SYNTHESIS OF 1-DEOXY-N,N-BIS-DEMETHYL-PYRROMYCIN (10), METHYL 3-N-(BENZYL-CARBOXAMIDO)-2,3,4,6-TETRADEOXY-DL-THREO-HEXOPYRANOSE (78) AND ITS 3-EPIMER (80)

by

© YULIN YAN

B.Sc. (Equivalent) China Pharmaceutical University

A Thesis Submitted to the Faculty of Graduate Studies
and Research of the University of Manitoba
in Partial Fulfillment of the Requirement for
the Degree of Master of Science
in the Department of Chemistry

Sept., 1988

Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission.

L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur (titulaire du droit d'auteur) se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation écrite.

ISBN 0-315-51643-7

SYNTHESIS OF 1-DEOXY-N,N-BIS-DEMETHYL-PYRROMYCIN (10), METHYL 3-N-(BENZYL-CARBOXAMIDO)-2,3,4,6-TETRADEOXY-DL-THREO-HEXOPYRANOSE (78) AND ITS 3-EPIMER (80)

BY

YULIN YAN

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

© 1989

Permission has been granted to the LIBRARY OF THE UNIVERSITY OF MANITOBA to lend or sell copies of this thesis, to the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film, and UNIVERSITY MICROFILMS to publish an abstract of this thesis.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

ACKNOWLEDGEMENTS

The author expresses his sincere thanks to Dr. C. M. Wong for his guidance and patience while doing the work presented in this thesis. The author would also like to express his thanks to Dr. Peter H. Y. Lam, from the University of Manitoba's Department of Medicine, for the biological activity tests. Thanks are also due to Ms. Huiyin Liu who developed some of the processes in the aminosugar synthesis.

The financial support by the University of Manitoba is deeply appreciated by the author.

ABSTRACT

A new anthracycline, 1-deoxy-N,N-bis-demethyl-pyrromycin (10) was synthesized by coupling natural aklavinone (55) with protected 1-bromo- (49) and 1-chloro-aminosugar (50) under Koenigs-Knorr condition. The coupling process is highly stereoselective presumably due to the formation of the 7-membered ring nitrobenzoyloxonium intermediate (59) to give the α-oriented glycoside (56) as the predominant product.

Aklavinone (55) was obtained by acid hydrolysis of a pigment compound isolated from the culture of Streptomyces Galilaeus Var. Siwenensis (68). The structural assignment of (55) was achieved by comparison of all chemical and physical data with literature values and further proved by high resolution p.m.r. spectrum (Fig. 1B) which is not available in the literature. The structures of compounds 56 and 57 were also unambiguously assigned by their two dimensional p.m.r. spectrum (Figs. 5B), ^{13}C spectrum (Fig. 5D) and high resolution p.m.r. spectrum (Fig. 6B) as well.

1-Deoxy-N,N-bis-demethyl-pyrromycin (10) shows remarkable antitumor activity comparable to those of adriamycin (2) and aclacinomycin A (6), (1).

Compound methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-DL-threo-hexopyranose (78) and its 3-epimer 80, are considered as intermediates for the total synthesis of novel aminosugars 2,3,4,6-tetradeoxy-3-N-aminomethyl-DL-threo-hexopyranose (92) and 2,3,4,6-tetra-3-N-aminomethyl-DL-erythro-hexopyranose (93). Intermediates

78 and 80 were also converted to new aminosugars 86 and 87.

The structures, relative stereochemistry and the preferred conformations of the four anomers, 82, 83, 84 and 85 of the key intermediates 78 and 80 were positively determined by detailed examination of their infrared spectra, (Figs. 14A, 15A, 17A and 18A), high resolution p.m.r. spectra (Figs. 14B, 15B, 17B and 18B) and mass spectra, (Figs. 14C, 15C, 17C and 18C), including high resolution mass spectra, (Figs. 14D, 15D, 17D, 18D).

TABLE OF CONTENTS

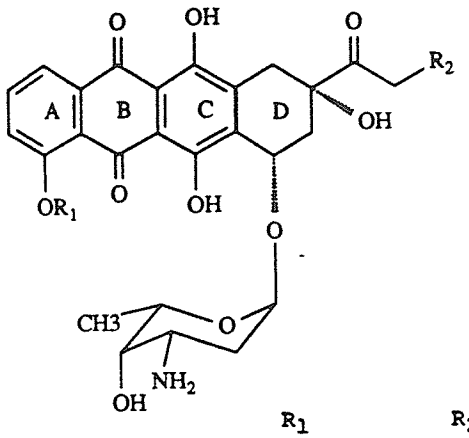
1. Introduction
2. Results and Discussions
   1) Part A: Synthesis of 1-Deoxy-N,N-bis-demethyl pyrromycin (10).
   2) Part B: Synthesis of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-DL-threo-hexopyranose (78) and its 3-Epimer (80).
3. Experiment
   1) Part A: Synthesis of 1-Deoxy-N,N-bis-demethyl-pyrromycin (10).
   2) Part B: Synthesis of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-DL-threo-hexopyranose (78) and its 3-epimer (80).
4. Spectra
   1) Infrared
   2) Nuclear Magnetic Resonance
   3) Mass Spectra
5. Reference

INTRODUCTION

The work on the antitumor anthracyclines can be traced back to the late 1950's (2). Later on, rhodomycin A (4) and B (5), pyrromycin (9), cinerubins A (8), aklavin (7), and other anthracyclines were separated and characterized (3). Among this class of pigmented substances antibiotic and antitumor activity was only displayed by the glycosides. A detailed review of studies of the anthracyclines during 1950's was given by Brockmann (3).

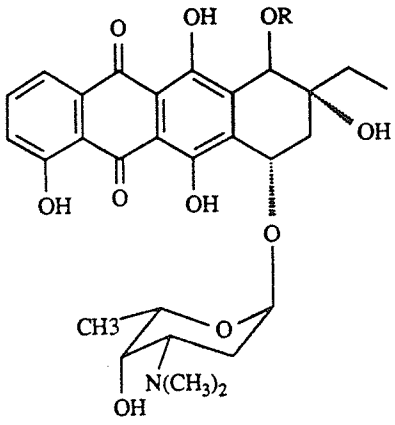
In 1961, daunomycin (1) was isolated from a culture of Streptomyces peucetius (4). Some other daunomycin related analogues had also been isolated from microbial culture, such as carminomycin (3), (7), and duborimycin (8). The biological antiyumor activities of daunomycin (1), which demonstrated superiority over the previously known anthracyclines such as rhodomycin A (4) and B (5), motivated the elucidation of the structural features of daunomycin and its analogues.

The research strategy, concerned with the development of new anthracycline analogues with more potent antitumor activities and/or less cardiotoxicities than daunomycin (1), were originally carried out on two lines: one having purpose of investigations of structure, stereochemistry and structure-activity relationship; and the other having the purpose of search for new biosynthetic analogues in cultures. These approaches essentially resulted in the isolation and characterization of adriamycin (2) in 1968 (5), aclacinomycin A (6) in

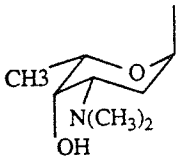


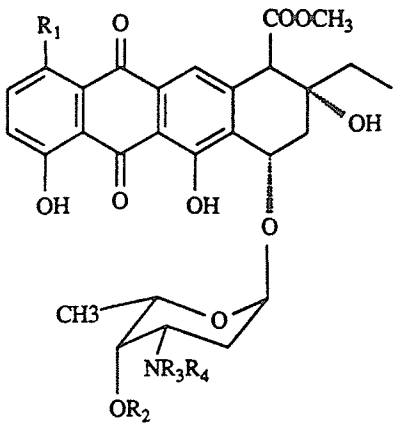
		R_1	R_2
Daunomycin	(1)	CH_3	H
Adriamycin	(2)	CH_3	OH
Carminomycin	(3)	H	H

		R
Rhodomycin A	(4)	X
Rhodomycin B	(5)	H



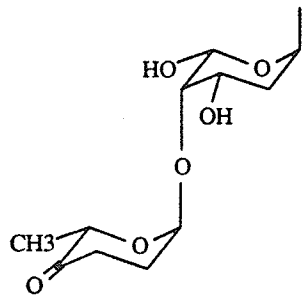
X =





		R_1	R_2	R_3	R_4
Aclacinomycin A	(6)	H	Y	CH_3	CH_3
Aklavin	(7)	H	H	CH_3	CH_3
Cinerubin A	(8)	OH	Y	CH_3	CH_3
Pyrromycin	(9)	OH	H	CH_3	CH_3
1-Deoxy-N,N-bis-demethyl-pyrromycin	(10)	H	H	H	H

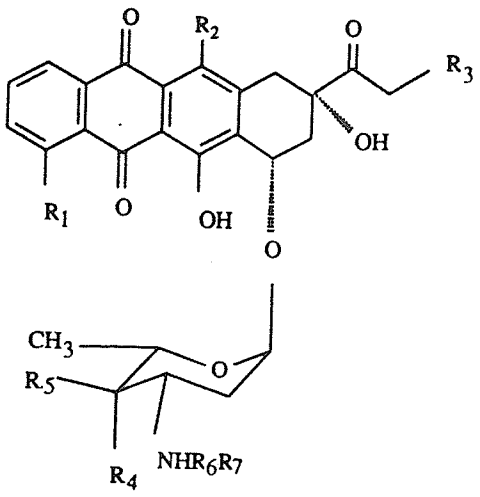
Y =



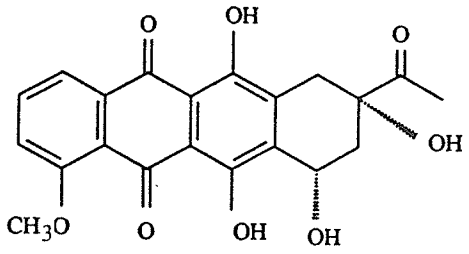
1975 (6) and the development of some new synthetic analogues which displayed better biological antitumor activities and/or less cardiotoxicities than daunomycin (1).

Daunomycin (1), as well as adriamycin (2) and aclacinomycin A (6), are being used clinically in the treatment of acute leukemia and solid tumors in man. Unfortunately, these compounds have some undesirable side effects, the most serious being dose-related cardiotoxicities to various extents (9), which hampers their therapeutical applications. However, such dose related cardiotoxicities are not observed with the administration of other anticancer drugs (10).

The mechanisms of antitumor properties of these drugs seem to be related with anthracycline glycoside-DNA intercalation (11), bioreductive alkylation (12), and anthracycline glycoside-cell surface interaction (13). The X-ray diffraction study of the daunomycin-DNA complex revealed that the daunomycin aglycone chromophore in the DNA complex is oriented at right angles to the long axis between base pairs of DNA. The ring D rests in the minor groove of the double helix (14). The oxygen on C_7 is in a quasi axial position in which the oxygen is projected further away. Consequently the oxygen on C_7 and the hydroxy hydrogen on C_9 can no longer form an intramolecular hydrogen bond, in contrast to those observed in other crystal structures of anthracycline antibiotics, (14, 15). The C_9-OH forms hydrogen bonds with the nitrogens of an adjacent base in DNA double helix (14). Interestingly, in a series of related anthracycline glycoside drugs, the cardiotoxicity can not be related



		R_1	R_2	R_3	R_4	R_5	R_6	R_7
4-Demethoxy-daunomycin	(11)	H	OH	H	OH	H	H	H
4-Demethoxyadriamycin	(12)	H	OH	OH	OH	H	H	H
11-Deoxydaunomycin	(13)	OCH_3	H	H	OH	H	H	H
11-Deoxy$_a$driamycin	(14)	OCH_3	H	OH	OH	H	H	H
4'-Epidaunomycin	(15)	OCH_3	OH	H	H	OH	H	H
4'-Epiadriamycin	(16)	OCH_3	OH	OH	H	OH	H	H
4'-Deoxydaunomycin	(17)	OCH_3	OH	H	H	H	H	H
4'-Deoxyadriamycin	(18)	OCH_3	OH	H	H	H	H	H
N,N-Dimethyl-daunomycin	(19)	OCH_3	OH	H	H	H	CH_3	CH_3



(20)

to the antitumor activity, suggesting a unique mode of action (16). Some reports suggested correlation of this cardiotoxicity with an anthracycline glycoside-mitochodrial membrane interaction (17). In terms of biochemistry, the cardiotoxicity is assumed to be a consequence of a redox process involving the quinone moiety of the aglycone which generates superoxide and hydroxide radicals.

These radicals are highly toxic to the heart cell in which superoxide dismutase or catalase concentration is very low compared to the liver and kidney cells (18). In addition, mutagenicity did not seem to relate with the cytotoxic activity, e.g. antitumor activity, (19).

The chemical investigation of new analogues of daunomycin (1) mainly focuses on four objectives: 1) structural and stereochemical investigations of anthracyclines; 2) modification and total syntheses of aglycone moieties; 3) modification and total syntheses of aminosugar moieties; and 4) methodology of coupling aglycones with aminosugar moieties. So far more than 500 analogues of daunomycin have been synthesized or isolated from nature and tested for biological activity, (20).

1) Structural and Stereochemical Investigations of Anthracyclines

The structural and stereochemical investigations of the anthracyclines show that the site on C_9 in ring D bears the two different carbon atom side chain, either an acetyl group in daunomycin or an ethyl group with an extra carbomethoxy side chain on C_{10} in aclacinomycin A. In addition, a tertiary hydroxy on C_9 and secondary hydroxy on C_7 have a cis geometry relationship with each

other. All of these are characteristic features of the anthracyclines with significant antitumor activity. Furthermore, ring D contains the site of sugar attachment, e.g. the benzylic position at C_7. Therefore two asymmetric centers at C_9 and C_7 reside in ring D of daunomycin (1) and adriamycin (2). Aclacinomycin A (6) has an extra asymmetric centre at C_{10}. The formation of a hydrogen bond between the hydroxy group on C_9 and oxygen atom on C_7, which was confirmed by X-ray diffraction studies of anthracycline, apparently stabilized the half-chair conformation (24) of ring D (Fig.1).

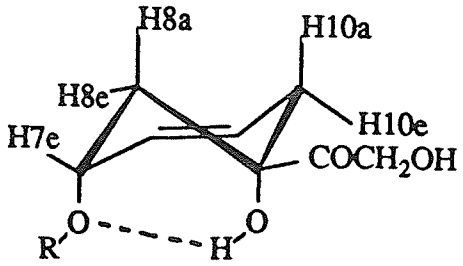
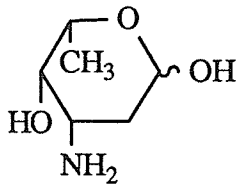
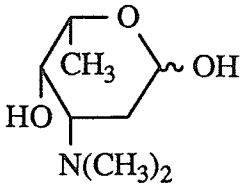


Fig. 1 The Half-chair Conformation of Ring D in Adriamycin (15).

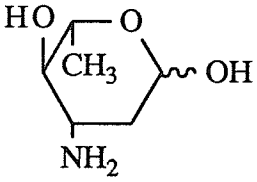
Daunosamine (21) and rhodosamine (22), present in anthracycline antibiotics, are L-lyxo-hexoses with α-glycosidic linkages to the C7 benzylic position. Four chiral centers in these aminosugars are assigned the 1'(R), 3'(S), 4'(S) and 5'(S) configuration corresponding to α-glycosidic linked glycosides. The α-glycosides may show



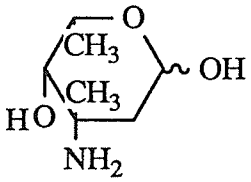
daunosamine (21)



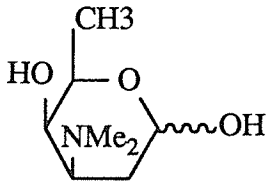
rhodosamine (22)



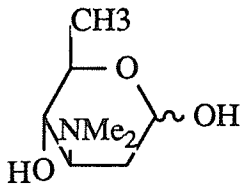
acosamine (23)



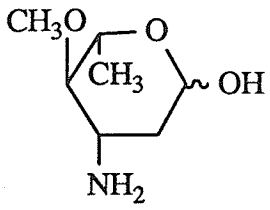
vancosamine (24)



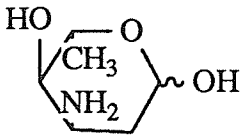
L-megosamine (25)



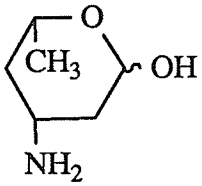
angolosamine (26)



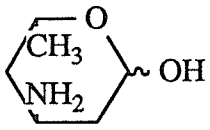
actinosamine (27)



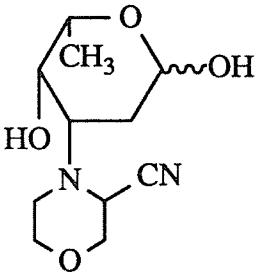
ristosamine (28)



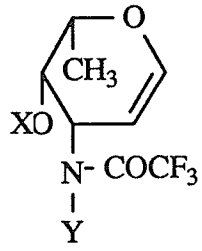
4-deoxydaunosamine (29)



4-deoxyristosamine (30)



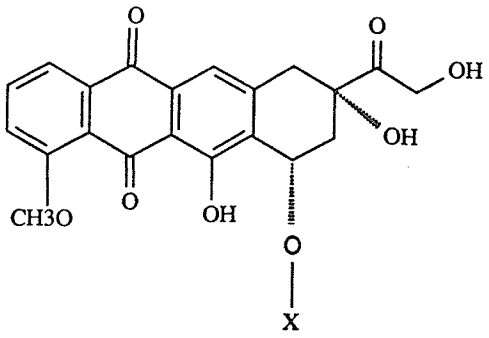
(31)



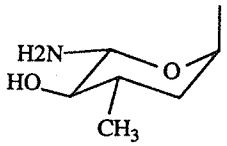
(32)

X = $COC_6H_4NO_2$

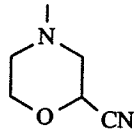
Y = Me

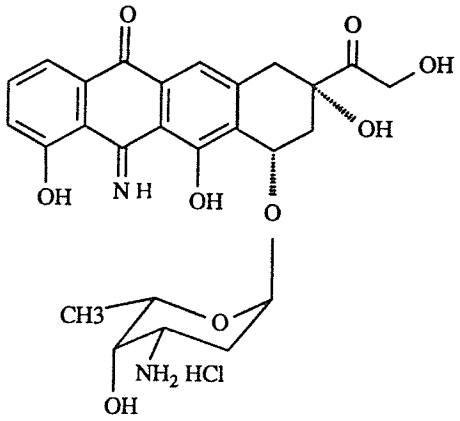


(19a) X =



(19b) X =



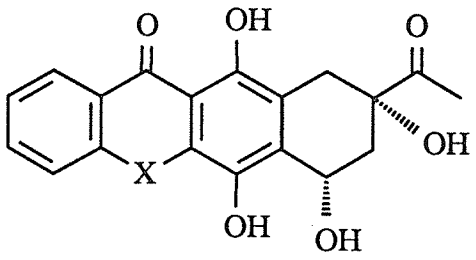


(19c)

antitumor activities while β-isomers are only weakly active or completely inactive (40). The special feature of Daunosamine (21), (3-amino-2,3,6-trideoxy-L-lyxo-hexopyranose), with the configurational and structural differences from other aminosugars present in anthracyclines, seems to account for the remarkable antitumor activity of the anthracycline glycosides, such as daunomycin (1) and adriamycin (2). In addition to daunosamine (21), other 3-amino-2,3,6-trideoxy hexoses such as L-rhodosamine (22), 3-N,N-dimethylamino-2,3,6-trideoxy-L-lyxo-hexopyranose, from rhodomycin and Aklavin (6); acosamine (23), 3-amino-2,3,6-trideoxy-L-arabino-hexopyranose, from actinoidin; vancosamine (24), 3-amino-3-C-methyl-2,3,6-trideoxy-L-lyxo-hexopyranose, from vancomycin; L-megosamine (25), 3-dimethylamino-2,3,6-trideoxy-L-ribo-hexopyranose, from megalomycins; angolosamine (26), 3-dimethylamino-2,3,6-trideoxy-D-arabino-hexopyranose, from angolamycin; actinosamine (27), 3-amino-4-O-methyl-2,3,6-trideoxy-L-arabino-hexopyranose, from actinoidin and ristosamine (28); 3-amino-2,3,6-trideoxy-L-ribo-hexopyranose, from ristomycin, have been found as components of other antibiotic molecules.

