Californium-252 Plasma Desorption Mass Spectrometry as an Aid in the Synthesis of a Series of Adenosine and Xanthine Conjugates

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Two series of biologically active purine derivatives have been analyzed using californium-252 plasma desorption mass spectrometry. The series of compounds are adenosine agonists (N^6-phenyladenosine derivatives) and antagonists (8-phenyl-1,3-dipropylxanthine derivatives) at extracellular purine receptors. Included are receptor probes synthesized through successive chain elongation reactions. Positive and negative ion spectra are often dominated by signals from the quasimolecular ion [$(M + H)^+$, $(M + Na)^+$, $(M - H)^-$] or by peaks resulting from loss of whole molecules such as ribose. The simplicity of sample preparation, spectrometer operation, and interpretation of spectra from the non-heated sample are demonstrated.

In recent years many mass spectral methods have become available to the synthetic organic chemist for assistance in the stepwise preparation of complex organic compounds. These include, besides the more commonly recognized electron and chemical ionization techniques (e.i. and c.i.), fast atom or ion bombardment (f.a.b. or f.i.b.),[1] secondary ion mass spectrometry (s.i.m.),[2] thermospray,[3] and californium-252 plasma desorption mass spectrometry (p.d.m.s.).[4] All of these methods vary considerably in both the complexity of their associated equipment and the sample preparation they entail.

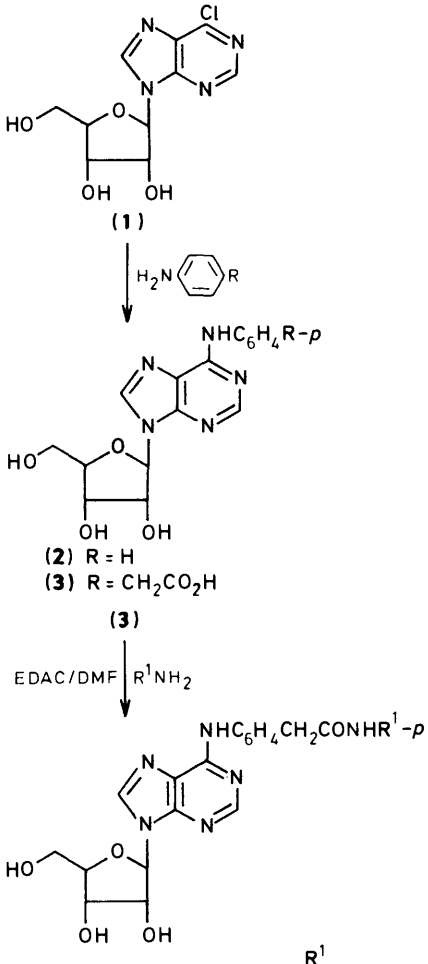
In the synthetic sequence described below, we emphasize the p.d.m.s. technique, calling attention to its ease of use and the straightforward interpretation of the resulting spectra. In most cases these spectra are characterized by high ion intensities in the molecular weight region followed by simple fragmentation with loss of easily discerned molecular entities.

Results and Discussion

The purine derivatives described below were prepared for pharmacological studies; their synthesis has been described earlier in detail.[5–8] These adenosine and xanthine conjugates exemplify the application to extracellular adenosine receptors of the 'functionalized congener' approach for drug design.[5–9] By this approach, a drug analogue is synthesized with the inclusion of a chain terminating in a chemically reactive group, such as a carboxylic acid or an amine. This chain is located at a biologically insensitive position of the drug and the reactive group then coupled to a 'carrier', to provide a particular pharmacological or chemical property. The attached 'carrier' may contain a peptide[8] [*e.g.* (**5b**)], a protein,[7] a fluorescent marker [*e.g.* (**7h,i**)], a protein labelling moiety [*e.g.* (**7e,f,g,i**),(**8r**)], a spin label (**7k**), an electron opaque marker[10] (**8r**), or a chelating group (**8s**), *etc.*

Many of these derivatives are soluble only in solvents such as dimethylformamide and dimethyl sulphoxide and often crystallized with water and solvent of crystallization. Thus it was often necessary to use mass spectroscopy rather than combustion analysis for complete characterization.

The first members of the *p*-substituted N^6-phenyladenosine series[6] (**1**)—(**4d**) provide fairly satisfactory NH_3 or CH_4 c.i. mass spectra. For example, Figure 1 shows the NH_3 c.i. spectrum of compound (**3**) where a detectable $(M + H)^+$ ion is present at *m/z* 402. Extensive cleavage of the ribose (Rb) occurs to form an ion at *m/z* 270, but in practice loss of this large moiety is not as much of an inconvenience as certain other fragmentations. For example, loss of formic acid, both from



(**4a**) Me
(**4b**) CH_2CO_2Et
(**4c**) C_6H_4Me-*p*
(**4d**) $C_6H_4CF_3$-*p*
(**4e**) $C_6H_4CH_2CO_2Me$-*p*

(**5a**) C_6H_4-(*p*)-CH_2—$CH(NHCOCF_3)CO_2Me$ (L)

