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Free Radical Chemistry of Nucleosides and Nucleotides. Ring Opening of C4'-Radicals

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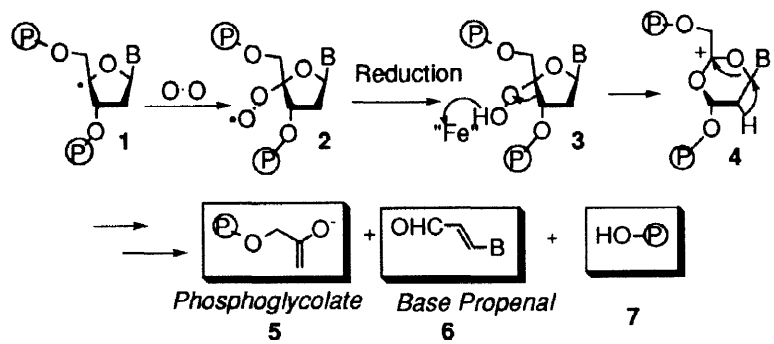
Abstract. It is demonstrated that nucleotide C4' radicals may be generated from a C4'-thiolester on treatment with tributyltin hydride. When the reaction is conducted in benzene at reflux the C4' radical expels the C3'-phosphate group to give a radical cation. This species undergoes deprotonation to an allylic radical which suffers cleavage of the deoxyribose ring. Similar reactions are observed when the reaction is conducted with tris(trimethylsilyl)silane in place of the stannane. In methanolic benzene the radical cation is trapped by methanol to give a new C4' radical which is quenched before ring opening. The behavior of C4' radicals toward ring opening is discussed in terms of the conformations imposed by the substituents at C3'.

The chemistry of nucleotide C4' radicals is currently an area of intense interest owing to the central role played by these species in the cleavage of oligonucleotides by various antitumor antibiotics, notably the bleomycins, and the continually expanding class of enediynes and related substances.[1-4] Following early work by Giloni,[5] Stubbe and coworkers have delineated most of the steps in the Fe.BLM mediated cleavage of DNA with the aid of an elegant series of labelling and kinetic experiments.[1,2] The essential steps in this mechanism (Scheme 1) are the quenching of the C4' radical (**1**) by molecular oxygen to give a C4' peroxy radical (**2**) which, following reduction to a hydroperoxide (**3**), undergoes a Criegee type rearrangement with scission of the C3'-C4' bond. Subsequent fragmentations of (**4**) provide the three products: phosphoglycolate (**5**), base propenal (**6**), and 5'-phosphate (**7**).



Scheme 1

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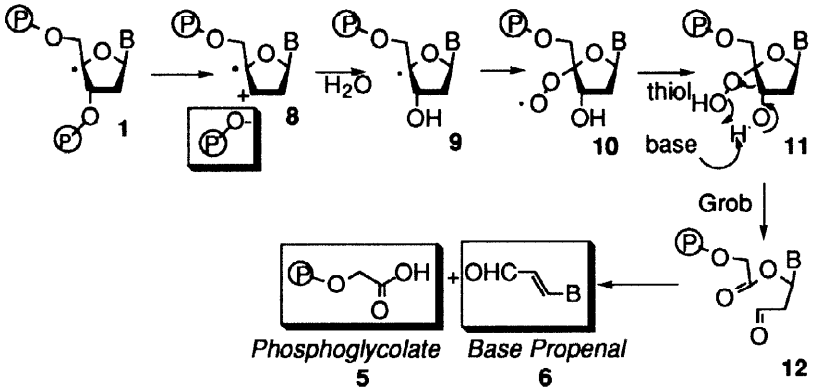
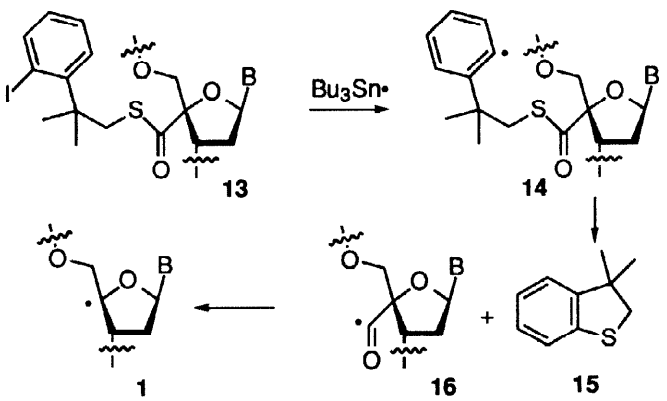
Following extensive studies with a series of unambiguously generated C4' radicals, and building upon the foundations laid by the Schulte-Frohlinde and von Sonntag groups,[6-9] Giese and coworkers have advanced an alternative mechanism (Scheme 2).[10,11] The key points in this mechanism are: i) cleavage of the C3'-O3' bond in **1** with expulsion of the phosphate to give a C3'C4' radical cation **8** before trapping of the initial radical by oxygen; ii) nucleophilic attack by water giving the new C4' radical **9**; iii) quenching by oxygen to give the peroxy radical **10**; iv) reduction to the hydroperoxide **11**; v) Grob-type fragmentation with cleavage of the C3'C4' bond giving **12**.



Scheme 2

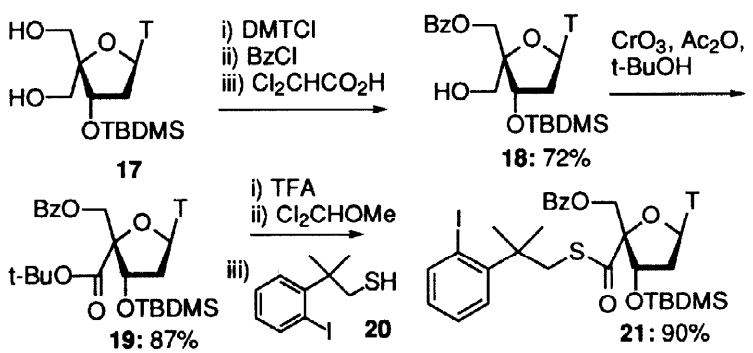
The two mechanisms differ in almost all aspects, but the most important divergences are the timing of incorporation of oxygen at C4', and the mode of cleavage of the carbon-carbon bond. The Giloni-Stubbe mechanism invokes a Criegee rearrangement, a process known to be catalyzed by strong acid, whereas the Giese mechanism revolves around the base catalyzed Grob fragmentation. In the case of BLM-mediated DNA cleavage, the requirement for strong acid catalysis of the Criegee mechanism is thought to be met by the iron, of undetermined oxidation state, in the spent BLM. No such Lewis acid is available when the C4' radical is generated with an enediyne, which necessarily suggests that the Giloni-Stubbe BLM mechanism is not general. The picture is further confused by conflicting results from the Giese laboratory in which the chemistry of the C4' radical is found to vary according to the source of the C4' radical.[11] Thus, when C4'-*tert*-butyl ketones are employed as precursors, in a Norrish type I process, the C4' radical is trapped by oxygen, à la Giloni-Stubbe, before expulsion of the C3'-phosphate. When generation is achieved by photolysis of C4'-selenides, fragmentation to the radical cation (Scheme 2) precedes trapping by oxygen. Somewhat implausibly, it is suggested that the bulky PhSe· radical, in the initial radical pair arising from photolysis of the selenide shields the C4' radical from attack by molecular oxygen and provides the time for fragmentation to occur. In the Norrish I process the molecule of CO is considered to separate the nucleotide radical from the bulky *tert*-butyl radical thus enabling oxygen to diffuse in.[11] Doubtless, other explanations are possible for this divergent behavior, not the least of which is the use of different bases in the two systems. Furthermore, and convenient though the two Giese precursors may be, they obligatorily give rise to equimolar quantities of *tert*-butyl• or PhSe• radicals which necessarily complicate the system and, especially, the analysis of any kinetic measurements involving the use of precise concentrations of a radical trap.