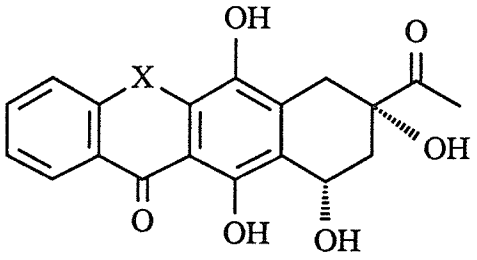
2). Modification and Total Synthesis of Aglycone Moieties

A huge number of chemically modified analogues of the antitumor anthracyclines have been obtained by exploration of the reactivity of analogues modified in ring D substitution and those modified in the anthraquinone chromophore (21), such as 19c. The modifications of ring D were mainly focused on: the modification of the side chains on C_{13} and C_{14} by oxidative degradation; the variation of the side



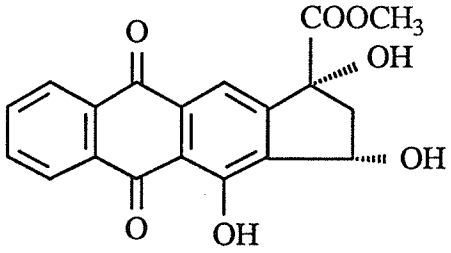
(33) X = SO_2

(33a) X = SO



(34) X = SO_2

(34a) X = SO



(35)

chains at C_9 and C_{10}; the substitution at C_8; and construction of a new skeleton of ring D. Modification of the quinone moiety and the anthraquinone chromophore at positions of C_4, C_6 and C_{11} were also investigated extensively by: a) synthesis of 4-demethoxy-daunomycin (11), 4-demethoxyadriamycin (12), 11-deoxydaunomycin (13), 11-deoxyadriamycin (14), the 6-O-methyl and the 11-O-methyl derivatives; b) replacement of 4-O-methyl with other alkyl groups; and c) the modification of the quinone moiety (21) and the skeleton of ring D (22, 23).

Some significant results of structure-activity-toxicity study are worth mentioning. Alternation of the groups attached to C_9 produced profound effects on the biological antitumor activities (20). Absence of 4-methoxy or 11-hydroxy groups seemed to display higher anticancer activity and/or lower cardiotoxicity (25). The quinone moiety seems to be responsible for both antitumor activity and cardiotoxicity (20b). A summary of studies on anthracyclines during the 1960's and 1970's was given by F. Acarmone (4c).

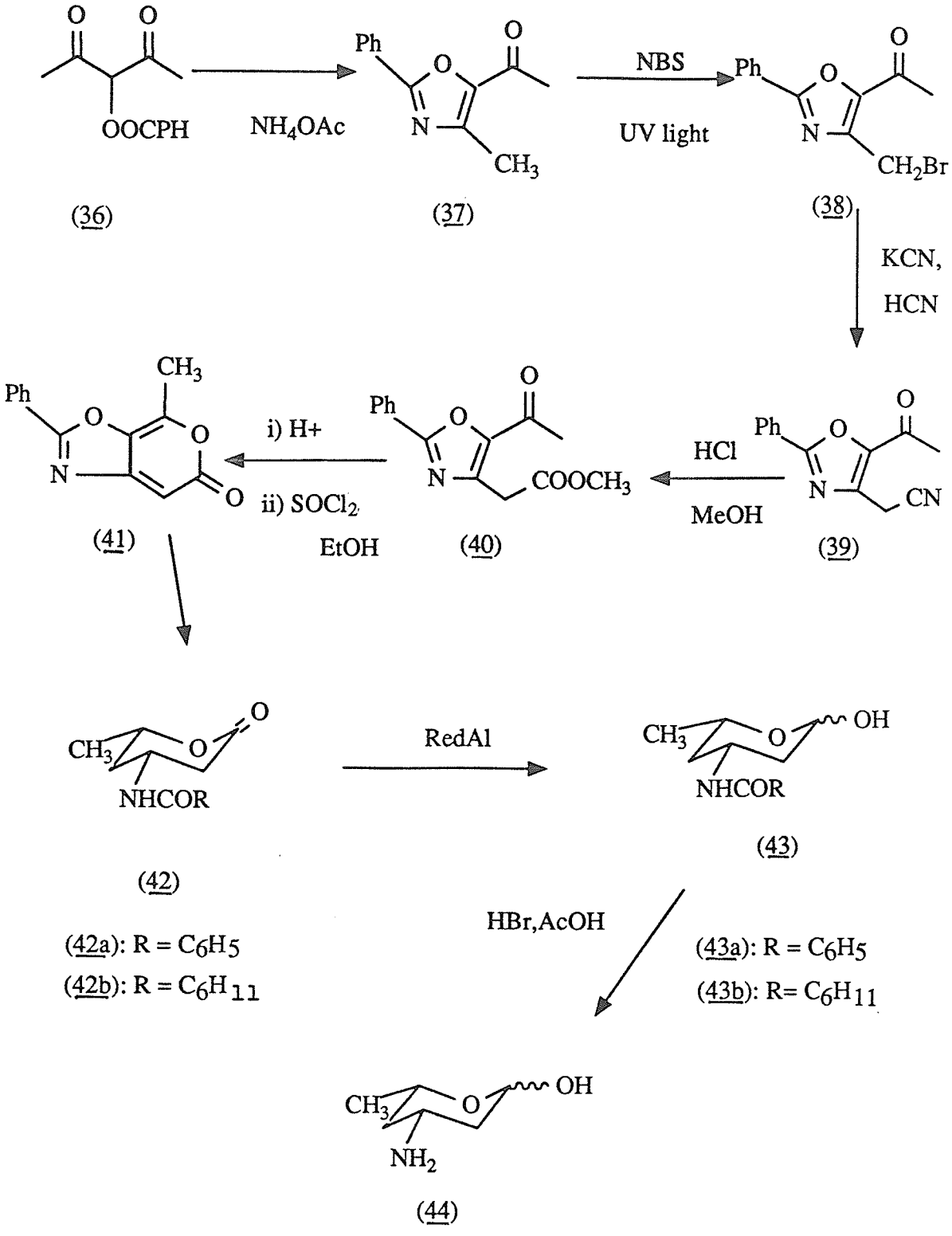
The total syntheses of the aglycone moieties of daunomycin, adriamycin, aclacinomycin A and their analogues have also been extensively studied (4c, 26-36). The first total synthesis of daunomycinone (20) in 1971 and heteroanthracyclinones, such as, 6,7,9,11-tetrahydroxy-9-acetyl-7,8,9,10-tetrahydrobenzo(b)thioxanthen-12-one-5-oxide (33a), the 5-dioxide analogue 33; their corresponding regioisomers 34a and 34 in 1984; and the aglycone 35 with five membered ring D in 1987 were reported by Wong and coworkers, (22, 33, 37, 38). The total syntheses of anthracyclinones, while quite different in

conception, all involved the construction of either ring B or C from a bicyclic precursor as a key step. The challenge related to the construction of the aglycone is: the regiospecific connection of the ring A to the CD unit; the functionalization of ring D; and the stereospecific introduction of two hydroxy groups on C_9, C_7 and $-COOCH_3$ on C_{10} in the case of aklavinone.

3) Modification and Total Syntheses of Aminosugar Moieties

The presence of the aminosugar residue in the anthracycline antibiotics is an important structural requirement for biological activity, since biological activity of the isolated aglycone moiety has never been reported. The results also indicate that the mutagenesis of anthracycline is closely related to the structure difference in the sugar moiety (19). The 3'-amino groups in the anthracycline analogues are involved in electrostatic interaction between the aminosugar and the phosphate groups of DNA (39) and therefore are responsible for significant antitumor activities (43). As such it has been the object of extensive synthetic investigation.

A number of modified amino sugar analogues have been comprehensively evaluated through the investigation of structure-activity relationship of a number of systems including: 4'-epidaunomycin (15), 4'-epiadriamycin (16), 4'-deoxydaunomycin (17), 4'-deoxyadriamycin (18), 4'-methyl derivatives, 4'-C-methylated analogues, and other configurational analogues such as L-ribo analogues, L-xylo-analogues, configurational analogues belonging to the D-series and N-acyl derivatives, N,N-dimethyl daunomycin (19) and non sugar derivatives (4c).



(36) (37) (38) (39) (40) (41) (42) (43) (44)

(42a): R = C_6H_5
(42b): R = C_6H_{11}

(43a): R = C_6H_5
(43b): R= C_6H_{11}

Scheme 1 Synthesis of (R, S) 4-Deoxydaunosamine (44) from 3-benzoyl-2,4-pentanedione (48).

The structure-activity-toxicity study revealed that 4'-deoxydaunomycin (17), 4'-epidaunomycin (15), 4'-deoxyadriamycin (18) and 4'-epiadriamycin (16) all have lower cardiotoxicity, presumably due to a lower level of superoxide produced, which was directly related to the absence of 4'-deoxy groups of these compounds, (41, 42). N,N-diakylation of daunomycin (19) seems to enhance the efficacy against test tumour cells but it was found, however, to be markedly more cardiotoxic than adriamycin (2) (44). This conclusion could be further supported by the biological antitumor activity of 3'-deamino-3'-(3-cyano-4-morpholinyl)-adriamycin (19b), which was much more active than daunomycin (1) but with extraordinarily higher cardiotoxicity (45) than that of daunomycin (1). Configurational analogues belonging to D-series, such as 7-O-(3-amino-2,3,6-trideoxy-D-arabino-hexopyranosyl)-daunomycin (19a), show weak activity (46).

The syntheses of both racemic and chiral daunosamine, 4-deoxydaunosamine and other related analogues have been reported. The idea of using 4-deoxydaunosamine was originally suggested by Wong et al., who first presented the total synthesis of (R,S) daunosamine and (R,S) 4-deoxydaunosamine from non-sugar precursors in 1975 and 1978 respectively (47, 48). Wong's synthesis of 4-deoxy-DL-daunosamine is illustrated in Scheme 1.

Oxazolino-γ-pyrone (41), obtained in four steps from compound (36), was hydrogenated in the presence of Adam's catalyst to a mixture of (42a) and (42b), or to only (42b), the relative stereochemistry of the products being ascertained by the analysis of the

p.m.r. spectra. Both (42a) and (42b) were converted to 3-amino-2,3,4,6-tetradeoxy-D-threo-hexopyranose (44) by reduction of the lactone function to the hemiacetal as in (43a) and (43b), followed by hydrolysis with hydrogen bromide.

Since then, a great number of reports dealing with total syntheses of racemic daunosamine (49) and L-daunosamine have been published (50). Various efforts have been made to increase stereoselectivity in the synthetic processes.

The first total synthesis of optically active daunosamine from L-fucose was accomplished by Marsh et al (51). Various other chiral materials were also employed as precursors, such as less expensive D-sugars (52), fermentation intermediate (53), D-threonine and tartaric acid (54) and other sugar precursors (55). Sugar precursors were also used to synthesize 4-deoxydaunosamine (56). Dyong and Weiman (50) reported the first use of asymmetric induction to accomplish a chiral total synthesis of daunosamine. Other novel asymmetric synthesis (57) and the chiral pool syntheses of derivatives of daunosamine (58) were also achieved.

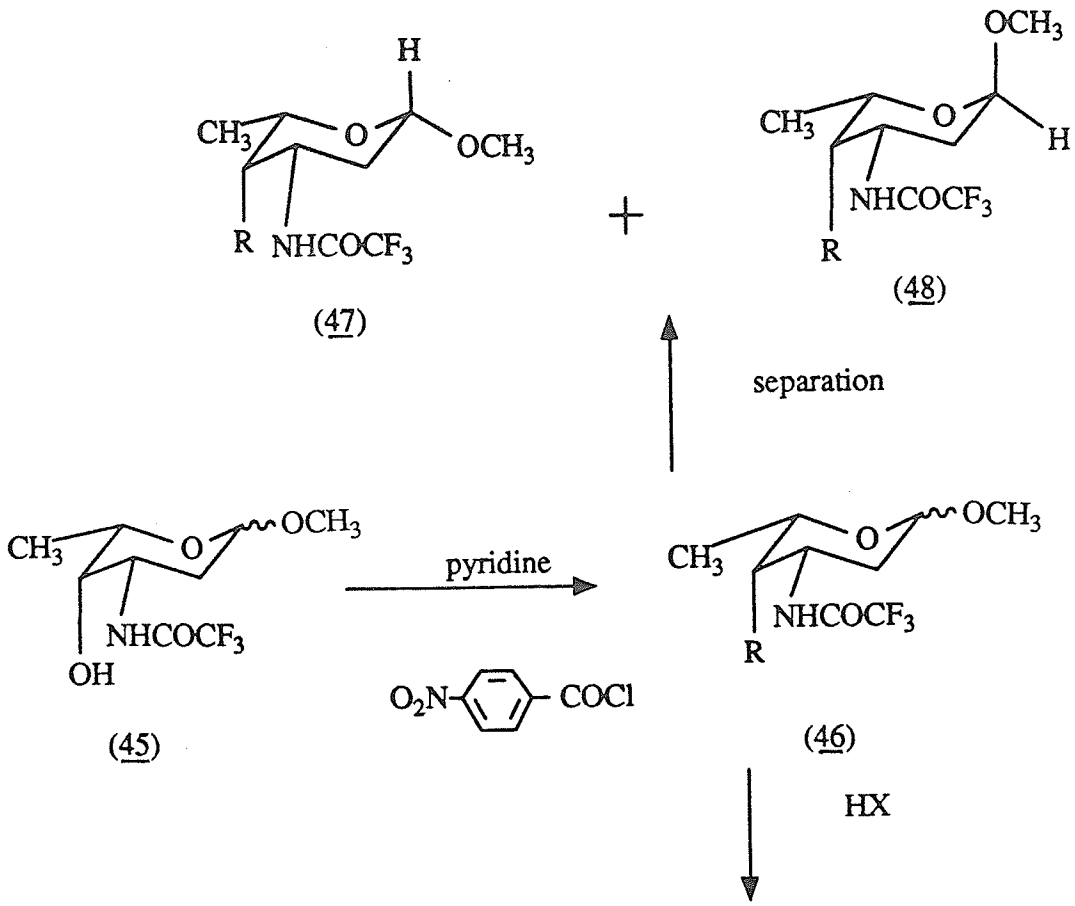
L-Daunosamine, prepared through asymmetric synthesis via an enantioselective intramolecular [3+2] cycloaddition of a nitrone to an olefine, was reported by Peter M. Wovkulich et al. (57). Frank M. Hauser et al. carried out a stereoselective synthesis of N-trichloracetyl derivatives of (R, S) daunosamine starting from simple acyclic precursor via a Pummerer rearrangement of the corresponding sulfoxide isomers (59). L-Daunosamine obtained from the optically

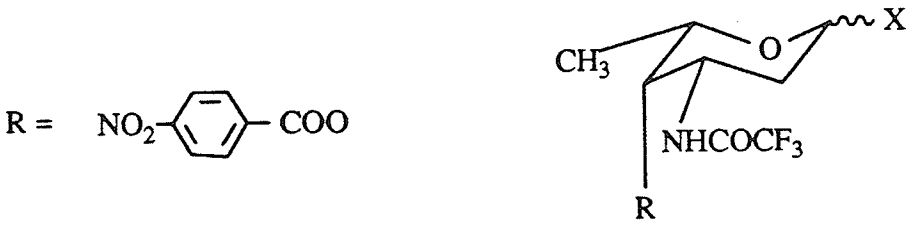
active synthetic intermediate, 7-oxa-bicyclo-[2.2.1]-hept-5-enes was reported by Pierre Vogel et al. (60), in 21.8 % overall yield.

4) Methodology of Coupling Aglycones with Aminosugar Moieties

The first glycosidic coupling of daunomycinone with protected daunosamine under Koenigs-Knorr reaction was reported by Acton et al. (61). The presence of silver trifluoromethane sulfonate, which assisted glycosidation of aglycone with sugar halide, has been proved to be particularly useful for the preparation of glycosides (62). To date, almost all glycosidation processes involved in the chemical syntheses of the anthracyclines have been carried out by the classical Koenigs-Knorr reaction or modified procedure (62). The acid catalyzed condensation of an aglycone and a protected hex-1-enopyranose, such as 32, was carried out in the presence of p-toluenesulfonic acid at room temperature to give, stereoselectively, only the α-glycoside (63). The high-yield enzymatic glycosidation of aklavinone (55) with corresponding sugars to give aclacinomycin A (6), was achieved by H. Umezawa and coworkers (65).

Based on these extensively fundamental studies, many new anthracycline analogues, both of natural and synthetic origin, such as 4-demethoxydaunomycin (11), 4-demethoxyadriamycin (12), 4'-deoxyadriamycin (18), 4'-deoxydaunomycin (17), 11-demethoxydaunomycin (13), 11-deoxyadriamycin (14), 4'-epiadriamycin (16), and aclacinomycin A (6), exhibit greater antitumor activity and/or less cardiotoxicity (45, 65). Some of them are being used in clinical studies.

