FOR RENEWAL DATE 1/15/89

DOE/ER/60526--001

A NEW APPROACH TO THE ANALYSIS OF RADIOPHARMACEUTICALS

PROGRESS REPORT

for the period November 1 1987 - October 1 1988

Department of Energy Grant No. DE-FG-2-87 ER60526

DISCLAIMER

HARVARD MEDICAL SCHOOL SITE

F 4620.1
(7-85)

U.S. Department of Energy

Grant Application Budget Period Summary

(See Reverse for Definitions and Instructions)

OMB Approval
No. 1910-1400

Please Print or Type

janization: Department of Radiology Harvard Medical School	Period Covering:	FOR DOE USE ONLY
	From: 1/15/89	Proposal No:
icipal Investigator (P.I.)/Project Director (P.D.): Alun G. Jones, PhD	To: 1/14/90	Award No.:

	DOE Funded Person-Mos.			Funds Requested By Applicant
NIOR PERSONNEL PI/PD Co PIs, Faculty and Other Senior Associates ist each separately with title, A 6 show number in brackets ach separate sheet, if required.)	Cal.	Acad	Sumr.	$
1. Alun G. Jones PhD (20%)	2.4			16,496
2. James F. Kronauge PhD (25%)	3			10,406
) TOTAL SENIOR PERSONNEL				
HER PERSONNEL (SHOW NUMBERS IN BRACKETS)				
) POST DOCTORAL ASSOCIATES				
) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)				
) GRADUATE STUDENTS				
) UNDERGRADUATE STUDENTS				
5.) SECRETARIAL-CLERICAL -Administrative Alice Carmel (5%)				1,500
) OTHER				
OTAL SALARIES AND WAGES (A + B)				28,402
C. INGE BENEFITS (IF CHARGED AS DIRECT COSTS) 23% SW at HMS				6,532
OTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				34,934
UIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM) D.				
AL EQUIPMENT				
E. AVEL 1. DOMESTIC (INCL. CANADA AND U.S. POSSESSIONS)				800
2. FOREIGN 1 person, partial support				800
F. HER DIRECT COSTS				
1 ATERIALS AND SUPPLIES Chemicals, disposable labware, HPLC supplies				5,016
2 UBLICATION COSTS/PAGE CHARGES				750
3 ONSULTANT SERVICES				-0-
4 OMPUTER (ADPE) SERVICES				
5 ONTRACTS AND SUBGRANTS Service contract - Rainin/Apple HPLC System				765
6 THER Office supplies, telephone, postage, xerox				560
TAL OTHER DIRECT COSTS				7,091
G. TAL DIRECT COSTS (A THROUGH F)				43,625
IndIRECT COSTS (SPECIFY RATE AND BASE)				
H. AL INDIRECT COSTS 67.7% MTDC at HMS				29,535
I. AL DIRECT AND INDIRECT COSTS (G & H)				73,160
J. PLICANT'S COST SHARING (IF ANY)				-0-
K. TAL AMOUNT OF THIS REQUEST (ITEM I LESS ITEM J)				73,160

D TYPED NAME & SIGNATURE Alun G. Jones Ph.D. Alun G. Jones	DATE 11/3/88
T. REP. TYPED NAME & SIGNATURE	DATE

1. **Project Title:** A New Approach to the Analysis of Radiopharmaceuticals

Principal Investigator: Alun G. Jones, PhD
Organization: Department of Radiology
Harvard Medical School
Address: Harvard Medical School, 50 Binney Street, Boston, MA 02115
Telephone: 617-732-2184

Co-Principal Investigator: Alan Davison, PhD
Organization: Department of Chemistry
Massachusetts Institute of Technology
Address: Room 6-435, Massachusetts Insitute of Technology,
77 Massachusetts Avenue, Cambridge, MA 02139
Telephone: 617-253-1794

Co-Principal Investigator: Catherine E. Costello, PhD
Organization: Department of Chemistry
Massachusetts Institute of Technology
Address: Mass Spectrometry Facility, Room 56-029
Massachusetts Institute of Technology
77 Massachusetts Avenue, Cambridge, MA 02139

2. Key Investigators

Investigator	Percentage of Time
Alun G. Jones, PhD	20%
Alan Davison, PhD	15% (academic)
Catherine E. Costello, PhD	5%

3. Curriculum Vitae

The curriculum vitae of the principal investigators are included in the original grant submission.

4. Progress Report

This research is designed to investigate analytical techniques that may be used in the study of both the basic chemistry and the radiopharmaceutical chemistry of the transition metal element technetium. The original submission funded in 1987 described possible new uses in this field of high pressure liquid chromatography, mass spectrometry, and resonance Raman spectroscopy. Since then we have added the significant capability of MS/MS measurements on isolated technetium complexes, making possible the study of individual species and fragments observed in the spectra (Figures 1 and 2). Another important technique which we began to use last year and have extended during this reporting period is the use of ^{99}Tc NMR. Below is a brief outline of the investigations carried out in the past year, concentrating mainly upon MS and NMR.

FABMS and FAB MS/MS Investigations of technetium and rhenium complexes

One of the more important aspects of technetium chemistry is as thorough an understanding of the structure and reactivity of new complexes as possible. To this end, the fragmentation pathways of technetium and rhenium coordination compounds have been explored by recording the metastable (MS/MS) and collision induced decomposition (CID MS/MS) spectra of molecular ions, cluster ions and fragment ions that appear in the fast atom bombardment mass spectra (FABMS) of three series of related compounds. Ultimately this should provide insight into the effects that variations in the ligands, the metal core and the oxidation state of the the metal have upon ion structure and chemistry. The three series are chloro(1,2-dioxolato)oxo-(1,10-phenanthroline)technetium(V) complexes, sterically hindered arene thiolates of the general structure Tc(III)$(SAr)_3L_2$, and hexakis aryl isonitrile technetium(I) cations.

FABMS and MS/MS spectra obtained with or without the use of collision gas show that the behavior of these complexes appears indeed to reflect changes in properties that accompany modifications of ligand, oxidation state and metal core. Furthermore, because MS/MS spectra are free of matrix-related ions, they are simpler to interpret than the FABMS spectra. They are also useful to clarify the many fragmentation pathways because they permit observation of products originating from individual species.

Chloro(1,2-dioxolato)oxo-(1,10-phenanthroline)technetium(V) complexes have proven to be a useful source of the TcO^{3+} core for ligand exchange reactions (1,2). $TcOCl_3$(phen) reacts with olefins to produce <u>cis</u>-diols. The fragmentation pattern shown in Figure 3 was deduced on the basis of MS/MS spectra obtained for six members of the series, chosen as representative of the structural variations among more than ten 1,10-phenanthroline analogs and many more analogs containing other aromatic ligands for which the two-sector spectra have been recorded. Facile chlorine loss is always observed (Figs. 4,5) but there is a minor pathway which retains the chlorine atom while the ethanedioxalate ligand is lost. Fragmentation may occur within the ethanedioxalate ligand or it may be lost completely. Only after loss of other ligands is bond-breaking within the phenanthroline ligand observed, and then it may be fragmented or stripped away entirely to leave Tc(I) (Figs. 6,7).

Complexes of technetium with sterically hindered arene thiooxalates, Tc(III)(SAr)$_3$L$_2$, can exchange their ligands without distrubing the Tc(SAr)$_3$ core (3,4). MS/MS spectra of common ions derived from complexes that vary in the axial ligand, and obtained with or without the presence of collision gas, are indistinguishable thereby suggesting the formation of identical intermediates (Figs. 8-11). The spectra obtained from analogs that contain rhenium, however, show quite different fragmentation patterns that reflect the difference in the oxidation potentials of the two elements. Like the first group of complexes, the arene thiooxalates form dimers in their FAB mass spectra. However, while the CID MS/MS spectrum of (2M-Cl)$^+$ Tc(V) dioxolato species indicates that the dimer most readily collpases back to the monomer, the (2M-L$_n$)$^+$ ions of the Tc(III)(SAr)$_3$L$_2$ complex form a variety of product ions by sequential loss of ligands but with retention of both Tc atoms (Fig. 12).

