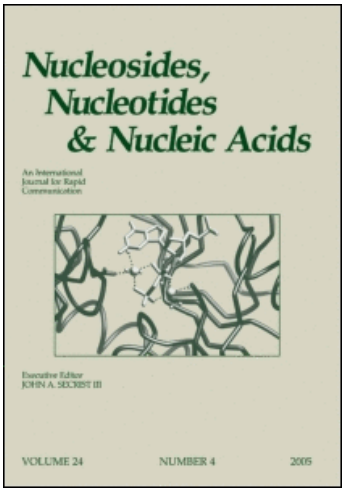

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PHOTOCHEMICALLY INDUCED RNA AND DNA ABASIC SITES

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□ *Two phosphoramidite building blocks were synthesized that can easily be deprotected by UV light to reveal natural abasic sites in oligoribonucleotides as well as in oligodeoxyribonucleotides. Another building block which releases a 2′-O-methylated abasic site upon UV radiation is also described.*

Keywords RNA; DNA; natural abasic sites; UV radiation

The design of potential assays using true abasic site containing oligonucleotides demands the final deprotection step to release the potentially unstable abasic lesion to occur fast, under mild conditions and merely quantitative in order not to interfere unnecessarily with the assay conditions. Moreover the deprotection conditions of a chosen protecting group have to be orthogonal to standard oligonucleotide chemistry to ensure a wide usability of the designed phosphoramidite building blocks.

The 2-nitrobenzyl moiety (NB) has been used for the protection of the 1′-*O*-position of a deoxyribose unit before.[1] The NB unit, also used to cage the 2′-hydroxy position of the furanose ring, has shown not to be fully stable when treated with fluoride ions used in standard RNA deprotection of 2′-*O*-TBDMS groups.[2] Clearly, a 2-nitrobenzyl derivative with higher stability against fluoride treatment was needed.

The 1-(2-nitrophenyl)ethyl (1NPE) unit was shown to be fully stable under the deprotection conditions mentioned above. Besides this, deprotection of the 1NPE unit gives rise to the less reactive 2-nitrosoacetophenone compared to the biologically toxic 2-nitrosobenzaldehyde that arises from 2-nitrobenzyl deprotection. 1NPE adducts are additionally known to photolyse 20 times more rapidly than the NB adducts.[3]

The synthesis of the RNA abasic site precursor (Scheme 1) started with the formation of the acetal **5** under Vorbrüggen conditions using

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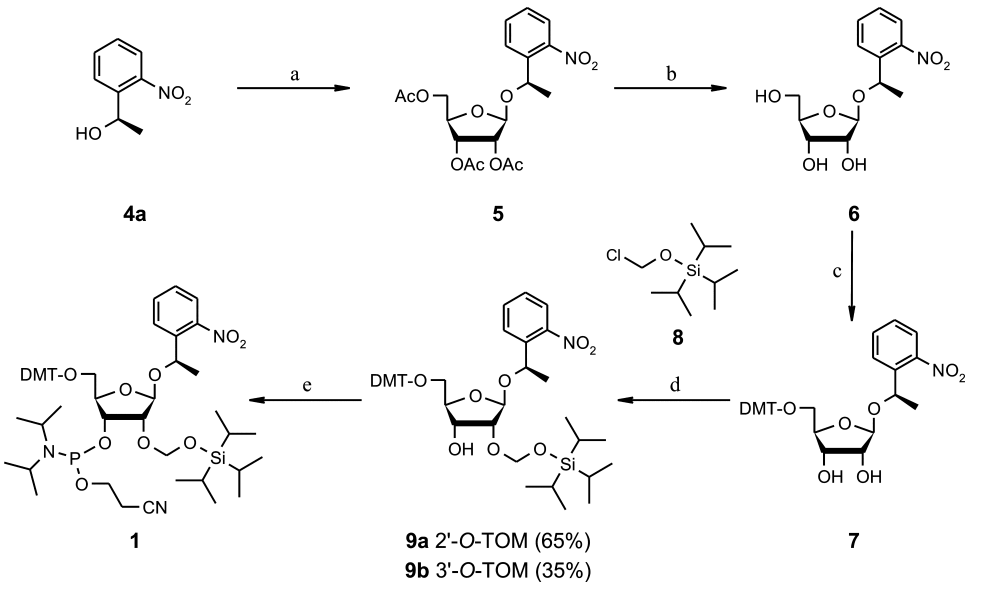
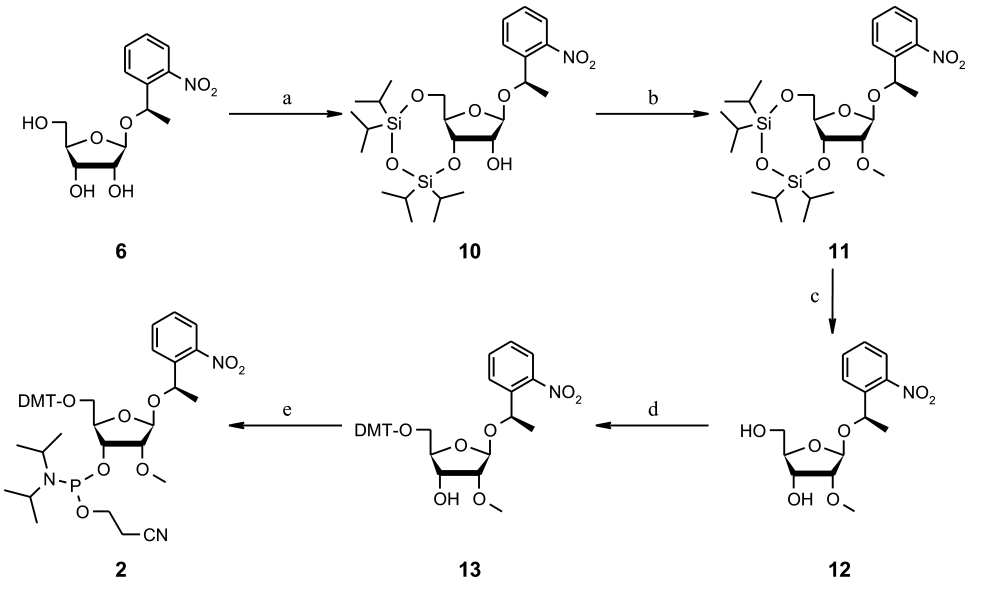
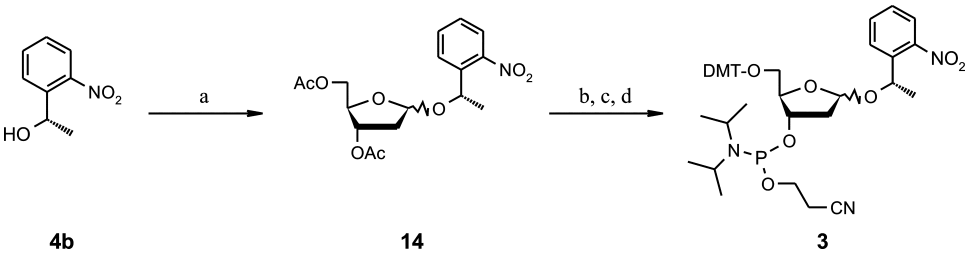