In furtherance of our own studies on the chemistry of β-(phosphatoxy)alkyl radicals,[12,13] of which nucleotide C4' radicals are but a particular example, we were driven to design a different, unambiguous source of C4' radicals which did not function through formation of a radical pair and so is not susceptible to many of the problems inherent in the Giese system. After some experimentation, we settled on an intramolecular homolytic process at sulfur in a thiol ester[14] as illustrated in Scheme 3. In this chemistry the initiating aryl radical (**14**) is generated from the aryl iodide **13** with a stannyl or silyl radical. In the cyclization reaction, a dihydrobenzthiophen (**15**) is formed with displacement of the acyl radical **16** which subsequently undergoes rapid decarbonylation to give the desired C4' radical (**1**). A potential, additional advantage of this scheme is the generation of the heterocycle **15**, in the same yield as the acyl radical **16**, which, at least in principle, can serve as a marker for the efficiency of the acyl radical generation process. Here we describe our initial experiments conducted with a view to determining the suitability of such thiol esters (**13**) for the generation of nucleotide C4' radicals, their compatibility with standard phosphoramidite coupling reactions, and some very interesting substituent effects on the ring opening of nucleoside C4'-radicals.



Scheme 3

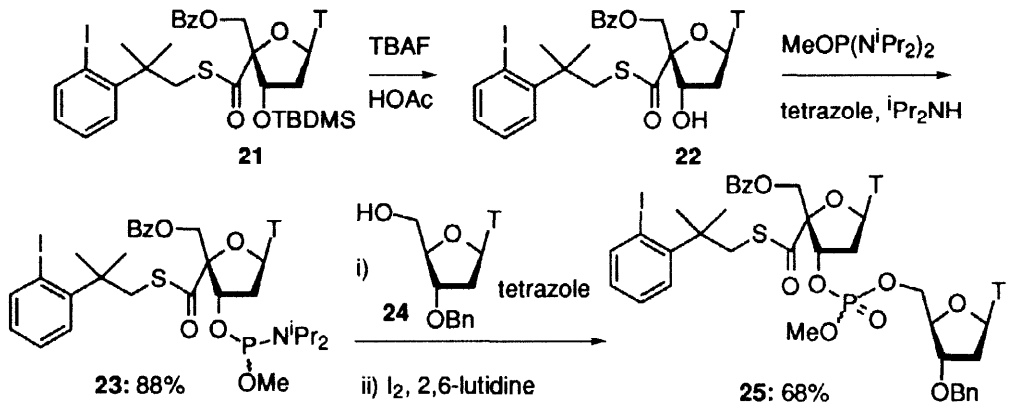
As described in Scheme 4, a suitably orthogonally protected 4'-thioacylnucleoside **21** was prepared uneventfully from the known[15,16] substrate **17**.



Scheme 4

Selective monoetherification with dimethoxytrityl chloride (DMTCl) took place on the more reactive (α) of the two hydroxymethyl groups:[17] this was followed by benzoylation and then removal of the DMT group

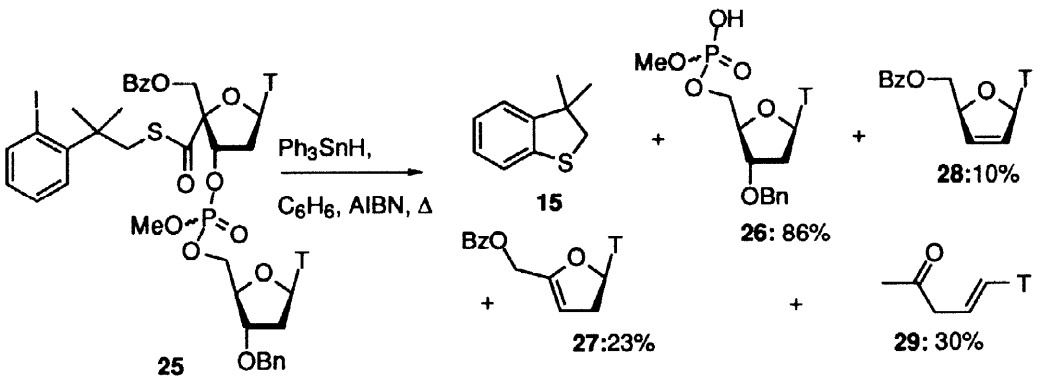
to give **18**. Oxidation with CrO_3 in the presence of *tert*-butanol and acetic anhydride[18] provided the 4'α-*tert*-butyloxycarbonyl nucleoside **19**. On sequential treatment with trifluoroacetic acid, 1,1-dichloromethyl methyl ether, and thiol **20**, **19** provided the target nucleoside **21**. Exposure of **21** to TBAF buffered with acetic acid furnished **22**, which was converted to phosphoramidite **23** by treatment with bis(diisopropylamino)-methoxyphosphine[19] in the presence of diisopropylammonium tetrazolide in 88% yield. Coupling of **23** to 3'-*O*-benzylthymidine (**24**),[20] with freshly sublimed tetrazole in acetonitrile, gave a phosphite which was oxidized *in situ* with I_2 in methanol to give the phosphate **25** in good overall yield (Scheme 5). Thus, the thiol ester function is fully compatible with the standard coupling protocols used in automated oligonucleotide synthesis. Indeed, far higher yields for the coupling sequence can be anticipated for automated solid phase synthesis than those obtained here in the solution phase with only moderate excesses of reagents.