The hexakis alkylisonitrile Tc(I) cations generally produce FABMS and MS/MS spectra that are characterized by multiple alkyl or alkene losses to form Tc[CN(H)]$_n$(CNR)$_{6-n}$ species. The spectrum of the hexakis aryl isonitrile cation is quite simple, being dominated by CNAr losses. Upon CID, the doubly charged ion is formed from the singly charged ion by two routes of apparently equal probability: loss of a second electron and loss of a proton (Fig. 13). CID of the doubly charged cation leads predominantly to (M-CNAr$_n$)$^{++}$ species; the few singly-charged ions formed show loss of Ar and/or CNAr. The substitution of aryl for alkyl groups affects both the spectra and the chemistry of these complexes.

^{99}Tc NMR Studies

^{99}Technetium is one of the most sensitive nuclei known for NMR spectroscopy with a receptivity relative to ^{1}H of 0.275, a value which is 2.134 times that of ^{13}C. The ^{99}Tc nucleus is quadrupolar, having a nuclear spin I = 9/2 and a magnetogyric ratio of 6.0211 x 10^7 rad.s^{-1}.T^{-1}. A further advantage is that samples are monoisotopic in technetium. The standard reference sample used in this work is taken as (NH^4)[TcO$_4$] in H$_2$O/D$_2$O which resonates with Ξ = 22.508.304 (5), which is chosen for its sharp resonance ($\Delta\nu_{1/2}$ = 3Hz) near the centre of the known chemical shift range. Because of the high sensitivity and convenient resonance frequency of this nucleus together with the increasing number of diamagnetic complexes being discovered, particularly in oxidation states +7, +5, and +1, we now believe this form of spectroscopy will prove very useful in the study of technetium chemistry.

In the previous progress report we reported early studies that indicated shifts varying over a range of >7000 ppm. It is now clear that there is a correlation between the chemical shift and the oxidation state. The technetium(V) species investigated all resonate downfield of TcO_4^- indicating that the nucleus is heavily deshielded. This effect is also noted in the ^{95}Mo spectra of the isoelectronic molybdenum(V) species and is in accord with the Griffith and Orgel theory (6). Because of quadrupolar relaxation mechanisms, the bandwidth of the resonances depend directly on the electric field gradient experienced by the Tc nucleus. Therefore in symmetrical complexes such as TcO_4^- and $Tc(CNR)_6^+$ narrow lines are observed allowing for accurate dterminatiion of chemical shifts. With less symmetrical molecules, for example, $TcO_2(en)_2^+$ the lines are >2000 Hz wide at half-height limiting the information available.

Some examples of the kind of data that may be obtained now follow. It has long been known that the green color of $TcOCl_4^-$ intensifies upon dissolution in coordinating solvents, suggesting an interaction <u>trans-</u> to the oxo ligand. Investigation of the NMR resonance of this compound in MeOH and CH_2Cl_2 (coordinating and noncoordinating solvents, respectively) shows significant changes in both the position and width of the spectral lines. Such experiments should permit measurement of chemical exchange and equilibria in solution.

The complex $Tc(CNBu^t)_4(bpy)^+$ has been prepared and is nominally a technetium(I) complex, being a derivative of $Tc(CNBu^t)_6^+$. The NMR resonance for this complex at -844 ppm does not occur in the expected region for Tc(I), but rather in that of known Tc(III) complexes. Inspection of the X-ray crystal structure of this ion reveals that that the isonitrile ligands are distinctly nonlinear, with the CNC bond angle of one being 148° while the others are 165°, 167°, and 168°. Based upon both the magnetic and structural information, therefore, the complex can almost be regarded as trivalent.

We have also studied the effect of small changes in the ligands in order to determine whether this technique is capable of differentiating closely related complexes. A large number of coordination compounds of the form $Tc(CNR)_6^+$ were prepared and their ^{99}Tc NMR shifts determined. As shown in the table on next page, the change in chemical shift as a function of R is small compared to that between different oxidation states; the narrow linewidths of t70 Hz, however, permit clear differentiation between these species. The sensitivity and specificity of this technique for the technetium(I) isonitrile complexes is now being investigated for the study of speciation in biological tissue.

^{99}Tc chemical shifts* for $[Tc(CNR)_6]PF_6$ complexes

R	δ (ppm)	R	δ (ppm)
Ph	+24.7	$C(Me)_2CO_2Me$	-22.7
2,6-Me_2Ph	+20.4	i-Bu	-24.8
t-Bu	0.0	Cy	-24.8
i-Pr	-1.5	$C(Me)_2CH_2C(Me)_3$	-30.8
Et	-2.4	CH_2CO_2(t-Bu)	-32.0
Me	-2.5	$CH_2C(Me)_2OMe$	-36.3
n-Pr	-14.7	$CH(Et)CO_2Me$	-38.2
n-Bu	-16.3	$CH(i\text{-}Pr)CO_2Me$	-49.8

* Shifts quoted with $[Tc(CNBu^t)_6]PF_6$ set arbitrarily to 0.0. This complex resonates at -1914 ppm relative to TcO_4^-.

Literature Cited

1. Pearlstein RM. Applications of ligand exchange reactions of technetium complexes. PhD Thesis, Massachusetts Institute of Technology, May 1988.

2. Pearsltein RM, Lock CJL, Faggiani R, Costello CE, Zeng C-H, Jones AG, Davison A. Synthesis and characterization of technetium(V) complexes with amine, alcoholate, and chloride ligands. Inorg Chem. (submitted).

3. De Vries NH. Synthesis and study of complexes of technetium with sterically hindered arene thiolates. PhD thesis, Massachusetts Institute of Technology, May 1988.

4. De Vries N, Dewan JC, Jones AG, Davison A. Complexes of technetium(III) sterically hindered arenethiolates and their interactions with small π-accepting molecules. Inorg Chem. 1988; 27: 1574-1580.

5. Buckingham MJ, Hawkes GE, Thornback JR. Inorg Chim Acta 1981; L4: 56.

6. Griffith JS, Orgel LE. Trans Faraday Soc. 1957; 53: 601.

5. Aims for the next period

The specific aims for the final year of this cycle remain unchanged from those described in the original application. The only addition is the continued study of the applications of ^{99}Tc NMR to the problems outlined.

Publications

The following is a listing of publications describing research conducted either wholly or in part with support from this grant.

1. Piwnica-Worms D, Kronauge JF, Holman BL, Lister-James J, Davison A, Jones AG. Hexakis(carbomethoxyisopropylisonitrile)technetium(I) [Tc-CPI], a new myocardial perfusion agent: Binding characteristics in cultured chick heart cells. J Nucl Med. 1988; 29: 55-61.

2. De Vries N, Dewan JC, Jones AG, Davison A. Complexes of technetium(III) sterically hindered arenethiolates and their interactions with small π-accepting molecules. Inorg Chem. 1988; 27: 1574-1580.

3. Davison A, Kronauge JF, Jones AG, Pearlstein RM, Thornback JR. ^{99}Tc NMR spectroscopy of technetium(I) phosphine and phosphite complexes. Inorg Chem. 1988; 27: 3245-3246.

4. Piwnica-Worms D, Kronauge JF, Holman BL, Davison A, Jones AG. Comparative myocardial binding characteristics of hexakis(alkylisonitrile)technetium(I) complexes: Effect of lipophilicity. Invest Radiol. (in press).

5. Davison A, Faggiani R, Franklin KJ, Jones AG, Lock CJL, Sayer B, Schrobilgen GJ, Trop HS. NMR studies of d^2 systems: trans-$[TcO_2(CN)_4]^{4-}$. Inorg Chem. (submitted).