SCHEME 1 a) Tetra-*O*-acetyl-ribofuranose (1.2 eq.), TMSOTf (0.35 eq.) added in 4 portions over 90 minutes, CH_3CN, 2 hours, −20°C, 77%. b) Na_2CO_3 (1 eq.), MeOH, 22 hours, r.t., 90%. c) DMT-Cl (1.2 eq.), pyridine, 1.5 hours, r.t., 94%. d) 1) Bu_2SnCl_2 (1.1 eq.), $^{i}Pr_2NEt$ (3.5 eq.), $(CH_2Cl)_2$, 1 hour, r.t., 2) **(8)** (1.1 eq.), 30 minutes, 80°C, 94% for both isomers. e) $[(^{i}Pr_2N)(NCCH_2CH_2O)P]Cl$ (1.45 eq.), $^{i}Pr_2NEt$ (2.9 eq.), THF, 14 hours, r.t., 87%.



SCHEME 2 a) TIPDS-Cl (1:2 eq.), pyridine, 18 hours, r.t., 82%. b) AgO (8 eq.), MeI, 72 hours, 50°C, 90%. c) TBAF (3 eq.), acetic acid (1.6 eq.), THF, 30 minutes, r.t., 85%. d) DMT-Cl (1.2 eq.), pyridine, 1.5 hours, r.t., 94%. e) $[(^{i}Pr_2N)(NCCH_2CH_2O)P]Cl$ (1.45 eq.), $^{i}Pr_2NEt$ (2.9 eq.), THF, 4 hours, r.t., 78%.



4b **14** **3**

SCHEME 3 a) Tri-*O*-acetyl-2′-deoxyribofuranose (0.7 eq.), TMSOTf (0.2 eq.) added dropwise, CH_3CN, 1 hour, −20°C, 84%. b) Na_2CO_3 (1 eq.), MeOH, 22 hours, r.t., 91%. c) DMT-Cl (1.2 eq.), pyridine, 1.5 hours, r.t., 74%. d) [($^iPr_2N$)($NCCH_2CH_2O$)P]Cl (1.45 eq.), $^iPr_2NEt$ (2.9 eq.), THF, 1 hour, r.t., 77%.

enantiopure 1-*(R)*-(2-nitrophenyl)ethanol **4a**, synthesized after the method of Corrie et al.,[4] and commercially available (1,2,3,5)-tetra-*O*-acetyl-ribofuranose. The pure β-anomer **5** was deacetylated and DMT-protected by standard methods to give nucleoside **7**. Preparation of [(triisopropylsilyl)oxy]methyl) chloride **8** and subsequent alkylation of the 2′-hydroxy group of the ribose was performed in analogy to known methods.[5] The ratio of the 2′- and 3′- regioisomers **9a** and **9b** was determined to be 65% in favor of the 2′-*O*-TOM protected nucleoside **9a**, according to ^{1}H-NMR analysis. Repeated column chromatography yielded 37% of the pure 2′-*O*-TOM nucleoside and 57% of an inseparable 2′-, 3′-*O*-TOM mixture. Reaction of **9a** with 2-cyanoethyl diisopropylchlorophosphoramidite finally gave the abasic RNA building block **1**.

