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Heterolytic C,O-Bond Cleavage of 4'-Nucleotide Radicals

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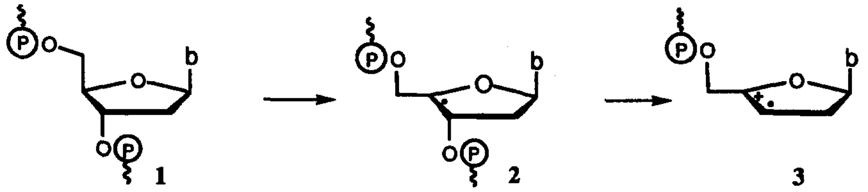
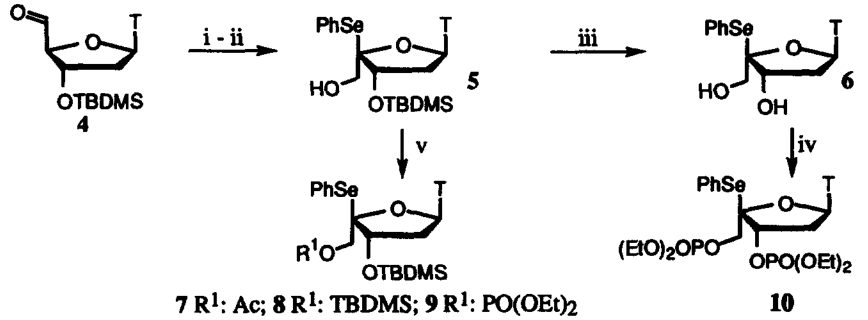
Abstract: Photolysis of selenoacetals **7-10** and acylselenides **13** and **14** generates 4'-deoxyribonucleotide radicals. Photocurrent experiments show that radicals with a phosphate group in β-position give rise to a heterolytical bond cleavage.

An important intermediate of the radical induced DNA cleavage is deoxyribonucleosyl radical **2** with the radical center in the 4'-position.[1] Pioneering experiments of Schulte-Frohlinde et al.[2] as well as recent studies of Giese et al.[3] indicate that 4'-deoxyribonucleosyl radicals can undergo a heterolytic cleavage of the carbon-phosphate bond. Such a heterolytic cleavage of the DNA backbone yields radical cation **3** which reacts with nucleophiles or electron donors.[3]



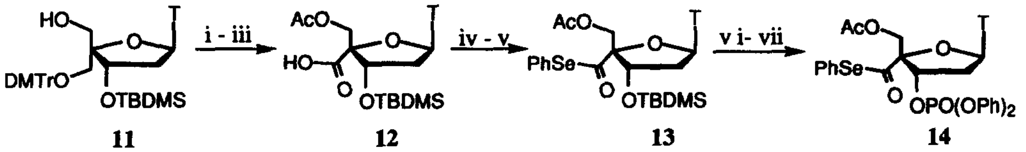
We have now found the first direct proof for the heterolytic C,O bond cleavage of suitably substituted 4'-deoxyribonucleosyl radicals utilizing the transient photocurrent method. This method is widely used for detecting of charged species in irradiated solutions.[4] The intensity of the measured current is directly proportional to the concentration of ions[5] which appear as intermediates upon laser flash photolysis.

As substrates for these photocurrent studies we chose nucleotide derivatives bearing a selenoacetal or selenoester functionality in the 4'-position. These functional groups undergo homolytic bond cleavage upon irradiation to generate alkyl radicals. The key intermediate for the synthesis of all nucleotides was the thymidine 5'-aldehyde **4**.[6] The first step in the synthesis of the selenoacetals **7-10** was the reaction of **4** with phenylselenenyl chloride in the presence of Et_3N. The subsequent reduction with DIBAL gave a single diastereomer. NOE studies of **5** proved the L-*threo* configuration at C-4'.[7] Simple protective group manipulations at C-3' and C-5' gave the derivatives **7-10**.[8]



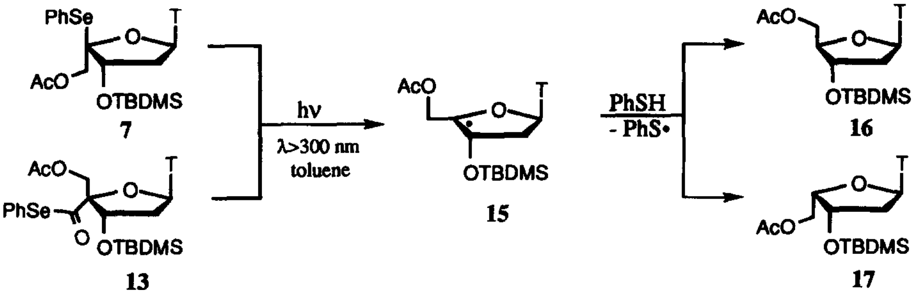
i) PhSeCl, NEt_3, CH_2Cl_2, -78° → 0°, 84%; ii) DIBAL, THF, -78°, 80%; iii) TBAF(1M), THF, 0°, 82%; iv) $(EtO)_2POCl$, NMI, CH_2Cl_2, 0°, 81%; v) 5→7: Ac_2O, Py, 0°, 73%; 5→8: TBDMSCl, Im, DMF, 92%; 5→9: $(EtO)_2POCl$, NMI, CH_2Cl_2, 0°, 83%.

