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SYNTHESIS OF AN ARABINONUCLEIC ACID (tANA)

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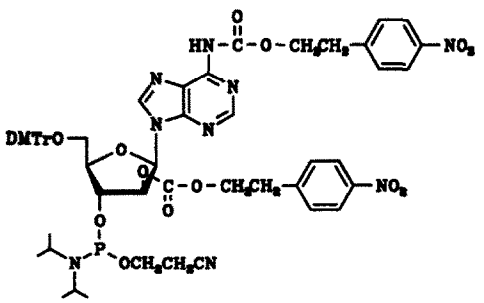
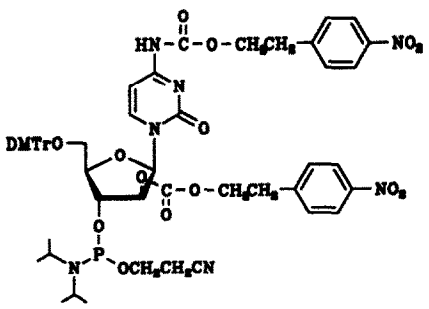
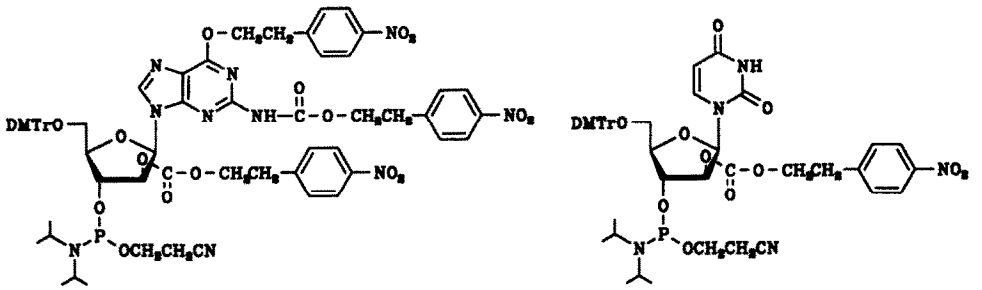
The introduction of the p-nitrophenylethyl- (NPE) and p-nitrophenylethoxycarbonyl- (NPEOC) group for amino and hydroxy protection of purine and pyrimidine arabinofuranosides and their interconversion into the corresponding 3'-phosphoramidite building blocks provided very effective starting materials for solid support oligoarabinonucleotide syntheses. A structural analogue of the Phage T5 histidine tRNA consisting of 78 nucleotide units has been synthesized in excellent yield and high purity.

The "antisense approach"[1] has recently stimulated oligonucleotide chemistry to a large extent due to a high potential of chemotherapeutic effects. Modifications of the base moiety, the sugar part and the phosphate backbone are the common structural changes which have been performed in a great variety in the last few years. Little attention has been focussed towards arabinonucleosides as starting materials due to their high price and inaccessibility, respectively. A few short oligoarabinonucleotides have recently been synthesized[2,3] applying a solid support approach and using acyl protecting group combinations.

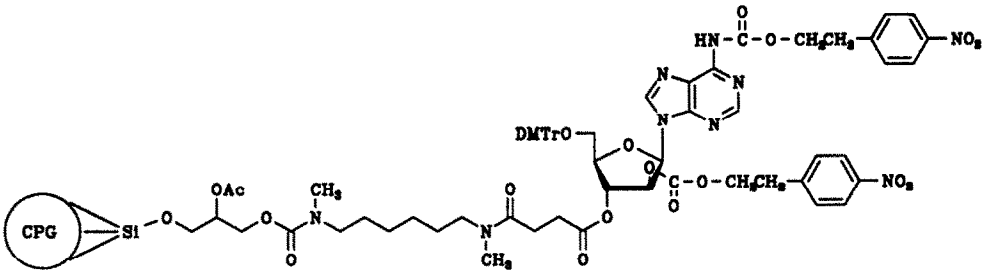
Encouraged by the excellent results achieved in oligodeoxyribonucleotide synthesis[4] based upon the NPE/NPEOC strategy we decided to extend this methodology to a convenient synthesis of arabinonucleotide oligomers. The synthesis of the four monomeric arabinonucleoside 3'-(2-cyanoethyl N,N-diisopropylphosphoramidite) building blocks was performed from 3',5'-disilyl protected aUrd, aCyd, aAdo and aGuo in the usual manner[5] by blocking the O^6 position in aGuo by the NPE group and the amino functions as well as the 2'-OH groups by the NPEOC group.







