d, J = 2.4 Hz), 5.60−5.64 (1 H, m), 6.71 (1 H, t, J = 6.6 Hz), 7.50−7.55 (2 H, m), 7.60−7.63 (1 H, m), 8.02−8.05 (2 H, m), 8.14 (1 H, s), 8.80 (1 H, s), 9.16 (1 H, br s). ^{13}C NMR δ: 16.5 (2 C), 38.7, 64.7 (2 C), 75.6, 85.3, 88.8, 124.0, 128.3 (2 C), 129.3 (2 C), 133.3, 133.9, 141.4, 150.2, 152.0, 153.4, 158.9, 165.0. ^{31}P NMR δ: −3.44, −3.40. ^{17}O NMR δ: 76.42. ESI-MS (*m*/*z*, relative intensity): 473 (M, 71), 474 (M + 1, 32), 475 (M + 2, 100), 476 (M + 3, 24).

^{17}O-Labeled 6-*N*-Benzoyl-9-(5-deoxy-3-*O*-diethylphosphoryl-2-*O*-methyl-*β*-D-*glycero*-pent-4-enofuranosyl)adenine (19). In a manner similar to that described above for **18**, the ribonucleotide **19** was prepared as a colorless gum in 57% yield from 6-*N*-benzoyl-9-(5-deoxy-2-*O*-methyl-*β*-D-*glycero*-pent-4-enofuranosyl)adenine.[8] ^{1}H NMR δ: 1.35−1.40 (6 H, m), 3.49 (3 H, s), 4.14−4.28 (4 H, m), 4.66 (1 H, d, J = 2.7 Hz), 4.73 (1 H, d, J = 2.6 Hz), 4.95−5.00 (1 H, m), 5.47−5.52 (1 H, m), 6.33 (1 H, d, J = 7.6 Hz), 7.50−7.55 (2 H, m), 7.60−7.65 (1 H, m), 8.00−8.05 (2 H, m), 8.13 (1 H, s), 8.81 (1 H, s), 9.20 (1 H, br s). ^{13}C NMR δ: 16.5 (2 C), 59.1, 64.6, 64.8, 73.4, 80.8, 87.3, 91.0, 124.2, 128.3 (2 C), 129.3 (2 C), 133.3, 133.9, 142.2, 150.2, 152.4, 153.5, 156.9, 165.0. ^{31}P NMR δ: −3.29, −3.25. ^{17}O NMR δ: 76.10. ESI-MS (*m*/*z*, relative intensity): 503 (M, 65), 504 (M + 1, 35), 505 (M + 2, 100), 506 (M + 3, 24).

General Protocol for the Reaction of Monofunctional Thiols with Exocyclic Glycals 6 and 7 and Their ^{17}O-Labeled Analogues 18 and 19 (Table 1). A solution of glycal (0.06 mmol) and thiol (30 equiv) in the appropriate solvent (0.6 mL) was degassed by sparging with Ar at 0 °C for 5 min and then warmed to 40 °C. DBPO (Table 1) in the reaction solvent (0.25 mL) was added, and the reaction was monitored by TLC or NMR as appropriate. In the cases when little or no reaction took place, further DBPO was added periodically over the course of the reaction up to the total amount given in Table 1. The products were isolated by preparative TLC (eluent: 20/1 $CHCl_3$/MeOH) following direct deposition of the reaction mixture onto the plate. The results are presented in Table 1, and the spectral data of the products are recorded below.

6-*N*-Benzoyl-9-(2,3,5-trideoxy-5-phenylthio-*β*-D-*glycero*-pent-3-enofuranosyl)adenine (12).[8] ^{1}H NMR δ: 2.93 (1 H, d, J = 17.1 Hz), 3.32−3.45 (1 H, m), 3.63 (1 H, d, J = 15.9 Hz), 3.72 (1 H, d, J = 15.0 Hz), 5.02−5.06 (1 H, m), 6.90 (1 H, dd, J = 3.2, 9.2 Hz), 7.20−7.68 (8 H, m), 8.00−8.05 (2 H, m), 8.11 (1 H, s), 8.80 (1 H, s), 9.04 (1 H, br s).

6-*N*-Benzoyl-9-(3,5-dideoxy-2-*O*-methyl-5-phenylthio-*β*-D-*glycero*-pent-3-enofuranosyl)adenine (13).[8] ^{1}H NMR δ: 3.44 (3 H, s), 3.69 (1 H, d, J = 15.2 Hz), 3.81 (1 H, d, J = 15.2 Hz), 4.76 (1 H, s), 5.28 (1 H, d, J = 1.8 Hz), 6.63 (1 H, s), 7.20−7.65 (8 H, m), 8.00−8.05 (2 H, m), 8.06 (1 H, s), 8.83 (1 H, s), 9.17 (1 H, br s). IR (film) ν: 1695, 1655 cm^{-1}.