6. Pearsltein RM, Lock CJL, Faggiani R, Costello CE, Zeng C-H, Jones AG, Davison A. Synthesis and characterization of technetium(V) complexes with amine, alcoholate, and chloride ligands. Inorg Chem. (submitted).

Abtracts

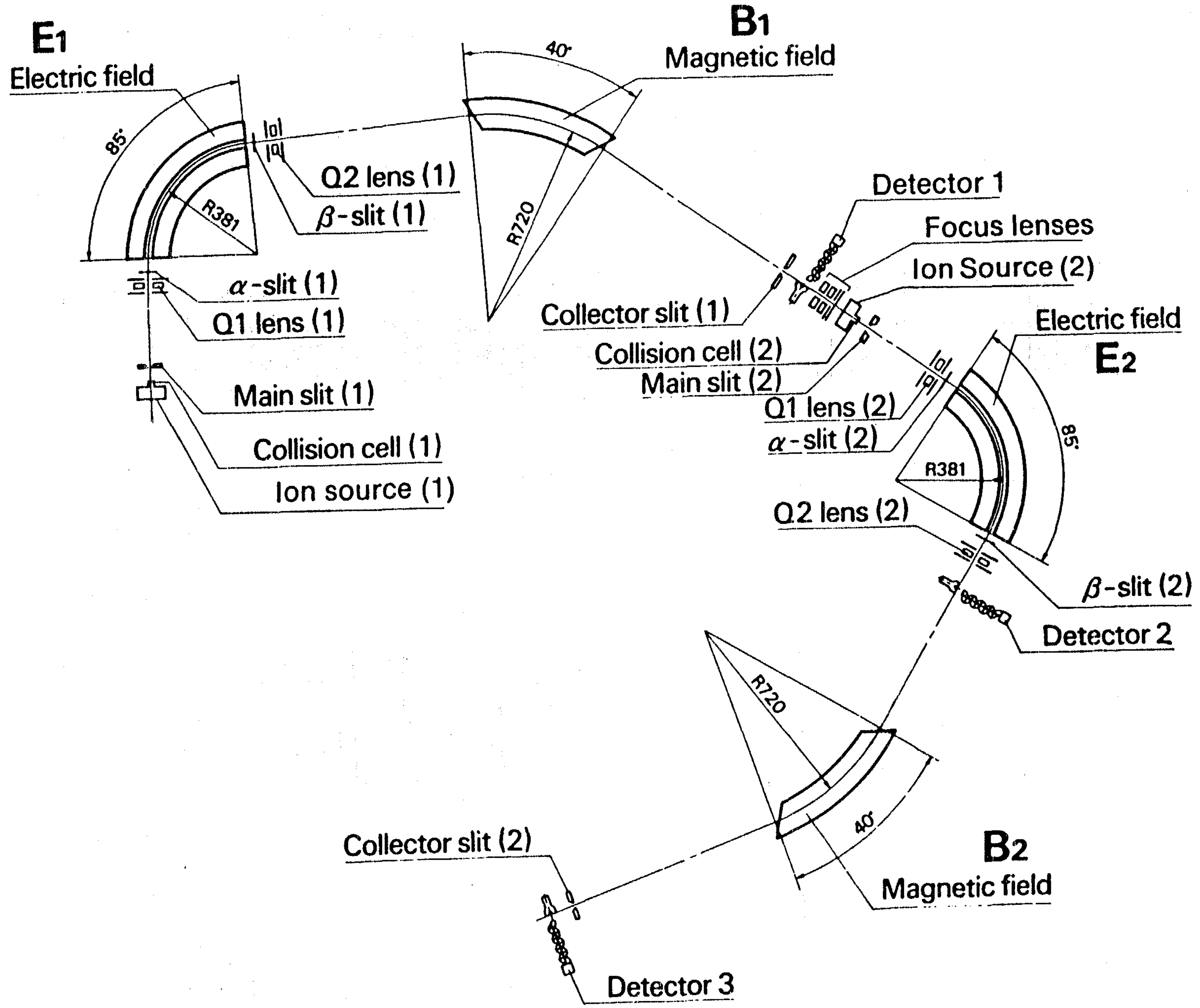
1. Costello CE, Zeng C-H, de Vries NH, O'Connell LA, Pearlstein RM, Davison A, Jones AG. FABMS and FAB MS/MS investigations of technetium and rhenium complexes. Presented at the 36th American Society of Mass Spectrometry Conference on Mass Spectrometry and Allied Topics, San Francisco, June 1988.

2. Davison A, Kronauge JF, O'Connell L, Pearlstein RM, Thornback JR, Jones AG. Applications of ^{99}Tc NMR

spectroscopy to the identification of technetium complexes in vitro and in vivo. Presented at the Seventh International Symposium on Radiopharmaceutical Chemistry, Groningen, The Netherlands, July 1988.

3. Davison A, de Vries N, Jones AG. New aspects of technetium nitrosyl chemistry. - ibid -.

FIGURE 1.



JEOL HX110/HX110 Tandem Mass Spectrometer

FIGURE 2.