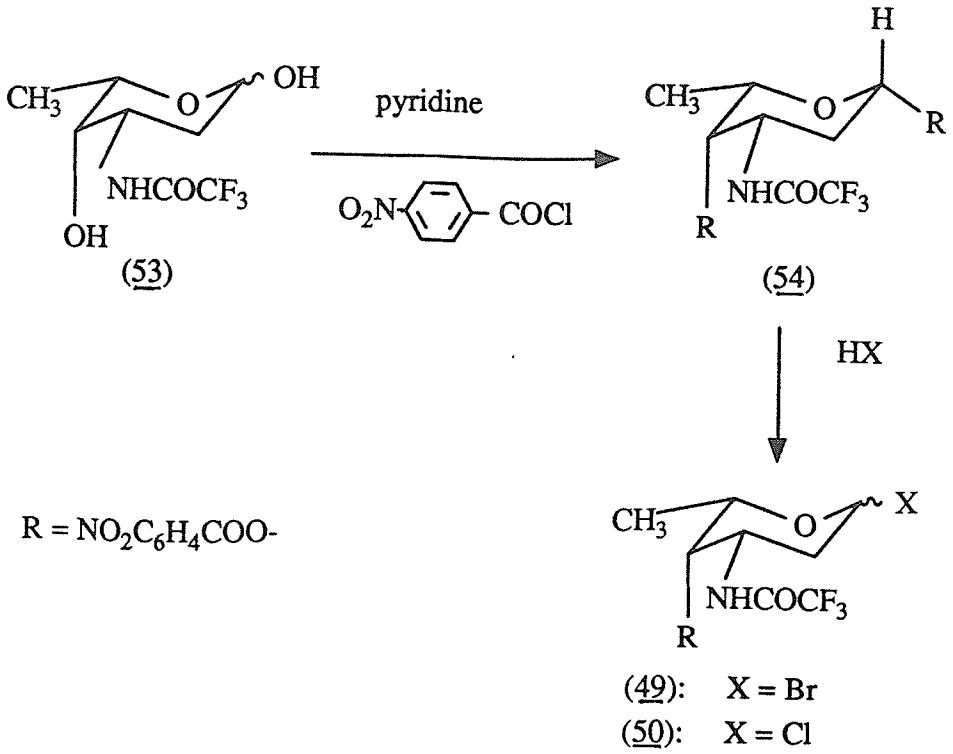




(49): X = Br

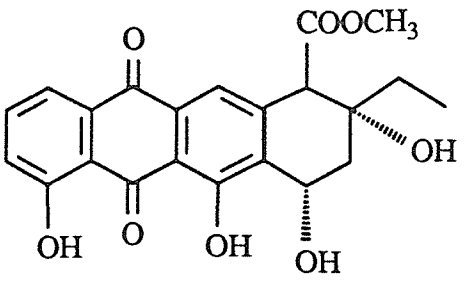
(50): X = Cl

Scheme 2 Synthesis of 1-Bromo-aminosugar (49) and aminosugar (50)



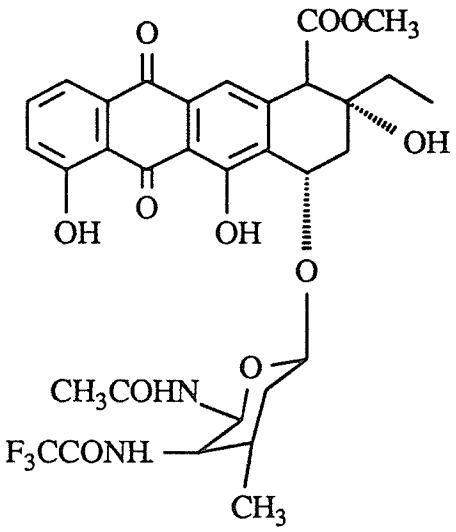
Scheme 3 Synthesis of 1-bromo-Aminosugar (**49**) and 1-chloro-aminosugar (**50**) from N-Trifluoroacetyl-daunosamine (**53**).

Scheme 4 Synthesis of 1-Deoxy-N,N-bis-demethyl-pyrromycin (10).



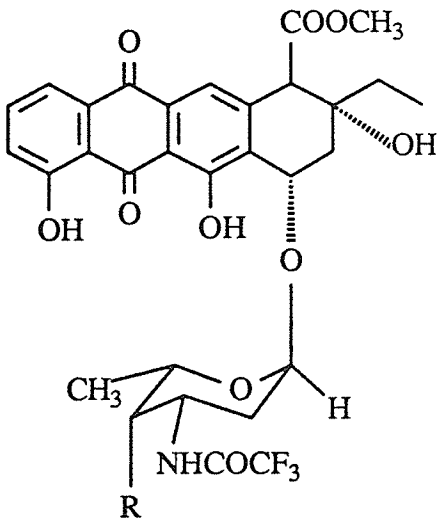
(56)

1) (50) or (49)
2) silver trifluoro-methanesulfonate
3) dichloromethane

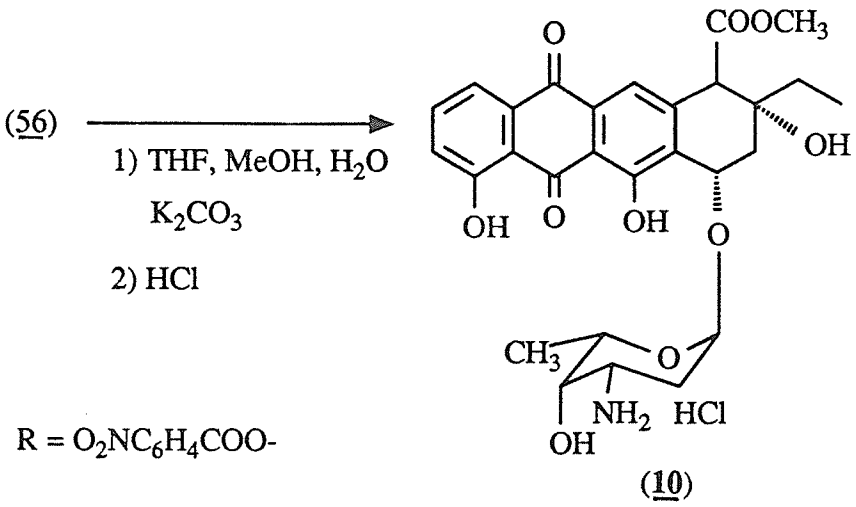


(57)

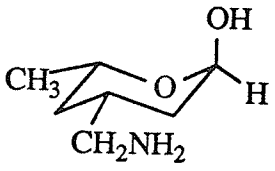
+



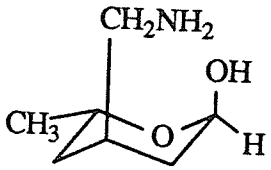
(56)


(56)
1) THF, MeOH, H_2O
K_2CO_3
2) HCl
R = $O_2NC_6H_4COO$-
O
$COOCH_3$
OH
OH
O
OH
O
CH_3
O
NH_2 HCl
OH
(10)

It was observed that removal of the 11-hydroxy function group reduces cardiotoxicity as in the cases of aclacinomycin A (6), 11-deoxydaunomycin (13) and 11-deoxyadriamycin (14) analogous. The daunomycin derivative, in which daunosamine (21) was substituted with its natural analogue rhodosamine (22), reduced mutagenicity and enhanced antitumor activity, but was markedly more cardiotoxic. It was also observed that 4'-deoxydaunosamine (29) was responsible for the increase in efficacy. In the case of 4'-deoxydaunomycin (17) and 4'-deoxyadriamycin (18) analogues, which have not been found in natural products, hence are superior to daunomycin (1) and adriamycin (2) themselves.



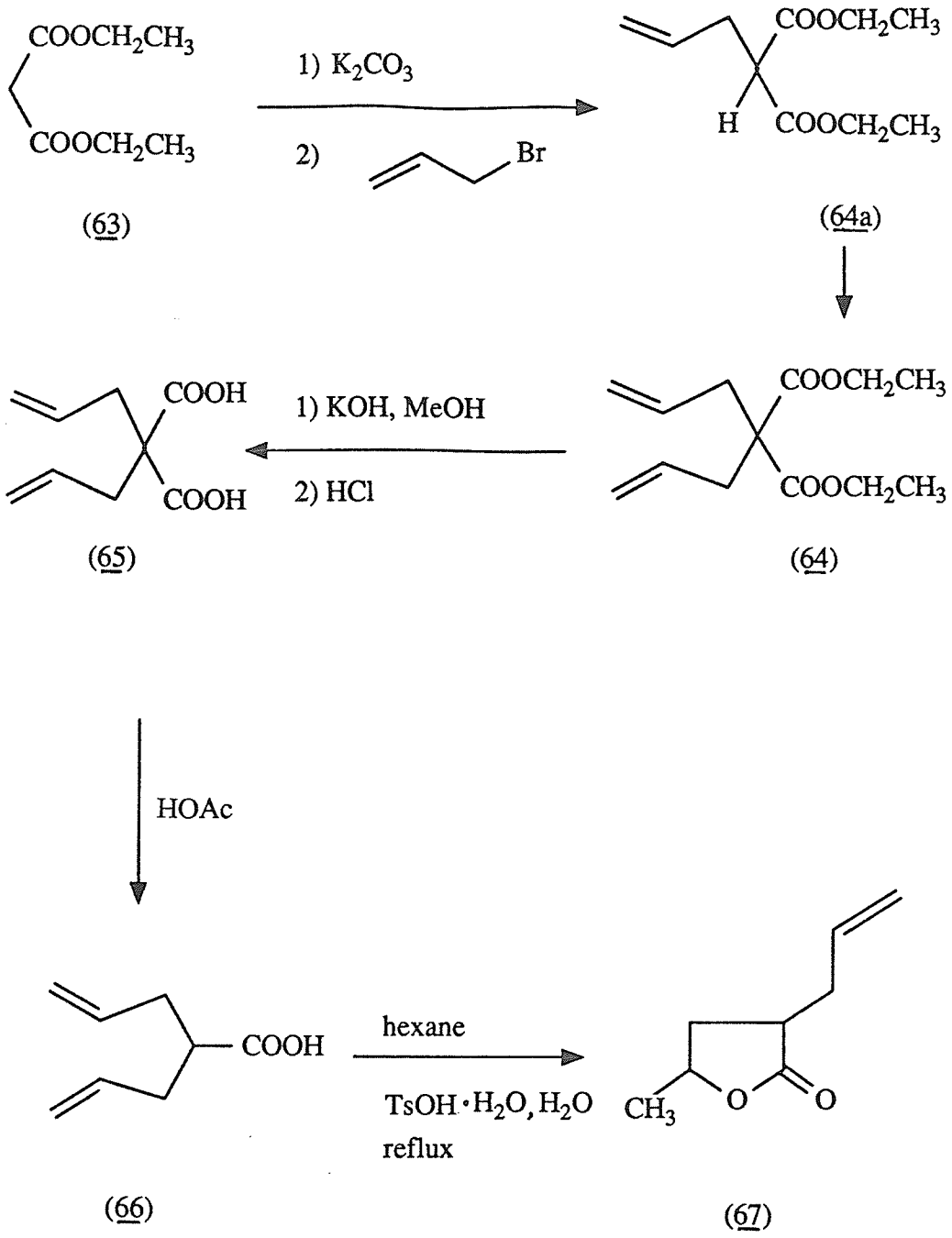
(92)



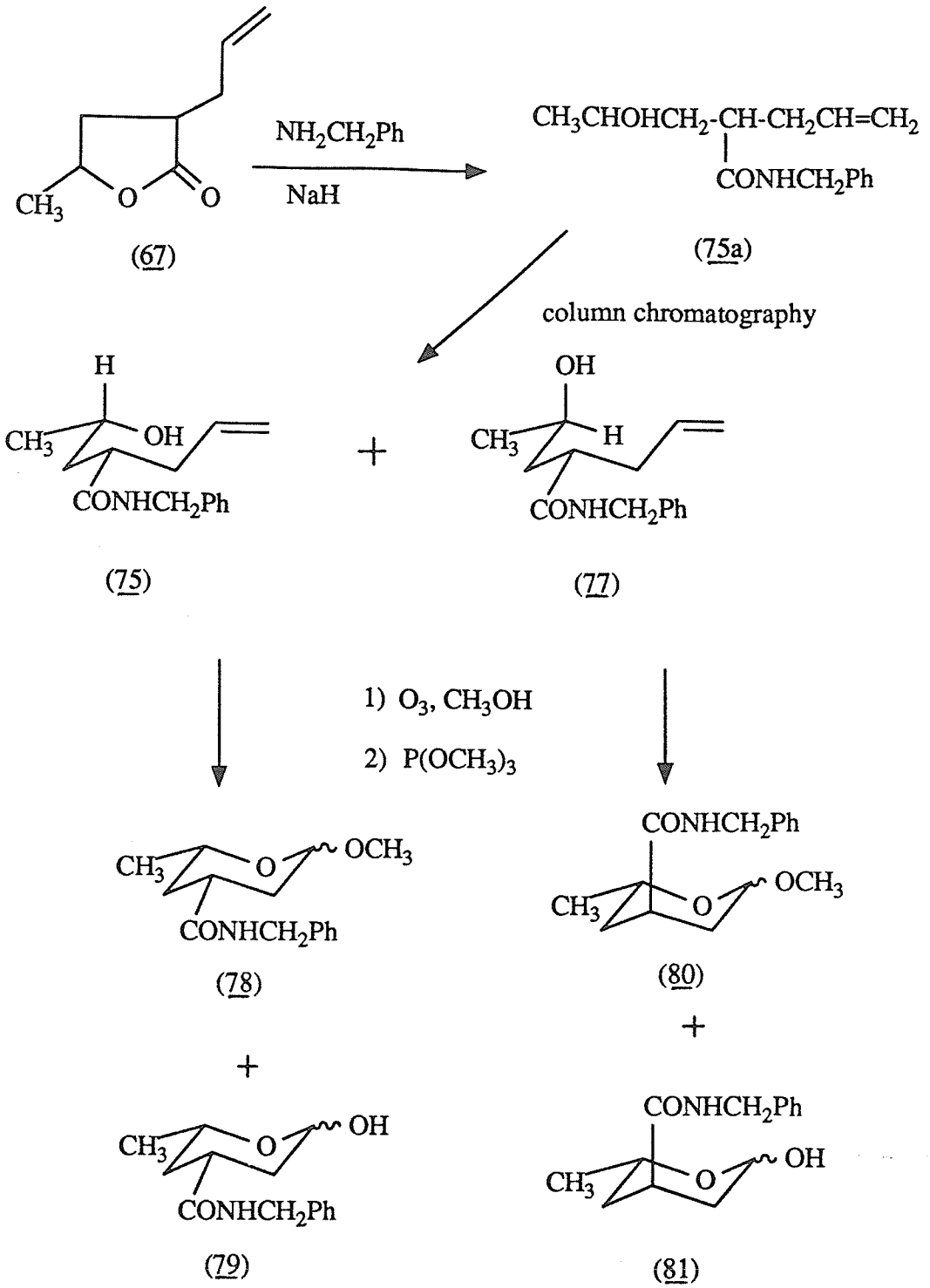
(93)

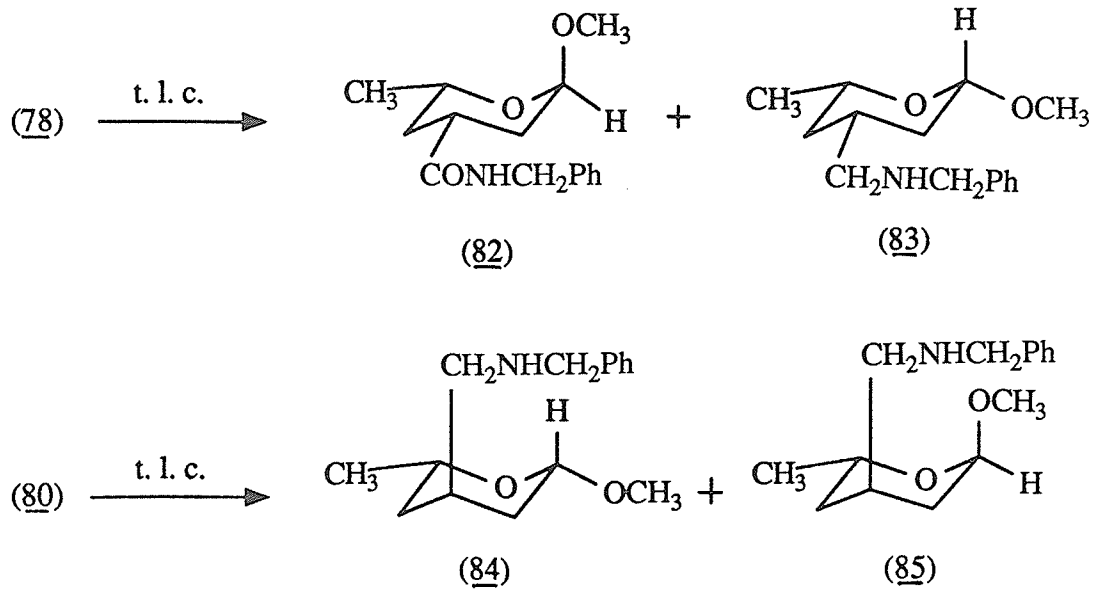
Therefore, it is of great interest to further investigate the structure-activity relationship of 1-deoxy-N,N-bis-demethyl-pyrromycin (10). However, based on literature survey, its physical and its preparation data were not reported before. Its biological property was only very casually mentioned as mutagenic in the S. typhimurium test by K. Umezawa et al. (90). It is also of great value to create

Scheme 5 Synthesis of 2-Allyl-4-hydroxy-4-methyl-γ- butyrolacton (67).



Scheme 6 Synthesis of methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-DL-threo-hexopyranose (78) and its 3-epimer 80





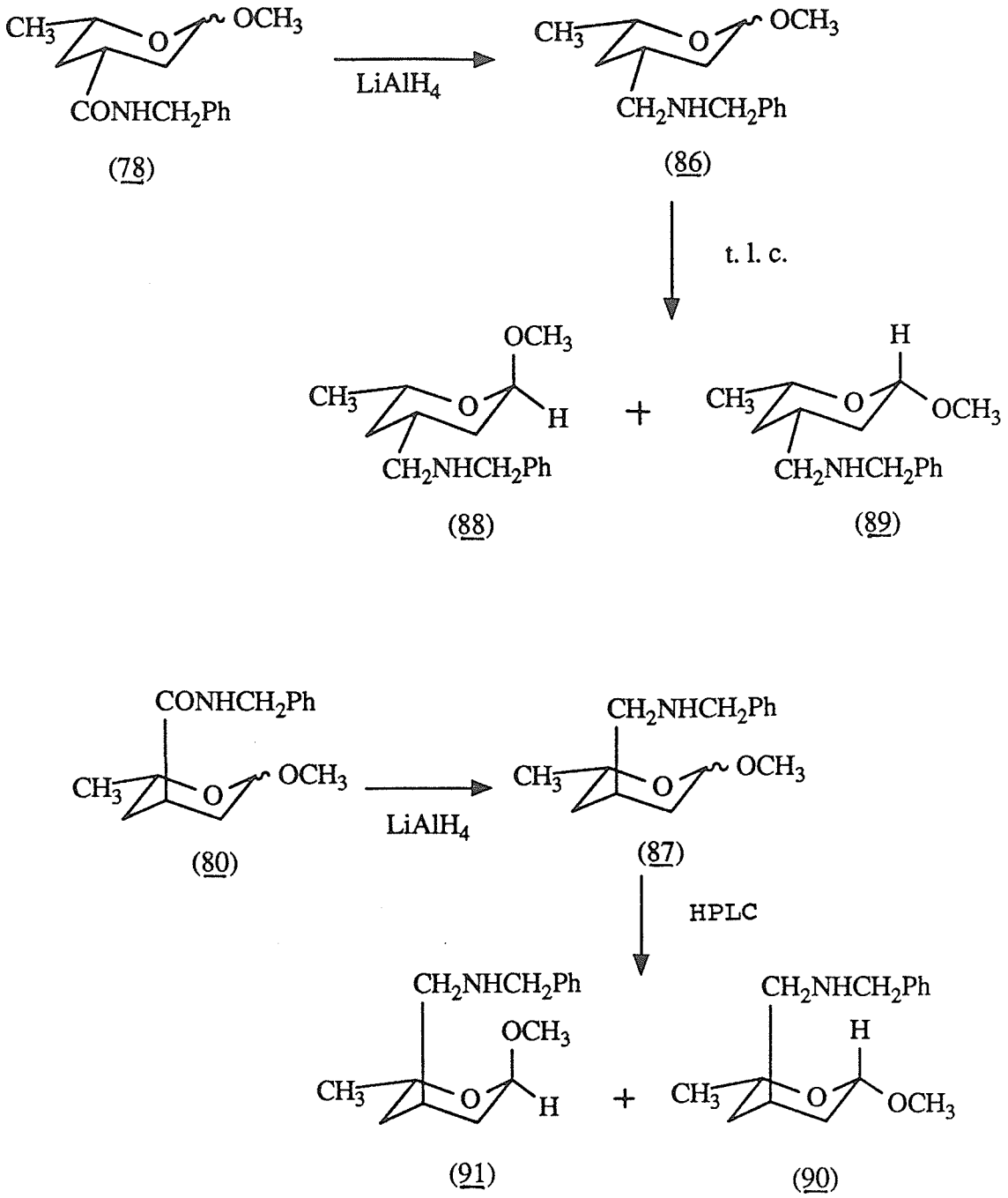
Scheme 7 Isolation of the Anomers (**82**), (**83**), (**84**) and (**85**) from methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-DL-threo-hexopyranose (**78**) and its 3-epimer **80**

an approach for total syntheses of 78 and 80, which are the intermediates for the new aminosugars 3-N-aminomethyl-2,3,4,6-tetra deoxy-DL-threo-hexopyranose (92) and its 3-epimer, 3-N-aminomethyl-2,3,4,6-tetradeoxy-DL-erythro-hexopyranose (93). Compound 78 and 80 were also converted to the new amino sugars 86 and 87. These new amino sugars will be coupled with different aglycone moieties for additional study of structure-activity relationship.