6-*N*-Benzoyl-9-(2,3,5-trideoxy-5-ethylthio-*β*-D-*glycero*-pent-3-enofuranosyl)adenine (38). ^{1}H NMR (CD_3OD) δ: 1.21 (3 H, t, J = 7.4 Hz), 2.57 (2 H, q, J = 7.4 Hz), 3.15 (1 H, m), 3.30 (2 H, m), 3.45 (1 H, m), 5.16 (1 H, m), 6.94 (1 H, dd, J = 3.9, 9.0 Hz), 7.55 (2 H, m), 7.65 (1 H, m), 8.07 (2 H, m), 8.55 (1 H, s), 8.73 (1 H, s). ^{13}C NMR (CD_3OD) δ: 16.7, 25.0, 26.7, 36.1, 84.7, 96.3, 128.0, 128.3, 132.5, 141.7, 152.0, 154.0. HRFAB-MS: calcd for $C_{19}H_{20}N_5O_2S$, 382.1338; found, 382.1357 $(M + H)^+$.

6-*N*-Benzoyl-9-(3,5-dideoxy-5-ethylthio-2-*O*-methyl-*β*-D-*glycero*-pent-3-enofuranosyl)adenine (39). ^{1}H NMR δ: 1.30 (3 H, t, J = 7.4 Hz), 2.62−2.67 (2 H, m), 3.35 (1 H, d, J = 15.0 Hz), 3.42 (1 H, d, J = 15.0 Hz), 3.54 (3 H, s), 4.81 (1 H, s), 5.34 (1 H, d, J = 2.4 Hz), 6.65 (1 H, d, J = 1.4 Hz), 7.55−7.60 (2 H, m), 7.62−7.67 (1 H, m), 8.05−8.08 (2 H, m), 8.28 (1 H, s), 8.85 (1 H, s), 9.10 (1 H, br s). ^{13}C NMR δ: 14.4, 26.6, 28.1, 52.4, 88.3, 89.4, 98.3, 123.0, 128.1 (2 C), 129.1 (2 C), 133.1, 133.6, 140.3, 149.6, 152.6, 158.8, 160.8, 183.6.

6-*N*-Benzoyl 5′-Deoxy-5′-ethylthio-3′-*O*-diethylphosphoryl-2′-*O*-methyl-D-adenosine (40) and Its 4′-Epimer. 40. ^{1}H NMR δ: 1.25 (3 H, t, J = 7.4 Hz), 1.35−1.45 (6 H, m), 2.61 (2 H, q, J = 7.4 Hz), 2.96 (1 H, dd, J = 5.4, 14.2 Hz), 3.08 (1 H, dd, J = 5.6, 14.3 Hz), 3.51 (3 H, s), 4.15−4.25 (4 H, m), 4.50−4.55 (1 H, m), 4.80−4.85 (1 H, m), 5.05−5.10 (1 H, m), 6.11 (1 H, d, J = 5.6 Hz), 7.50−7.55 (2 H, m), 7.60−7.65 (1 H, m), 8.03−8.08 (2 H, m), 8.31 (1 H, s), 8.81 (1 H, s) 9.20 (1 H, br s). ^{13}C NMR δ: 14.7, 16.1, 27.2, 33.5, 58.8, 64.3, 75.5, 80.9, 83.2, 87.2, 120.8, 128.0, 128.9, 133.0, 133.4, 142.3, 149.5, 151.7, 152.2, 164.6. ^{31}P NMR δ: −3.03, −2.99. ^{17}O NMR δ: −77.35. HRESI-MS: calcd for $C_{24}H_{32}N_5O_7PSNa$, 588.1658; found, 588.1616 $(M + Na)^+$. **4′-Epi-40.** ^{1}H NMR δ: 1.27 (3 H, t, J = 7.4 Hz), 1.35−1.40 (6 H, m), 2.60 (2 H, q, J = 7.5 Hz), 2.85 (1 H, dd, J = 6.8, 13.8 Hz), 2.94 (1 H, dd, J = 7.0, 13.8 Hz), 3.47 (3 H, s), 4.20−4.30 (4 H, m), 4.95−5.00 (1 H, m), 5.10−5.15 (1 H, m), 5.30−5.35 (1 H, m), 5.96(1 H, d, J = 7.1 Hz), 7.50−7.55 (2 H, m), 7.60−7.65 (1 H, m), 8.02−