(**5b**) C_6H_4-(*p*)-CH_2—$CH(NHCO_2Bu^t)CONHCH_2CONHMe$ (L)

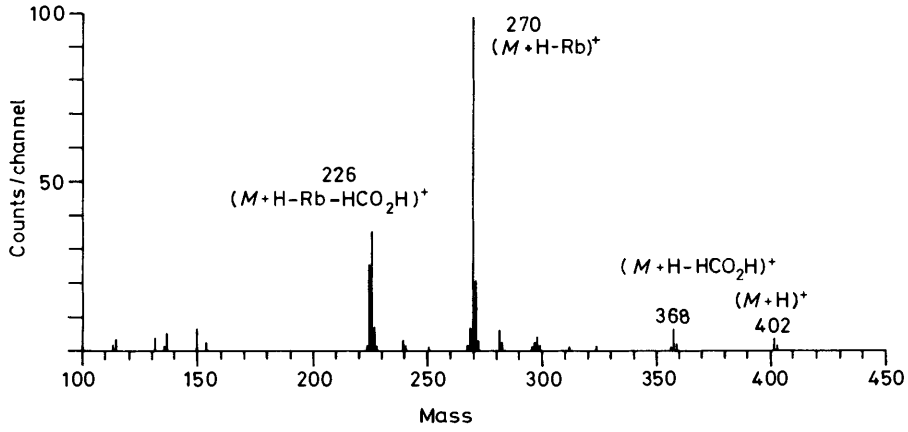
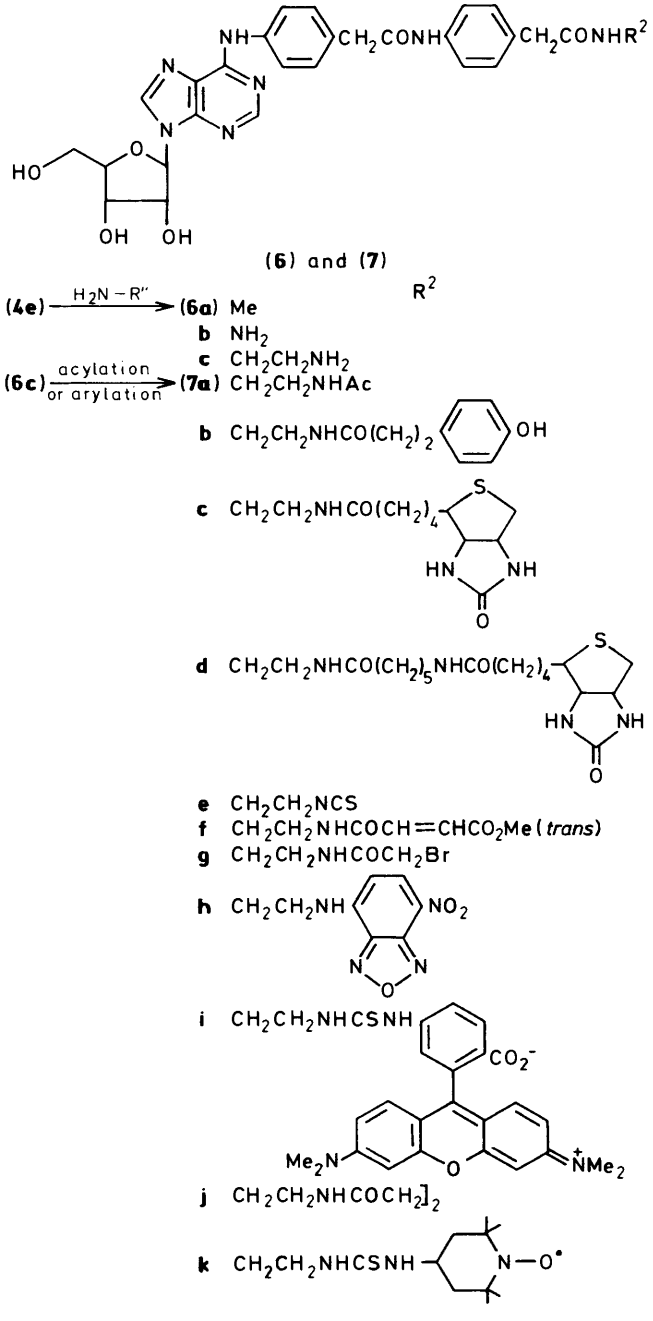


Figure 1. Compound (**3**) (Cl-NH_3)



the $(M + H)^+$ and $(M + H - Rb)^+$ ions might suggest the presence of the corresponding decarboxylated compound.

Further extension of the side chain (**4e**)—(**7j**) decreased volatility of the compounds to the point where c.i. spectra were no longer useful, and recourse was made to the p.d.m.s. method. The resulting spectra invariably show either the $(M + H)^+$ ion, or the $(M + Na^+)$ ion, or both, as easily detected peaks (Table 1), allowing us to follow the course of a given reaction and to detect by-products.