The preparation of protected 1-chloro- and 1-bromo-daunosamine 50 and 49 (Scheme 2 and 3), 1-deoxy-N,N-bis-demethyl-pyrromycin (10) (Scheme 4), the new aminosugar derivatives 78 and 80 (Scheme 5-6) and new aminosugars 86 and 87 (Scheme 5-8), are described in full detail in this thesis.

Aklavinone (55), used as a precursor, for the synthesis of 10 was obtained by acidic hydrolysis of a yellow pigment which was originally isolated from the cultured broth and mycelial cake of Streptomyces Galilaeus var. Siwenensis at Sichuan Industrial Institute of Antibiotics (China) (66). Its structure was positively identified by comparing all its physical and spectroscopic data with those reported in literature (67, 68).

Glycosidation of aklavinone (55) with 1-chloro-aminosugar 50 and 1-bromo-aminosugar 49 under Koenigs-Knorr condition gave α-glycoside 56 as a major product and β isomer 57 as the minor product. The results showed that the yield of α-glycosidation with 1-chloro-aminosugar was superior to that of 1-bromo-aminosugar. The structure of 56 is unequivocally established by detailed analysis of its two dimensional p.m.r. spectrum (Fig. 5B). Basic hydrolysis of



Scheme 8 Preparation of methyl 3-N-(benzylamino-methyl)-2,3,4,6-tetradeoxy- DL-threo-hexopyranose (86) and 3-epimer 87`

56 gave the final product 10, which displays strong antitumor activity paralleling that of adriamycin and aclacinomycin A, (Table 2), (1).

The preparation of methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-DL-threo-hexopyranose (78) and its 3-epimer 80, are presented in part B of the experiment section. The syntheses of 78 and 80 started from diethylmalonate (63) (Scheme 5-6). Alkylation of diethylmalonate with allyl bromide followed by acidic hydrolysis and decarboxylation gave compound 66. Cyclization of 66 in the solution of hexane, p-toluenesulfonic acid and water eventually gave 2-allyl-4-methyl-γ-butyrolactone (67) as a mixture of two diastereomers which failed to be separated from each other. Each of these diastereomers contains a pair of enantiomers which have been not resolved. The reaction of 67 with benzylamine and sodium hydride gave two diastereoisomers, 75 and 77 (Scheme 6).

Ozonolysis of 75 and 77 in anhydrous methanol followed by treatment with reducing agent trimethyl phosphite gave compounds 78 and 80, respectively (Scheme 6). Their anomeric isomers 82, 83, 84 and 85 were isolated upon column chromatography over silica, (Scheme 7), and their structures, relative stereochemistry and preferred conformations were, positively identified by detailed examination of the infrared spectra, (Figs. 14A, 15A, 17A and 18A), high resolution p.m.r. spectra (Figs. 14B, 15B, 17B and 18B) and mass spectra, (Figs. 14C, 15C, 17C and 18C), including high resolution mass spectra, (Figs. 14D, 15D, 17D, 18D).

Reduction of 78 and 80 with lithium aluminum hydride ($LiAlH_4$), gave new aminosugars 86 and 87 respectively, (Scheme 8). Their four anomers, 88, 89, 90 and 91, were also isolated by preparative layer chromatography upon silica gel and HPLC.

RESULTS AND DISCUSSIONS

PART A: SYNTHESIS OF 1-DEOXY-N,N-BIS-DEMETHYL-PYRROMYCIN (10)

The synthesis of 1-chloro-2,3,6-trideoxy-4-O-(p-nitrobenzoyl)-3-N-trifluoroacetamido-L-lyxo-hexopyranose, [1-chloro-4-O-(p-nitrobenzoyl)-3-N-trifluoroacetyl-daunosamine] (**50**) was achieved by two routes.

Treatment of 3-N-trifluoroacetyl daunosamine (**53**) with p-nitrobenzoyl chloride in methylene chloride in presence of pyridine, gave 1,4-di-O-(p-nitrobenzoyl)-N-trifluoroacetyl daunosamine (**54**), as a crystalline solid, in 80 % yield. The product was further purified by recrystallization from chloroform as white crystals, m.p. = 195-197 °C, Lit. 197.0-198.5 °C, (53), (Scheme 3).

This process was developed by modification of the similar one reported by Smith et al. (70) in order to obtain an acceptable yield in small scale reparation.

Conversion of β-1,4-di-O-(p-nitrobenzoyl)-N-trifluoroacetyl daunosamine (**54**) to the very unstable 1-chloro-4-O-(p-nitrobenzoyl)-N-trifluoroacetyl daunosamine (**50**) was achieved by anhydrous hydrogen chloride followed by filtration to remove the insoluble p-nitrobenzoic acid. Since **50** is very moisture sensitive, the whole reaction procedure was conducted in a sealed flask under anhydrous condition.

An alternative approach, starting from methyl N-trifluoroacetyl daunosamine (45), for preparation of 1-chloro-aminosugar (50), was also explored, (Scheme 3).

Reaction of 45 with p-nitrobenzoyl chloride in a solution of methylene chloride and pyridine gave methyl 4-O-(p-nitrobenzoyl)-3-N-trifluoroacetyl-daunosamine (46) as an amorphous solid from ether, m.p. = 70-74 °C, in 96.4 % yield.

The i.r. spectrum of 46, (Fig. 3A), reveals the carbonyl stretching at 1730 cm^{-1}, the aromatic C=C at 1610 cm^{-1}, the NO_2 bending vibration at 1530 cm^{-1} and OCH_3 at 2850 cm^{-1}.

The further preparative layer chromatography of 46 upon silica gel gave two anomers, 47 and 48, as the syrupy compounds, (Solvent A). The compound 47 and 48 were assigned to α and β glycoside respectively. Their p.m.r. and mass spectra are presented in Figs. 3B, 4B, 3C and 4C.

The p.m.r. spectrum of 47, Fig. 3B, shows the C_5-CH_3 at 1.20 ppm as a doublet and the two methylene protons on C_2 at 2.00 ppm as a multiplet. A three proton singlet at 3.40 ppm corresponds to the C_1-OCH_3. The C_5-H appears at 4.20 ppm as a quartet. The presence of a multiplet at 4.70 ppm is assigned to the C_3-H.

A one proton broad singlet at 4.95 ppm with W_H = 5.6 Hz is attributed to the hydrogen on C_1. Its small coupling indicates the equatorial orientation of the anomeric hydrogen, (70).

One methine proton on C_4 is at 5.45 ppm, as a barely resolved doublet. As expected, it is shifted to lower field than other methine protons in the molecule, most likely due to the desheilding effect of

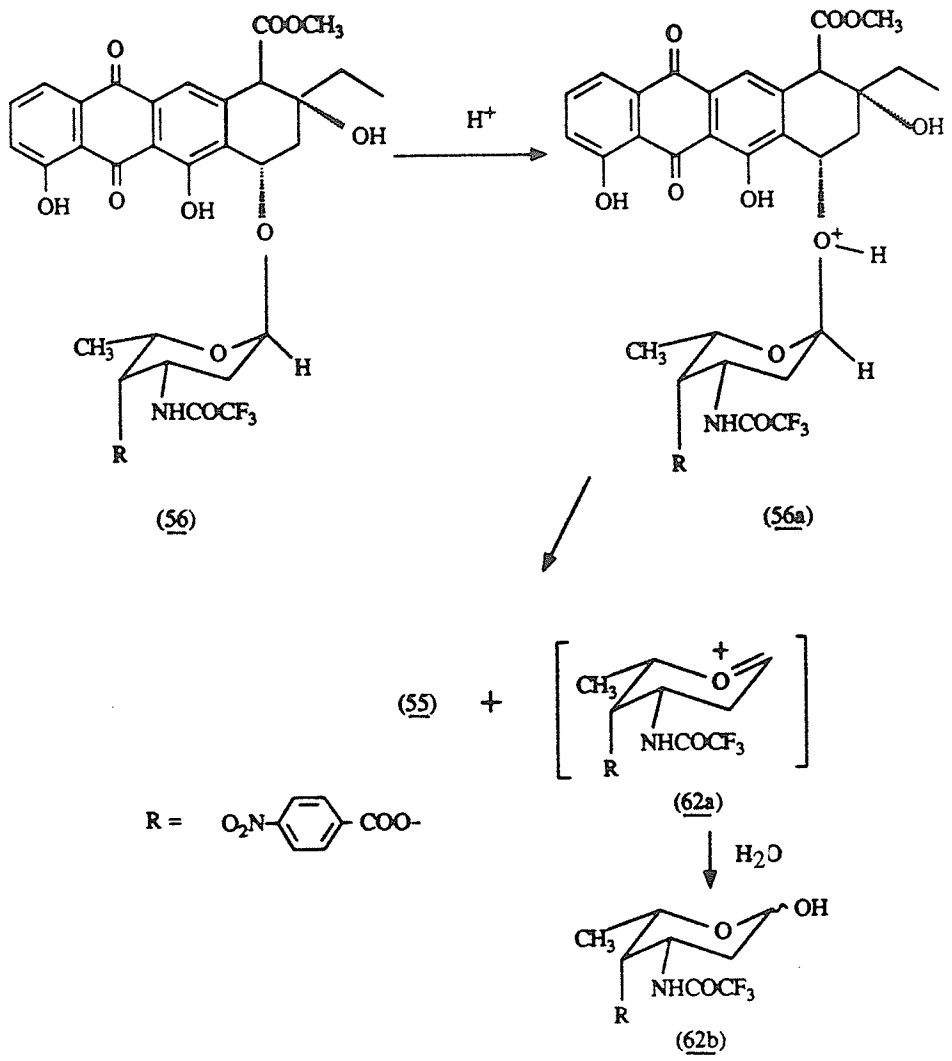
the carboxyl group on C_4. One proton signal at 6.37 ppm, as a broad doublet, corresponds to the NH proton. The four protons on p-nitrobenzoyl group appears at 8.28 ppm as a typical AA'BB' spin system.

In the mass spectrum of **47**, Fig. 3C, loss of CH_3O from the molecular ion gives the fragment M^+/z 375. The fragment at M^+/z 150 is due to the p-nitrobenzoyl ion.

The p.m.r. spectrum of **48**, Fig. 4B, shows three methyl protons, at 1.25 ppm, as a doublet and two methylene protons on C_2, at 1.85 ppm and 2.15 ppm, as two sets of a multiplet. The methoxy protons appear at 3.58 ppm as a singlet. One proton signal at 3.87 ppm as a quartet and one proton signal at 4.40 ppm as a multiplet may be assigned to the two methine protons on C_5 and C_3 respectively. The axial proton on C_1 occurs at 4.56 ppm as a double of doublets. One proton on C_4 is at 5.36 ppm as a poorly resolved doublet. One proton signal at 6.35 ppm, as a barely resolved doublet, corresponds to the NH. The four aromatic protons are at 8.30 ppm as the symmetric multiplet of a typical AA'BB' spin system.

The mass spectrum of **48**, Fig. 4C, shows that the fragment losing one proton from the molecular ion is at M^+/z 405. The fragment of the p-nitrobenzoyl ion is at M^+/z 150.

Further treatment of **46** with dry hydrogen chloride in methylene chloride solution at temperature of 0 °C for 4 hrs followed by evaporation of the solution to dryness gave **50**, (Scheme 2), as a foamy amorphous solid from ether. The residue was dissolved in methylene chloride, evaporated to dryness. These operations was



Scheme 9 The Hydrolysis of 7-O-3'-N-(Trifluoroacetyl)-4'-O-(p-nitrobenzoyl)-α-daunosaminyl-aklavinone (56) under Acidic Condition.

repeated once more until the residue was free of hydrogen chloride. It is a precaution against possible cleavage of the glycosidic bond under acidic condition, (Scheme 9). This residue was permitted to couple with aklavinone without further purification.

Aklavinone (55) was obtained by acidic hydrolysis of a yellow amorphous solid, which exhibited potent antitumor activity, (66). This solid was separated from the cultured broth and mycelial cakes of Streptomyces Galilaeus var. Siwenensis. This amorphous solid was further purified by preparative layer chromatography upon silica gel (Solvent A) and recrystallized from chloroform. The purified yellow crystal shows that its physical and spectroscopic properties are parallel those of Aclacinomycin A, reported by H. Umezawa et al. (71).

The purified yellow pigment was hydrolyzed with 0.3 M sulfuric acid to give orange crystalline needles, after recrystallization from chloroform. This orange material was identified as aklavinone (55) by comparing its physical and spectroscopic data with those reported in literature (67, 68), and further confirmed by the high resolution p.m.r. spectrum which was not available in literature (Fig. 1B-1).

In the p.m.r. spectrum of 55, Fig. 1B-1, shows the small coupling between the proton on C_7, at 2.35 ppm and the two protons on C_8, at 2.45 ppm and the long range coupling between C_8-H_e and C_{10}-H_e of 55 based on a expended p.m.r. spectrum.

Double irradiation studies (Fig. 1B-2) further revealed the relationship among C_7-H_e, C_8-H_aH_e and C_{10}-H. When frequency sweep double irradiation was applied at 5.38 ppm, corresponding to

the H_7-H_e, spectral simplification due to the decoupling of C_8-H_aH_e from the C_7-H_e was observed. Decoupling of the proton of the C_8-H_e from the C_{10}-H_e was also observed by the double irradiation on the C_{10}-He at 4.13 ppm.

This operation converts the original broad doublet at 2.10 ppm, corresponding to the C_8-H_a, to a fairly sharp doublet which clearly indicates the decoupling from the C_{10}-H_e. All these p.m.r. spectroscopic data can only be accommodated to the half chair form of the preferred conformation of D ring as shown in Fig. 2.

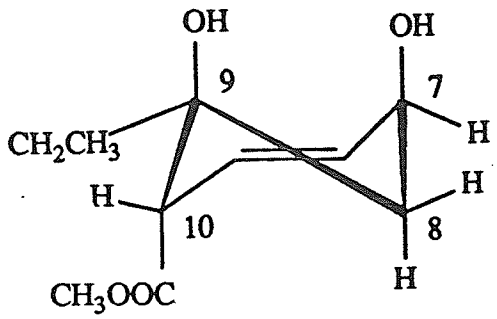


Fig. 2 The Half-Chair Conformation of D ring of Aklavinone (55)

Thus these spectroscopic features readily distinguished aklavinone from other epimers, e.g. aklavinone I and aklavinone II which may also be isolated from the culture medium, (67).

The reaction of aklavinone (55) with 50 in the presence of silver trifluoromethane sulfonate, followed by preparative layer chromatog-

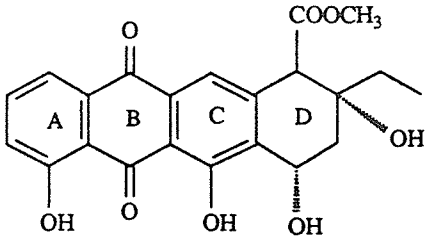
raphy upon silica gel, gave 7-O-4'-O-(p-nitrobenzoyl)-3'-N-trifluoroacetyl-α-daunosaminyl-aklavinone (56) as predominant product in 40 % yield, and a small amount of the β-isomer (57).

Introducing the 4-O-p-nitrobenzoyl group in a sugar moiety for improving stereoselectivity of coupling reaction under Koenigs-Knorr conditions, was reported by Dejten-Juszyuski, (73, 74).

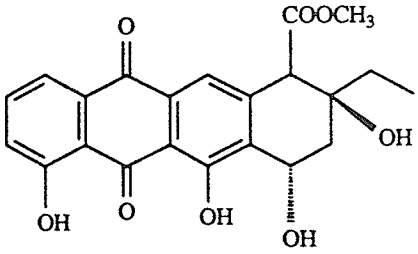
Thus it is assumed that the high stereoselectivity of the Koenigs-Knorr reaction of 50 and 49 with aklavinone (55) is attributed to the presence of the p-nitrobenzoyl group at C-4. The p-nitrobenzoyl group most likely participates in this reaction to form stable 7-membered ring nitrobenzoyloxonium intermediate (59). This intermediate probably facilitates the axial attack by the hydroxyl group in the formation of α-anomeric compound (56), as illustrated in Scheme 10, (74).

Acton et al. (75) obtained the glycoside with more stereoselectivity with 4-O-p-nitrobenzoyl-3-N-trifluoroacetyl-daunosaminyl bromide over the chloro sugar. However our experiment results shows that 1-chloro-aminosugar (50) seems to be superior to 1-bromo-aminosugar (49) in coupling with aklavinone (55) to give -isomer (56) in 40.0 % yield, as opposed to the 26.9 % yield obtained from 1-bromo-aminosugar. These results parallel those contributed by Acarmone et al. in the preparation of analogues of adriamycin and daunomycin (76). The phenomenon may be well explained by the HSAB principle (77).