Desilylation and subsequent dimethoxytritylation at the 5'-OH groups and phosphitylation afforded the anticipated monomers in high yields. Appropriate solid supports with a nucleoside loading of 19-25 μmol/g were obtained by derivatization of 500 and 1000 Å LCAMA-CPG material[4], respectively, with 5'-O-dimethoxytrityl-N^6,2'-O-di-[2-(4-nitrophenyl)-ethoxycarbonyl]-3'-O-succinoyl-arabinoadenosin mediated by O-{[(cyano)ethoxycarbonyl]-amino-1,1,3,3-tetramethyl}uronium tetrafluoroborate (TOTU)[6] and N-methylmorpholine and subsequent blocking of unreacted hydroxy and amino functions by acetylation with acetic anhydride / DMAP / pyridine[7].



Several homoarabinonucleotide oligomer syntheses up to 10-mers as well as mixed sequences up to 21-mers were performed as model studies indicating high coupling yields and formation of very pure materials. For verification of this strategy and proof of its universal applicability the synthesis of the anticipated arabino analogue of the Phage T5 histidine transfer RNA consisting of 78 nucleotide units was undertaken. The natural tRNA contains two pseudouridine moieties in position 39 and 55 and one ribothymidine in position 54 all three of which have been replaced by arabinouridine to give the following sequence:

5'-ara(UGU GGC UAU AUC AUA AUU GGU UAA UGG UCC UGA UUG UGA AUC AGG CCU AUG UGG AUU CGA AUU CUA CUA GCC ACA CCA)-3'

The automated synthesis of the 78-mer oligoarabinonucleotide was achieved on an Applied Biosystems DNA-Synthesizer Model 392 using the following repetitive cycle (modified ABI 1 μmol RNA cycle) with a condensation time of 650 sec each.

Step	Reagents	Time [sec]
Detritylation	3% Trichloroacetic acid, Dichloromethan	40
Washing	Acetonitrile	30
Coupling	0.11 M Phosphoramidite, 0.5 M 1H-Tetrazole	650
Capping	Acetic anhydride/N-Methylimidazole/2,6-Lutidine, THF	20
Oxidation	I_2, Pyridine/Water/THF	30
Washing	Acetonitrile	30

During the coupling step reagent solution and solid support were mixed by short flush pushes. Absorption monitoring of the released dimethoxytrityl solutions indicated high average coupling efficiency of 99.3 % giving 58.7 % calculated overall yield of the correct sequence. Total deprotection of the trityl off oligonucleotide still attached to the support was achieved by treatment with 1 M DBU solution in acetonitrile on the synthesizer[8]. The 2-cyanoethyl as well as the NPE and NPEOC groups were completly removed within 11 hours[9] and washed away separating the protecting group residues easily from the deblocked oligomer which was finally cleaved from the solid support by treatment with concentrated ammonia at room temperature for 2 hours and yielding directly 220 OD_{260} units of crude material free of any by-products.

This material was analysed by HPLC on an ion exchange column[10] (fig. 1) and by polyacrylamide-gelelektrophoresis indicating one main product and nearly no failure sequences. The correct nucleoside composition of the 78-mer oligoarabinonucleotide was checked by enzymatic digestion[11] with snake venom phosphodiesterase from *crotalus durissus* and alkaline phosphatase. The HPLC chromatogram[12] of the nucleoside mixture of the degraded oligomer and the calculated and found values of the arabinonucleoside ratio is shown in fig. 2.

