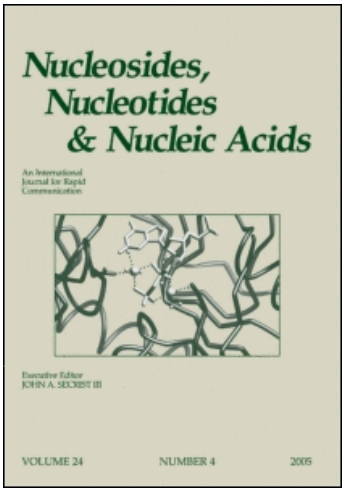

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TIME-OF-FLIGHT SECONDARY ION MASS SPECTROMETRY OF BRANCHED RNA FRAGMENTS: MESSENGER RNA SPLICING INTERMEDIATES

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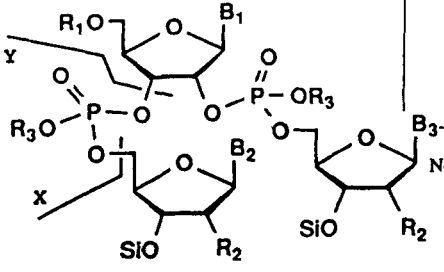
Abstract: The negative ion mass spectra generated by a reflecting time-of-flight mass spectrometer are reported for a series of protected oligonucleotides. Quasimolecular and sequence ions have been detected, and the location and nature of protecting groups have been confirmed.

Secondary ion mass spectrometry (SIMS) with a time-of-flight (TOF) mass analyzer is a powerful analytical tool for studies of non-volatile and thermally sensitive biomolecules. We have investigated the use of TOF-SIMS for characterization of a series of protected trinucleotides and a tetranucleotide (see below), all containing an unusual feature: vicinal 2′-5′ and 3′-5′



COMPOUND	R1	R2	R3	B1	B2	B3
$G^{U}U$	H	OSi	Me	$_{Bz}G^{NPE}$	U	U
$A^{C}C$	H	OSi	CE	A^{Bz}	C	C
$A^{T}T$	H	H	Me	A^{Bz}	T	T
$A^{U}U$	H	OSi	Me	A^{Bz}	U	U
$UA^{U}U$	*U	OSi	Me	A^{Bz}	U	U

Notes: Si = t-Bu$(CH_3)_2$Si-
Me = CH_3
CE = $NC\text{-}CH_2\text{-}CH_2$-
Bz = C_6H_5CO- (on N^2 of G and N^6 of A)
NPE = p-$NO_2C_6H_4CH_2CH_2$- (on O^6 of G)
*U = Uridine-2'-OSi-3'-methyl phosphate

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phosphodiester linkages. These branched RNA fragments, synthesized at McGill University[1], are key constituents of lariats which are believed to play an essential role in the splicing of messenger RNA precursors[2].

Negative ion spectra were obtained with the Manitoba TOF II mass spectrometer which incorporates an ion mirror[3]. Sample preparation and instrumentation have been described elsewhere[4].

All compounds show prominent quasimolecular ion signals, although the loss of one or more protecting groups from nucleoside or phosphate oxygens gives ions with 39% to 56% of the $[M-H]^-$ ion abundance. One of the main fragmentation pathways involves cleavage of glycosyl bonds with charge retention on the nucleobases. With the exception of UA^UU, the abundance of base ions originating from the branching nucleotide is always greater than the total contribution of both peripheral bases i.e. $B_1^- > (B_2^- + B_3^-)$. The ratio $(B_2^- + B_3^-)/B_1^-$ (see Fig.1) increases according to $G^UU < A^CC < A^TT < A^UU < UA^UU$. This supports a rationale involving the assistance of 2'-phosphate oxygen in cleaving the glycosyl bond of the branching nucleotide[5]. In addition, for the trinucleotides, 5'-phosphate sequence ions (Y^-, Fig.1) are always more abundant than 3'-phosphate sequence ions (X^-), and the Y^-/X^- abundance ratio increases according to $A^UU < A^TT < A^CC < G^UU$. In the case of UA^UU, sequence ions produced by cleavage of sugar-phosphate bonds of the branching nucleotide are more abundant than those of terminal nucleotides. These results further demonstrate the utility of time-of-flight SIMS as an analytical tool for the analysis of oligonucleotides. We are currently extending this work to deprotected nucleotides.

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