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FAST IDENTIFICATION BY FAB-MASS SPECTROMETRY OF BUILDING BLOCKS FOR OLIGONUCLEOTIDE SYNTHESIS

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Abstract: Negative ion FAB-MS is recommended for the rapid routine identification of monomeric as well as oligomeric building blocks used in the phosphotriester synthesis of DNA fragments.

During the past few years the chemical synthesis of DNA fragments has become progressively more important for modern biosciences. This has mainly been made possible by some major progress in the synthesis technology, in particular the use of better coupling and deprotection methods, the employment of suitable solid supports and refined isolation and characterization techniques. A recent development in our laboratory for the simultaneous synthesis of large numbers of oligonucleotides on segmental solid supports ("the filter approach")[1] has further increased our interest in rapid and reliable characterization techniques for the nucleotide building blocks. Fisher and Caruthers reduced to some extent the error probability of the cyclic elongation reactions by introducing the use of triarylmethyl groups which have different colours in acid[2]. Although such a set of protecting groups may be useful for the monitoring of monomer additions during the synthesis of an oligonucleotide, it is of less importance for oligomer additions and does not rule out an unambiguous characterization of any building block. We have applied NMR[3] and MS[4] methods to characterize monomeric building blocks and to sequence oligomeric building blocks for the phosphotriester approach.
Fully deprotected oligonucleotides show a very simple fragmentation behaviour in the negative FAB-MS mode which was used to postulate a general rule for the sequence elucidation of oligonucleotides[5,6]. While our initial attempts to analyze fully protected building blocks by FAB-MS were unsuccessful, these substances became susceptible to FAB-MS analysis simply by liberating one (or more) phosphate charge(s). Such compounds with one phosphate charge, which may be directly used in the phosphotriester synthesis method, generally show fragmentation patterns similar to those obtained with the fully deprotected oligonucleotides[5]. However, the spectra are more complex due to some extra bond breakages which occur next to the protecting groups[7]. A full spectrum of a partially protected dinucleoside diphosphate is given in Figure 1. We have recorded spectra of all 16 possible dimers (see Table 1). In principle, we need to see only the molecular ion and the nucleoside-5',3'-diphosphate ion, which both give rise to intense peaks, to determine the base sequence (bold faced in table). The 5'-OH-nucleoside monophosphate and the monomethoxytrityl protected nucleoside-3'-monophosphate ions can be used to confirm the sequences.