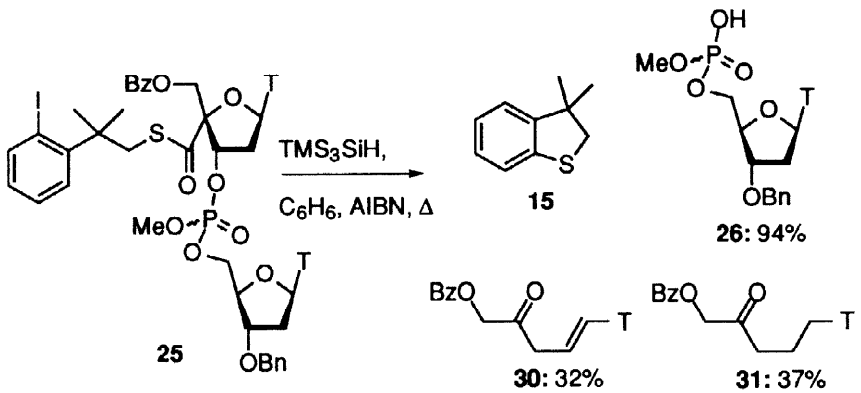
Scheme 5

To determine the suitability of the thiol ester for C4' radical generation, nucleotide **25** was dissolved in benzene at reflux and treated with triphenyltin hydride (0.045M) and a catalytic quantity of AIBN. After 2 h at reflux, ^{31}P-NMR spectroscopy revealed complete consumption of the substrate in favor of a single new phosphate ester. Column chromatography over silica gel enabled isolation of the 5'-phosphate **26** in 86% yield, along with two 3'-deoxy-unsaturated nucleosides (**27**) and (**28**) in 23 and 10% yields, respectively, and an unanticipated acyclic nucleoside **29** in 30% yield (Scheme 6). Heterocycle **15** was isolated admixed with triphenyltin residues and was consequently not quantified. When the experiment was repeated with the poorer hydrogen atom donor tris(trimethylsilyl)silane (TTMS)[21] in place of the stannane a significantly different spectrum of products was isolated. These comprised the phosphate **26** (94%), and the two acyclic nucleosides (**30**) and (**31**) in 32 and 37% yields, respectively (Scheme 7). The heterocycle **15** was again formed as anticipated, but was not quantified.

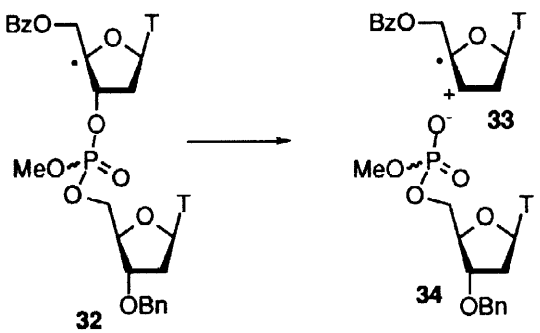
The results outlined in Schemes 6 and 7 are satisfactorily rationalized in terms of efficient ionic fragmentation of the C4'-radical (**32**) to give the radical cation **33** and the phosphate anion **34** (Scheme 8) which then evolve along different pathways according to the nature of the reductant.



Scheme 6



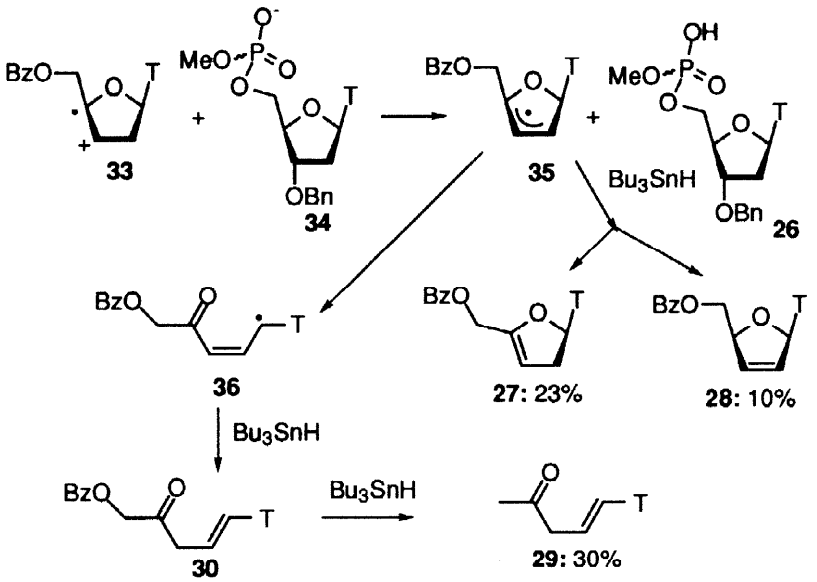
Scheme 7



Scheme 8

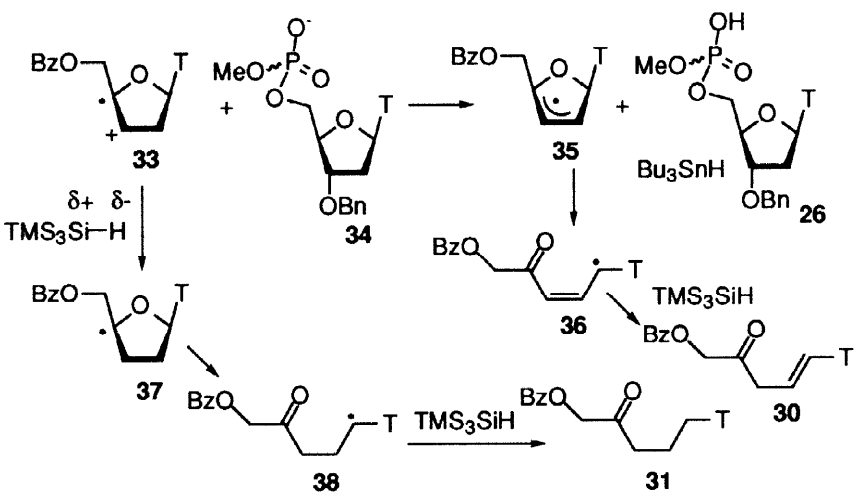
In the presence of triphenyltin hydride, proton transfer occurs to give the isolated phosphate **26** and the allylic radical **35**. Proton transfer within a closely analogous radical cation/phosphate anion pair has also been noted recently by Giese and coworkers.[22] The allyl radical is then quenched at either terminus by the stannane to give **27** and **28**, in competition with a fragmentation reaction leading to the acyclic radical **36**. This is quenched by the stannane to give **30**, which undergoes deoxygenation to provide the observed product **29** (Scheme 9). Interestingly, quenching of the allyl radical **35** at its C4'-terminus takes place with a high degree of stereoselectivity on the face opposite to the base giving predominately the indicated, known stereoisomer **28**. Retro-5-endo-trigonal fragmentations, such as **35** → **36**, are expected to be slow but are by no means

unknown.[23] Likewise, the proposed deoxygenation of the benzoate is not a common reaction but, nevertheless, one for which there is ample precedent,[24] especially when a resonance stabilized radical results.[25]