For the preparation of the selenoester precursor **14**, the 5'-aldehyde **4** was first transformed into the known 4'-hydroxymethyl substituted nucleoside **11**.[9] Acetylation, removal of the trityl group, and oxidation of the primary alcohol afforded the carboxylic acid **12**. Treatment with α,α-dichloromethyl methyl ether yielded the acid chloride which was converted to the selenoester **13**. Deprotection of the 3'-position and subsequent phosphorylation gave the desired nucleotide **14**.[10]



i) Ac_2O, Py, 0°; ii) HOAc/THF (4/1), 25°, 91% (2 steps); iii) PDC (10 eq.), DMF, 25°, 78%; iv) Cl_2CHOCH_3, CH_2Cl_2, 0°; v) PhSeH, Py, THF, 0°, 72% (2 steps); vi) $BF_3 \cdot Et_2O$, CH_2Cl_2, 40°; vii) $(PhO)_2POCl$, NMI, CH_2Cl_2, 0°, 91% (2 steps).

In order to check whether selenoacetals and acylselenides are suitable radical precursors for the photocurrent experiments, compounds **7** and **13** were irradiated in the presence of thiophenol as radical scavenger. These reactions gave 1:2 mixtures of isomers **16** and **17** in 70-75% yield, proving the formation of radical **15** as the common intermediate.



Photocurrent experiments were carried out with the radical precursors **8**-**10** and **14**.[11] Fig. 1 and 2 show that radical precursors **9**, **10** and **14** with phosphate groups at C-3' and/or C-5' generate current. By contrast, with nucleoside **8** bearing silyloxy substituents at C-3' and C-5' no photocurrent is detectable.

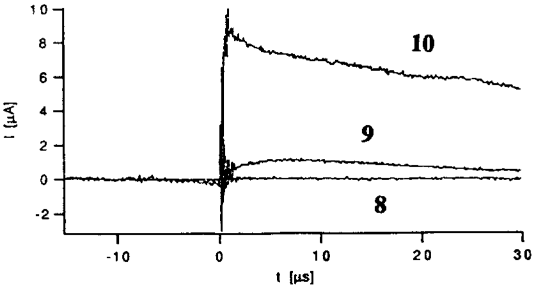


Fig. 1: Photocurrent measurements of the precursors 8-10

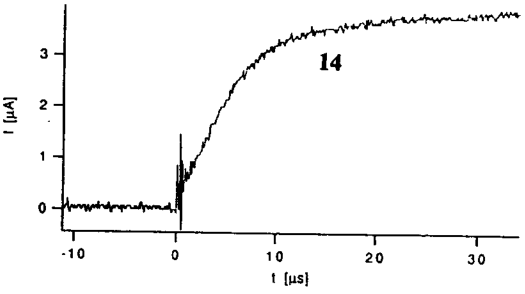
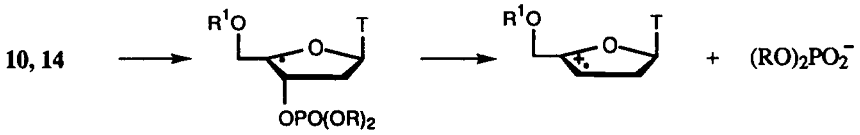


Fig. 2: Photocurrent measurements of the precursor 14

The observation of a photocurrent proves the appearance of charged species. The first step of the photocurrent experiments with compounds **8**-**10** and **14** is the formation of a 4'-nucleotide radical. The radical then fragments into ions if the phosphate group is located in the β-position. As expected, the photocurrent is larger for the diphosphate **10** compared to the mono phosphate **9**, bearing the phosphate group at the primary C-5' position.

These experiments provide strong evidence that 4'-nucleotide radicals **18** with good anionic leaving groups like phosphates cleave the β-C,O bond heterolytically to give a phosphate anion and a radical cation **19**. In earlier experiments we have already shown that radical cations like **19** can be trapped by nucleophiles or electron donors.[3]



18 **19**

Conclusion: 4'-Nucleotide radicals with a phosphate substituent in the β-position cleave the β-C,O bond heterolytically. These model reactions are in accord with a heterolytical phosphate elimination in radical induced DNA strand cleavage under anaerobic conditions.

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7. The NOE-experiments showed an enhancement of the 1'-H on irradiation of 5'-CH_2.
8. All new compounds gave satisfactory analytical and spectroscopic data (^{1}H NMR, ^{13}C NMR, MS, elemental analysis). The introduction of the selenoacetal resulted in a characteristical downfield shift (10-15 ppm) of the C4'-signal in the ^{13}C NMR (**8**: 98.0 ppm; **9**: 94.7 ppm; **10**: 92.1 ppm). In the phosphorylated nucleotides **9** and **10** a coupling of the C-4' with the ^{31}P-core of the phosphates in the 3' and/or 5'-positions are observed (**9**: J=11.1 Hz; **10** J=11.3;8.1 Hz).
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10. The nucleotide **14** bearing a radical precursor functionality at C-4' is especially interesting as a potential building block of oligonucleotides because it has the 'natural' stereochemistry at C-4'. First experiments have proved that **14** is compatible with the reaction conditions used by solid phase oligonucleotide synthesis.
11. In a typical experiment degassed solutions (10 mM) of the radical precursor in acetonitrile were irradiated (λ=355 nm) in a custom-made photocurrent cell[12] with a YAG laser (*JK-Lasers* model 2000) at the frequency-tripled wavelength (the fundamental and the doubled beams were eliminated by a prism). The pulse energy was measured as 8 mJ, the pulse duration being about 22 ns. The Pt-electrode area was 3.6 mm^2 and the inner electrode distance was 5 mm. A constant voltage of 500 V was applied between the electrodes. All experiments were carried out at 20 ± 2 °C.
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