Figure 2 illustrates a typical spectrum for one of the simpler members of the series (**4e**). The most intense peaks by a factor of 2—10 are invariably the highly conjugated alkylphenyladenine nuclei (*m/z* 224) and its reduced molecular ion counterpart (*m/z* 225), presumably surviving from decompositions of higher mass ions. In Table 1 these ions are omitted because of their tendency to dominate the spectra. As in the case of c.i., another abundant ion usually represents the loss of ribose from the $(M + H)^+$ ion; it appears here at *m/z* 417. The $(M + Na)^+$ ion, as expected, is proportionately less prone to decompose in this manner and as a result is often the most abundant ion in the molecular ion region. This stability does not necessarily extend to the sodium replacement ion $(M + 2Na - H)^+$, *i.e.* the $(M + Na)^+$ of the sodium salt, and in several cases (**4c**),(**4e**),(**6a**),(**6b**),(**7d**),(**7e**) this species appears especially prone to lose ribose (Table 1).

The low resolution apparent in these spectra is often a manner of concern to those not familiar with time-of-flight mass spectrometers. While the inherent resolution of our spectrometer is over 800 $M/\Delta M$, the presence of metastable ions and neutrals, which are all detected in this mass spectrometer, is an important cause of broadening of the base of the peaks. These ions can be removed, but at the price of considerable sensitivity, and we and others using the technique are accustomed to comparing the measured centroid of the unresolved isotope cluster with the mass calculated on the basis of the chemist's atomic weights (C = 12.01). We have not found this to be as disconcerting as one might suppose, and the masses in Tables 1 and 2 are compared on this basis. One compensating factor is the reliability of the spectrometer mass scale which is automatically calibrated on each run, requiring only the H^+ and Na^+ always adventitiously present in the sample.

Further extension of the side chain in the alkylphenyladenosine series as in (**5a**) (Figure 3) does not change this spectral pattern noticeably; even minor peaks are usually explained satisfactorily. Moreover, impurities when present were readily detected. For example, the presence of starting (**4e**) in the adenosine amine congener (**6c**) was easily detected as a minor impurity when the aminolysis reaction was not carried fully to completion.

Negative ion spectra of these compounds, although always run for confirmatory reasons, are much less useful. Figure 4

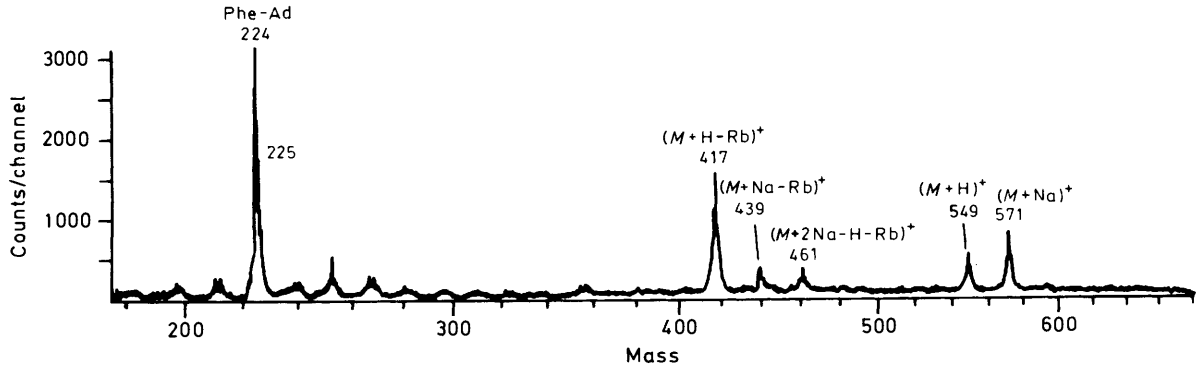


Figure 2. Compound (**4e**) (positive ions)

Table 1.

Compound	M. wt.(av.)	$(M + 2Na - H)^+$	$(M + Na)^+$	$(M + H)^+$	$(M + 2Na - H - Rb)^+$	$(M + Na - Rb)^+$	$(M + H - Rb)^+$	Other ions
(**4b**)	486.5	531 (40)	509 (100)	486 (10)	399 (31)	377 (44)	355 (44)	
(**4c**)	490.5	535 (6)	513 (73)	491 (24)	403 (18)	381 (26)	359 (100)	
(**4d**)	544.5	589 (3)	567 (31)	545 (24)		435 (7)	413 (100)	
(**4e**)	548.6	593 (4)	571 (48)	549 (26)	461 (13)	439 (18)	417 (100)	
(**5a**)	673.6	718 (4)	696 (65)	674 (26)	585 (7)	564 (15)	542 (100)	
(**5b**)	733.8	779 (4)	757 (100)	735 (9)		525 (25)		657(37, $M + Na - C_4H_8 - CO_2$) 701 (45, $M + Na - C_4H_8$)
N-6-Phenyl-adenosine	343.3	388 (2)	366 (17)	344 (13)	256 (5)	234 (9)	212 (100)	
(**6a**)	547.6	592 (14)	570 (100)	548 (2)	460 (59)	438 (35)	416 (11)	
(**6b**)	548.6	593 (28)	571 (98)	549 (6)	461 (100)	439 (30)	417 (28)	
(**6c**)	576.6	621 (9)	599 (100)		489 (60)	467 (47)	445 (48)	
(**7a**)	618.7	663 (7)	641 (100)	619 (2)	531 (24)	509 (24)	487 (17)	
(**7b**)	724.7	769 (4)	747 (100)	725 (5)	638 (7)	615 (5)	594 (15)	
(**7c**)	802.9	848 (7)	826 (100)		715 (4)	693 (14)	672 (4)	
(**7d**)	916.1	962 (15)	939 (100)	917 (2)	830 (45)	807 (65)	785 (18)	762 (85, imp.)
(**7e**)	618.7	664 (23)	641 (95)		532 (100)	509 (40)		
(**7f**)	688.7		712 (66)			580 (24)	557 (33)	600 (65), 446 (100)
	725.2		748 (65)			616 (20)		
(**7g**)	697.6		721 (100)			589 (30)		642 (25, $M + Na - Br$)
(**7h**)	738.6	785 (18)	762 (100)	741 (5)		630 (11)	608 (22)	746 (7, imp.)
(**7i**)	1 018.1	1 065 (2)	1 042 (12)	1 020 (7)				445 (100), 428 (67), 412 (37) 402 (75), 386 (52)
(**7j**)	1 235.2		1 258 (w)					
(**7k**)	789.2			758 (100, $M - OCH_3$)			626 (15, 758 − Rb)	

