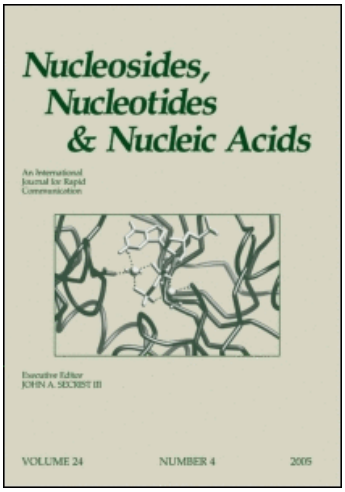

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Synthesis and Application of 3'-Amino-Dye-Terminators For Dna Sequencing

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SYNTHESIS AND APPLICATION OF 3′-AMINO-DYE-TERMINATORS FOR DNA SEQUENCING

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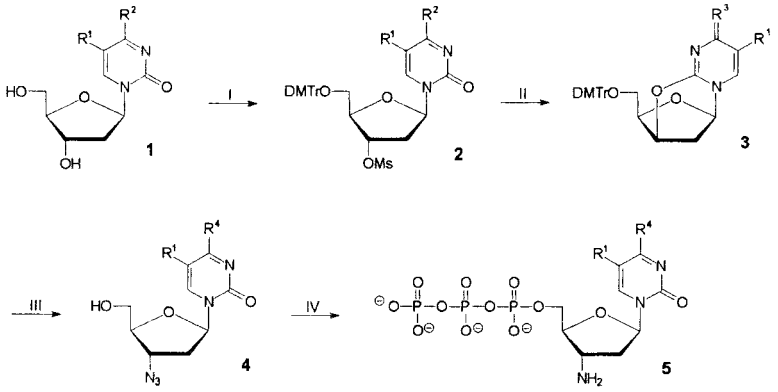
ABSTRACT: The synthesis of 3′-aminomodified nucleoside 5′-triphosphates and their coupling with oligoaminoacid-linked dyes is described. Application for DNA dye-terminator sequencing was investigated.

The rapidly growing field of genetic analysis demands powerful methods for sequencing of DNA with low error rates. Unlike dye-primer sequencing where primers are labeled with dyes at the 5′-end, dye-terminator sequencing is characterized by the dye attached to the terminator. This leads to exclusive detection of correctly terminated DNA-fragments and therefore offers the possibility for more accurate sequencing data. We have established 3′-aminonucleotides as potent chain terminators in DNA sequencing that are readily accepted in the enzymatic polymerisation reaction[1].

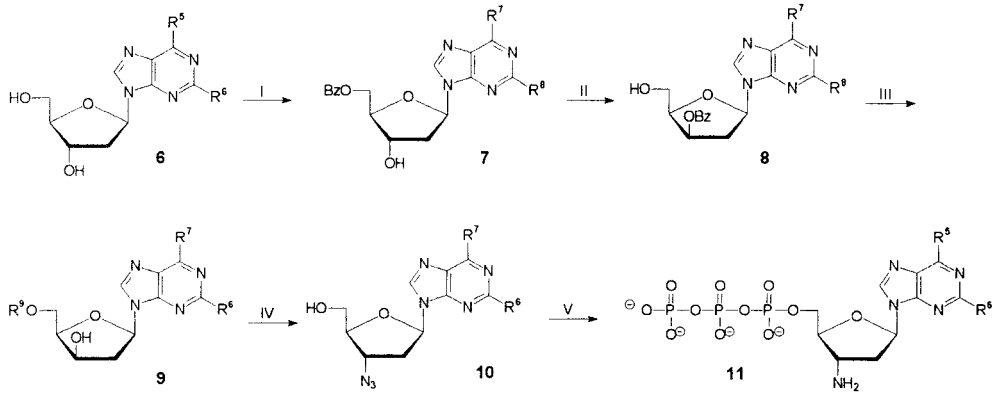
We started our syntheses of 3′-amino-2′,3′-dideoxynucleoside 5′-triphosphates **5** and **11** with double inversion of the configuration at C-3′. Different strategies for this step were performed for the purine and the pyrimidine nucleosides. The latter were inverted by an intermediate cyclic anhydro compound **3** which was reopened by nucleophilic attack of an azide ion as shown in Fig. 1[2]. The azidonucleosides of the pyrimidine bases T and C were obtained in 5 respectively 6 steps in good yields. Alternatively, the T nucleoside was synthesized by a tandem Mitsunobu reaction according to Czernecki[3].

In order to improve the synthesis of the guanine nucleoside we evaluated different strategies and compared them with the existing method of transglycosylation[4]. The procedure of Herdewijn[5] describing an intramolecular benzoyl-rearrangement from C-5′ to C-3′ with inversion of configuration at C-3′ proved to be most suitable. We obtained azidodeoxyguanosine **10** in 7 steps with an overall yield of 15% starting with deoxyguanosine **6** (Fig. 2). The same procedure was applied for the adenine nucleoside.