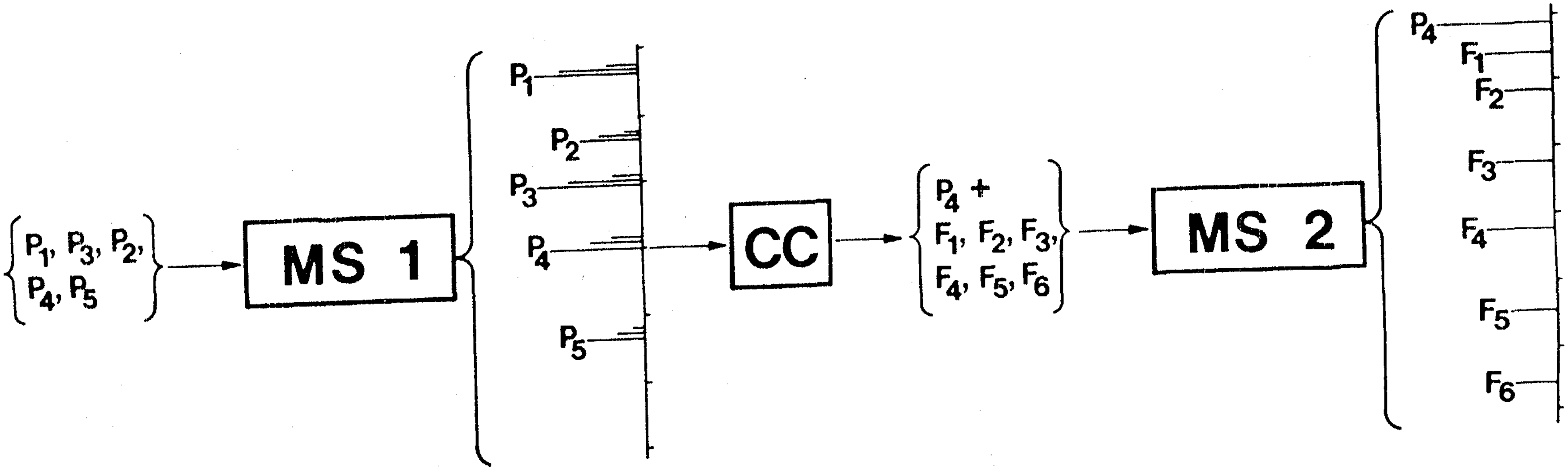
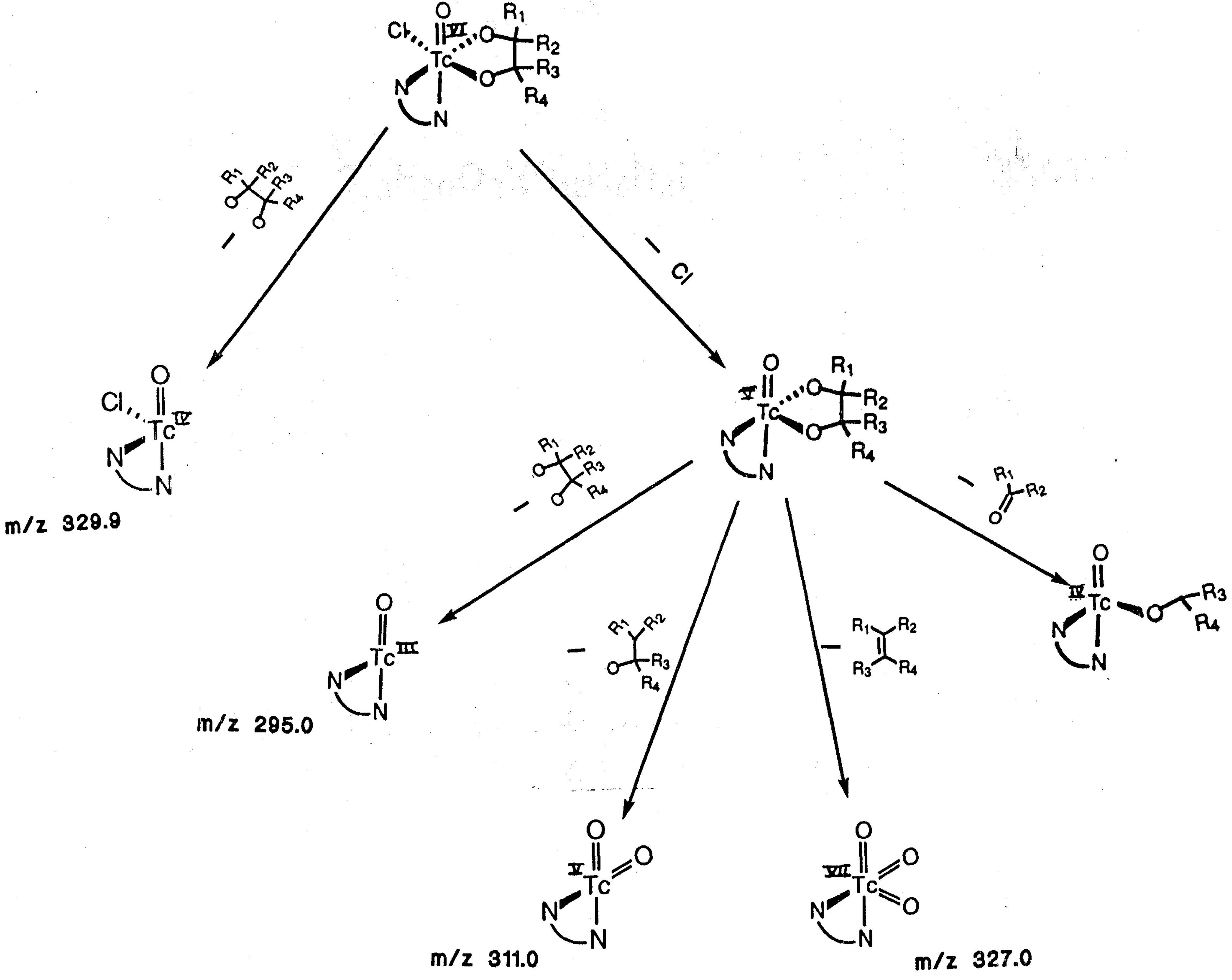

P_1, P_3, P_2, P_4, P_5
MS 1
P_1
P_2
P_3
P_4
P_5
CC
P_4 +
$F_1, F_2, F_3, F_4, F_5, F_6$
MS 2
P_4
F_1
F_2
F_3
F_4
F_5
F_6

FIGURE 3.



Cl
O
VI
Tc
R1
R2
R3
R4
N
N
− Cl
V
m/z 329.9
IV
m/z 295.0
III
m/z 311.0
V
m/z 327.0
VII
IV

FIGURE 4.