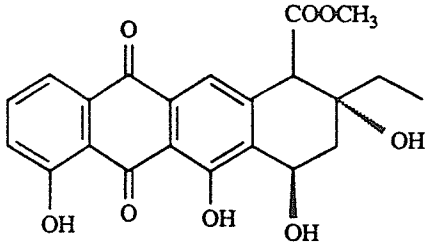
According to the HSAB principle, the C_1 in 49 is a less hard acidic centre than that in 50. Consequently the carbonyl oxygen,



aklavinone (55)

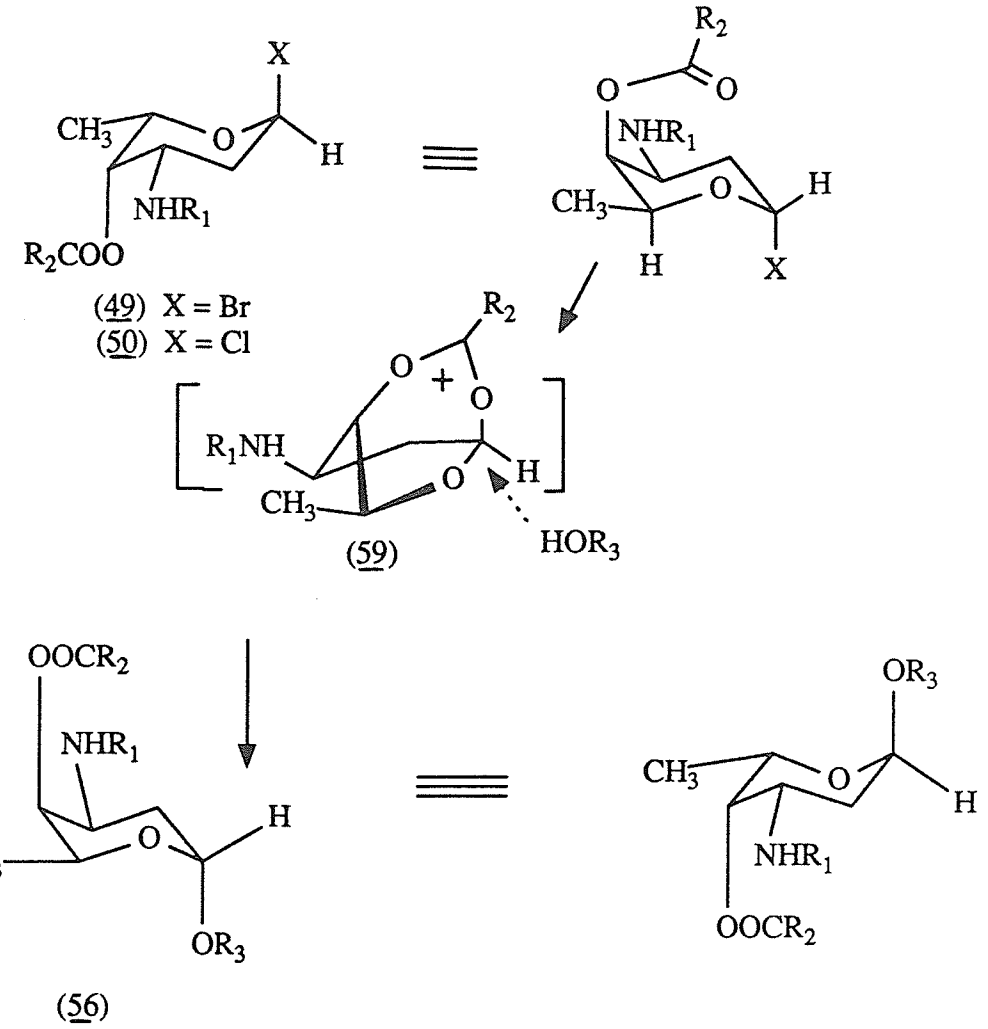


aklavinone I (55a)



akavinone II (55b)

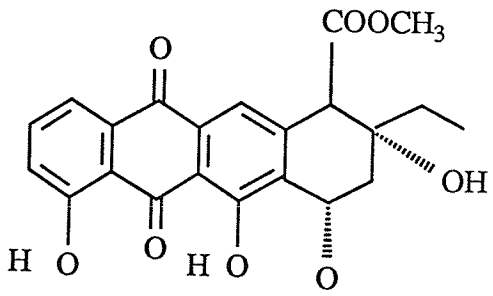
Scheme 10 Highly Stereoselective Glycosidation of Aklavinone (55) with Aminosugar (49) and (50)



$R_1 = COCF_3$

$R_2 = C_6H_5NO_2$

R3 =



which is considered as a hard base, prefers to attack the harder acidic centre at the C_1 of 1-chloro-aminosugar to form 7-membered nitrobenzoyloxonium intermediate (59). Therefore the choice of 1-chloro-aminosugar (50) may provide a more stereoselective approach to give the α-glycoside (56) in the higher yield than that of 1-bromo-aminosugar (49).

The stereochemistry of both 56 and 57 are positively established by detailed analysis of their two dimensional p.m.r. spectrum (Fig. 5B), ^{13}C n.m.r. spectrum (Fig. 5D) and high resolution p.m.r., (Figs. 6B).

The i.r. spectrum of 56, Fig. 5A, shows the OH stretching absorption (non hydrogen bonding) at 3530 cm^{-1}. The NH stretching is at 3430 cm^{-1} and chelated phenolic OH is at 3500-3000 cm^{-1}. The carbonyl stretching absorptions of $-COOCH_3$, PhCOO, and $NHCOCF_3$ occur at 1735 cm^{-1} as a broad and intense band due to overlapping with each other. The quinone carbonyl stretching appears at 1680 cm^{-1} and the chelated carbonyl stretching is at 1630 cm^{-1}. The aromatic C=C stretching and the NO_2 absorption are at 1620 cm^{-1} and 1535 cm^{-1} respectively.

The two dimensional p.m.r. spectrum of 56, Fig. 5B, shows one proton singlet at 5.64 ppm with W_H = 6 Hz, assignable to the equatorial hydrogen on C'_1, whereas the proton on C'_1 of 57 (Fig. 6B) is assigned to the axial orientation on the basis of one proton signal at 5.35 ppm, as a quintet, with W_H = 13 Hz, (76).

In the compound 56, the three proton triplet at 1.11 ppm with J = 6.2 Hz corresponds to the $C_{13}-CH_3$. Three methyl protons (C_5-

CH_3) are at J = 1.26 ppm as a doublet with J = 7.0 Hz due to the coupling with C'_5-H.

Two chemically and magnetically non equivalent protons on C_{13} occur at 1.54 ppm and 1.79 ppm as the AB part of an ABC_3 spin system due to the influence of the adjacent chiral centre (C_9).

The two methylene protons (C'_2-H_aH_e) appear at 2.10 ppm as a multiplet as the AB part of an ABXY system. This conclusion is supported by the analysis of the two dimension spectrum (Fig. 5B) which shows C'_2-H_aH_e coupling with C'_1-H at 5.70 ppm and C'_3-H at 4.46 ppm. The C_8-H_a is at 2.34 ppm as a broad doublet with J_{8a8e} = 15 Hz and J_{8a7e} = 1 Hz. The C8-H_e appears at 2.62 ppm as a doublet of doublet with J_{8a8e} = 15 Hz and J_{7e8e}= 5 Hz. The two dimensional spectrum indicates the coupling of H_{8a} with H_{8e} and both coupling with H_{7e}. The small coupling of J_{8a7e} is due to the proton of C_9-OH forming a hydrogen bond with the oxygen of C_7-O-sugar; consequently the half chair form of the D ring is slightly twisted expanding the H_{8a}-C_8-C_7-H_{7e} dihedral angle to about 75°, (15, 24).

The three protons of C_{10}-$COOCH_3$ appear at 3.70 ppm as a singlet. A broad singlet at 3.84 ppm, which exchanges with deuterium, is assigned to the C_9-OH. The two dimensional spectrum also reveals the coupling of C_9-OH with C_{10}-H. One proton singlet at 4.15 ppm is assigned to the C_{10}-H_e. This signal, in an expanded spectrum, reveals broadening which indicates the presence of a weak long range coupling. This assumption is also clearly verified by the two dimensional spectrum, which indicates the hydrogen on C_{10} (H_e)

W-type long-range coupling with the C_8-H_e. Thus the coupling phenomenon is in agreement with the conformation of the D ring, in which the hydroxy groups on C_7 and C_9 have a relationship of the quasi-diaxial cis orientation, and the C_{10}-$COOCH_3$ group has a quasi-axial orientation in cis position to the C_9-CH_2CH_3.

The C'_3-H_a overlaps C'_5-H_a at 4.47 ppm and the two dimensional p.m.r. clearly shows that the C'_3-H_a is coupled to both the C'_2-H_aH_e which appear at 2.10 ppm as a multiplet due to its weak coupling to the C'_4-H_e. The C'_5-H_a appears only as a quartet with $J_{CH3,5a} = 6$ Hz. The C'_4-H_e is observed at 5.47ppm as a broad singlet.

The signal of C_7-H_e occurs at 5.38 ppm as a broad singlet; a result of a weak coupling to both the C_8-H_a and C_8-H_e. One proton signal which appears at the lowest field, 5.70 ppm, among the methine protons, is assignable to the C'_1-H_e, (76). The NH at 6.20 ppm appears as a broad doublet as a consequence of fast exchange of the N-H proton (78), restricted rotation of the trifluoroacetamide function and quadropole relaxation of the nitrogen (79). The aromatic proton on C_{11} is at 7.73 ppm as a singlet with a small coupling with the C_{10}-H_e. The C_3-H is at 7.33 ppm with $J_{2,3} = 8$ Hz and 7.85 ppm with $J_{1,3} = 1$ Hz due to coupling with the C_2-H and the C_1-H respectively. The C_2-H, which appears at 7.72 ppm as a triplet, is recognised as the coupling with the C_1-H and C_3-H. The typical AA'BB' pattern at 8.33 ppm is assigned to the four aromatic protons. Two phenolic proton signals in the lowest field at 12.00 ppm and 12.78 ppm corresponds to C_4-OH and C_6-OH respectively.

The structure of 56 was further confirmed by ^{13}C n.m.r. spectrum (Fig. 5D). The assignments of the carbons were listed in Table 1-1 and Table 1-2, (80). The FAB mass spectrum (1) of 56 shows the molecular ion at $M^+ = 786$.

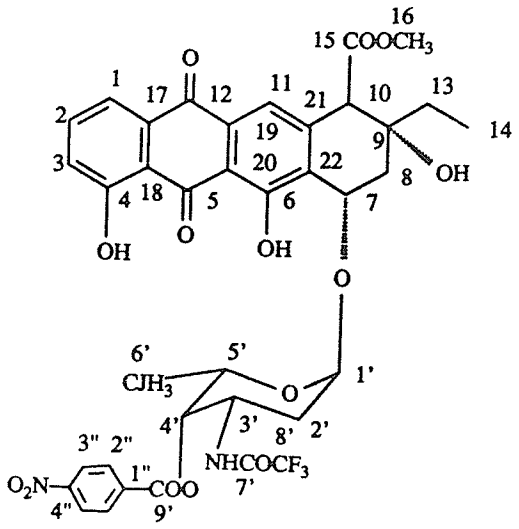
Removal of the protecting groups was achieved by the treatment of 56 in a mixture of tetrahydrofuran, methanol, potassium carbonate solution while stirring at mild temperature. Then the reaction solution was adjusted to pH 9 with dilute hydrochloric acid and usual workup afforded a dark orange residue. This compound was subjected to preparative layer chromatography upon silica gel to give an amorphous solid (10) in 37.9% yield,
m.p. = 132-143 °C (decomposed).

The concentration of base and time for the hydrolysis are crucial factors. Under stronger basic conditions, and longer reaction time, many other side products were observed, which might be caused by the cleavage of ester bond and glycosidic bond of the glycoside. One of these by-products was identified as compound 55a. (Scheme 11).

The i.r. spectrum of 10 (1) shows the OH stretching at 3600 cm^{-1} and 3520 cm^{-1}. The primary NH_2 stretching is at 3470 cm^{-1} and 3430 cm^{-1}. The chelated OH stretching absorbs at 3500-3100 cm^{-1}. The quinone carbonyl stretching of $COOCH_3$ appears at 1735 cm^{-1} and the carbonyl stretching absorption is at 1680 cm^{-1}. The chelated carbonyl stretching is at 1580 cm^{-1}.

The tumour cell growth inhibition assay of 10 shows its significant antitumor activity comparable with Adriamycin (2) and Aclacinomycin A (6) (Table 2).

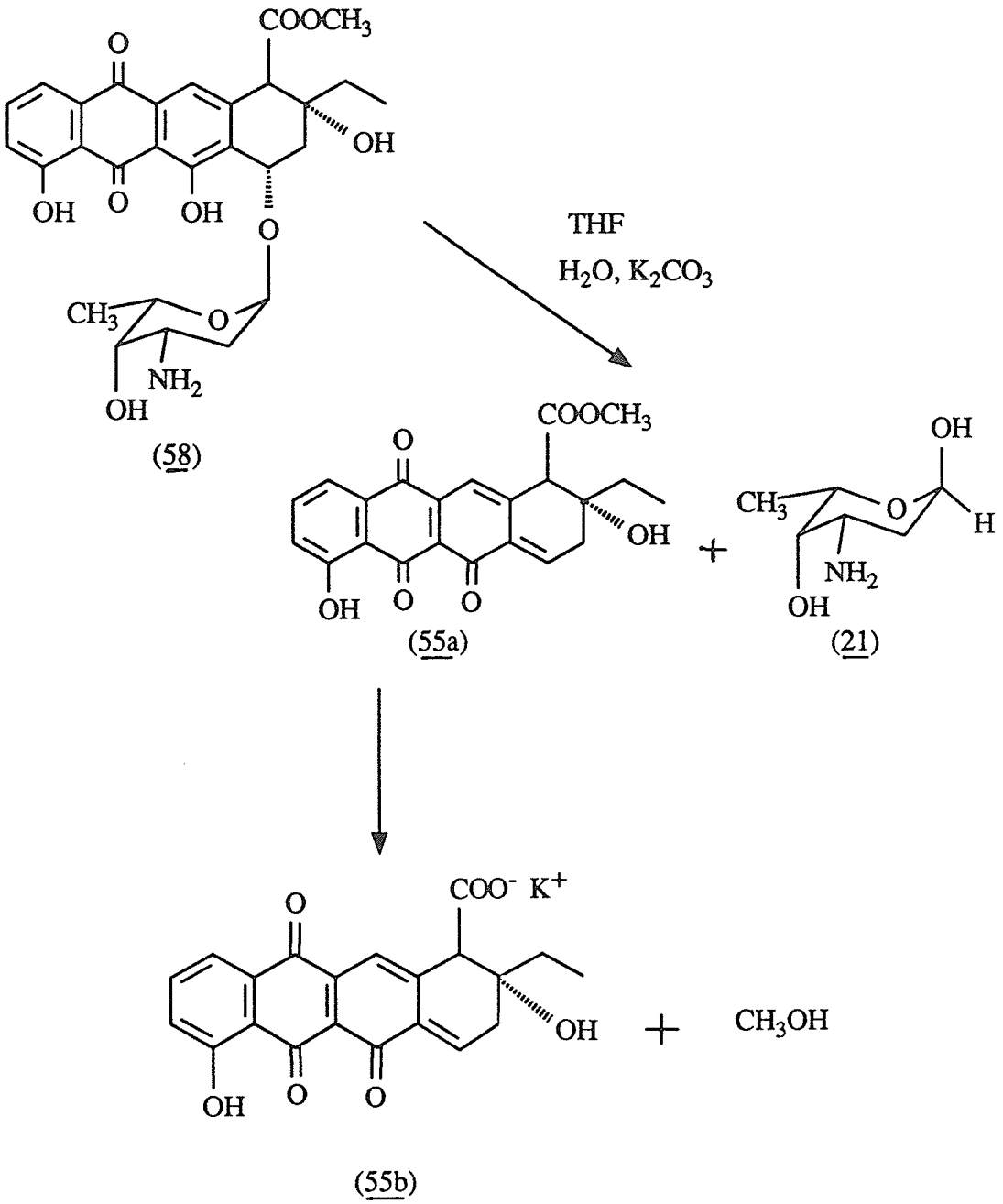
Table 1-1 Interpretation of ^{13}C N. m. r. Spectrum of 7-O-3'-N-(Trifluoroacetyl)-4'-O-(p-nitrobenzoyl)-α-daunosaminyl-aklavinone (56), (Fig. 5D), (80).



ppm	Assignment	ppm	Assignment
125.0	C_1	131.0	C_{20}
126.0	C_2	143.0	C_{21}
121.0	C_3	135.0	C_{22}
157.5	C_4	101.0	C'_1
192.0	C_5	30.0	C'_1
157.0	C_6	58.0	C'_3
67.0	C_7	72.5	C'_4
32.0	C_8	72.0	C'_5
73.0	C_9	17.0	C'_6

Table 1 -2.

ppm	Assignment	ppm	Assignment
47.0	C_{10}	171.0	C'_7
122.0	C_{11}	115.0	
181.0	C_{12}	116.0	C'_8
35.0	C_{13}	117.0	
14.0	C_{14}	118.0	
165.0	C_{15}	161.5	C'_9
53.0	C_{16}	161.0	C''_1
134.0	C_{17}	138.0	C''_2
131.5	C_{18}	132.0	C''_3
133.5	C_{19}	152.0	C"4



Scheme 11 The Hydrolysis of 1-Deoxy-N,N-bis-demethyl-pyrromycin (10) under Strong Basic Conditions

Table 1. ID_{50} values of Adriamycin (2), Aclacinomycin A (6) and compound **10** (1).

	P388/S	P388/Al
Adriamycin (2)	6×10^{-8} M	3×10^{-6} M
Aclacinomycin A (6)	7×10^{-7} M	6×10^{-6} M
Compound **10**	3×10^{-7} M	5×10^{-6} M

RESULTS AND DISCUSSIONS

PART B: SYNTHESIS OF METHYL 3-N-(BENZYL-CARBOXAMIDO)-2,3,-4,6-TETRADEOXY-DL-THREO-HEXOPYRANOSE (78) ANDITS 3-EPIMER (80)

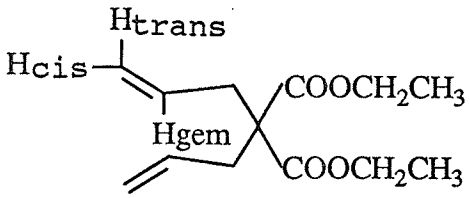
Compounds methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-DL-threo-hexopyranose (78) and its 3-epimer 80, which are considered as intermediates for the total syntheses of novel aminosugars, 3-aminomethyl-2,3,4,6-tetradeoxy-DL-threo-hexopyranose (92) and 3-aminomethyl-2,3,4,6-tetradeoxy-DL-erythro-hexopyranose (93), are prepared in the acceptable overall yield starting from diethylmalonate (63). Compound 78 and 80 were also converted to new aminosugars, e.g. methyl 3-N-(benzylamino-methyl)-2,3,4,6-tetradeoxy-DL-threo-hexopyranose (86) and methyl 3-N-(benzylamino-methyl)-2,3,4,6-tetradeoxy-DL-erythro-hexopyranose (87).

The carbanion of diethylmalonate (63), which was generated by treatment of ethylmalonate with finely ground potassium carbonate in acetone, reacted with allyl bromide to give 2,2-diallyl diethylmalonate (64) as a colorless liquid after distillation under reduced pressure at 87-89 °C/1.5 mm in 95% yield.