Synthesis of the 2′-O-Me RNA abasic site precursor (Scheme 2) started with intermediate **6** that was TIPDS protected to give **10.** The 2′-hydroxyl group was then methylated followed by deprotection of the TIPDS group with TBAF to yield diol **12.** Protection with DMT-chloride and phosphitylation finally gave the 2′-OMe-abasic building block **2.**

If for both synthetic pathways a mixture of diastereomers of **6**, arising from the use of an enantiomeric mixture of 1(2-nitrophenyl)ethanol is used, the two isomers can be separated by column chromatography at the stage of intermediate **7** or **10**, respectively. Enantiopure 1-(2-nitrophenyl)ethanol as starting material is therefore not needed in order to get diastereopure products.

The synthesis of the DNA abasic site precursor (Scheme 3) started with (1′,3′,5′)-Tri-*O*-acetyl-2′-deoxy-D-ribofuranose,[6] which was transformed into acetal **14** with enantiopure 1-*(S)*-(2-nitrophenyl)ethanol **4b** as described for the RNA abasic site precursor with comparable yields. The remaining steps were performed with the mixture of anomers although at the stages of the intermediates **14** and **15** the anomers were separable according to TLC. Phosphoramidites **1–3** were incorporated in oligonucleotides using standard nucleic acid synthesis procedures. The modified phosphoramidites were allowed to couple for 6 minutes and 12 minutes for DNA

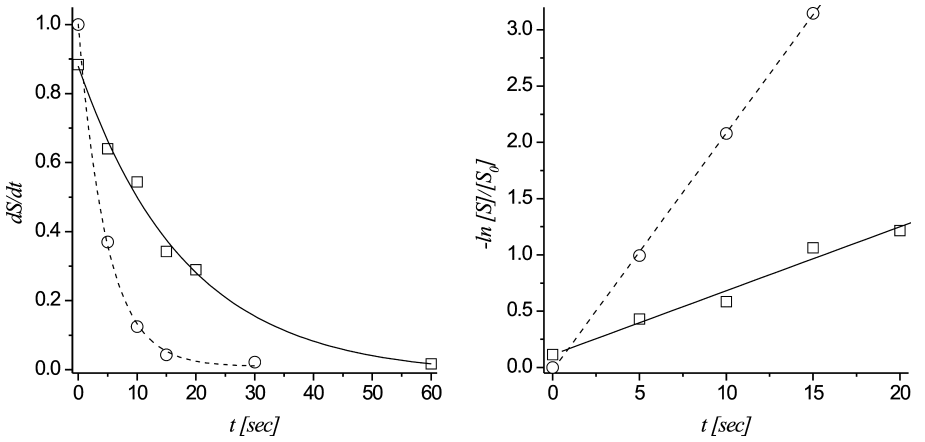


FIGURE 1 Heptamers 5′-AGG-**1**-UUC-3′ (□) and 5′-AGG-**3**-TTC-3′ (○) are deprotected with a UV lamp (150 W). Deprotection kinetics show half-life times of 12.5 seconds and 3.4 seconds for the deprotection of RNA and DNA abasic sites, respectively. Logarithmic plots prove the first order character of the reactions and give the rate constants $k = 5.68 \cdot 10^{-2}s^{-1}$ for RNA and $k = 2.11 \cdot 10^{-1}s^{-1}$ for DNA for the deprotection reaction.

and RNA, respectively. 2′-*O*-TOM groups were removed using the normal silyl deprotection procedure of oligoribonucleotides.

In order to measure deprotection kinetics, phosphoramidite building blocks **1** and **3** were incorporated into the heptamers 5′-AGG-**1**-UUC-3′ and 5′AGG-**3**-TTC-3′. Aqueous solutions of the oligonucleotides at a concentration of 1 $OD_{260}mL^{-1}$ in a quartz cuvette were irradiated with a UV lamp (medium-pressure mercury vapor lamp, 150 W). The deprotection was followed by RP-HPLC. From the exponential fits, half-life times of 12.5 seconds ($k = 5.68 \cdot 10^{-2}s^{-1}$) and 3.4 seconds ($k = 2.11 \cdot 10^{-1}s^{-1}$) for the deprotection of RNA and DNA abasic site were determined. The collected data show the deprotection reactions to be of first order kinetics. Oligonucleotides containing building blocks **1–3** were also deprotected in almost quantitative yield by exposition to the glass-filtered light of a slide projector (tungsten lamp, 250 W) for 6 minutes. Especially the latter method shows a very mild way to produce abasic sites from the presented building blocks with a very low risk of inducing photolesions in oligonucleotides.

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