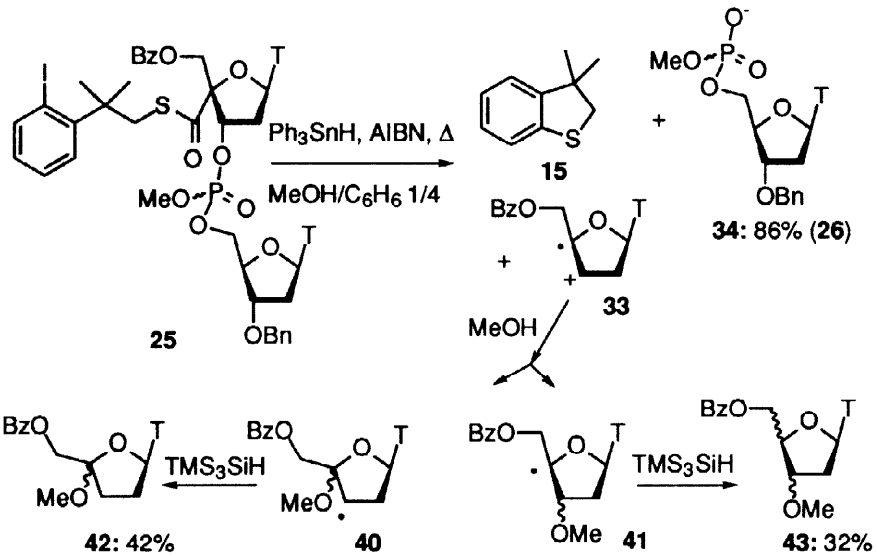
Scheme 9

With TTMS as reductant it appears that proton transfer from the radical cation **33** to the phosphate anion **34** is, in part, overcome by TTMS which acts as a hydride donor to the radical cation at the C3'-position giving the tetrahydrofuranyl radical **37**. As in the case of triphenyltin hydride, competing proton transfer gives the dihydrofuranyl radical **35**. Cleavage of **35** and its saturated analog **37** takes place to give, after chain transfer, the observed products **30** and **31** (Scheme 10). Trialkyl and triarylsilanes are, of course, well known as hydride donors toward carbenium ions[26] and, thus, the reduction of the radical cation by TTMS is none too surprising. In full agreement with the poorer hydrogen atom donating capabilities of TTMS, as compared to triphenyltin hydride,[21] ring opening is more efficient in the presence of the latter reagent. Interestingly, the final deoxygenation observed with triphenyltin hydride (**30** → **29**) is not seen with the silane, despite other silanes having been reported as efficient reagents for the deoxygenation of simple carboxylate esters.[27]



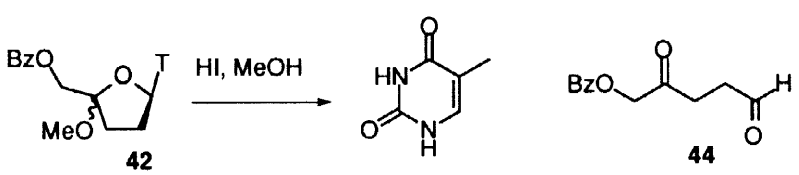
Scheme 10

Subsequently, **25** was treated with TTMS in benzene/methanol (4/1) at reflux. In this experiment phosphate **26** was again formed in near quantitative yield indicating that the C4' radical is generated and undergoes clean ionic fragmentation to the radical cation **33**. This is now quenched by methanol at either the C3' or C4'-positions to give tetrahydrofuranyl radicals **40** and **41** which are quenched, before ring opening, by the stannane to give the observed products **42** and **43**, which were isolated in 42 and 32% yields, respectively (Scheme 11). Product **42** was isolated as a 4:3 mixture of the two possible stereoisomers and **43** as a 3:2 mixture of two of the possible three diastereomers. No attempt was made to assign stereochemistry to any of the isomers of **42** or **43**, in view of the complex spectra and low selectivities observed.



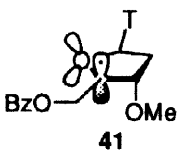
Scheme 11

The reduction of **25** with TTMS in benzene/methanol (4/1) was repeated under photochemical conditions at room temperature. The results closely paralleled those obtained at reflux with the exception that **42** was not observed in the crude reaction mixture on examination by ^{1}H-NMR spectroscopy. However, the presence of thymine and the aldehyde **44** was indicated. We rationalize this different outcome in terms of the hydrolysis of the byproduct TTMS-I to HI by methanol (Scheme 12). Under refluxing conditions HI is continually expelled from the reaction mixture, whereas in the photochemical experiment, at room temperature, it accumulates in the solution and promotes the degradation of **42** to aldehyde **44** and thymine. We attempted to overcome this problem by working in the presence trimethyl orthoacetate which, it was felt, would effectively capture HI. Under these conditions, neither aldehyde **44**, nor thymine, were formed, but neither was **42**. On the other hand the yield of the regiosiomer **43** increased to around 90% as determined by ^{1}H-NMR spectroscopy of the crude reaction mixture. It appears that trimethyl orthoacetate **23** itself transfers MeO to the radical cation and, being more bulky than methanol, does so with greater regioselectivity at the least substituted site.

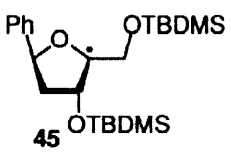


Scheme 12

It is of some interest that the methoxytetrahydrofuranyl radical **41**, which can be thought of as a model for any C3'-oxygen substituted C4' radical such as the C3'-hydroxy radical (**9**) in the Giese fragmentation mechanism (Scheme 2), does not undergo ring opening under conditions where its deoxy and didehydrodeoxy analogs (**35**) and (**37**), respectively, did so cleanly (Schemes 9 and 10). We rationalize this change in behavior in terms of the extended anomeric effect[28-31] which causes the radical **41** to adopt a conformation in which the C3'-O3' bond, the singly occupied orbital and the lone pairs on the ring oxygen are effectively coplanar. Such a conformation sets the singly occupied orbital and the O4'-C1' bond close to orthogonal and so precludes the possibility of ring opening. Indeed, recent ESR work by Giese indicates that a closely related radical (**45**) adopts such a conformation.[22]



41



45

Finally, we note that quantification, by isolation, of the dihydrobenzthiophene **15**, as a marker for the efficiency of C4' acyl radical generation is not practical. This is due to the relatively volatile nature of **15** which ensures that some is always lost on removal of the solvent under vacuum. However, this does not preclude the eventual use of **15** as a marker by HPLC or GLC analysis of the reaction mixture.

In conclusion, we have demonstrated that C4' thioacyl derivatives are efficient sources of nucleotide C4' radicals under both thermal and photochemical conditions. Moreover, the thioacyl group is fully compatible with typical conditions for oligonucleotide synthesis. C4' radicals of a simple dinucleotides undergo very efficient fragmentation of the C3'-O3' bond giving a radical cation, even in benzene solution. This radical cation evolves along several different pathways dependent upon the milieu with deprotonation to an allyl radical being the preferred mode in the absence of nucleophiles.

General. Melting points were recorded on a Thomas hotstage microscope and are uncorrected. Unless otherwise noted, ^{1}H, ^{13}C, and ^{31}P NMR spectra were run in $CDCl_3$ at 300, 75, and 121 MHz, respectively. ^{1}H and ^{13}C chemical shifts are downfield from tetramethylsilane as internal standard. ^{31}P chemical shifts are quoted with respect to external H_3PO_4. IR spectra were recorded with a Perkin Elmer 1605 FTIR spectrophotometer. Specific rotations were recorded with a Perkin Elmer 241 polarimeter. All solvents were dried and distilled by standard procedures. All reactions were run under a dry nitrogen or argon atmosphere. THF was distilled, under N_2, immediately prior to use from sodium benzophenone ketyl. Ether refers to diethyl ether. Microanalyses were conducted by Midwest Microanalytical, Indianapolis.