$TcOCl(C_{10}H_{20}O_2)(C_{12}H_8N_2)$ M_r 502.1

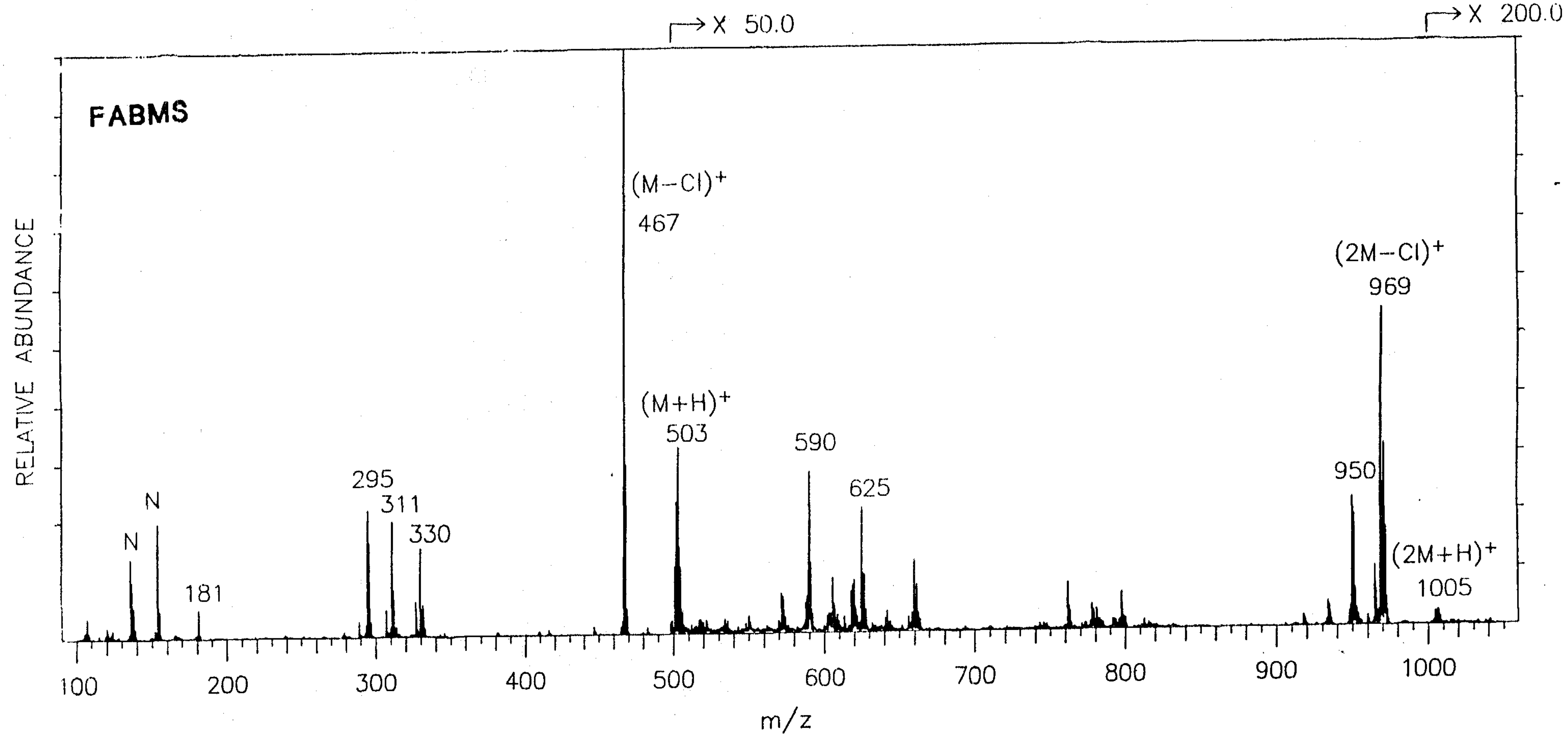


FIGURE 5

$TcOCl(C_{10}H_{20}O_2)(C_{12}H_8N_2)$ M_r 502.1

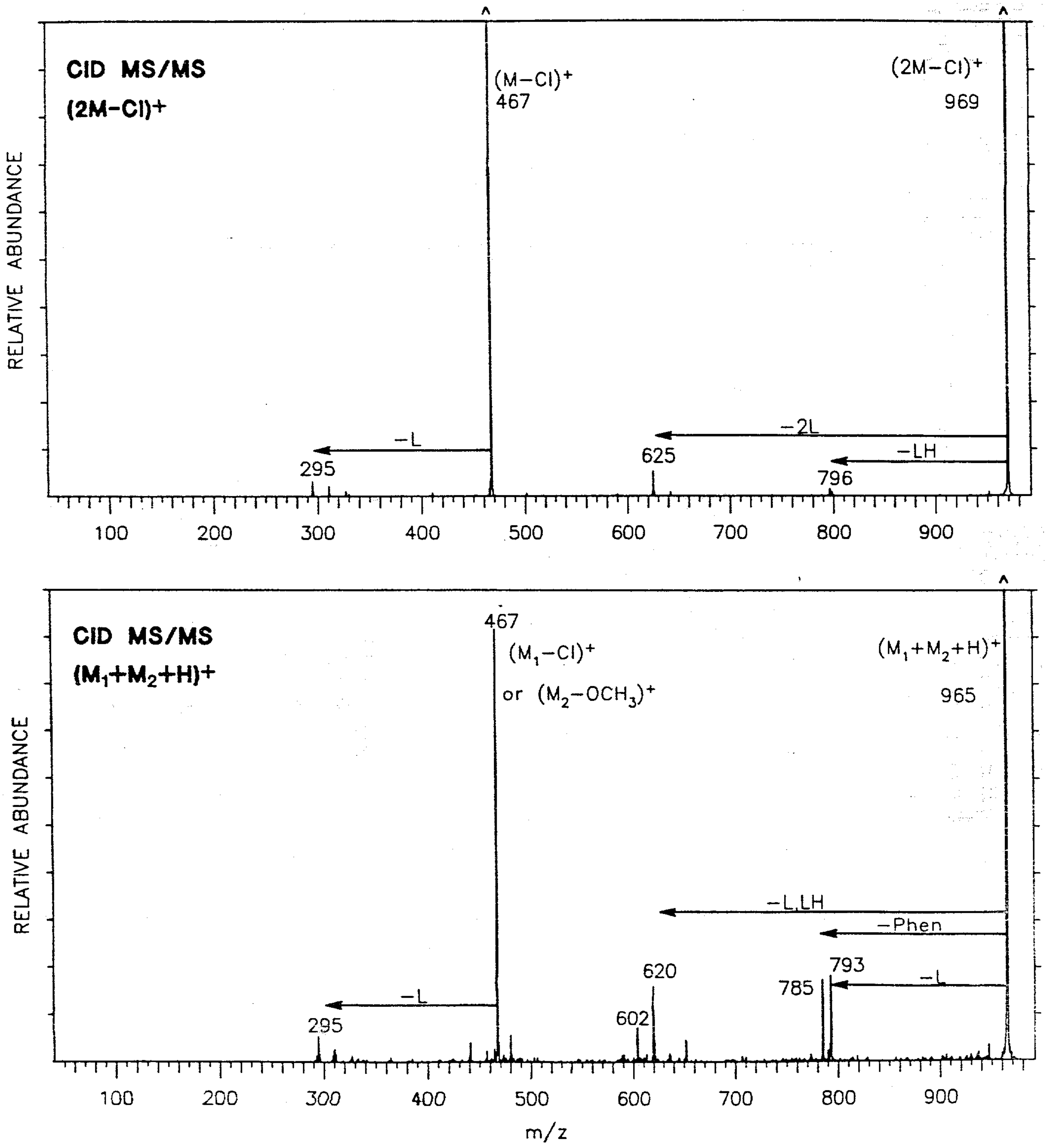
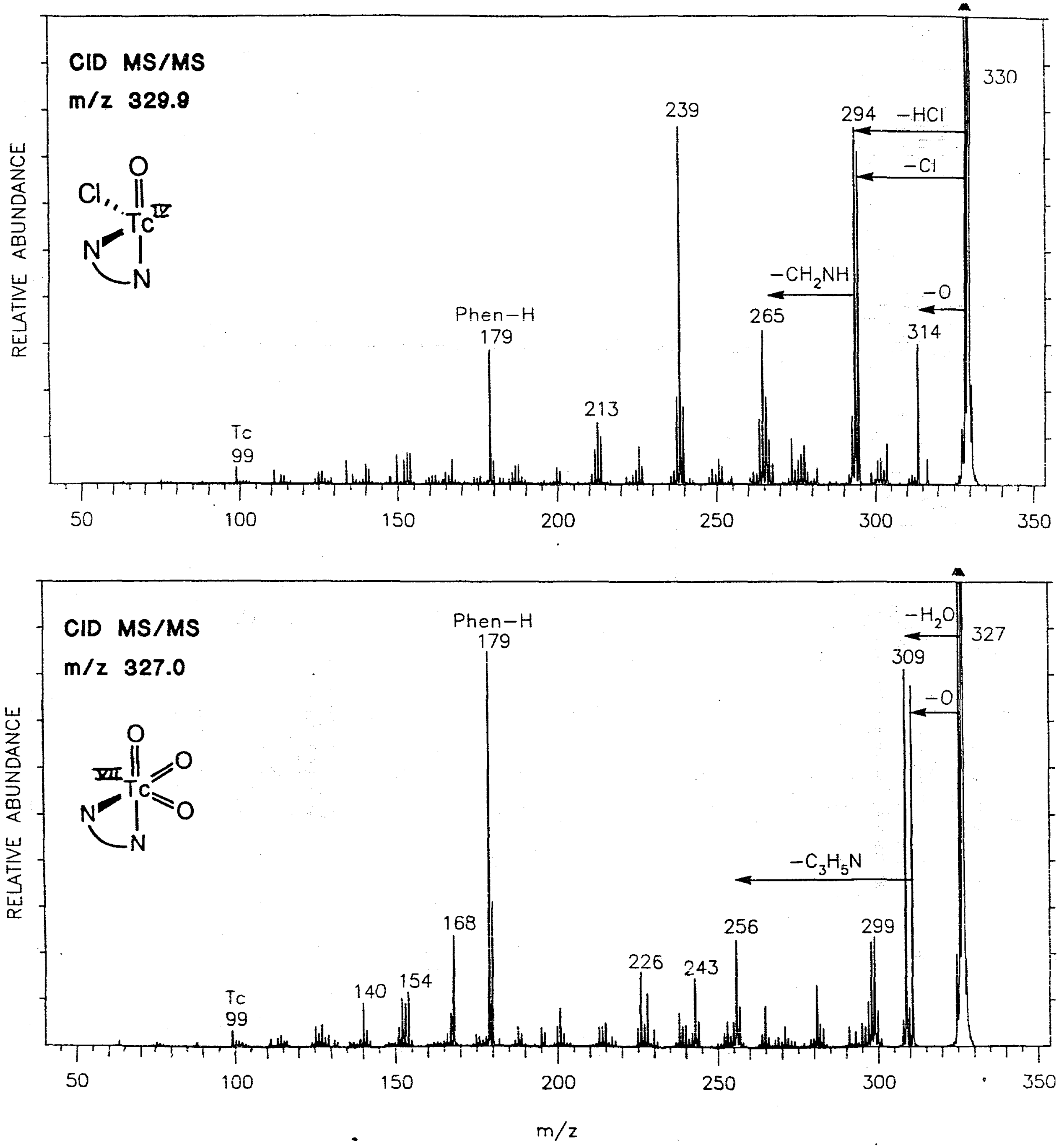
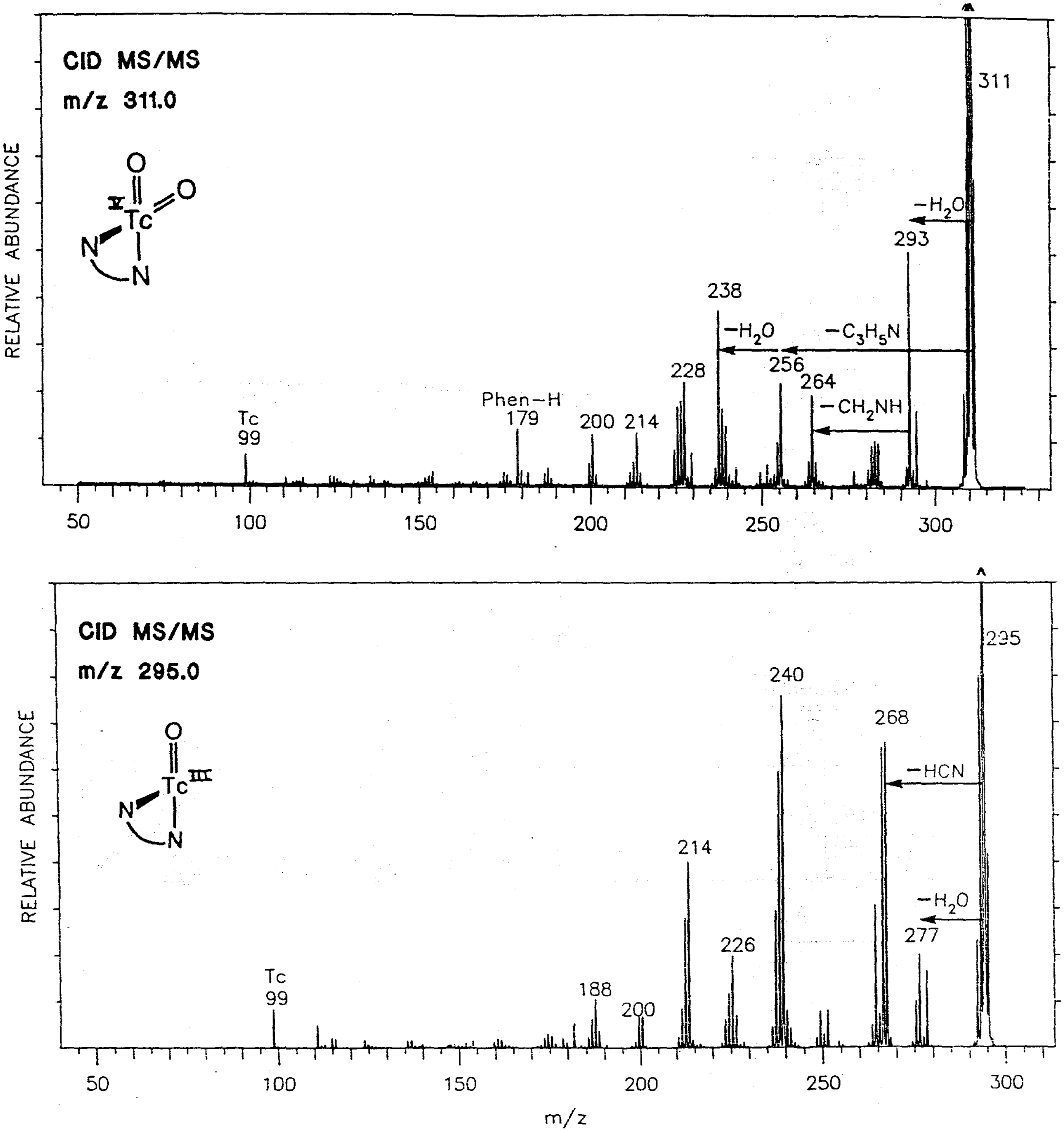


