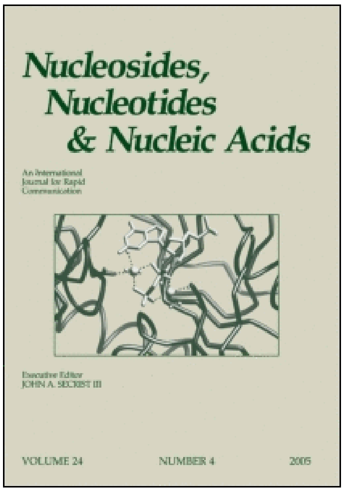

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Isolation and Characterization of Diastereomeric 2'-Oeoxy-ribonucleoside-3'-phosphoramidites

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ISOLATION AND CHARACTERIZATION OF DIASTEREOMERIC 2'-DEOXYRIBONUCLEOSIDE-3'-PHOSPHORAMIDITES

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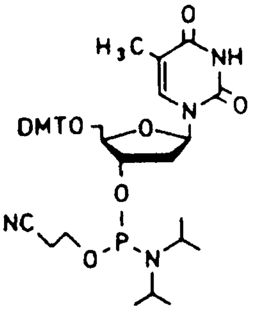
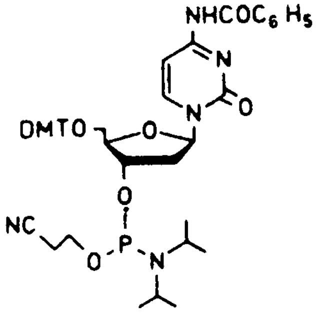
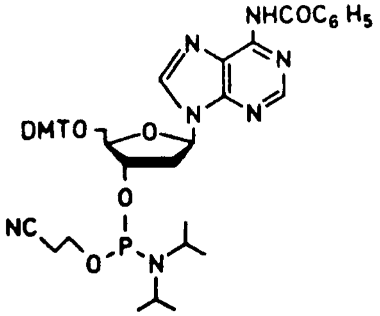
Abstract: The commonly used four 2'-deoxyribonucleoside-3'-(O-β-cyanoethyl, N-diisopropyl)-phosphoramidites have been separated into the two diastereomers by chromatographical means. The ^{1}H-NMR spectra of the two components show characteristic differences, which were utilized to assign the absolute configurations of the P(III)-centers.

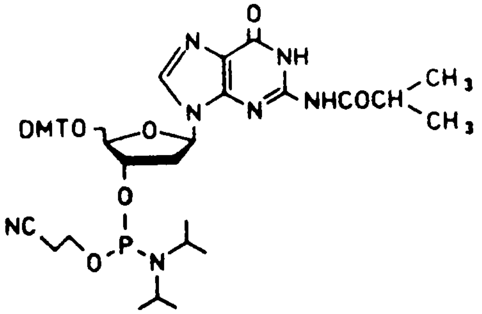
Recent investigations with phosphorothioate analogues of 2',5'-oligoadenylate have shown that three of the four diastereomeric trimer cores with RpRp, RpSp and SpRp internucleotide linkages are able to activate RNase L (1,2). The order of RNase L activation was the same for the core analogues and their 5'-monophosphates (RpRp > SpRp > RpSp). The SpSp and pSpSp analogues, however, inhibit the activation process and the latter derivative is so far the most effective inhibitor of RNase L able to shut down the antiviral mode of action of the system.

Since the chemical synthesis of a thiophosphodiester function is commonly achieved by the phosphoramidite method (3,4) diastereomeric mixtures are always obtained affording further tedious chromatographical separations to get the stereochemicall pure components (5). A stereospecific approach is therefore needed to form directly chiral thiophosphodiesters of known absolute configuration. Recent stereochemical studies on these lines indicated that the forma-