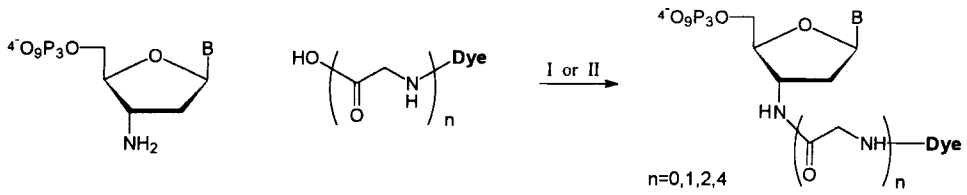
I) a) 4,4′-dimethoxytritylchloride, dimethylaminopyridine, NEt_3, pyridine b) $(CH_3SO_2)Cl$, pyridine II) potassium phthalimide, DMF III) a) LiN_3, DMF b) 80% HOAc IV) a) 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one, $P_2O_7^{4-}$, I_2 b) PPh_3, NH_3; thymine: $R^1 = CH_3$, $R^2 = R^4 = OH$, $R^3 = O$; cytosine: $R^1 = H$, $R^2 = NHBz$, $R^3 = NBz$, $R^4 = NH_2$

FIG. 1: Synthesis of pyrimidine 3′-aminonucleotides



I) guanine: a) $((CH_3)_3Si)_2N$, $(iPrCO)_2O$, pyridine, b) CH_3OH c) benzoylchloride, pyridine; adenine: a) Me_3SiCl, benzoylchloride, pyridine, b) CH_3OH c) benzoylchloride, pyridine II) a) $(CF_3SO_2)_2O$, CH_2Cl_2/pyridine b) H_2O III) guanine: a) 4,4′-dimethoxytritylchloride, dimethylaminopyridine, NEt_3, pyridine b) NH_3/CH_3OH; adenine: $NaHCO_3$, CH_3OH IV) guanine: a) LiN_3, PPh_3, CBr_4, DMF b) 80% HOAc; adenine: a) $(CF_3SO_2)_2O$, LiN_3, pyridine b) NH_3/H_2O V) a) 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one, $P_2O_7^{4-}$, I_2 b) PPh_3, NH_3; guanine: $R^5 = R^7 = OH$, $R^6 = NH_2$, $R^8 = NH(ibu)$, $R^9 = DMTr$; adenine: $R^5 = NH_2$, $R^6 = R^8 = H$, $R^7 = NHBz$, $R^9 = Bz$

FIG. 2: Synthesis of purine 3′-aminonucleotides



I) TSTU, DIPEA, DMF/dioxane/H_2O
II) HOBT, DIC, DIPEA, pyridine/dioxane/$NaHCO_3$

FIG. 3: Dye labeling of aminonucleotides

After introduction of the azido function the triphosphates were prepared using 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one and pyrophosphate according to the strategy of Ludwig and Eckstein[6] followed by reduction of the azido group by catalytic hydrogenation or the Staudinger reaction.

Dyes were coupled either directly or to an oligoaminoacid spacer in a solid phase supported strategy[7]. Dye-linker labeling of the aminonucleotides was performed as shown in Fig. 3. Activation of the carboxyl groups during the coupling step was achieved involving either TSTU or HOBT/DIC. The reaction was pursued by HPLC for several hours indicating incomplete coupling for the former conditions but quantitative yield for the latter.

Dyes with different emission maxima from 520 nm (fluoresceines) up to 660 nm (rhodamines) were employed[8] (Fig. 4). As we have shown before[9], a strong dependence of the spacerlength between dye and nucleotide upon quantum yields and absorption coefficients exists. Best results were indicated with the longest spacer arm. T-lane DNA sequencing

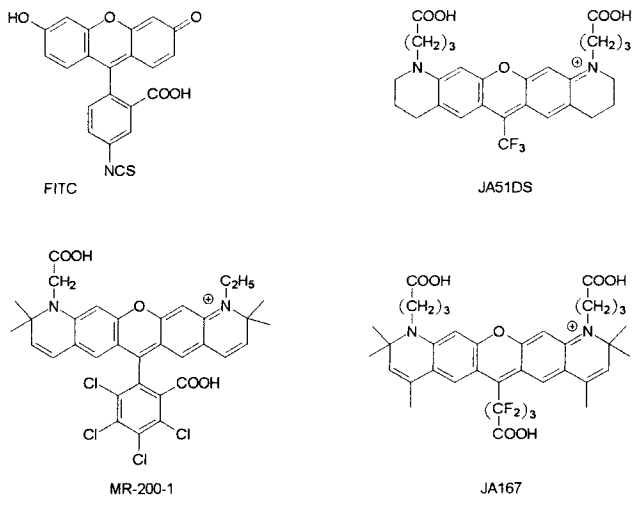


FIG. 4: Fluorescein- and rhodamine-derivatives coupled to aminonucleotides

experiments with T7 DNA Polymerase and Mn^{2+} as catalyst with M13mp18(+)ss as template demonstrate the ability of the compounds to act as potent chain terminators. The quality of termination is equal to dideoxyterminators for all tested compounds.

In summary, convenient routes for all four 3′-aminonucleoside 5′-triphosphates were carried out. Synthesis of the guanine derivative succeeds in a much better overall yield compared with the former synthetic route. Coupling efficiency with the aminoacid functionalized dyes is almost quantitative. Sequencing experiments with aminomodified nucleotides show the high potential of terminating properties.

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