5'-*O*-Benzoyl-3'-*O*-*tert*-butyldimethylsilyl-4'-(hydroxymethyl)thymidine (18). To a solution of the diol **17**[15,16] (1.70 g. 4.40 mmol) in pyridine (50 mL) were added with stirring 4,4'-dimethoxytrityl chloride

(1.56 g, 4.60 mmol) and DMAP (54 mg, 0.44 mmol). After stirring for 12 h at room temperature, benzoyl chloride (615 μL, 5.3 mmol) was added to the reaction mixture and stirring continued for another 2 h. The reaction mixture was concentrated under reduced pressure and the residue partitioned between ethyl acetate (50 mL) and water (50 mL). The organic layer was separated and the aqueous layer extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were washed with sat. NH_4Cl, dried (Na_2SO_4) and concentrated. This crude product was then dissolved in dry dichloromethane (200 mL), and treated with a solution of dichloroacetic acid in dichloromethane (9% v/v, 100 mL). After stirring for 30 min, the reaction was quenched with sat. $NaHCO_3$ (200 mL). The aqueous layer was separated and extracted with dichloromethane (2 x 50 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. Column chromatography (SiO_2, eluant: dichloromethane then dichloromethane /EtOAc 1:1) gave the title compound (1.56 g, 72 %) as a white foam. ^1H NMR, δ: 0.115 (3H, s), 0.123 (3H, s), 0.91 (9H, s), 1.68 (3H, s), 2.33 (1H, dt, J = 13.7, 6.9 Hz), 2.47 (1H, ddd, J = 4.2, 6.4, 13.7 Hz), 3.82 (1H, d, J = 12.2 Hz), 3.92 (1H, d, J = 12.2 Hz), 4.53 (1H, d, J = 12.0 Hz), 4.58 (1H, d, J = 12.1 Hz), 4.64 (1H, dd, J = 4.1and 6.9 Hz), 6.29 (1H, t, J = 6.6 Hz), 7.23 (1H, s), 7.47 (2H, t, J = 7.7 Hz), 7.61 (1H, t, J = 7.4 Hz), 8.02 (2H, d, J = 7.8 Hz), 8.73 (1H, bs); ^{13}C NMR, δ: -5.3, -4.7, 12.2, 17.8, 25.6, 41.3, 63.1, 64.8, 73.4, 84.9, 87.2, 111.1, 128.6, 129.3, 129.4, 133.5, 135.1, 150.2, 163.8, 166.1. Anal. Calcd for $C_{24}H_{34}N_2O_7Si \cdot 1/2H_2O$: C, 57.70; H, 7.06; Found: C, 57.91; H, 7.15.

(*tert*-Butoxycarbonyl)-3'-*O*-(*tert*-butyldimethylsilyl)thymidine (19). To a stirred suspension of CrO_3 (6.0 g, 60 mmol) in a mixture of dichloromethane /DMF (4:1, v/v, 50 mL) was added pyridine (9.6 mL, 120 mmol). After stirring for 15 min, **18** (1.03 g, 2.1 mmol) in dichloromethane /DMF (4:1, v/v, 20 mL) was added by syringe followed by acetic anhydride (11.3 mL, 120 mmol) and *tert*-butanol (19.2 mL, 200 mmol). The reaction mixture was stirred at room temperature under an Ar atmosphere for 4 days before MeOH (10 mL) was added. After stirring for 15 min, the reaction mixture was diluted with ethyl acetate (150 mL), washed with water (150 mL), brine, and dried (Na_2SO_4). The organic extract was filtered through a short pad of silica gel eluting with 2 portions of EtOAc (50 mL). The filtrate was then concentrated and column chromatographed on silica gel (eluant: dichloromethane /EtOAc 5:1 then 3:1) to give **19** (1.03 g, 87.5 %) as a white foam. ^1H NMR, δ: 0.110 (3H, s), 0.125 (3H, s), 0.90 (9H, s), 1.50 (9H, s), 1.64 (1H, d, J = 1.1 Hz), 2.39 (1H, ddd, J = 4.0, 8.2 and 13.5 Hz), 2.60 (1H, dt, J = 14.3 and 7.5 Hz), 4.63 (1H, d, J = 12.2 Hz), 4.74 (1H, t, J = 7.6 Hz), 4.89 (1H, d, J = 12.2 Hz), 6.35 (1H, dd, J = 4.0 and 7.8 Hz), 7.14 (1H, bs), 7.45 (2H, t, J = 7.6 Hz), 7.59 (1H, t, J = 7.4 Hz), 8.00 - 8.03 (2H, m), 8.29 (1H, bs). ^{13}C NMR, δ: -5.0, -4.9, 12.1, 18.0, 25.6, 28.0, 39.8, 64.0, 72.5, 83.0, 87.0, 88.8, 110.8, 128.6, 128.7, 129.4, 129.5, 133.4, 136.3, 149.7, 163.6, 165.8, 168.1. Anal. Calcd for $C_{28}H_{40}N_2O_8Si$: C, 59.98; H, 7.19. Found: C, 59.62; H, 7.13.

5'-*O*-Benzoyl-3'-*O*-(*tert*-butyldimethylsilyl)-4'-[(2-methyl-2-(2-iodophenyl)propylthio)-carbonyl]thymidine (21). To a solution of **19** (561 mg, 1.0 mmol) in dichloromethane (8 mL) was added trifluoroacetic acid (3.2 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. The solvent and volatiles were co-evaporated with benzene (2 x 10 mL). The crude acid was then taken up with benzene (15 mL), treated with 1,1-dichloromethyl methyl ether (1.8 mL). The reaction mixture was heated to gentle reflux

for 1 h. After cooling to room temperature, the solvent and volatiles were removed under vacuum. The resulting acid chloride was taken up with dry dichloromethane (15 mL), and treated with thiol **20**[14] (306 mg, 1.05 mmol) followed by DMAP (153, 1.25 mmol). After stirring at room temperature for 12 h, the solvent was removed and the crude reaction mixture column chromatographed on silica gel (eluant: dichloromethane then dichloromethane/EtOAc 4:1) yielding **21** (700 mg, 90 %) as a solid. M.p. 104 - 106 °C. ^{1}H NMR, δ: 0.08 (3H, s), 0.11, 0.88 (9H, s), 1.58 (3H, s), 1.60 (3H, s), 1.72 (3H, d, *J* = 1.0 Hz), 2.14 (1H, ddd, *J* = 4.8, 9.2 and 13.8 Hz). 2.39 (1H, ddd, *J* = 1.3, 5.0 and 12.9 Hz), 3.55 (1H, d, *J* = 13.4 Hz), 3.90 (1H, d *J* = 13.4 Hz), 4.53 (1H, d, *J* = 3.9 Hz), 4.60 (1H, d, *J* = 11.7 Hz), 4.80 (1H, d, *J* = 11.7 Hz), 6.56 (1H, dd, *J* = 5.0 and 9.3 Hz), 6.84 (1H, dt, *J* = 1.6 and 7.4 Hz), 7.23 - 7.35 (3H, m), 7.45 (2H, t, *J* = 7.6 Hz), 7.61 (1H, t, *J* = 7.4 Hz), 7.97 -8.00 (3H, m), 8.06 (1H, bs). ^{13}C NMR, δ: -5.3, -5.1, 12.0, 17.9, 25.5, 27.9, 28.1, 38.6, 39.9, 41.2, 66.2, 74.8, 87.1, 94.6, 94.9, 111.4, 128.0, 128.3, 128.6, 128.7, 129.0, 129.6, 133.6, 135.0, 143.7, 146.4, 149.7, 163.2, 165.9, 199.2. Anal. Calcd for $C_{34}H_{43}IN_2O_7SSi$: C, 52.44; H, 5.57. Found: C, 52.39; H,5.68.