Table 2.

Compd. (**8**)	M. wt.(av.)	$(M + Na)^+$	$(M + H)^+$	Other ions	$(M - H)^-$	$(M - R)^-$	Other ions
f	428.5	451 (54)	429 (100)		427 (15)	326/7 (100)	
g	414.5	437 (2)	415 (100)	458 ($M + 2Na - H$) 414 (50, $M^{+\cdot}$)	413 (74)	326/7 (100)	
h	475.5	498 (22)	476 (100)		474 (36)	326/7 (100)	
i	477.5	500 (4)	478 (100)		476 (85)	326/7 (100)	
j	413.5	436 (61)	414 (100)	458 (30, $M + z\,Na - H$)	412 (20)	326/7 (100)	
k	512.7	535 (19)	513 (100)		512 (32)	326/7 (100)	
l	592.7		593 (100)	468 (71, $M + H - C_6H_{11}NCO$)	592 (19)	326/7 (100)	467 (10, $M - H - C_6H_{11}NCO$)
m	470.5	493 (100)	471 (98)		470 (63)	326/7 (100)	492 ($M + Na - 2H$)
n	654.8	677 (100)	655 (54)		654 (30)	326/7 (100)	
o	566.6	607 (100)	585 (98)		583 (1)	326/7 (100)	
p	676.7	700 (100)	678 (36)		675 (5)	326/7 (100)	
q	542.6	565 (100)	543 (60)		541 (25)	326/7 (100)	
r	812.3	775 (15, $M - OAc + Na - H$)	753 (22, $M - OAc$)	597 (59, $M + 2Na - H - HgOAc$), 575 (100, $M + Na - HgOAc$)	552 (12, $M - HgOAc$)	326/7 (100)	
s	802.8	827 (14)	805 (5)	444 (100)			

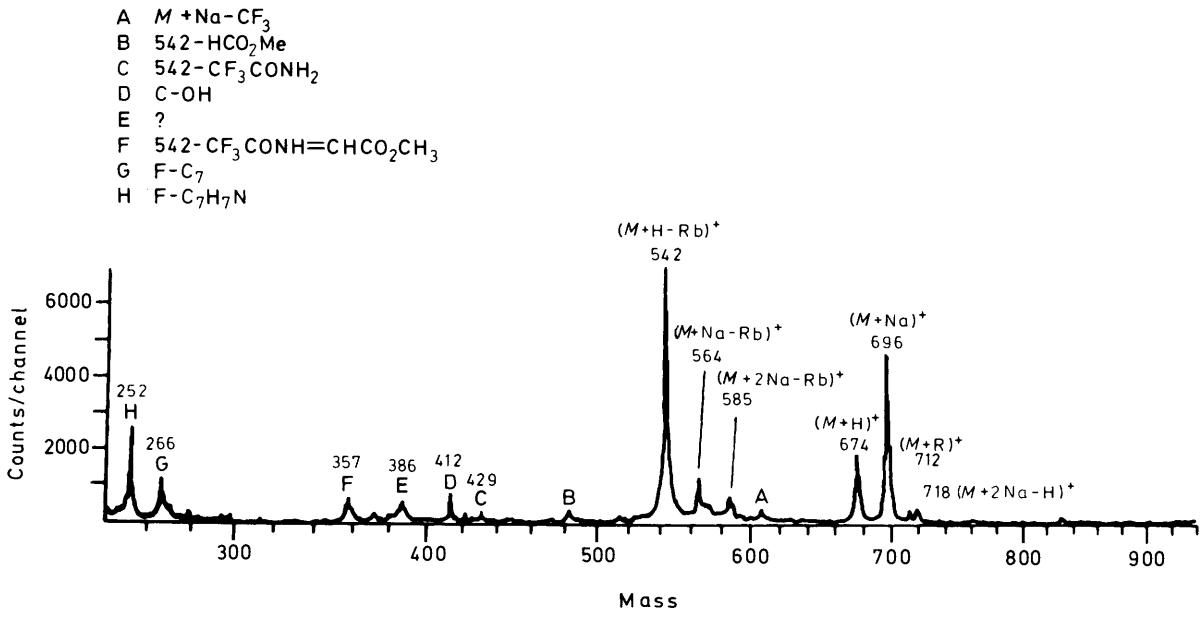


Figure 3. Compound (**5a**) (positive ions)