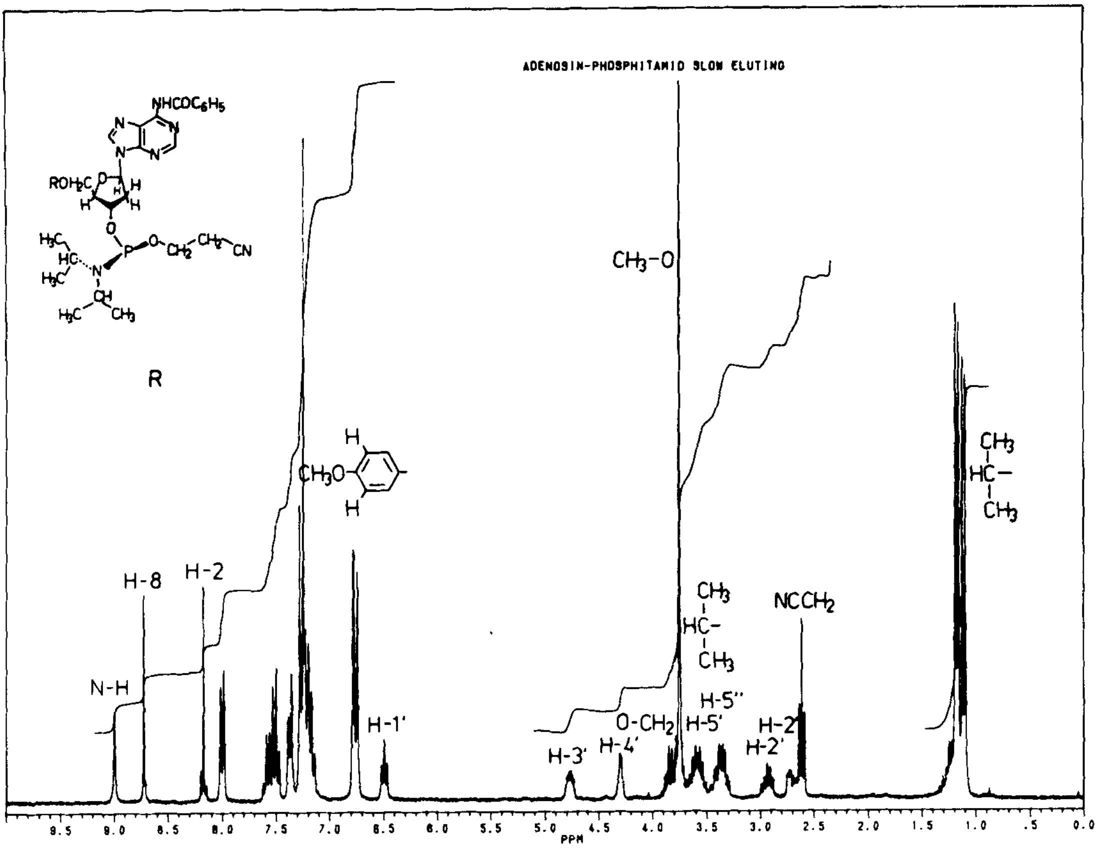


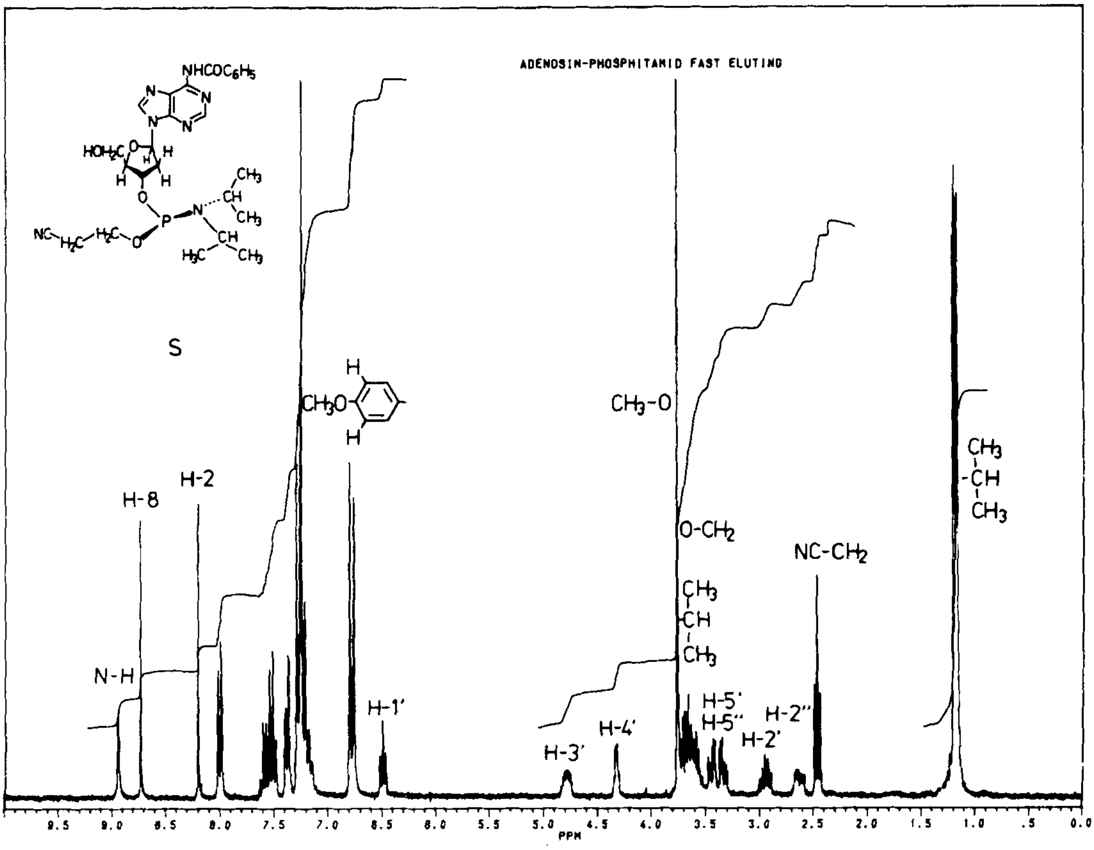


tion of chiral internucleotide linkages by phosphoramidite-coupling in the solid-phase synthesis of oligodeoxyribonucleotide analogues gave on 1H-tetrazole catalysis the intermediary phosphites with with epimerization at phosphorus (6,7). The subsequent conversion of the phosphite moieties into P-chiral-modified internucleotide linkages led on sulfurization with S_8 consequently to diastereomeric mixtures.

Despite these results we decided to separate the diastereomeric mixtures of each of the four commonly protected 2'-deoxyribonucleoside-3'-(O-ß-cyanoethyl, N-diisopropyl)-phosphoramidites into the pure components.

The separation was achieved in preparative scale by chromatographical means using a low-pressure technique (8) on si-


ADENOSIN-PHOSPHITAMID SLOW ELUTING
NHCOC6H5
ROH2C
R
CH3-O
CH3O
H
N-H
H-8
H-2
H-1'
H-3'
H-4'
O-CH2
H-5'
H-5''
CH3
HC-
CH3
NCCH2
H-2'
H-2''
CH3
HC-
CH3
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0.0
PPM


ADENOSIN-PHOSPHITAMID FAST ELUTING
NHCOC6H5
HOH2C
S
CH3-O
CH3O
H
N-H
H-8
H-2
H-1'
H-3'
H-4'
O-CH2
CH3
-CH
CH3
H-5'
H-5''
H-2'
H-2''
NC-CH2
CH3
-CH
CH3
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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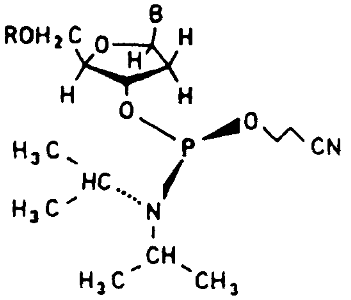
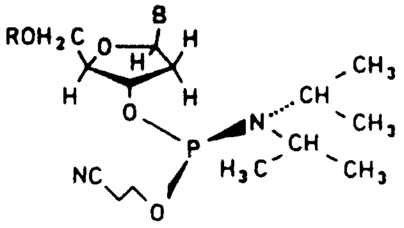
1.0
.5
0.0
PPM



R



S