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Table 2. Intramolecular Capture of Radical Cation

substrate	solvent	DBPO (mol %)	thiol	fragmentation (NMR/isolated yield)	thiol adduct (NMR/isolated)	spirocycle (NMR/isolated)	recovered substrate (NMR/isolated)
7	CD_3OD	150	2-mercaptoethanol	**27** (95/76)			
7	CD_3CN	150	2-mercaptoethanol	**27** (12/7.3)	**37** (15/3.5)		(53/12)
7	C_6D_6	150	2-mercaptoethanol	**27** (24/2.4)	**37** (44/1.8)		nd/nd
6	C_6D_6	100	2-hydroxy-2-methylpropanethiol			**36** (nd[a]/5.2)	nd/nd
6	C_6H_6	100	2-mercaptoethanol			**32** (nd[a]/3.3)	nd/nd

[a] The yields of **32** and **36** in the reaction could not be accurately determined because of the complexity of the mixtures; nevertheless, both **32** and **36** were the major products before attempted isolation.

8.07 (2 H, m), 8.10 (1 H, s), 8.80 (1 H, s), 9.25 (1 H, br s). ^{13}C NMR δ: 15.1, 16.6, 27.0, 30.7, 59.6, 64.7, 76.2, 82.1, 83.3, 88.7, 124.3, 128.4, 129.4, 133.5, 133.7, 143.6, 150.0, 152.0, 152.4, 164.9. ^{31}P NMR δ: −2.77, −2.73. ^{17}O NMR δ: −77.35. HRESI-MS: calcd for $C_{24}H_{32}N_5O_7PSNa$, 588.1658; found, 588.1668 $(M + Na)^+$.

6-*N*-Benzoyl-9-(5-*tert*-butylthio-3,5-dideoxy-2-*O*-methyl-*β*-D-*glycero*-pent-3-enofuranosyl)adenine (41). ^{1}H NMR δ: 1.38 (9 H, s), 3.38 (1 H, d, J = 14.6 Hz), 3.47 (1 H, d, J = 14.6 Hz), 3.52 (3 H, s), 4.77 (1 H, s), 5.38 (1 H, d, J = 2.4 Hz), 6.66 (1 H, d, J = 1.3 Hz), 7.54−7.58 (2 H, m), 7.60−7.65 (1 H, m), 8.05−8.10 (2 H, m), 8.29 (1 H, s), 8.86 (1 H, s), 9.15 (1 H, br s). HRESI-MS: calcd for $C_{22}H_{25}N_5O_3SNa$, 462.1576; found, 462.1580 $(M + Na)^+$.

General Protocol for the Reaction of 2-Hydroxyalkanethiols with Exocyclic Glycals 6 and 7 (Table 2). A solution of **6** or **7** (ca. 0.01 mmol) and hydroxythiol (30 equiv) in CD_3OD or C_6D_6 (ca. 0.6 mL) was degassed by sparging Ar at 0 °C for 5 min and then allowed to warm to 40 °C under argon. DBPO (100 and 150 mol % for **6** and **7**, respectively) was then added, and the reaction mixture was stirred for 45 min at 40 °C. The resulting mixture was analyzed directly by ^{1}H NMR spectroscopy with 1,3,5-tri-*tert*-butylbenzene as internal standard.

6-*N*-Benzoyl-9-[3,5-dideoxy-2-*O*-methyl-5-(2-hydroxyethylthio)-*β*-D-*glycero*-pent-3-enofuranosyl]adenine (27). The product was isolated by preparative TLC on silica gel (eluent: 20/1 $CHCl_3$/MeOH). ^{1}H NMR δ: 2.81 (2 H, t, J = 5.8 Hz), 3.35 (1 H, d, J = 15.0 Hz), 3.42 (1 H, d, J = 15.0 Hz), 3.50 (3 H, s), 3.79 (2 H, m), 4.82 (1 H, s), 5.36 (1 H, d, J = 1.9 Hz), 6.61 (1 H, d, J = 0.9 Hz), 7.50−7.55 (2 H, m), 7.60−7.63 (1 H, m), 8.02−8.07 (2 H, m), 8.34 (1 H, s), 8.83 (1 H, s), 9.08 (1 H, br s). ^{13}C NMR δ: 28.2, 35.4, 56.6, 60.7, 88.8, 89.5, 98.7, 122.6, 128.0 (2 C), 128.9 (2 C), 133.0, 133.3, 140.3, 149.3, 151.1, 152.7, 160.3, 164.7. IR (film) ν: 2360, 2339, 1698, 1669 cm^{-1}. HRESI-MS: calcd for $C_{20}H_{21}N_5O_4S$, 427.1314; found, 427.1311 (M^+).