FIGURE 6.

$TcOCl(C_{10}H_{20}O_2)(C_{12}H_8N_2)$ M_r 502.1



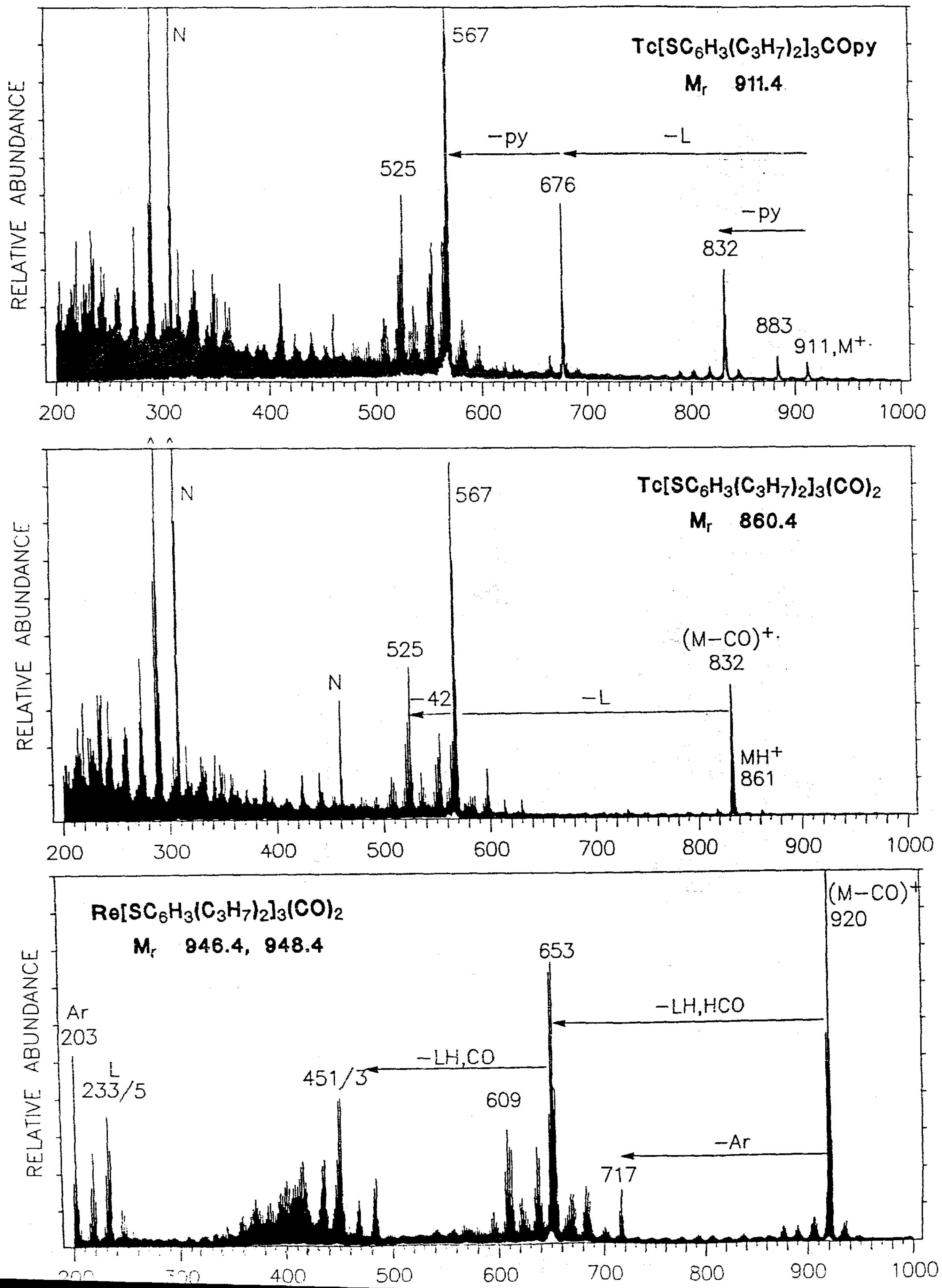
$TcOCl(C_{10}H_{20}O_2)(C_{12}H_8N_2)$ M_r 502.1