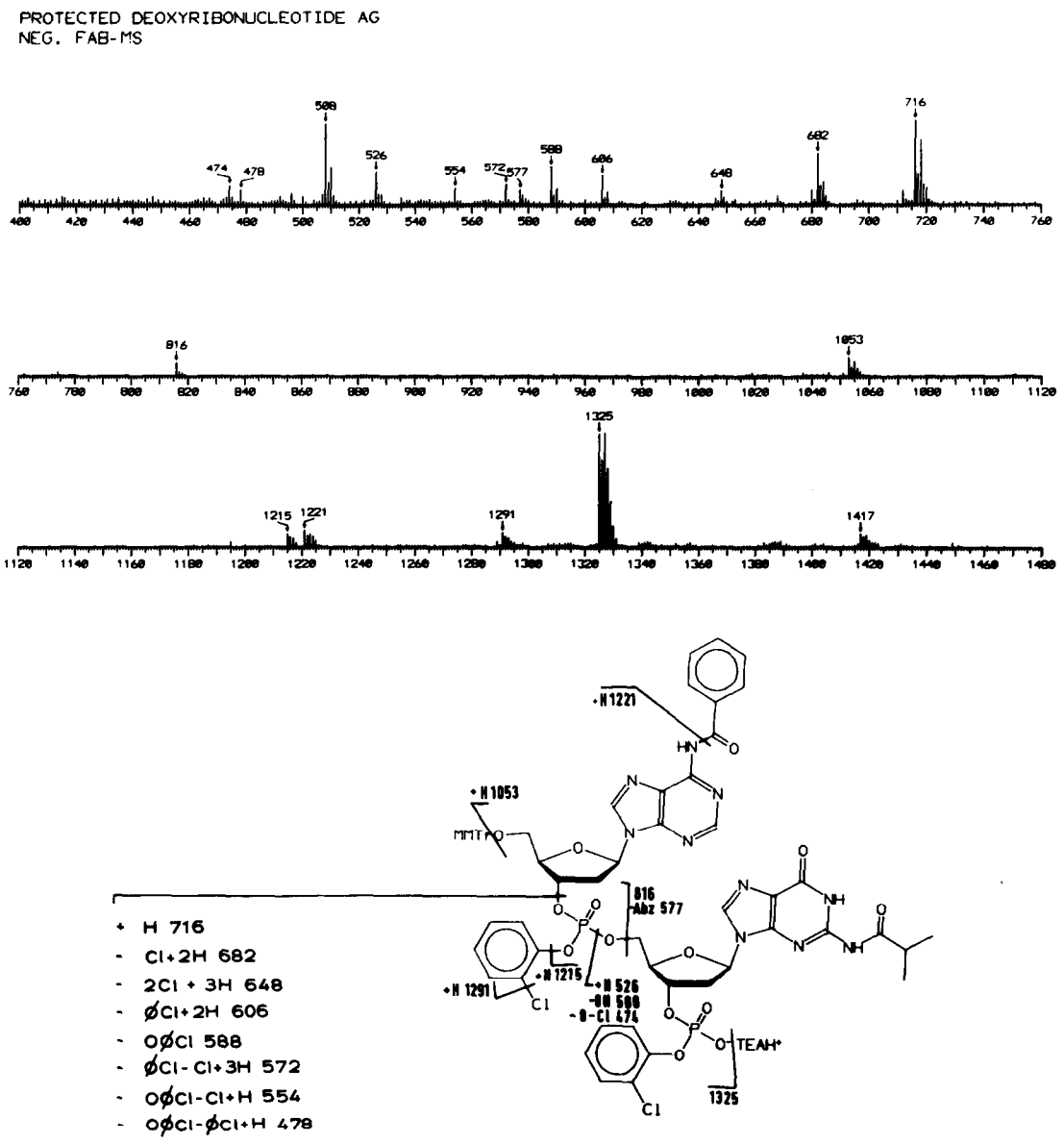

Figure 1: Negative ion FAB mass spectrum and structure formula of d[MMTr $A^{bz}p_{oCP}G^{ib}p_{oCP}O^{-}$]. The interpretation of the spectrum is given in the figure. Mass spectra were recorded with a KRATOS MS 50 S mass spectrometer with a KRATOS FAB-source. Xenon was used for the atom gun at 8-9 kV. The matrix was glycerol for the dimers and a 1:1 mixture of glycerol/triethyleneglycol for the higher oligomers.

X \ Y	A	C	G	T
A	M 1343 pAp 734 Ap 544 MMTrAp 816	M 1349 pCp 740 Cp 550 MMTrAp 816	M 1325 pGp 716 Gp 526 MMTrAp 816	M 1230 pTp 621 Tp 431 MMTrAp 816
C	M 1349 pAp 734 Ap 544 MMTrCp 822	M 1355 pCp 740 Cp 550 MMTrCp 822	M 1331 pGp 716 Gp 526 MMTrCp 822	M 1236 pTp 621 Tp 431 MMTrCp 822
G	M 1325 pAp 734 Ap 544 MMTrGp 798	M 1331 pCp 740 Cp 550 MMTrGp 798	M 1307 pGp 716 Gp 526 MMTrGp 798	M 1212 pTp 621 Tp 431 MMTrGp 798
T	M 1230 pAp 734 Ap 544 MMTrTp 703	M 1236 pCp 740 Cp 550 MMTrTp 703	M 1212 pGp 716 Gp 526 MMTrTp 703	M 1117 pTp 621 Tp 431 MMTrTp 703

Table 1: Sequence ions of protected deoxyribodinucleotides type: M = d[MMTr Xp_{oCP} $Yp_{oCP}O^-$]; X,Y: A^{bz},C^{an},G^{ib},T