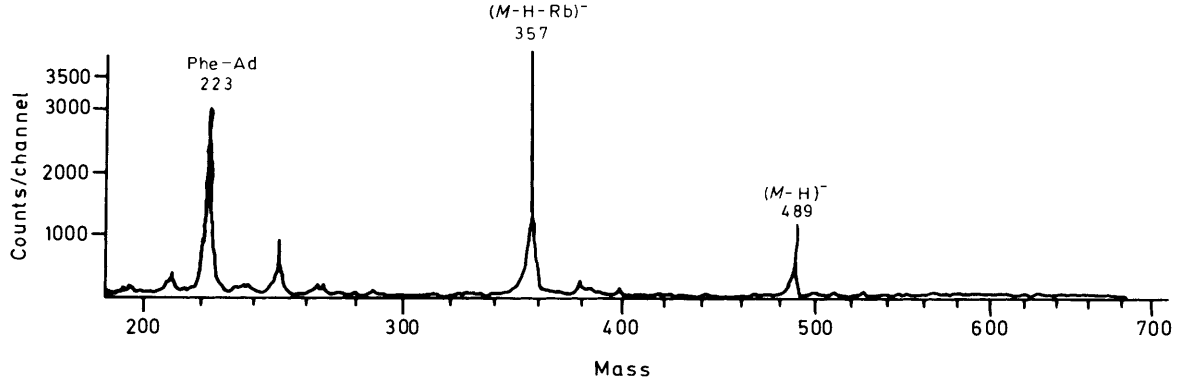


Figure 4. Compound (**4c**) (negative ions)

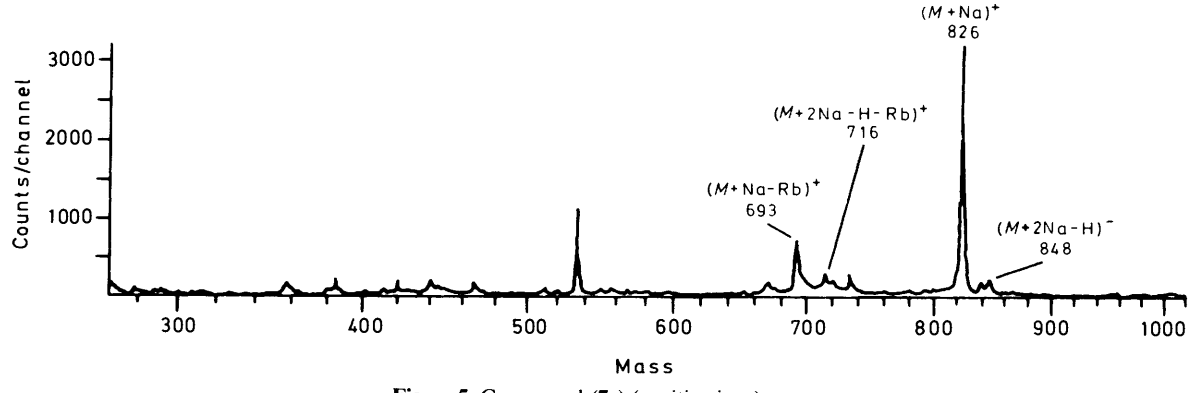
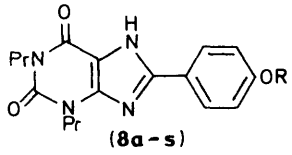


Figure 5. Compound (**7c**) (positive ions)

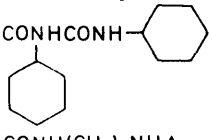
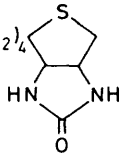
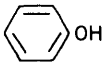
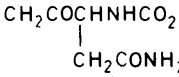
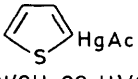
shows one particularly favourable case where compound (**4c**) exhibits a relatively abundant $(M - H)^-$ ion at m/z 489. As with positive ions, loss of ribose from this species is prominent. Various unsubstituted alkylphenyladenine negative ion nuclei appear again from m/z 222—224, and they are often even more abundant proportionally in this mode than in the positive ion mode.