$M[SC_6H_3(C_3H_7)_2]_3COX$

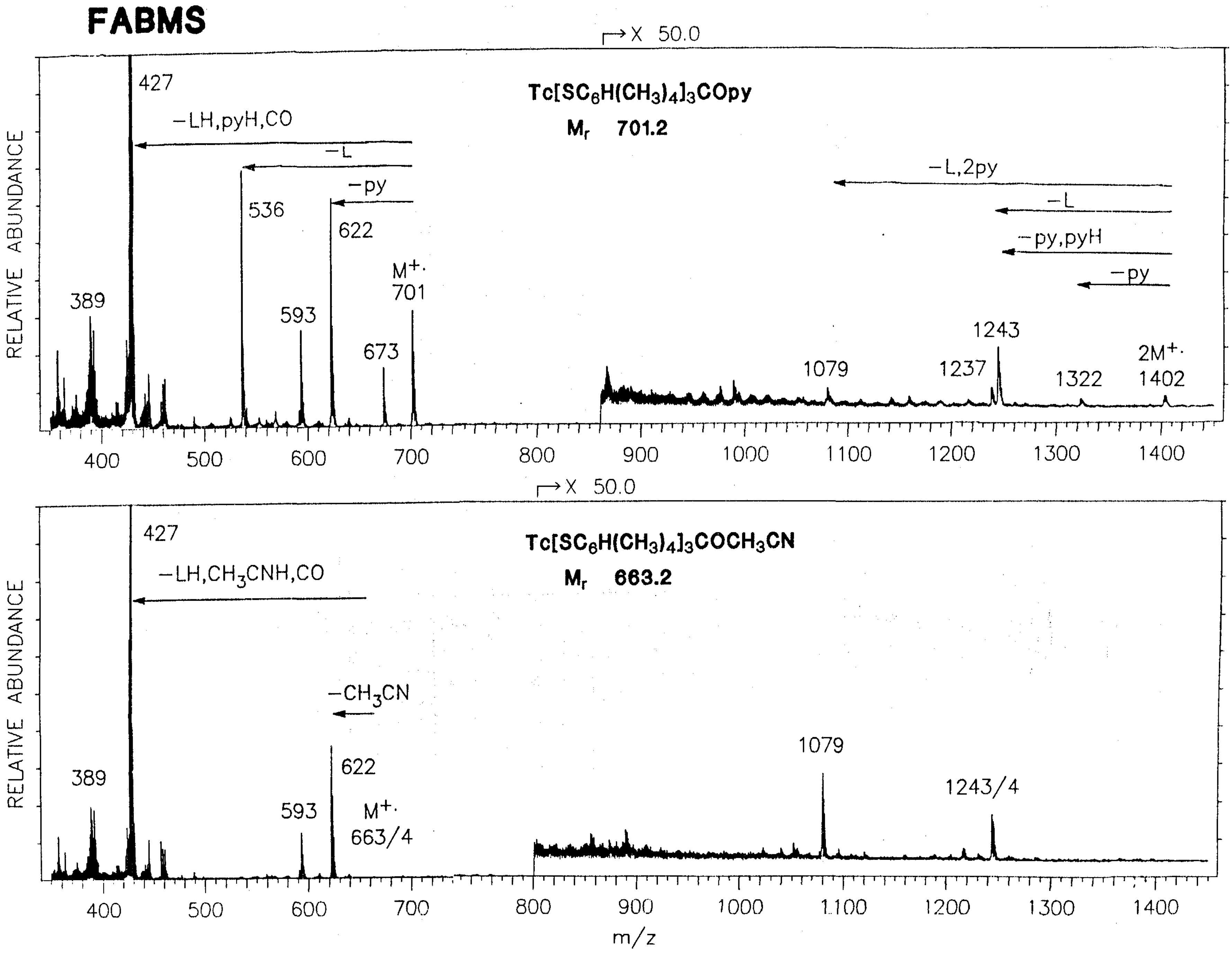
FIGURE 8.

FABMS



$Tc[SC_6H(CH_3)_4]_3COX$

FIGURE 9.



MS/MS

FIGURE 10

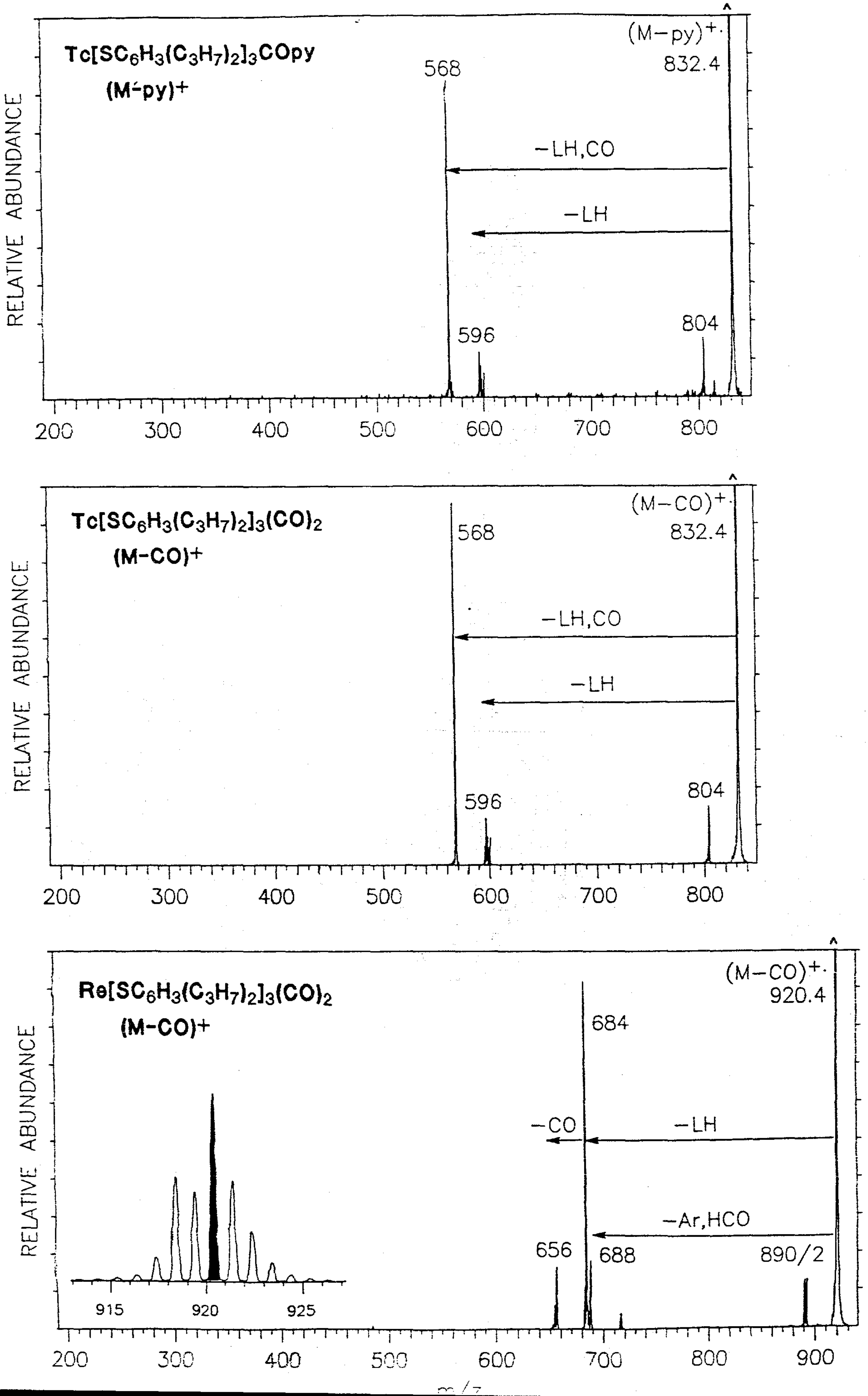


FIGURE 11.