5'-*O*-Benzoyl-4'-[(2-methyl-2-(2-iodophenyl)propylthio)carbonyl] thymidine 22. To a solution of **21** (685 mg, 0.88 mmol) in THF (10 mmol) were added acetic acid (0.25 mL, 4.4 mmol) and TBAF (2.7 mL, 1.0 M in THF, 2.7 mmol). After stirring for 12 h, another portion of TBAF (0.9 mL, 1.0 M in THF, 0.9 mmol) was added and stirring continued for another 6 h. Removal of the solvent and column chromatography (SiO_2,eluant: dichloromethane /EtOAc 2:1 to 2:3) afforded **22** (543 mg, 93 %) as a white foam. ^{1}H NMR, δ: 0.08 (3H, s), 1.60 (3H, s), 1.61 (3H, s), 1.70 (1H, d, *J* = 1.1 Hz), 2.20 (1H, m), 2.49 (1H, dd, *J* = 6.9 and 13.5 Hz), 3.77 (1H, d, *J* = 13.5 Hz), 3.84 (1H, d, *J* = 13.6 Hz), 4.56 (1H, d, *J* = 11.8 Hz), 4.64 (1H, m), 4.75 91H, d, *J* = 11.8 Hz), 6.51 91H, dd, *J* = 5.2 and 9.0 Hz), 6.86 (1H, dt, *J* = 1.8 and 7.5 Hz), 7.26 -7.36 (3H, m), 7.46 (2H, t, *J* = 7.6 Hz), 7.61 (1H, t, *J* = 7.5 Hz), 7.98 - 8.01 (3H, s), 8.03 (1H, bs). ^{13}C NMR, δ: 12.0, 27.9, 28.0, 38.4, 39.4, 40.2, 66.2, 77.1, 86.5, 94.9, 111.6, 128.0, 128.4, 128.7, 128.8, 129.6, 133.7, 134.8, 143.9, 145.9, 149.6, 162.9, 165.8, 202.0. Anal. Calcd for $C_{28}H_{29}N_2O_7S$: C, 50.61; H, 4.40. Found: 50.30; H, 4.68.

5'-*O*-Benzoyl-3'-*O*-(*N*,*N*-diisopropylmethylphosphoramidyl)-4'-[(2-methyl-2-(2-iodophenyl)propylthio)carbonyl]thymidine (23): To a solution of the nucleoside **22** (432 mg, 0.65 mmol) in dry dichloromethane was added *N*,*N*-diisopropylethylamine (517 mg, 4.0 mmol) and *N*,*N*-diisopropylmethylphosphonamidic chloride (360 mg, 1.82 mmol) under Ar. After stirring for 12 h, MeOH (0.5 mL) was added. After stirring for another 15 min, the reaction mixture was poured into sat. $NaHCO_3$ (15 mL) and extracted with dichloromethane (2 x 5 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. Column chromatography (SiO_2,eluant: dichloromethane then dichloromethane /EtOAc 5:1, with 1 % triethylamine) afforded **23** (475 mg, 88.5 %) as a white foam. ^{31}P NMR, δ 150.89, 152.55. Owing to the instability of this compound, further characterization was not attempted.

Dinucleotide 25. A mixture of **23** (415 mg, 0.5 mmol) and 3'-*O*-benzylthymidine (**24**)[20] (249 mg, 0.75 mmol) in a vacuum-dried flask was purged with Ar and treated with freshly distilled MeCN (10 mL) and resublimed tetrazole (210 mg, 3.0 mmol). After stirring at room temperature for 12 h, an 0.5 M I_2 solution in