Table 1 summarizes other spectra of the alkylphenyladenosine series in which a complex member is the biotin-containing compound (**7c**).[7] With this substance (Figure 5), the $(M + Na)^+$ ion at m/z 826 is 60% as abundant as the alkylphenyladenosine nucleus at m/z 224. The highest molecular weight compound run successfully is the polyamide dimer (**7j**), which showed a very small but clearly distinguishable $(M + Na)^+$ peak at m/z

1 258. The methyl fumarate derivative (**7f**) showed an apparent $(M + Na)^+$ ion at *m/z* 748, 36 mass units higher than expected, suggesting the addition of HCl which was present during the synthesis. Ions were also present at *m/z* 712, either representing (**7f**) itself or the elimination of HCl from *m/z* 748. The nitroxyl



(**8a–s**)

	R
(**8a**)	H
b	CH_2CO_2H
c	CH_2CONH_2
d	$CH_2CONHNH_2$
e	
f	$CH_2CONH(CH_2)_2NH_2$
g	CH_2CO_2Et
h	$CH_2CONHC_6H_4Me$-*p*
i	$CH_2CONHC_6H_4OH$-*p*
j	CH_2CONMe_2
k	$CH_2CONH(CH_2)_8NH_2$
l	
m	$CH_2CONH(CH_2)_2NHAc$
n	
o	
p	 (L)
q	$CH_2CONH(CH_2)_2NHCOCH(NH_2)CH_2CONH_2$ (L)
r	
s	$CH_2CONH(CH_2)_2NHCOCH_2N(CH_2CO_2H)(CH_2)_2N(CH_2CO_2H)$-$(CH_2)_2N(CH_2CO_2H)_2$

radical (**7k**) showed a weak peak at *m/z* 758 corresponding to loss of CH_3OH from a hypothetical $(M + H)^+$ ion. Such losses have been observed in the e.i. spectra of similar compounds.[11] A model compound, the corresponding phenethylthiourea, also showed an ion at *m/z* 303 for this loss (35%), but provided abundant (100%) $(M + H)^+$ and $(M - H)^-$ ions at *m/z* 335 and 333 as well.

The phenylxanthine series[5] (**8a—s**), is considerably less polar, both because the ribose moiety is absent and because the xanthine NH groups in the 1 and 3 positions are substituted by propyl groups. As a result, compounds (**8a—g**) provide satisfactory NH_3 or CH_4 c.i. spectra, and Figure 6 shows the CH_4 c.i. spectrum of (**8f**). Although abundant $(M + H)^+$ and $(M + Et)^+$ ions are present, loss of water from both species, might indicate the presence of an impurity due to partial cyclization of the terminal amino group with the amide carbonyl. As seen from Table 2, no dehydration from $(M + H)^+$ or $(M + Na)^+$ ion again dominates (Table 2). In most cases it is pounds (**8h—s**) failed to give quasimolecular ions in c.i. and the p.d.m.s. technique was once again required for satisfactory molecular weight information.

The most noticeable feature of the spectra of (**8h—l**) compared to those of the previous (phenyladenosine) series is the relatively greater abundance of $(M + H)^+$ as exemplified by (**8i**) (Figure 7 and Table 2). This was not unexpected, since the ribose moiety, with its high hydroxy content and resulting affinity for Na^+ is absent in this series. However, in (**8m—s**) the $(M + Na)^+$ ion again dominates (Table 2). In most cases it is not easy to discern logical structures for lower mass fragment ions in this series, although $(M + H - C_6H_{11}NCO)^+$ is apparently responsible for the 72% abundant ion at *m/z* 468 in (**8l**).

Unlike the alkylphenyladenosine series, the xanthine derivatives also provide useful negative ion spectra (Table 2). Compound (**8i**) is typical (Figure 8): cleavage of the phenyl ether side chain invariably occurs to give the xanthine phenoxy ion (*m/z* 327) and its one-H-less counterpart at *m/z* 326. Although the latter is the most abundant ion in all spectra, the $(M - H)^-$ ion is easily discerned in every example except (**8r**) and (**8s**). As in the positive ion spectrum, (**8l**) loses $C_6H_{11}NCO$ from the $(M - H)^-$ ion.

Compound (**8q**) illustrates especially well the value of the p.d.m.s. method. Investigated with NH_3 c.i., only very weak peaks were seen in the *m/z* 400—600 region. When inspected using selected ion monitoring however, distinct peaks arose simultaneously (Figure 9) at *m/z* 543 $(M + H)^+$, *m/z* 526 $(M + H - NH_3)^+$, *m/z* 429 $(M + H - Asn)^+$ in the intensity ratio 0.013, 0.17, 1.0 (note values for number of ions captured on each peak in Figure 9). Although the peak representing loss of asparagine rose simultaneously with the $(M + H)^+$ peak, suggesting that ion as its source (because of the discrepancy in

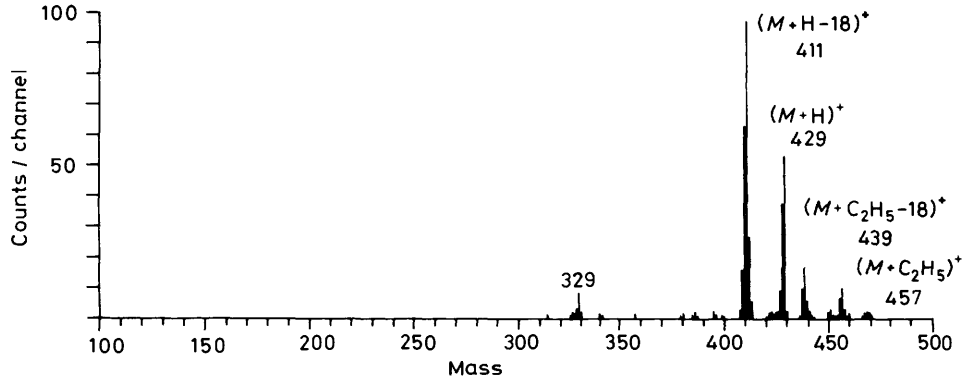


Figure 6. Compound (**8f**) (Cl–CH_4)