CID MS/MS

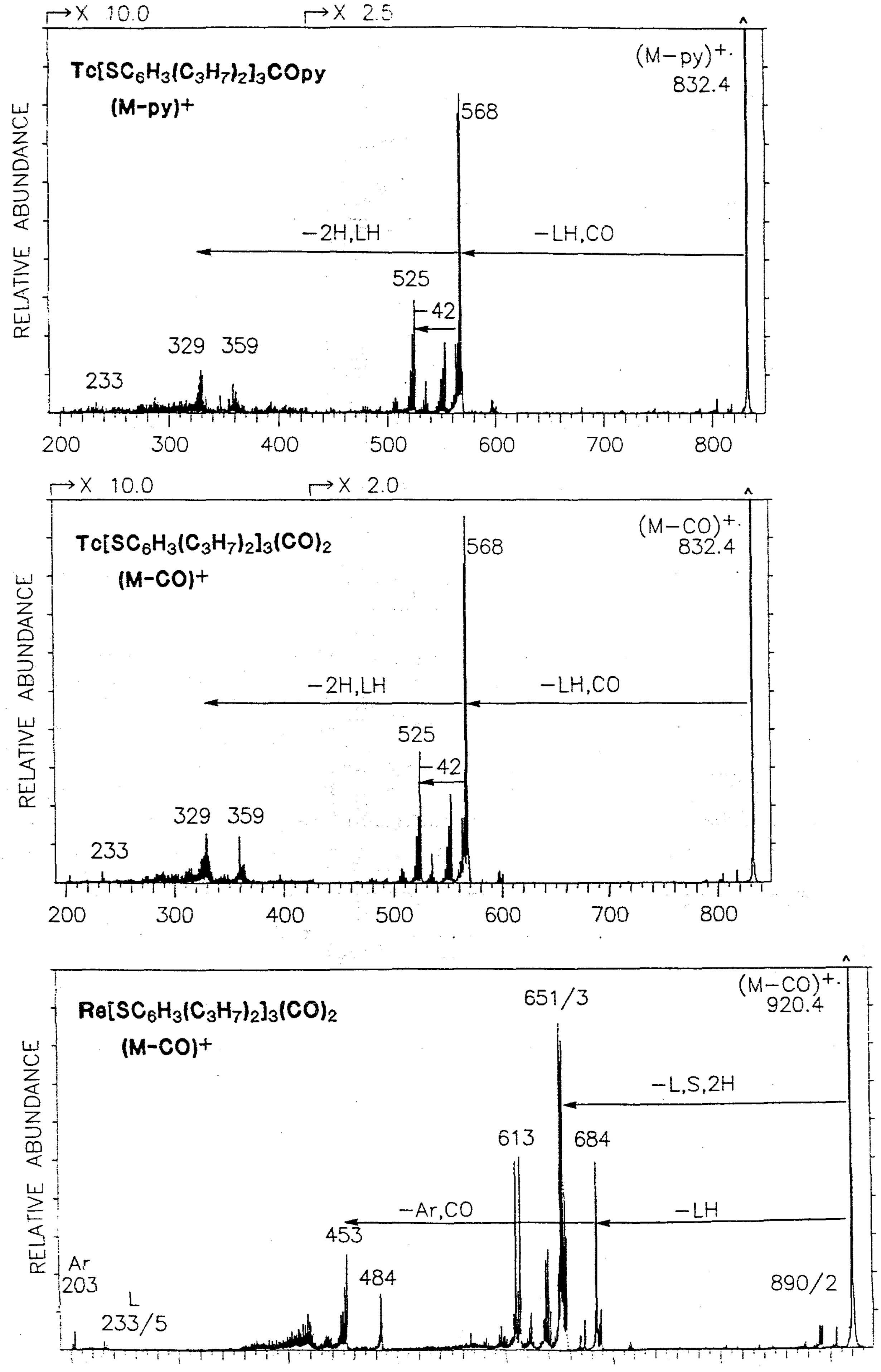
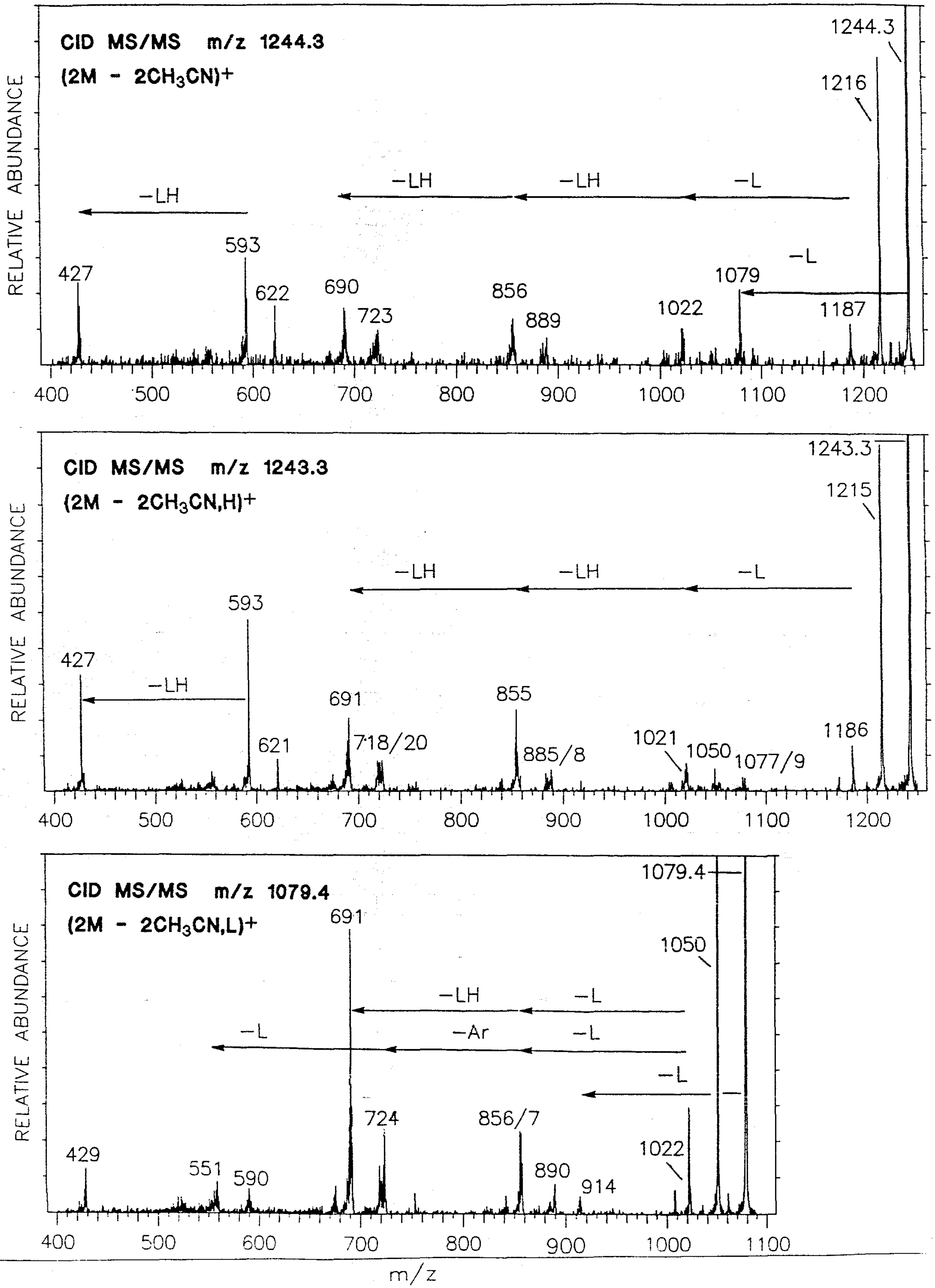


FIGURE 12.

$Tc[SC_6H(CH_3)_4]_3COCH_3CN$ M_r 663.2



$Tc[CN(C_6H_3(CH_3)_2]_6^+\ PF_6^-$ C^+ 885.4