2,6-lutidine/THF/H_2O (2:2:1) was added dropwise with stirring to the reaction mixture until a purple color persisted. Sat. $NaHCO_3$ solution was then added and the reaction mixture was extracted with EtOAc (3 x 20 mL). The combined organic extracts were washed with aqueous $NaHSO_3$, brine, dried (Na_2SO_4), and concentrated. Column chromatography (SiO_2,eluant: DCM then DCM/EtOAc1:3 to 0:1) afforded **25** (365 mg, 68 %) as an unassigned diastereomeric mixture of two isomers **a** and **b**. Further chromatographic separation (SiO_2,eluant: EtOAc) gave 128 mg of **25a** (R_f = 0.53), 62 mg of **25b** (R_f = 0.40) and 164 mg of a mixture of **25a** and **25b** (**25a/25b** = 1/2). **25a**: M.p. 121 - 122 °C. ^1H NMR, δ: 1.57(3H, s), 1.58 (3H, s), 1.67 (3H, d *J* = 0.9 Hz), 1.91 (3H, d, *J* = 0.9 Hz), 2.15 (1H, m), 2.28 (1H, m), 2.44 (1H, dd, *J* = 11.3 and 3.9 Hz), 2.66 (1H, dd, *J* = 12.5 and 5.6 Hz), 3.72 (1H, d, *J* = 13.4 Hz), 3.80 (1H, d, *J* = 11.5 Hz), 3.80 (3H, d, *J* = 13.4 Hz), 4.22 (4H, m), 4.50 (1H, d, *J* = 11.7 Hz), 4.58 (1H, d, *J* = 11.9 Hz), 4.68 (1H, d, *J* = 11.9 Hz), 4.76 (1H, d, *J* = 11.7 Hz), 5.25 (1H, bt, *J* = 5.4 Hz), 6.27 (1H, t, *J* = 6.9 Hz), 6.37 (1H, dd, *J* = 8.7 and 5.4 Hz), 6.86 (1H, dt, *J* = 1.9 and 7.8 Hz), 7.18 (1H, bs), 7.24 - 7.36 (9H, m), 7.46 (2H, t, *J* = 7.7 Hz), 7.61 (1H, bt, *J* = 7.5 Hz), 7.95 - 7.99 (3H, m), 8.96 (1H, b). ^{13}C NMR, δ: 12.0, 12.4, 27.8, 27.9, 37.1, 38.4, 38.9, 40.1, 55.0 (d, *J*= 5.4 Hz), 66.0, 67.4 (d, *J* = 6.5 Hz), 77.9, 78.7, 82.4 (d, *J* = 8.7 Hz), 85.5, 86.8, 93.1, 93.2, 94.5, 111.3, 111.8, 127.6, 127.9, 128.1, 128.5, 128.7, 129.6, 133.9, 134.7, 135.7, 137.1, 143.8, 146.0, 149.8, 150.2, 163.3, 163.6, 165.6, 197.9. ^{31}P NMR, δ -0.85. IR ($CDCl_3$, cm^{-1}): 3391, 1691, 1274. **25b**: .M.p. 116 - 117 °C. ^1H NMR, δ: 1.55 (3H, s), 1.56 (3H, s), 1.68 (3H, d *J* = 0.9 Hz), 1.94 (3H, d, *J* = 0.9 Hz), 2.03 - 2.14 (1H, m), 2.35 (1H, ddd, *J* = 14.2 , 8.8 and 5.5 Hz), 2.48 (1H, bdd, *J* = 13.5 and 6.0 Hz), 2.73 (1H, bdd, *J* = 12.5 and 5.5 Hz), 3.68 (1H, d, *J* = 13.4 Hz), 3.79 (3H, d, *J* = 13.4 Hz), 3.80 (1H, d, *J* = 13.5 Hz), 4.23 - 4.30 (4H, m), 4.54 (1H, d, *J* = 11.6 Hz), 4.60 (1H, d, *J* = 11.7 Hz), 4.66 (1H, d, *J* = 11.9 Hz), 4.78 (1H, d, *J* = 11.9 Hz), 5.28 (1H, bt, *J* = 5.5 Hz), 6.33 (1H, dd, *J* = 8.0 and 5.9 Hz), 6.47 (1H, dd, *J* = 8.7 and 5.3 Hz), 6.85 (1H, m), 7.22 (1H, bs), 7.26 - 7.41 (9H, m), 7.45 (2H, t, *J* = 7.7 Hz), 7.61 (1H, bt, *J* = 7.4 Hz), 7.95 - 7.99 (2H, m). ^{13}C NMR, δ: 12.0, 12.5, 27.8, 27.9, 37.3, 38.6, 39.1, 40.0, 54.8 (d, *J* = 6.5 Hz), 66.0, 67.6 (d, *J* = 5.4 Hz), 71.6, 78.4, 78.7, 82.6 (d, *J* = 7.8 Hz), 85.1, 86.5, 93.0. 93.1, 94.5, 111.4, 111.9, 127.6, 128.0, 128.1, 128.5, 128.9, 129.6, 133.9, 134.6, 135.3, 137.1, 143.7, 146.0, 149.9, 150.2, 163.3, 163.6, 165.6, 198.2. ^{31}P NMR, δ: -0.90. IR ($CDCl_3$, cm^{-1}): 3392, 1694, 1274. Anal. Calcd for $C_{45}H_{48}IN_4O_{14}PS$: C, 51.05; H, 4.57. Found: C. 50.96; H, 4.61.

Reaction of 25 with Ph_3SnH in Benzene at Reflux. A solution of **25** (32 mg, 0.03 mmol), Ph_3SnH (16 mg, 0.045 mmol) and AIBN (1mg, 0.006 mmol) in benzene (1 mL) was heated to reflux for 2 h. After cooling to room temperature, the solvent was removed under vacuum. ^1H NMR spectroscopy of the crude reaction mixture indicated complete conversion of **25**. The crude products were taken up with MeCN and MeOH (3 mL, 2:1 v/v) and washed with hexane (2 x 3 mL). The residue from the hexane layer was shown by ^1H NMR spectroscopy to contain mainly the heterocycle **15** and organotin residues. The MeCN/MeOH layer was concentrated and purified by repeated column chromatography (SiO_2,eluant: dichloromethane then dichloromethane /EtOAc 2:1 to 1:1), to give **27** (2.3 mg, 23%), **28** (0.9 mg, 10%) and **29** (2.0 mg, 30%). Further elution with EtOAc/MeOH(1:1) gave phosphate **26** (11 mg, 86%). **27**: ^1H NMR, δ: 1.81 (3H, d, *J* = 1.2 Hz), 2.62 (1H, m), 2.67 (1H, m), 3.31 (1H, ddq, *J* = 17.5, 9.9 and 1.8 Hz), 4.90 (1H, d, *J* = 13.6 Hz),

4.98 (1H, d, J = 13.2 Hz), 5.24 (1H, bs), 6.76 (1H, dd, J = 3.9 and 9.7 Hz), 7.08 (1H, q, J = 1.8 Hz), 7.46 (2H, J = 7.6 Hz), 7.59 (1H, d, J = 7.5 Hz), 8.05 (2H, m). ^{13}C NMR, δ: 12.4, 36.7, 58.4, 84.7, 99.4, 112.0, 128.5, 129.6, 133.5, 134.5, 149.6, 152.4, 163.0, 165.9. HRMS Calcd for $C_{17}H_{16}N_2O_5$ $(M + 1)^+$: 329.11375. Found: 329.11494. **28**[32]: ^{1}H NMR, δ: 1.51 (3H, d, J = 1.1 Hz), 4.57 (1H, dd, J = 2.9 and 12.5 Hz), 4.63 (1H, dd, J = 3.7 and 12.5 Hz), 5.94 (1H, dq, J = 5.9 and 1.2 Hz), 6.42 (1H, dt, J = 6.1 and 1.7 Hz), 6.91 (1H, m), 7.08 (1H, q, J = 1.1 Hz), 7.46 (2H, bt, J = 7.6 Hz), 7.61 (1H, bt, J = 7.4 Hz), 8.02 (2H, dd, J = 1.3 and 8.4 Hz). **29**: ^{1}H NMR, δ: 1.98 (3H, d, J = 1.3 Hz), 2.22 (3H, s), 3.34 (2H, dd, J = 1.3 and 7.1 Hz), 5.75 (1H, dt, J = 14.6 and 7.1 Hz), 6.97 (1H, dt, J = 14.7 and 1.5 Hz), 7.35 (1H, bs), 8.21 (1H, bs). ^{13}C NMR, δ: 12.4, 29.7, 43.8, 111.1, 111.9 126.2, 135.6, 148.9, 163.0, 205.5. HRMS Calcd for $C_{10}H_{12}N_2O_3$: 208.08479. Found: 208.08378. **26**: ^{1}H NMR (CD_3OD), δ: 1.92 (3H, d, J = 1.0 Hz), 2.24 (1H, ddd, J = 13.7, 8.8 and 5.8 Hz), 2.39 (1H, bdd, J = 4.9 and 13.7 Hz), 3.56 (3H, d, J = 10.8 Hz), 4.02 (2H, m), 4.24 (1H, m), 4.34 (1H, bd, J = 5.6 Hz), 4.56 (1H, d, J = 11.9 Hz), 4.61(1H, d, J = 11.9 Hz), 6.33 (1H, dd, J = 8.8 and 5.7 Hz), 7.24 - 7.38 (5H, m), 7.81 (1H, q, J = 1.1 Hz). ^{13}C NMR (CD_3OD), δ: 12.3, 38.0, 52.9 (d, J = 5.6 Hz), 55.6 (d, J = 5.6 Hz), 72.0, 80.8, 85.1 (d, J = 8.4 Hz), 86.0, 111. 9, 128.5, 128.7, 129.2, 137.8, 139.2, 152.3, 166.3. ^{31}P NMR (CD_3OD), δ: 3.32.