Fig. 1: HPLC profile of the crude 78-mer

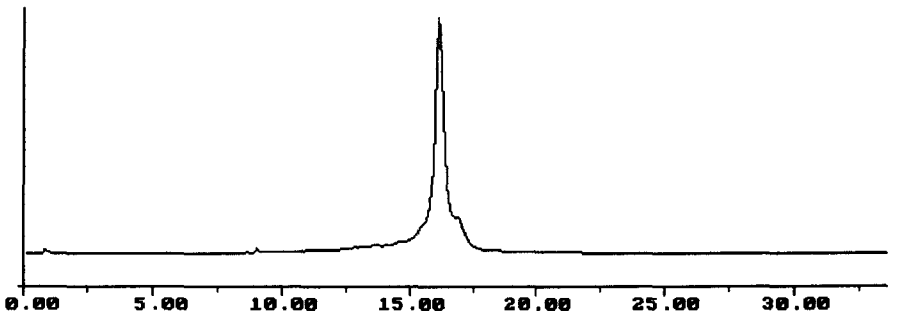
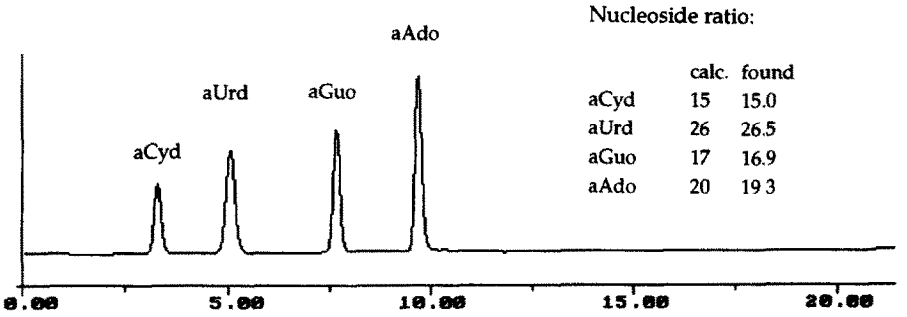


Fig. 2: HPLC profile of the enzymatic digest of the 78-mer



Nucleoside ratio:

	calc.	found
aCyd	15	15.0
aUrd	26	26.5
aGuo	17	16.9
aAdo	20	19 3

References and notes:

1. E. Uhlmann, A. Peyman Chem. Rev. **1990**, *90*, 543
2. M.J. Damha, N. Usman, K.K. Ogilvie Tetrahedron Lett. **1987**, *28*, 1633
3. M.J. Damha, N. Usman, K.K. Ogilvie Can. J. Chem. **1989**, *67*, 831
4. K.P. Stengele, W.Pfleiderer Tetrahedron Lett. **1990**, *31*, 2549
5. M. Resmini, W. Pfleiderer Helv. Chim. Acta **1993**, *31*, 2549
6. G. Breipohl, Hoechst AG, Frankfurt-Hoechst / Germany; personal communication
7. A mixture of 250 mg support, 150 µmol arabinonucleoside 3'-succinate, 375 µmol N-methylmorpholin, 150 µmol TOTU in 5 ml dry Acetonitrile was kept at room temperature for 4 - 24 hours. The support was filtered off, washed with DMF, MeOH, Et_2O and then capped by treatment with 1.5 ml of Ac_2O, 50 mg of 4-dimethylaminopyridine in 5 ml pyridine for 30 min.
8. The DBU bottle was attached to position No 12 at the ABI DNA-Synthesizer 392 and a suitable end procedure was created.
9. After 1 hour and then every 2 hours fresh DBU solution was pumped into the reaction chamber.
10. Gen-Pak FAX column (Millipore); linear gradient of 11 mM NaH_2PO_4, 0.11 M NaCl in H_2O/acetonitrile 4:1 to 11 mM NaH_2PO_4, 1.5 M NaCl in H_2O/acetonitrile 4:1 in 30 min, then held isocratically for 5 min; flow rate 0.75 ml/min.
11. 1 OD_{260} of the oligonucleotide, 50 µl buffer (50 mM tris HCl, 10 mM $MgCl_2$, pH 8), 3 µl snake venom phosphodiesterase and 5 µl alkaline phosphatase (enzymes from Boehringer Mannheim / Germany) were incubated at 37°C overnight. Then the mixture was heated to 80°C for 5 min, centrifugated and analysed.
12. Lichrospher 100 RP-18 column 5 µm, 4 x 125 mm (Merck); isocratically for 2 min 0.1 M triethylammonium acetate (TEAAc) pH 7, then linear gradient to 10% acetonitrile / 0.1 M TEAAc in 15 min, then to 50% acatonitrile / 0.1 M TEAAc in 5 min; flow rate 1 ml/min.

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