lica gel and an ethyl acetate/toluene 1:1 mixture as mobile physe. ^{1}H-NMR spectra of the various diastereomers reveal consistent features which will allow a straightforward assignment of the absolute configuration of the chiral P(III)-moiety. All fast eluting components show little chemical shift differences of the diastereotopical methyl doublets of the N-disopropyl group, whereas a distinct separation of the two doublets is clearly recognized in the slowly eluting isomers. Furthermore the methylene signals of the ß-cyanoethyl group always appear at higher field in the former and at lower field in the latter diastereomer. The other ^{1}H-signals are more or less identical as expected from structural analogies.

The configurational differences are also reflected in the ^{31}P-NMR spectra, but here the chemical shifts do not correlate with the chromatographical mobilities.

A speculative assignment of the absolute configuration of the P-center is based on the ^{1}H-NMR data indicating a stronger through-space interaction of the ring-O-atom of the 2'-deoxyribose ring on one of the isopropyl groups in the R-isomer, whereas the isopropyl groups of the S-diastereomer encounter much less disturbance from the ribosyl moiety.

We are currently trying to crystallize at least one of the diastereomeric phosphoramidites to prove our assumption by an X-ray analysis. Furthermore coupling reactions with the purified diastereomers are under investigation to find special conditions for a stereospecific built-up of thiophosphodiesters of high diastereomeric excess.

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