Predictably, introduction of a second allyl group was more difficult than that of the first one, since the first allyl group introduced was considered as an electron donating group which could decrease acidity of hydrogen to the carbonyl group. In addition, steric hindrance of the first group could also cause difficulty for

further alkylation. Consequently, high temperature and relatively long reaction time were required for completion of the reaction. Monitoring reaction process by t.l.c. revealed the concurrent consumption of the starting material (63) (Rf = 0.3) and the formation of a monoalkylated intermediate (63a), (Rf = 0.5), and final product (64), (Rf = 0.65), (Solvent G).

The infrared spectrum of 64, Fig. 8A, shows the intense carbonyl stretching vibration at 1720 cm^{-1}, the weak absorption of the C=C stretching vibration at 1640 cm^{-1}, and the C-O-C bending vibration at 1200 cm^{-1}, as a broad band.



(64)

In the p.m.r. spectrum of 64, (Fig. 8B), the six protons of two methyl groups in two chemically equivalent ethyl groups absorb at 1.32 ppm as a triplet. The four methylene protons in the two ethyl groups appears at 4.17 ppm as a quartet due to the coupling with methyl protons as an A_2X_3 spin system. The six olefinic protons in the two equivalent allyl groups absorb at 4.86-6.00 ppm as an ABX spin system. The absorption at 5.18 ppm as a broad singlet was

assigned to the olefinic proton in trans position to the proton on the same carbon with the alkyl side chain (H_{trans}) and the absorption at 5.00 ppm as a broad singlet belongs to the proton in cis position to the hydrogen on the same carbon with the alkyl side chain (H_{cis}). A multiplet at 5.35-6.00 ppm in a low field is assignable to the olefinic proton on the same carbon with the alkyl proton (H_{gem}). The four allylic protons absorb at 2.55 ppm as a broad doublet due to their coupling with the adjacent H_{gem} and long range coupling with the H_{trans} and H_{cis}.

2,2-diallyl-diethylethylmalonate (64) was hydrolyzed in a solution of potassium hydroxide (KOH), methanol and water under reflux followed by adding concentrate hydrochloric acid at low temperature (0°C) to neutralize the solution to pH 6-7 to gave corresponding 2,2-diallyl-malonic acid (65) in 91% yield, as a white crystalline product after recrystallization from chloroform. m.p. = 126-128°C. During the neutralization, if the local concentration of the acid in the solution was high and the solution was stirred under room temperature, unidentified by-products could be detectable by t.l.c.. This is probably caused by polymerization and/or decarboxylation.

The i.r. spectrum and p.m.r. spectrum of 65 are presented in Figs. 9A and 9B respectively. The i.r. spectrum shows the OH stretching vibration at 3550 cm^{-1} due to non hydrogen bonding monomer. The OH stretching absorption of a dimmer occurs at 3500-3000 cm^{-1}.

The carbonyl stretching absorption of a monomer is at 1760cm^{-1} and the carbonyl absorption of a dimmer appears in lower frequency at 1710 cm^{-1} due to intermolecular hydrogen bonding, (81). The C=C

absorbs at 1650 cm^{-1} as a weak band. As expected, the strong broad band at 1200 cm^{-1} corresponding to C-O-C bending vibration of the ester was nonapparent compared to the spectrum of 64, (Fig. 8A).

In the p.m.r. spectrum of compound 65, (Fig. 9B), the four allylic protons appear at 2.71 ppm as a doublet. The two equivalent olefinic protons (H_{gem}), in Fig. 5, are at 5.70 ppm as a multiplet. The two proton signal at 5.16 ppm, as a poorly resolved sextet, corresponds to the two olefinic protons (H_{cis}). The two protons (H_{trans}) appear at 5.22 ppm as a poorly resolved doublet. There are no signals at a lower field for the two hydroxyl protons probably due to the fast exchange of these protons.

In the mass spectrum of 65, (Fig. 9C), M^+/z 185 is visualized as a M+1 species in which the molecular ion may abstract one proton from the neutral molecule in the intermolecular process. M^+/z 166 is recognized as arising by the loss of H_2O from the molecular ion. The formation of the ion M^+/z 125 is due to the sequential loss of H_2O. The formation of the basic fragment ion M^+/z 79 presumably results from the sequential loss of H_2O, CO_2 and CO molecules from ion M^+/z 185 (M+1). These fragment patterns were confirmed by high resolution mass spectrum.

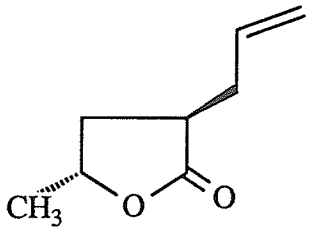
The decarboxylation of 65 proceeded at about 80 °C in a solution of acetic acid, water and N,N-dimethyl formamide (DMF) with the evolution of carbon dioxide. This eventually led to 66 as a colorless liquid in 95% yield after distillation under reduced pressure at 98-100 °C / 2.5 mm.

The infrared spectrum of 66, Fig. 10A, shows similarity with that of 65 in the region of carbonyl stretching absorption. But the carbonyl absorptions of monomer and dimmer are shifted to frequency lower than those of 66 at 1742 cm^{-1} and 1702 cm^{-1} respectively. The OH stretching of the monomer absorbs at 3500 cm^{-1} and OH stretching of dimmer is at a region of 3500-3000 cm^{-1}. The much more intense absorption of C=C is observed at 1650 cm^{-1} compared to that of compound 65 at 1640 cm^{-1}, (Fig. 9A). This is probably because of the diminishment of interplay of dipole-dipole interaction of the two carbonyl groups in 65 which tends to suppress bond polarization, (82).

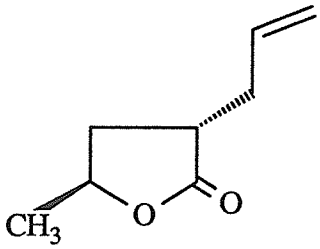
The p.m.r. spectrum of 66, Fig. 10B, shows the four allylic protons at 2.37 ppm as a broad doublet indicating their small coupling with the H_{gem} and the long range coupling with the H_{trans} and H_{cis}. The absorptions of the six olefinic protons in the two chemically equivalent allyl groups occur at 4.96 ppm (H_{cis}), 5.20 ppm (H_{trans}), and 5.83 ppm (H_{gem}) as an ABX spin system. The carboxylic proton occurs at a lower field, at 11.5 ppm, as a singlet.

In the mass spectrum of 66, (Fig. 10C), the molecular ion appears at M^+/z 140. The loss of -COOH from the molecular ion presumably gives the fragment M^+/z 95. The formation of fragment M^+/z 99 probably comes from the allylic fission of the molecular ion by the loss of CH_3-CH=CH_2. The basic ion occurs at M^+/z 43.

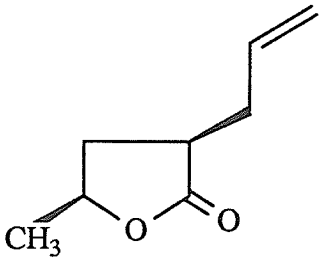
Cyclization of 66 in a solution of hexane, p-toluenesulfonic acid



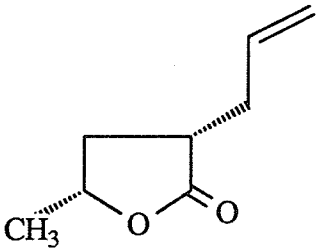
threo (68)
(2R,4R)



threo (69)
(2S,4S)



erythro (70)
(2R,4S)

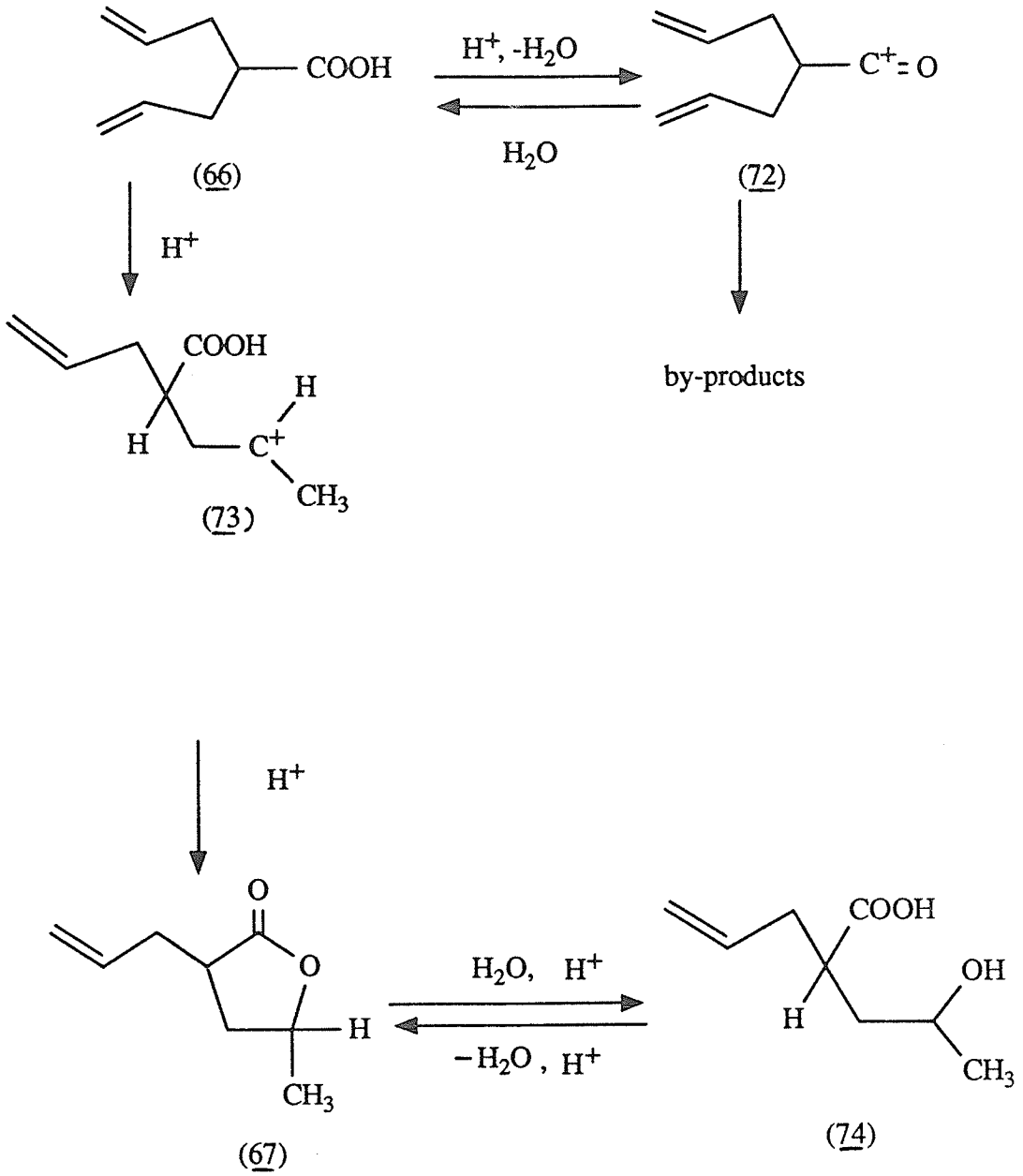


erythro (71)
(2S,4R)

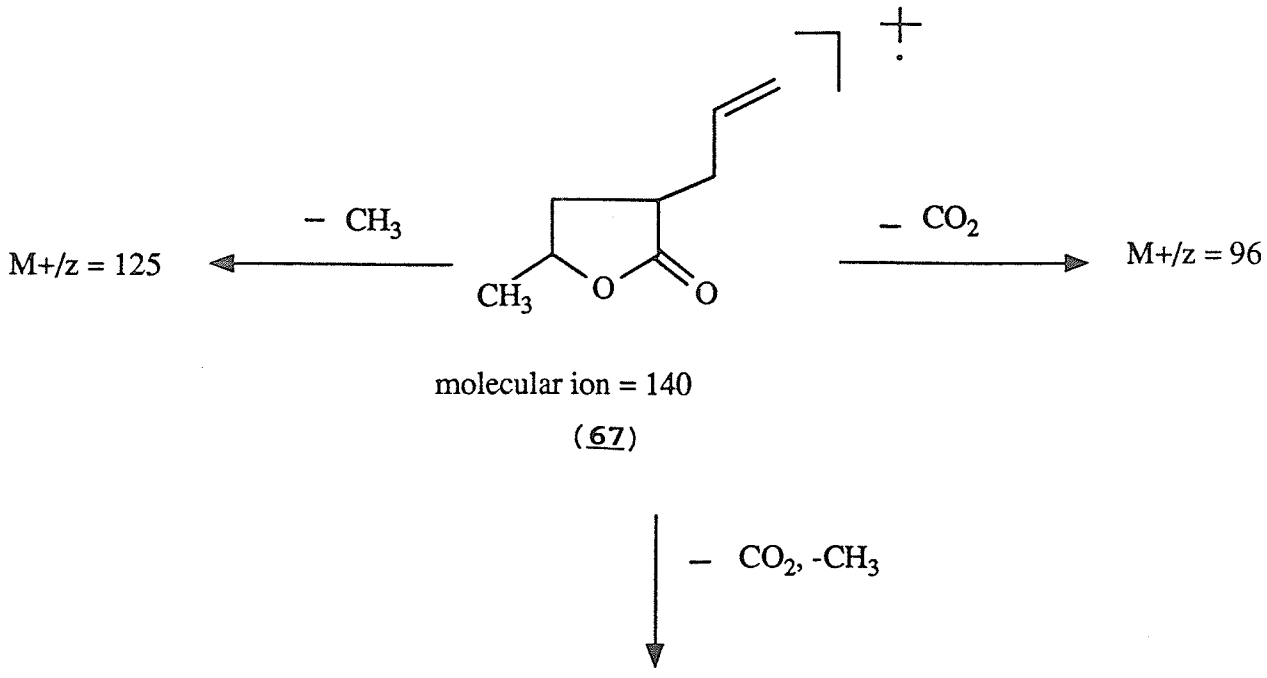
and proper amount of water under hexane-water azeotrope afforded 67 as a colorless liquid after distillation under reduced pressure at 87-90 °C/2.6 mm. The yield was 56.3 %.

The presence of certain amounts of water was a crucial factor for the formation of the ó-butyrolactone (67). In the absence of water the reaction gave, predominantly, high boiling by-product which was not characterized. It was suggested that water may prevent the formation of carbonium ion (72) which could inevitably undergo intramolecular and intermolecular nucleophilic reaction to give the undesirable by-product. In addition, the yield of the reaction was significantly improved by choosing hexane as a solvent over benzene. Because benzene ring could be attacked by the carbonium ion (73) under acidic conditions through a Friedel-Crafts like reaction mechanism to give the by-product.

The presumable mechanism of the reaction was elucidated in Scheme 12. In the presence of water, the reaction would favour the formation of -butyrolactone (67), even through water may cause the ring opening to give the carboxylic acid (74) in the meantime. Removal of water by hexane water azeotrope could eventually reverse the reaction to reform the γ-butyrolactone (67). Consequently, the intramolecular attack of the hydroxy group of 66 on either carbonium ion (73), created by protonation of two equivalent olefines in acidic condition, gave 67 as a mixture of two diastereoisomers. Each of these diastereomers contains a pair of enanteomers, e.g. threo isomer (2R,4S) 68 or (2S,4R) 69; and erythro isomer (2S,4S) 70 or (2R,4R) 71. The attempts to separate one



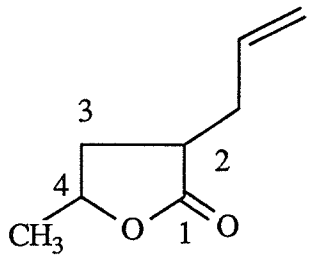
Scheme 12 Formation of 2-Allyl-4-hydroxy-4-methyl-γ-butyrolactone(67) by Cyclization of 2-Allyl-4-pentenoic acid (66).



Scheme 13 Possible Pathways for Formation of the Fragments in the Mass Spectrum of 2-Ally-4-hydroxyl-4-methyl-γ-butyrolactone (67).

diastereoisomer from the other failed. Their existence are only verified by the p.m.r. spectrum of 67, (Fig. 11B), which shows that the ratio of two diastereoisomers is about 2:1. The threo isomer is probably a major product since it is more thermodynamically stable than that of erythro isomer due to its less steric interaction between the allyl group and the methyl group, (83).

The i.r. spectrum of 67, Fig. 11A, shows the carbonyl stretching absorption at 1762 cm^{-1} as a strong sharp band and a weak C=C stretching absorption at 1640 cm^{-1}. The C-O-C asymmetric stretching absorbs at 1180 cm^{-1} as a strong sharp band.



(67)

The p.m.r. spectrum of 67, Fig. 11B, indicates three methyl protons at 1.38 ppm as a doublet. The three proton signal at 1.28 ppm is probably due to another diastereoisomer. The two allylic protons, the one methine proton on C_2, and the two methylene protons on C_3, occur at 2.00-3.00 ppm, as a multiplet. The one methine proton appears at 4.50 ppm as a sextet and the three olefinic protons is observed at 5.00-6.20 ppm as an ABX spin

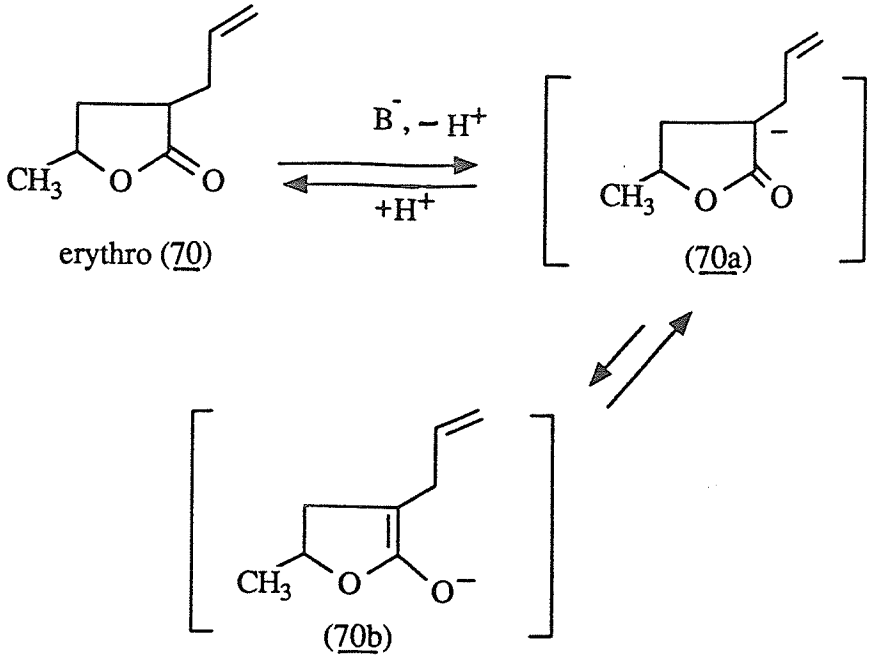
system. The chemical shift of these protons are coincident except the methyl protons of the two diastereoisomers.