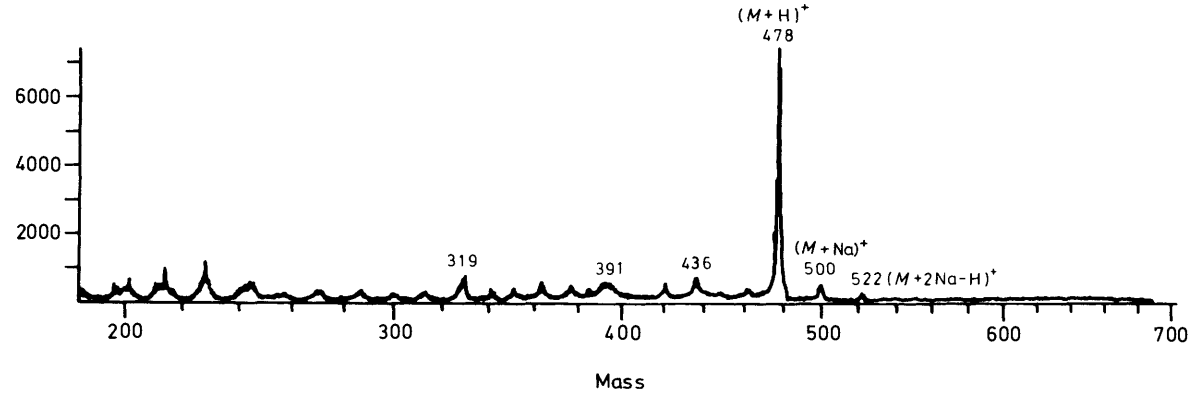


Figure 7. Compound (**8i**) (positive ions)

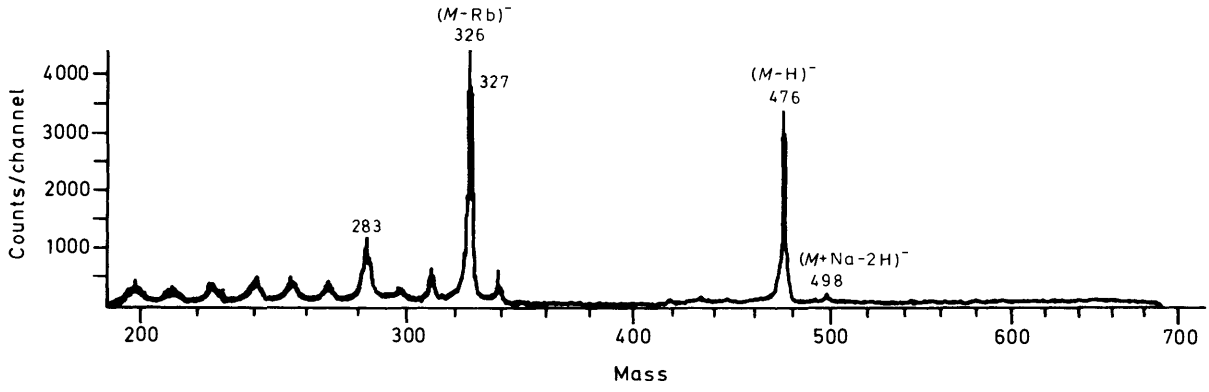


Figure 8. Compound (**8i**) (negative ions)

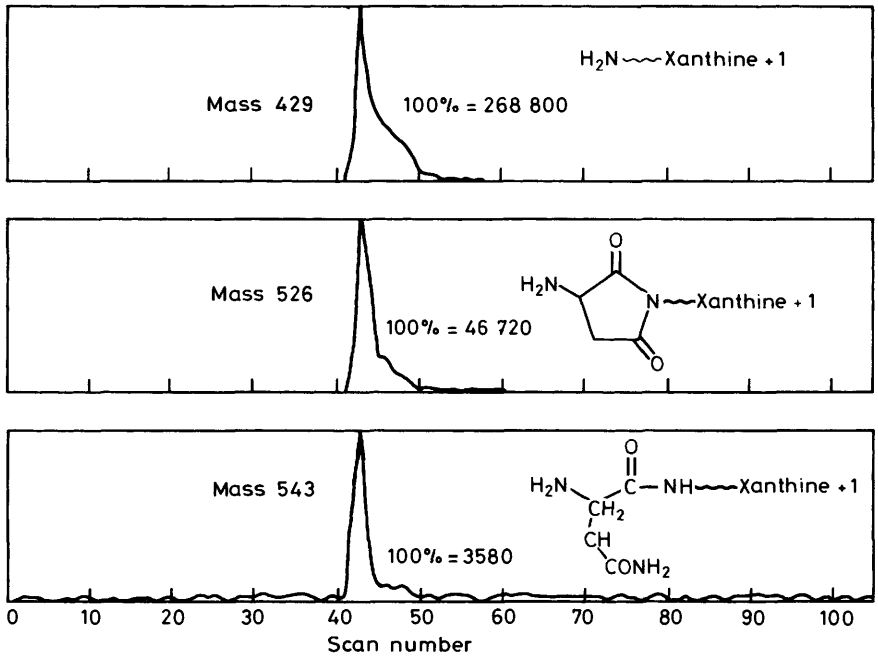


Figure 9.