2-(*N*-Benzoyl-9-adeninyl)-1,6-dioxa-9-thia-spiro[4,5]decane (32). **6** (31.7 mg, 0.067 mmol), 2-mercaptoethanol (141 μL, 2.01 mmol), and DBPO (15.7 mg, 0.067 mmol) were dissolved in benzene (13.4 mL) at 40 °C. After 45 min, the resulting reaction mixture was separated by preparative TLC on silica gel (eluent: 100/1/1 EtOAc/MeOH/Et_3N), resulting in the isolation of **32** in 3.3% yield as a mixture of isomers. **Isomer 1.** ^{1}H NMR (CD_3OD) δ: 2.20−2.25 (1 H, m), 2.32−2.40 (1 H, m), 2.50−2.60 (2 H, m), 2.74−2.78 (1 H, m), 2.80−2.90 (2 H, m), 3.16 (1 H, d, J = 13.7 Hz), 3.90−3.95 (1 H, m), 4.25−4.28 (1 H, m), 6.50−6.55 (1 H, m), 7.55−7.60 (2 H, m), 7.63−7.67 (1 H, m), 8.05−8.10 (2 H, m), 8.53 (1 H, s), 8.73 (1 H, s). **Isomer 2.** ^{1}H NMR (CD_3OD) δ: 2.20−2.25 (1 H, m), 2.32−2.40 (1 H, m), 2.40−2.48 (2 H, m), 2.74−2.78 (1 H, m), 2.80−2.90 (2 H, m), 3.13 (1 H, d, J = 13.6 Hz), 3.90−3.95 (1 H, m), 4.25−4.28 (1 H, m), 6.35−6.45 (1 H, m), 7.43−7.45 (2 H, m), 7.55−7.60 (1 H, m), 7.95−8.00 (2 H, m), 8.20 (1 H, s), 8.25 (1 H, s). **Unassigned Isomers 1 and 2.** ^{13}C NMR (CD_3OD) δ: 25.6, 30.4, 30.5, 32.3, 35.9, 36.0, 63.1, 85.1, 85.6, 104.2, 105.1, 128.4, 128.6, 128.8, 129.5, 132.9, 140.1, 152.2, 152.9. HRFAB-MS: calcd for $C_{19}H_{20}N_5O_3S$, 398.1287; found, 398.1280 $(M + H)^+$.

2-(*N*-Benzoyl-9-adeninyl)-7,7-dimethyl-1,6-dioxa-9-thia-spiro[4,5]-decane (36). The compound **36** was isolated in 5.2% yield by preparative TLC on silica gel (eluent: 100/1/1 EtOAc/MeOH/Et_3N). ^{1}H NMR (CD_3OD) δ: 1.59 (6 H, s), 2.05−2.15 (1 H, m), 2.42−2.48 (3 H, m), 2.77 (1 H, d, J = 13.7 Hz), 2.70−2.90 (2 H, m), 3.03 (1 H, d, J = 13.7 Hz), 6.52−6.55 (1 H, m), 7.55−7.60 (2 H, m), 7.62−7.66 (1 H, m), 8.05−8.10 (2 H, m), 8.52 (1 H, s), 8.73 (1 H, s). HRFAB-MS: calcd for $C_{21}H_{23}N_5O_3SNa$, 448.1419; found, 448.1417 $(M + Na^+)$.

6-*N*-Benzoyl-9-[5-deoxy-5-(2-hydroxyethylthio)-2-*O*-methyl-3-*O*-diethylphosphoryl-α-L-*lyxo*-furanosyl]adenine (37). The product was isolated by preparative TLC on silica gel (eluent: 20/1 $CHCl_3$/MeOH). ^{1}H NMR δ: 1.40−1.45 (6 H, m), 2.90−2.93 (2 H, m), 3.03 (1 H, dd, J = 5.4, 14.4 Hz), 3.19 (1 H, dd, J = 6.7, 14.4 Hz), 3.53 (3 H, s), 3.80−3.95 (2 H, m), 4.20−4.30 (4 H, m), 4.58−4.63 (1 H, m), 4.87−4.92 (1 H, m), 5.18−5.23 (1 H, m), 6.14 (1 H, d, J = 5.6 Hz), 7.55−7.60 (2 H, m), 7.64−7.68 (1 H, m), 8.05−8.10 (2 H, m), 8.40 (1 H, s), 8.85 (1 H, s). ^{31}P NMR δ: −2.95. HRESI-MS: calcd for $C_{24}H_{32}N_5O_8PSNa$, 604.1607; found, 604.1643 $(M + Na^+)$.

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