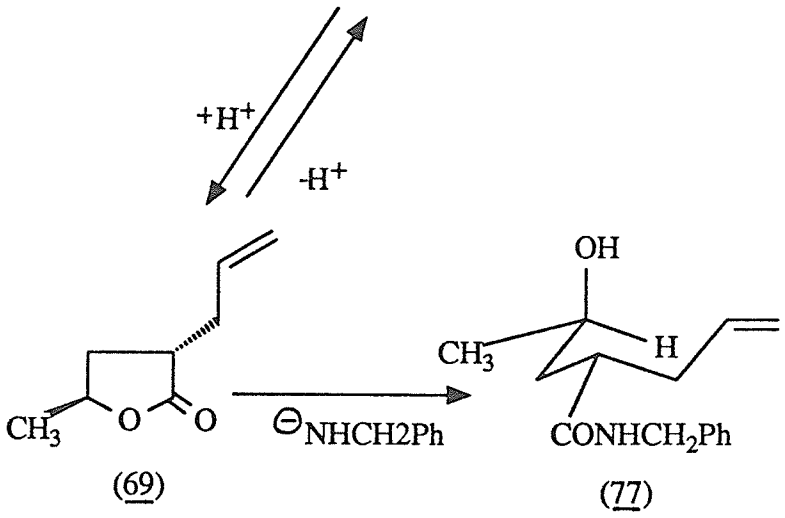
The mass spectrum of **67**, Fig. 11C, shows the molecular ion at $M^+/z = 140$ which is further proved by the high resolution mass spectrum (Fig. 11D). The basic fragment ion at $M^+/z = 81$, is due to the loss of both carbon dioxide and the methyl group. The loss of carbon dioxide probably gives the peak $M^+/z = 96$. The fragment M^+/z 125 is visualized as raising by loss of the methyl group. The presumed fragment patterns are elucidated in Scheme 13.

Compound **67**, as a mixture of the two diastereoisomers, was allowed to react with benzylamine and sodium hydride at 50 °C to afford a brown-yellow syrup. This syrup contained almost an equal amount of the two diastereomers visualized by t.l.c. The syrup was subjected to column chromatography over silica (Solvent I) to give, in order of elution, **75** as a slight yellow syrup in 19.5 % yield and **77** as white crystals in 25% yield, after solvent evaporation. The recrystallization of **77**, from a solution of ether and chloroform (10:1, v/v), gave a white crystalline solid as small needles, with m. p = 69-70 °C.

The i.r. spectrum and the p.m.r. spectrum of **75** and **77** are presented in Fig. 12A, 12B and 13A, 13B respectively. These molecules do not have one simple preferable conformation, but are, in general, a mixture of various conformers. As a result, the chemical shifts and the coupling constant obtained from the spectrum are the weighted averages of each conformer. However their relative stereochemistry could be finally induced from analysis

Scheme 14 The Epimerization of (R,S) 2-Allyl-4-hydroxyl-4-methyl-erythro-γ-butyrolacton (70)

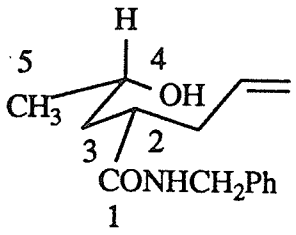




of the p.m.r. spectra of their corresponding cyclization products 82, 83 and 84, 85 obtained in the next step.

The same equivalents of benzylamine and sodium hydride were required to react with 68. Excess benzylamine and sodium hydride led to give more of 77 and less of 75 as judged by t.l.c.. The ratio of the two diastereoisomers remained unchanged by reducing the temperature to about 35 °C.

It is assumed that an excess of benzylamine anion would cause the epimerization of erythro 70 to give a more thermally stable threo isomer 69 through enolisation by losing hydrogen atom to the carbonyl group. The isomer 69 is eventualy attacked by benzylamido anion to afford 77, as a major compound, (Scheme 14). The ratio of 77 (Rf = 0.5) and 75 (Rf = 0.6) is about 5:1 as judged by t.l.c., (Solvent A). The infrared and p.m.r. spectra of 75 are presented in Fig. 12A and 12B.



(75)

The i.r. spectrum of 75 shows the stretching absorption of free hydrogen bonding OH at 3580 cm^{-1} and the NH stretching absorption at 3420 cm^{-1}. The stretching absorption of hydrogen

bonding OH is evident at 3320 cm^{-1} as a broad band. The stretching vibration of -CO-NH-, as a broad band, is at 1670 cm^{-1}. The C=C stretching vibration absorbs at 1620 cm^{-1} as a shoulder and the-HN-C bending vibration and amide II band are present at 1520 cm^{-1}.

In the p.m.r. spectrum of 75, Fig. 12B, the three methyl protons on C_5 are at 1.19 ppm, as a doublet with J = 6.2 Hz, due to vicinic coupling with one methine proton on C_4. The two allylic protons are both magnetically and chemically non-equivalent due to the influence of the adjacent chiral centre on C_2 and can be differentiated from the two methylene protons on C_3 by Shoolery equation, (84). Consequently, these protons occur at 1.57 and 1.80 ppm, as two sets of a doublet of triplets, as the AB pattern of an ABCD spin system. The hydroxyl proton appears at 1.97 ppm as a doublet with J = 4.5 Hz due to coupling with the adjacent methine proton on the C_4, while in the spectrum of 77, the OH absorption is at 2.10 ppm as a broad singlet. One methine proton on C_2, adjacent to the carbonyl group, appears at 2.20 ppm as a multiplet. The two methylene protons on C_3 are at 2.45 ppm, as a multiplet, as the AB pattern of an ABCD spin system. One methine proton on C_4 appears at 3.85 ppm as a multiplet. The two benzylic protons, which are magnetically and chemically non-equivalent because of their diastereotopic characters, absorb at 4.45 ppm, as a doublet of quartets of the typical AB pattern with J_{gem} = 15 Hz and $J_{CH3,NH}$ = 6 Hz. The two terminal olefinic protons absorb at 5.10 ppm as a broad singlet (H_{tans}) and 5.03 ppm as a broad doublet (H_{cis}). One olefinic proton (H_{gem}) occurs at 5.750 ppm as a multiplet in lower

field. These experimental data are in good agreement with the values, 4.97 ppm (H_{cis}), 5.03 ppm (H_{trans}), and 5.70 ppm (H_{gem}) calculated by an equation which is derived from an empirical correlation based on an compilation of experimental data, (85). The NH is at 6.00 ppm, as a broad singlet, and the five aromatic protons are observed at 7.30 ppm as a multiplet.

The mass spectrum of 75, (Fig. 12C), shows the molecular ion at M^+/z 247 and a basic peak corresponding to the tropylium ion at M^+/z 91. The loss of H_2O from the molecular ion probably gives the fragment ion M^+/z 229. The formation of the fragment ion M^+/z 189 (M-58) presumably came from the McLaffery rearrangement of the molecular ion. The fragment ion at M^+/z 202 is more likely attributable to the loss of CH_3CHOH by the cleavage of the molecular ion.

The i.r. spectrum and p.m.r. spectrum of 77 are presented in Fig. 13A and 13B. As expected, the i.r. spectrum is similar in character to that of 75 except for the weaker absorption at 3320 cm^{-1}, corresponding to intermolecular hydrogen bonding, and the minor difference at the finger print region when compared to the spectrum of 75. The free hydrogen bonding OH appears at 3580 cm^{-1} and the NH stretching absorption occurs at 3430 cm^{-1}. The carbonyl stretching absorption appears at 1670 cm^{-1} as a broad band and the C=C stretching appears at 1640 cm^{-1} as a shoulder (medium). The amide II band and the aromatic skeleton absorption are overlapped as a broad singlet at 1520 cm^{-1}.

The p.m.r. spectrum shows of 77 the three methyl protons on C_4, at 1.19 ppm, as a doublet with J = 6.2 Hz. The two allylic protons are observed at 1.60 ppm and 1.85 ppm as a quintet and a doublet of triplets as the AB part of an ABCD spin system. One broad singlet, at 2.08 ppm, is due to the hydroxyl group on C_4. The one methine proton on C_2, adjacent to the carbonyl functional group, appears at 2.25 ppm as a multiplet and two methylene protons on C_3 absorb at 2.35 ppm as a multiplet. The methine proton on C_4 occurs at 3.85 ppm as a multiplet. As expected, two magnetically and chemically non-equivalent benzylic protons are present, at 4.50 ppm, as a doublet of quartets of a typical AB spin system. Two proton signals; one at 5.10 ppm as a broad singlet, and the other at 5.03 ppm as a broad doublet, are assignable to the terminal olefinic proton H_{trans} and H_{cis} respectively. One olefinic proton (H_{gem}) is a multiplet at 5.75 ppm. The NH absorbs at 6.00 ppm as a broad singlet and the five aromatic protons are at 7.73 ppm as a multiplet.

The mass spectrum of 77, (Fig. 13C), shows the molecular ion at M^+/z 247. The lost of water from the molecular ion forms a fragment ion M^+/z 229 ($M-H_2O$). The fragment ion M^+/z 189 is obviously attributed to a McLafferty rearrangement of the molecular ion by the subsequent loss of neutral molecule $CH_3COH=CH_2$. The formation of the fragment ion M^+/z 106 ($NH-CH_2-Ph$) was recognized as the raising by cleavage of the molecular ion. A tropylium ion appears at M^+/z 91 as a basic peak due to benzylic fission (Scheme 15).

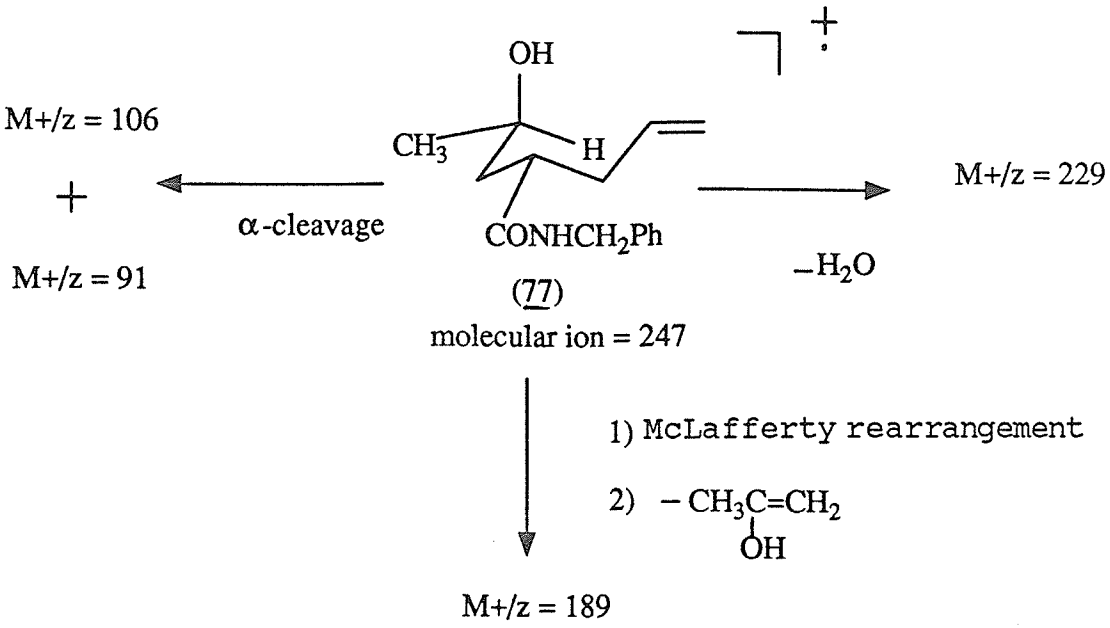
Ozonolysis of 75 in absolute methanol at -20° to -25 °C, followed by the treatment with trimethyl phosphite under reflux, gave a slight brown syrupy residue. It was subjected to column chromatography over silica (Solvent M) to give, in order of elution, a white amorphous solid (78) as the mixed acetals in 74% yield, and a minor amount of 79. Compound 78 could be further purified by recrystalization from ether and petroleum to give white amorphous solids, m.p. = 94-95 °C. Compound 79 was recrystallized from methylene chloride as white crystals, m.p. = 155-157 °C, which can be converted to 78 in absolute methanol, at pH = 3, almost quantitatively.

The original p.m.r. spectrum of 78 shows that the ratio of α-isomer 82 to β-isomer 83 is about 3 : 2. This phenomenon, in which stereochemistry unfavored α-isomer 82 was predominant, can be satisfactorily explained by anomeric effect principle.

Further preparative layer chromatography of 78 upon silica gel (Solvent K) gave 82 (Rf = 0.6), m.p. = 75-78 °C and 83 (Rf = 0.5), m.p. = 117-120°C, both as white crystals after being crystallized from ether and petroleum ether. They were unambiguously assigned to α and β anomers respectively by analysis of their p.m.r. spectra (Figs. 14B and 15B).

Over ozonolysis of 75 produced a series of side products, which were detectable by t.l.c., and caused extreme difficulty in purifying the product. These unidentified side products probably were produced by the oxidative breakage of the benzine ring in the molecule. For this consideration, the amount of ozone was carefully

controlled in a small scale preparation and the reaction process was monitored by t.l.c.. At each interval during t.l.c., dry nitrogen was



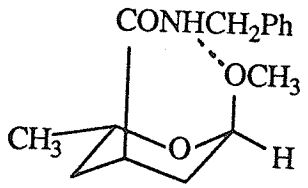
Scheme 15 The Possible Pathways for Formation of the Fragments in the Mass Spectrum of (R,S) 2-Allyl-4-hydroxyl-N-benzyl-threo-pentanamide (77).

bubbled into the reaction solution, instead of ozone, to quench the reaction temporarily.

Ozonolysis was considered completed when the spot correspondent to the starting compound had nearly disappeared on the t.l.c. plate, (Solvent A). The plate was viewed under UV lamp. Water soluble trimethyl phosphate evolved in the reaction, were readily removed by washing the methylene solution with 20% sodium carbonate solution.

The relative stereochemistry assignment of compounds **82**, **83**, **84** and **85** at C_5, C_3 and C_1 were achieved by the analysis of their p.m.r. spectra in Fig. 14B, 15B, 17B and 18B respectively. Each of these four isomers would be expected to adopt the two possible chair conformations, where isomers **82**, **83**, **84** and **85** are in their preferred conformations. The formation of intramolecular hydrogen bond facilitates the preferred conformation of **85**. One proton signal, at 2.71 ppm, as a broad multiplet with W_H = 28 Hz in the spectrum of **82**, and one proton multiplet signal, at 2.45 ppm with W_H = 26 Hz in the spectrum of **83**, are assignable to the axial protons on the C_3 respectively. This wide spacing of the multiplet, either in the spectra of **82** or **83**, clearly indicates the diaxial relationships with H_{2a} and H_{4a} and the axial-equatorial relations with H_{2e} and H_{4e}. By contrast, glycoside **84** and **85** displayed their equatorial C_3-H protons at 2.72 ppm and 2.70 ppm as both a multiplet with W_H = 15 Hz and W_H = 14 Hz respectively. These signals contain only small vicinal couplings due to the

equatorial-equatorial relations with H_{2e} and H_{4e} and the equatorial-axial relations with H_{2a} and H_{4a}.



(85)

The i.r. and p.m.r. spectra of 82 are presented in Figs 14A and 14B.

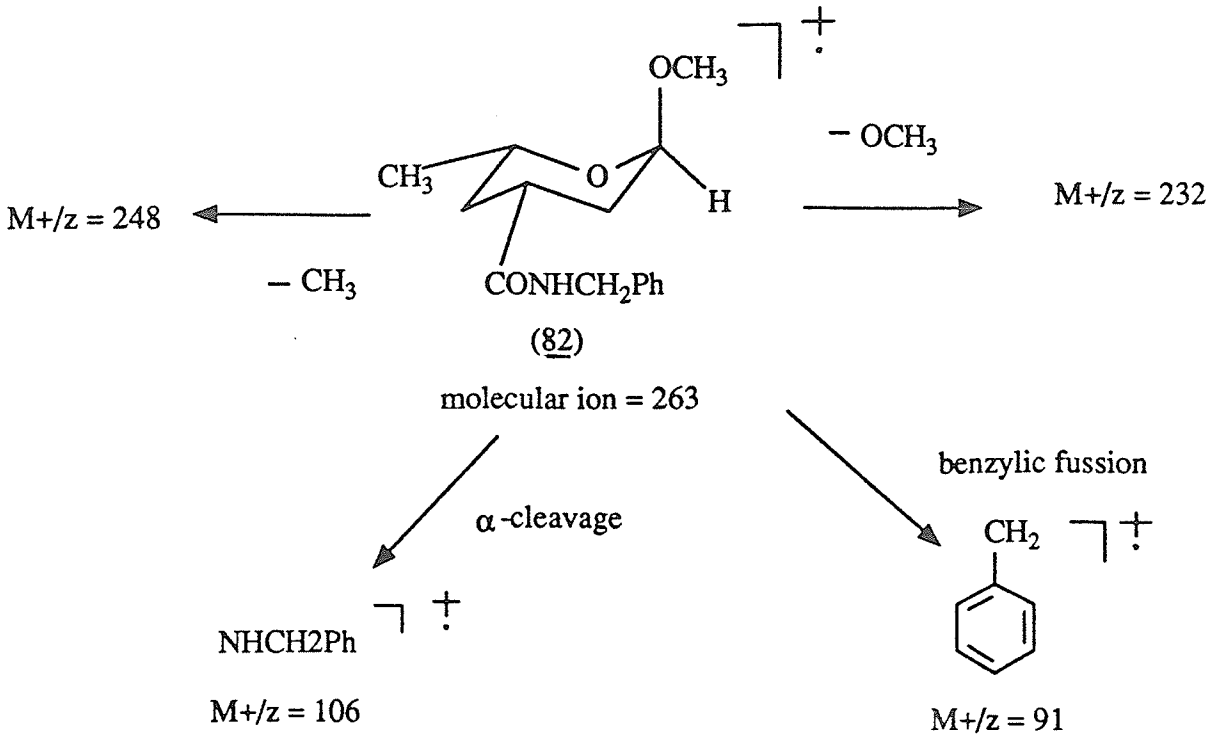
The i.r. spectrum of 82 indicates that the NH and amino carbonyl stretching vibrations are at 3450 cm^{-1} and 1670 cm^{-1} respectively. The aromatic C=C absorption and the amide II band are overlapped at 1520 cm^{-1} as a broad singlet.

The p.m.r. spectrum of 82 shows the three methyl protons (C_5-CH_3) absorption at 1.19 ppm as a doublet with J = 6.3 Hz due to the coupling with the methine proton (C_4-H). The one methine proton (C_1-H_e), at 4.80 ppm and as a poorly resolved triplet with W_H = 6 Hz, is assignable to the equatorial proton on C_1,. Its shifting to a lower field can be visualized as arising from the electronegative effect of two oxygen atoms and its equatorial orientation, (86, 87). Additionally, the small coupling indicates that the dihedral angle between H_e-C_1-C_2-H_aH_e is about 60 ° cor-

responding to the proton with equatorial orientation, (86b, 88). As expected, two diastereotopic methylene protons (C_2-H_aH_e) are observed at 1.85 ppm as a doublet of doublets with J_{2a2e}= 16 Hz as the AB pattern of an ABXY spin system, (84, 86, 89). For the two methylene protons on C_4, H_{4a} is at 1.55 ppm, as a quartet with J_{4a4e} = 12 Hz and H_{4e} appears at 1.75 ppm as a broad doublet with J_{4e4a} = 12 Hz, (84, 86, 89). One methine proton signal at 2.71 ppm, with W_H = 28 Hz, is assignable to the axial hydrogen on C_3. The three methoxy protons are at 3.34 ppm as a singlet. One proton multiplet, at 3.39 ppm with $J_{5a,CH3}$ = 6 Hz is assignable to the C_5-H. Compared to **83**, this signal shifts to a lower field as a consequence of the desheilding effect of the 1,3-diaxial interaction between the C_5-H and the C_1-OCH_3, (87). The two benzylic protons, appear at 4.43 ppm, as a doublet. Disappearance of the typical feature of a AB system is due to the decrease of the ratio of $\Delta\delta/J$ which is a response to the change of steric environment in **82** compared to those in **75**. The NH occurs at 5.77 ppm as a broad singlet. The five aromatic protons appear at 7.30 ppm as a multiplet.