mass and resulting volatility), no such assurance could be had for the $(M + H - NH_3)^+$ ion. The latter is, in fact, a logical impurity, and its absence as proved by the abundant $(M + Na)^+$ ion at m/z 565 in the p.d.m.s. spectrum (Table 2) is reassuring.

Discussion

From the structures of the foregoing compounds, it seems likely that fast atom bombardment (f.a.b.) mass spectrometry would also be useful in following the reactions involved in the preparations of these compounds. We also consider it likely that

the chemical ionization method, using properly designed and heated probes (so-called 'desorption CI'[12]) could have extended further into both of the foregoing series. We stress here, however, the simplicity of the Cf-252 p.d.m.s. apparatus and the spectra it produces. Thus, the apparatus itself consists simply of a pair of particle detectors in a hollow tube containing an acceleration grid, californium-252 source and sample holder. Unlike most f.a.b. and d.c.i. spectrometers, carefully regulated large magnets requiring calibration with standards are unnecessary. Nor are precise source, analyzer, and slit assemblies needed. Once a solution of the sample has been sprayed or evaporated on the foil and placed inside the apparatus, the spectrometer can be left to accumulate a statistically significant number of counts without further operator intervention, exactly in the manner of a liquid scintillation counter. No monitoring of sample evolution to decide upon proper scan time is involved and the sample is never subjected to heating, thus minimizing contamination of the spectrometer. The rugged nature of the system and its operation with minimal operator intervention seem to us to recommend it particularly for the organic laboratory.

On the other hand, as mentioned earlier, the comparatively low resolution of the analyzer and the detection of metastable ions does broaden the peaks and render isotope calculations difficult. By employing an ion reflection principle[13] however, the first of these problems may be ameliorated and the second used to distinct advantage to provide precursor-daughter relationships among ions.

Another disadvantage of p.d.m.s. is that samples are not run as quickly with this method as in the more established techniques. Again as in scintillation counting, the signal improves as the square root of time so that a quick survey of the sample can often be performed in a few minutes while reliable intensity measurements require 30 min or longer.

Regarding the spectra themselves, the $(M + H)^+$ or $(M + Na)^+$ ions are often among the most abundant ions at higher mass. Fragmentation near the quasimolecular ion usually follows reasonable lines involving loss of intact molecules such as ribose from the $(M + H)^+$ or $(M + Na)^+$ ion, in the manner of chemical ionization. Particularly important however, is the absence of loss of water or ammonia often seen in the c.i. and e.i. modes.

Recovery of sample often is not critical to the synthetic chemist, except perhaps in synthesis of labelled compounds, but, unlike f.a.b. where the sample is mixed with glycerol, this is entirely feasible in p.d.m.s. since only *ca.* 10^{-6} of the sample is used during analysis. In several cases we have found biological activity to be virtually unchanged after analysis. Certain simple chemical reactions (acetylation, Edmund degradation, *etc.*) can even be tested for their feasibility by allowing the reagent to contact samples while they reside on the foil (F. Field and B. Chait, Rockefeller University, personal communication), and this seems a particularly promising area for future investigation. The biological activity of the various adenosine receptor probes will be reported elsewhere.[14]

Experimental

The apparatus has been described in detail elsewhere.[4] Briefly, it consists of 15μCi of Cf-252 encapsulated between thin nickel sheets 1 cm in diameter placed behind the sample which in turn is deposited either by electrospray or evaporation from solvent on an aluminized Mylar foil disk. The whole assembly is placed in an evacuated ion flight tube housing with start and stop detectors to measure the elapsed flight time. Ions, after formation on the foil by the action of the californium fission fragments, are accelerated down a 42 cm flight tube by a grid-ground voltage difference of 10 kV. Flight times are automatically registered and converted into mass by a Perkin-Elmer 3220 computer system during the 15—30 min typically used for acquisition. Focussing of the ion beam is unnecessary in this type of spectrometer and up to 7 samples can be run in the batch mode with no operator attention. The only variable parameters are the acceleration voltage (and this is seldom changed), polarity, and acquisition time. The last may be chosen automatically depending on the ion production of the sample.

In the samples discussed below, the compounds were often quite insoluble in the usual solvents, particularly those most suitable for the electrospray method of application, and recourse was often made to addition of varying amounts of less easily vaporized solvents such as dimethylformamide. No deleterious effects were noted with their use.

References

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