Reaction of 25 with TMS_3SiH in Benzene at Reflux. Reaction of **25** (32 mg, 0.03 mmol) with TMS_3SiH (28 mL, 0.15 mmol) in benzene (1 mL) was conducted in exactly the same manner as its reaction with Ph_3SnH. Chromatography (SiO_2,eluant: dichloromethane then dichloromethane/EtOAc 1:1 to 0:1) of the crude reaction mixture gave products **30** (3.1 mg, 32%), contaminated by ~10% of an organosilicon residue, and which experienced significant decomposition upon further purification by prep. TLC, and **31** (3.7 mg, 37 %). Further elution with EtOAc/MeOH(1:1 then 1:2) gave phosphate **26** (12 mg, 94%). **30**: ^{1}H NMR, δ: 1.98 (3H, d, J = 1.2 Hz), 3.44 (2H, dd, J = 1.3 and 7.1 Hz), 4.94 (2H, s), 5.75 (1H, dt, J = 14.3 and 7.1 Hz), 6.76 (1H, dd, J = 3.9 and 9.8 Hz), 7.03 (1H, bd, J = 14.5 Hz), 7.46 -8.11 (5H, m), 8.32 (1H, bs). HRMS Calcd for $C_{17}H_{16}N_2O_5$: 328.10592. Found: 328.10619. **31**: ^{1}H NMR, δ: 1.92 (3H, d, J = 1.2 Hz), 2.03 (2H, quintet, J = 6.8 Hz), 2.60 (2H, t, J = 6.6 Hz), 3.74 (2H, d, J = 7.0 Hz), 4.88 92H, s), 7.03 (1H, bs), 7.47 (2H, bt, J = 7.5 Hz), 7.61 (1H, bt, J = 7.4 Hz), 8.08 (2H, dd, J = 1.3 and 8.3 Hz), 8.53 (1H, bs). ^{13}C NMR, δ: 12.2, 22.3, 34.8, 47.2, 52.1, 68.3, 111.0, 128.5, 128.9, 129.8, 133.6, 140.4, 150.8, 163.9, 165.9, 203.1. HRMS Calcd for $C_{17}H_{18}N_2O_5$: 330.12157. Found: 330.12154.

Reaction of 25 with TMS_3SiH in Benzene/MeOH at Reflux. A solution of **25** (32 mg, 0.03 moml), TMS_3SiH (28 mL, 0.15 mmol) and AIBN (1mg, 0.006 mmol) in benzene/MeOH (1 mL, 4:1 v/v) was heated to reflux for 2 h. After cooling to room temperature, the solvent was removed under vacuum and the residue was subjected to column chromatography (SiO_2,eluant: dichloromethane then dichloromethane /EtOAc 1:1 to 0:1), giving **42** (4.5 mg, 42%) as a 4:3 mixture of unassigned isomers and **43** (3.5 mg, 32%) as a 3:2 mixture of unassigned isomers. Further elution with EtOAc/MeOH (1:1 then 1:2) gave phosphate **26** (9 mg, 70%). **42**: ^{1}H NMR, δ: 1.61 - 1.66 (1H, m), 1.96 (3H, bs), 1.92 - 2.39 (2H, m), 2.42 - 2.47 (major) and 2.63 - 2.70

(minor) (1H, m), 3.40 (minor) and 3.43 (major) (3H, s), 4.39 (major) and 4.50 (minor) (1H, d, J = 12.0 Hz for the major and 11.8 Hz for the minor isomer), 4.61 (major) and 4.71 (minor) (1H, d, J = 11.9 Hz for major and 11.8 for the minor isomer), 6.29 (minor) and 6.51 (major) (1H, dd for the minor isomer, J = 4.6 and 7.2 Hz, t for the major isomer, J = 6.8 Hz), 7.12 (minor) and 7.39 (major) (1H, bs), 7.47 (2H, t, J = 7.5 Hz), 7.59 (1H, m), 8.03 - 8.06 (2H, m). ^{13}C NMR, δ: 12.2 (minor) and 12.7 (major), 29.8 (major) and 30.8 (minor), 32.6 (minor) and 34.7 (major), 49.8 (major) and 49.9 (minor), 62.7 (major) and 63.2 (minor), 85.1 (minor) and 86.2 (major), 107.7 (major) and 107.8 (minor), 111.3 (minor) and 111.6 (major), 128.5 (major) and 128.7 (minor), 129.3, 129.5 (minor) and 129.7 (major), 133.4 (major) and 133.6 (minor), 134.7 (minor) and 135.6 (major), 149.9 (minor) and 150.4 (major), 163.2 (minor) and 165.8 (major). HRMS Calcd for $C_{18}H_{20}N_2O_6$ (M + 1)$^+$: 361.13996. Found: 361.14164. **43**: ^{1}H NMR, δ: 1.63 (minor) and 1.66 (major) (3H, d, J = 1.4 Hz for minor and 1.2 Hz for the major isomer), 2.01 - 2.73 (2H, m), 3.39 (3H, s), 4.08 (1H, m), 4.36 (1H, m), 4.48 - 4.73 (2H, m), 6.25 - 6.31 (1H, m), 7.12 (minor) and 7.25 (major) (1H, d, J = 1.1 Hz for the minor and 1.2 for the major isomer), 7.47 (2H, t, J = 7.7 Hz), 7.62 (1H, m), 8.01 -8.06 (2H, m). HRMS Calcd for $C_{18}H_{20}N_2O_6$: 360.13213. Found: 360.13159.

Reaction of 25 with TMS_3SiH in Benzene/MeOH under Photolytic Conditions. A solution of **25** (32 mg, 0.03 mmol), TMS_3SiH (28 mL, 0.15 mmol) and AIBN (1mg, 0.006 mmol) in benzene/MeOH (1 mL, 4:1 v/v) was photolyzed at room temperature in a Rayonet photoreactor (254 nm through Pyrex) for 2 h. After removal of the solvent, ^{1}H NMR of the crude reaction mixture indicated complete conversion of **25** with formation of a complex mixture of products. Careful examination of this reaction mixture revealed the presence of aldehyde **44**, characterized by its partial ^{1}H NMR spectrum; δ 2.80 - 2.91 (4H, m, CH_2CH_2), 4.99 (2H, s, CH_2OBz), 9.82 (1H, s, CHO). Phosphate **26** (10.1 mg, 78%) was isolated by column chromatography, along with a trace amount of thymine which was identified with the aid of a commercial sample. In a separate experiment, the photolysis was conducted in the presence of trimethyl orthoacetate (19 mL, 0.15 mmol). A mixture of products (10.2 mg) containing mainly **43** (~90%) was obtained by column chromatography. Phosphate **26** (11.8 mg, 92%) was also isolated.

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