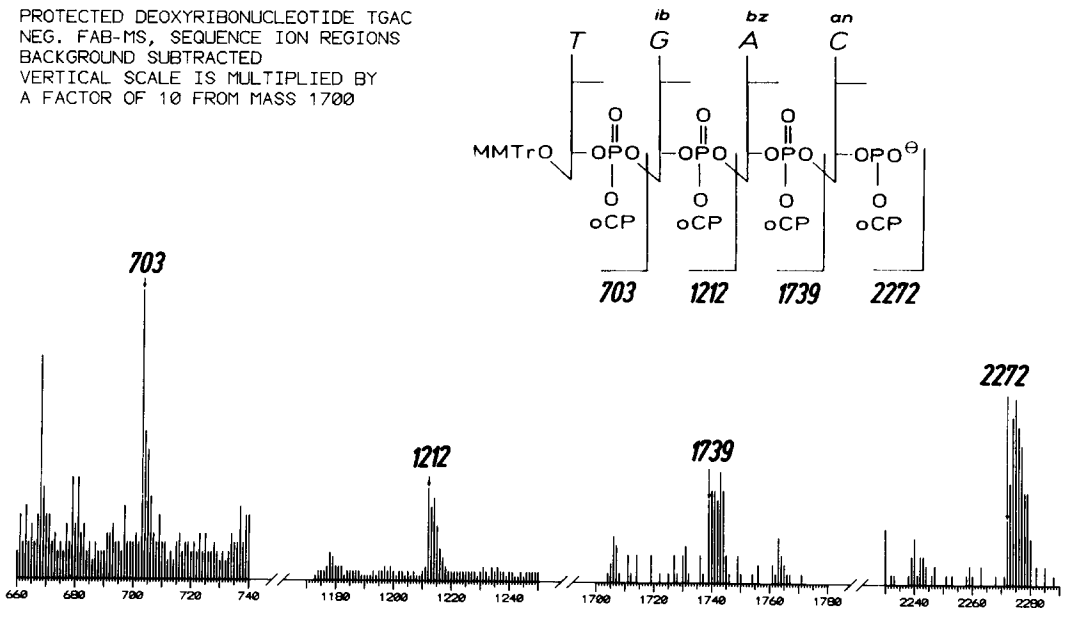

Figure 2: Relevant regions of the negative-ion FAB mass spectrum and shorthand structure of d[MMTr $Tp_{oCP}G^{ib}p_{oCP}A^{bz}p_{oCP}C^{an}p_{oCP}O^-$].

In addition we recorded spectra of many higher oligomers up to the hexamer level (mass ca. 3400). Shorthand structure and the relevant regions of a tetramer spectrum are given in Figure 2. Compared to the dinucleotides the protected oligomers show a less complex fragmentation behaviour, with the monomethoxytrityl protected 3'-phosphate fragment ions giving the most prominent peaks. From the examination of this set of ions the base sequence of the oligomer is easily deduced.

References and Footnotes:

1. R. Frank, W. Heikens, G. Heisterberg-Moutsis and H. Blöcker, Nucl. Acids Res. 11, 4365 - 4377 (1983).
2. E.F. Fisher and M.H. Caruthers, Nucl. Acids Res. 11, 1589 - 1599 (1983).
3. L. Ernst et al., submitted.
4. L. Grotjahn, R. Frank and H. Blöcker, presented at the 31st Annual Conference on Mass Spectrometry and Allied Topics, Boston, MA, USA, May 8-13; abstracts p. 644 (1983).
5. L. Grotjahn, R. Frank and H. Blöcker, Nucl. Acids Res. 10, 4671 - 4678 (1982).
6. Some exception from this rule have been observed especially for very short chains. This has recently also been reported by Panico et al., J. Am. Chem. Soc. 105, 5607 - 5610 (1983).
7. Similar complex fragmentation patterns of these compounds were described also for ^{252}Cf-plasma desorption mass spectrometry by McNeal et al., J. Am. Chem. Soc. 104, 972 - 975 (1982), and for secondary ion mass spectrometry by Ens et al., Anal. Chem. 54, 960 - 966 (1982) and Beavis et al. Int. J. Mass Spectrom. Ion Phys. 46, 475 (1983).

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