The mass spectrum of **82**, (Fig. 14C), shows the molecular ion at M^+/z 263 followed by the loss of the methoxyl group giving the fragment M^+/z 232. The loss of the methyl group from the molecular ion by cleavage gives the fragment m/z 248. The fragment ion M^+/z 106 likely attributes to the -NH-CH_2-Ph ion through the simple fission of the molecular ion. The basic benzylic fragment ion, predictably, appears at M^+/z 91, (Scheme 16).

Compound 83 was assigned to β-anomeric glycoside. Its i.r. spectrum and p.m.r. spectra are presented in Figs. 15A and 15B.



Scheme 16 The Possible Pathways for Formation of the Fragments in the Mass Spectrum of methyl 3-(N--benzyl-carboxamido)-2,3,4,6-tetradeoxy-α-DL-threo-hexopyranose (**82**)

The i.r. spectrum of 83 is similar to that of 82 except for the obviously difference in the finger print region. The NH stretching is present at 3450 cm^{-1}. The aminocarbonyl stretching is at 1680 cm^{-1}. The aromatic stretching and the amide II band are overlapped at 1520 cm^{-1} as a strong singlet. The absorption corresponding to the C-O-C bending vibration are at 1020 cm^{-1} and 1080 cm^{-1}.

The p.m.r. spectrum of 83, Fig. 15B, shows the three methyl protons at 1.276 ppm with J = 6.2 Hz as a doublet. For two diastereotopic methylene protons (C_4-H_aH_e), one (H_{4a}) is at 1.45 ppm, as a quartet, and the other one (H_{4e}), is at 1.55 ppm, as a quartet with J_{4e4a} = 12 Hz, as the AB pattern of an ABCD spin system. Their obviously different appearance with those of 82 is because of their different diastereotopic face of these two hydrogens. The H_{2a} shifts to a slight higher field, at 1.80 ppm compared to 1.84 ppm of 82 as a doublet, probably a result of the shielding effect of equatorial methoxy group on C_1, (84, 86, 89).

As expected, the signal of three methoxy protons on C_1, as a singlet at 3.50 ppm, shifts to a lower field than those of 82, (87). One methine proton (C3-H_a) is at 2.45 ppm as a multiplet with W_H = 26 Hz. One proton multiplet at 3.55 ppm is assignable to the C_5-H, which shifts to a higher field compared to that of 82, probably due to the absence of 1,3-diaxial interaction of the C_5-H and the C_1-OCH_3, (86, 87). Consequently assignment of the anomer for 83 is further confirmed.

As for the two methylene protons on C_2, C_2-H_e is at 1.80 ppm as a broad doublet with J_{gem} = 12 Hz. The C_2-H_a is at 1.45 ppm as a sextet with J_{gem} = 12 Hz. One axial proton (C_1-H_a) appears at 4.33 ppm as a doublet of doublets with W_H = 9 Hz. Predictably, this proton shifts to a higher field compared to that in 82, (87, 89). Two benzylic protons are at 4.45 ppm with J = 5.6 Hz as a doublet due to the coupling with the proton on nitrogen. The NH absorption appears at 5.70 ppm as a broad singlet. The five aromatic protons are at 7.25 ppm as a multiplet.

In the mass spectrum of 83, Fig. 15C, the molecular ion occurs at M^+/z 263. The fragment ion M^+/z 231 corresponds to the loss of the CH_3OH from the molecular ion. The formation of fragment ion M^+/z 106 (NH-CH_2-Ph) is presumably produced by cleavage of the molecular ion. A tropylium fragment ion appears at M^+/z 91 as a basic ion.

An analogous sequence of reactions described in the preparation of 78 was performed in the 3-epimeric isomer (77), (Scheme 7), giving a mixed acetal glycoside (80) as white amorphous crystals in 70 % yield and a small amount of 81. Compound 80 was recrystallized from ether and chloroform to give white amorphous crystals, m.p. = 57-59 ° C.

Compound 80 was subjected to column chromatography over silica (Solvent M) to afford 84, which was collected at earlier fraction, and 85 after solvent evaporation. Both 84 and 85 are white amorphous crystals, with m.p. = 57-58 ° C and m.p. = 55-56 °C respectively after being crystallized from ethyl acetate. Compound

84 and 85 were assigned based on the analysis of their p.m.r. spectra to β and α glycosides respectively. The infrared and p.m.r. spectra of 84 are present in Figs 17A and 17B.

The i.r. spectrum of 84, Fig. 17A, reveals the NH stretching vibration at 3450 cm^{-1} and the vibration of -CONH- at 1670 cm^{-1}. The bending vibration of aromatic C=C and amide II band is present at 1520 cm^{-1}. The absorption of C-O-C bending vibration appears as an intense sharp signal at 1050 cm^{-1}.

The p.m.r. spectrum of 84, Fig. 17B, shows the three methyl protons shifted to a higher field, as a doublet at 1.21 ppm, with J = 6 Hz compared to that of 85. The C_2-H_a is at 1.65 ppm, as a heptet with J_{gem} = 10 Hz. The C_2-H_e appears at 2.00 ppm, as a barely resolved heptet with J_{gem} = 12 Hz. The C_4-H_a appears at 1.55 ppm, as an octet with J_{gem} = 14 Hz. The C_4-H_e at 1.85 ppm, J_{gem} = 14 Hz, as a poorly resolved quintet. The C_3-H_e was observed at 2.72 ppm as a narrow multiplet with W_H = 15 Hz, which indicates its equatorial orientation. The three methoxy protons are at 3.43 ppm, as a singlet. The C_1-H_a at 4.76 ppm, with $J_{2a,\,1a}$ = 7.5 Hz and $J_{2e,\,1a}$ = 2.5 Hz (W_H = 12 Hz), as a doublet of doublets, shifts to a lower field than that of 85 and thus is assignable to β anomeric orientation. The C_5-H is observed at 3.98 ppm as a doublet of quintet which predictably appears at a lower field compared to 83 and 85, (86, 87). The two benzylic protons become a doublet at 4.37 ppm with J = 5.7 Hz and the NH occurs at 6.20 ppm as a broad singlet. The five aromatic protons are at 7.30 ppm as a multiplet.

In the mass spectrum of **84**, Fig. 17C, the molecular ion occurs at M^+/z 263. The loss of CH_3OH from the molecular ion probably produces the fragment ion M^+/z 231. A tropylium ion is at M^+/z 91. The basic fragment ion M^+/z 157 is presumably due to the loss of the $NH-CH_2-Ph$ group from molecular ion by cleavage. The molecular ion M^+/z 263 and fragment ion M^+/z 231 are confirmed by the high resolution mass spectrum (Fig.17D).

The infrared and p.m.r. spectra of **85** are presented in Figs. 18A and 18B. In the i.r. spectrum of **85**, Fig. 18A, the absorption of the NH stretching vibration shifts to a lower frequency, from 3450 cm^{-1} to 3320 cm^{-1}, compared to that of **84**.

The p.m.r. spectrum of **85**, Fig. 18B, reveals three methyl protons (C_5-CH_3) at 1.14 ppm as a doublet with J = 6.2 Hz due to coupling with C_5-H_a. The two methylene protons ($C_2-H_aH_e$) appear at 1.97 ppm as a sextet in the AB pattern of an ABCD spin system. The C_4-H_a, as an octet, is at 1.55 ppm with J_{gem} = 10 Hz, and the C_4-H_e, as a broad doublet, appears at 2.10 ppm with J_{gem} = 10 Hz. The one proton multiplet, at 2.72 ppm and with W_H = 14 Hz, is attributed to the C_3-H_e. Three methoxy protons appear at 3.18 ppm, as a singlet and the one methine proton on C_5 (C_5-H_1), appears at 3.80 ppm, as a doublet of sextets. The methoxy proton signal observably shifts to a higher field compared to those of **82**, probably because of the shielding effect of the benzene ring and its axial orientation, (85, 86). The two benzylic protons split at 4.40 ppm, as a doublet of quartets with J_{gem} = 15 Hz, and $J_{CH3,NH}$ = 4 Hz, of a typical AB spin system.

The equatorial proton on C_1 is at 4.70 ppm, a poorly resolved triplet with W_H = 8.5 Hz. The five aromatic protons are appears at 7.30 ppm as a multiplet. One proton signal corresponding to the NH, which shifted to a lower field compared to 83, clearly indicates hydrogen bonding with the oxygen atom of the methoxyl group.

The mass spectrum of 85, Fig. 18C, show the fragment ion M^+/z 231 corresponding to the loss of CH_3OH from the molecular ion, which is unequivocally identified by the high resolution mass spectrum, (Fig. 18D). It is not surprising that there is no signal of the molecular ion at M^+/z 263. Since the formation of the hydrogen bonding between the C_1-OCH_3 and the NH (Fig. 12C) facilitates the hydrogen rearrangement, the molecular ion is very unstable in the applied electron field and losts a methanol molecule to give fragment M^+/z 231. Formation of the fragment ion M^+/z 97 is presumably due to the loss of the CO-NH-CH_2-Ph group from fragment M^+/z 231. A tropylium ion is at M^+/z 91 as a basic peak.

Treatment of 78 with lithium aluminum hydride in methylene chloride eventually gave 86 as a syrup. Further preparative layer chromatography of 86 (solvent L) afforded two anomers. The compound, with Rf = 0.26, was assigned as the α-anomer (88) while the other one, with Rf = 0.17, was identified as the β-anomer (89).

The i. r., p.m.r. and mass spectrum of 88 are presented in Figs. 19A, 19B, 19C.

In the i.r. spectrum of 88, the C-N stretching is at 1130 cm

$^{-1}$ and the C-O stretching is at 1050 cm^{-1}. The aromatic C=C absorption appears at 1600 cm^{-1} as a weak band.

The p.m.r. spectrum of **88** shows the three methyl protons at 1.16 ppm as a doublet. The five proton signal at 1.75 ppm as a multiplet is probably attributed to the two protons on C_4, the two protons on C_2 and the one proton on C_3. The NH appears at 2.10 ppm as a broad signet. The two methylene protons adjacent to the nitrogen are present at 2.45 ppm. The three methoxy protons are at 3.34 ppm as a singlet. The two benzylic protons are at 3.76 ppm as a singlet. The proton on C_5 is at 3.85 ppm as a multiplet. The one equatorial proton on C_1 is at 4.75 ppm with W_H = 7.5 Hz. The five aromatic protons are at 7.34 ppm as a multiplet. The two benzylic protons shift to a higher field and turn to be a singlet at 3.76 ppm comparing to **82**.

In the mass spectrum of **88**, M + 1 fragment is at M^+/z 250. The loss of methyl group gave M^+/z 234. The fragment M^+/z 120 is presumably due to the fragment of the CH_2NHCH_2Ph. The tropylium ion is at M^+/z 91 as a basic peak.

In the i.r. spectrum of **89**, The C-O-C stretching is observed at 1080 cm^{-1} and the C-N-C stretching appears at 1130 cm^{-1}.

The p.m.r. spectrum of **89** shows the three methyl protons at 1.24 ppm as a doublet. The six proton signals, at 1.75 as a multiplet, are probably corresponding to the two methylene protons on C_2, the two methylene protons on C_4, the one methine proton on C_3 and the one proton on nitrogen. Predictably, the three protons on equatorial methoxyl group shift to a lower field, at 3.48 ppm , comparing to

those of 88. The two methylene protons adjacent to the nitrogen are at 2.55 ppm, which turn out to be a doublet comparing to that in 88. The axial proton on C_1 is present at 4.35 ppm as a doublet of doublets, with W_H = 14 Hz. The two benzylic protons are at 3.80 ppm as a singlet.

In the mass spectrum of 89, the molecular ion is at M^+/z 249, M-1 is at M^+/z 248. The loss of the methyl group is present at M^+/z 234. The fragment ion CH_2NHCH_2Ph is at M^+/z 120. The tropylium ion is at M^+/z 91. These fragment ion are confirmed by high resolution mass spectrum, (Fig.20D). The presumed pattern of fragment ions are illustrated in Scheme 17.

The treatment of 80 with the similar reduction procedure as for 78, (Scheme 9), gave 87 in 64.7 %, as a colorless syrup. After HPLC of 87 over silica (solvent: N), two anomers, 90 (Rf = 0.3), and 91 (Rf = 0.25), were obtained. The compound 90 was collected at early fraction followed by 91. Compounds 90 and 91 are assigned to β and α glycosides respectively based on the analysis of their p.m.r. spectra, Figs. 21B and 22B.

In the i.r. spectrum of 90, Fig. 21A, the strong C-O-C stretching absorption is present at 1050 cm^{-1} and the C-N-C stretching appears at 1110 cm^{-1}.

The p.m.r. spectrum of 90, Fig. 21B, show the three methyl protons at 1.25 ppm, as a doublet with J = 6 Hz. A five proton multiplet at 1.5-1.6 ppm is assumably due to the two protons on C_4, two protons on the C_2 and one proton on the NH. The equatorial proton on C_3 is at 2.15 ppm as a multiplet. A two proton signal, at

2.65 ppm as a doublet, is attributed to the two methylene protons adjacent to nitrogen. The three methoxy protons are at 3.44 ppm as a singlet. The two proton signal, at 3.81 ppm as a singlet, is due to the two benzylic protons. The one methine proton on C_4 is at 3.75 ppm as a multiplet and partially overlapped the benzylic protons. The one proton signal at 4.50 ppm, as a doublet and doublets, with W_H = 14 Hz, corresponds to the C_1-H_a. The five aromatic protons are at 7.30 ppm as a multiplet.

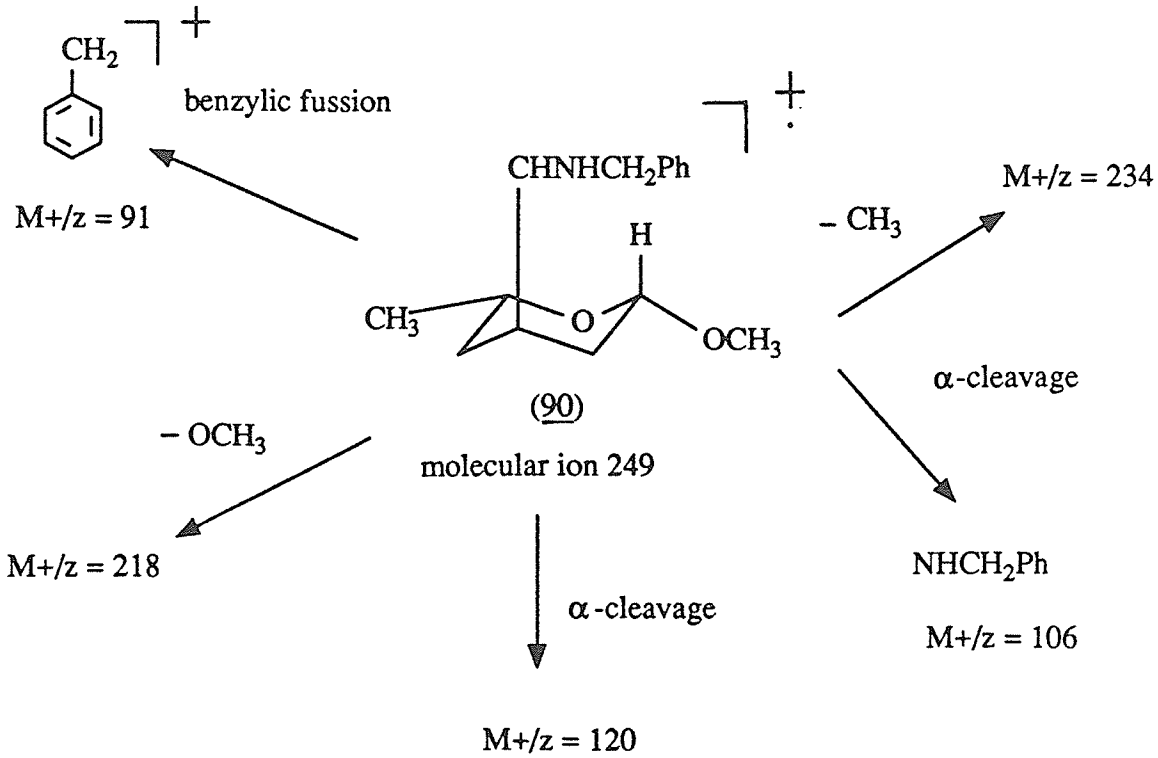
The mass spectrum of **90**, Fig. 21C, demonstrates a molecule ion at M^+/z 249. The M^+/z 234 and M^+/z 218 correspond to the loss of the methyl group and the methoxyl group respectively. The M^+/z 120 is due to the fragment CH_2NHCH_2Ph. The tropylium ion is at M^+/z 91. These fragments are all proved by high resolution mass spectrum (Fig. 21D) and the presumed fussion pattern is elucidated in Scheme 17.

The i.r. spectrum of **91** is similar to that of **90**. The strong C-N-C stretching vibration is at 1120 cm^{-1} and the C-O-C appears at 1050 cm^{-1}.

The p. m. r. spectrum of **91** shows the three methyl protons at 1.15 ppm as a doublet with J = 6 Hz. The two methylene protons on C_4 overlap the two methylene protons on C_2 and the proton on the nitrogen at 1.45-1.85 ppm. The two methylene protons adjacent to nitrogen, are evident at 2.80 ppm as a triplet which shift to a higher field comparing to that in **78**. The axial methoxy group, as expected, shifts to a higher field compared to its equatorial anomer (**90**), at 3.31 ppm, as a singlet. One proton singlet at 3.95 ppm is

due to the C_5-H_a. The equatorial proton on C_1 is present at 4.65 ppm as a broad singlet, with W_H = 7 Hz. The two benzylic protons are at 3.80 ppm as a singlet. A five proton multiplet signal, at 7.30 ppm, attributes to the aromatic protons.

The mass spectrum of **91** shows a similar fragment pattern to that of **90**. The molecular ion is at M^+/z 249. The M^+/z 234 and M^+/z 218 are visualized by arising as a consequence of losing the methyl and methoxyl group respectively. The M^+/z 120 corresponds to the fragment CH_2NHCH_2Ph. The M^+/z 91 is due to the tropylium ion.



Scheme 17 The Possible Pathways for Formation of the Fragments in the Mass Spectrum of Methyl-3-N-(benzylamino-methyl)-2,3,4,6-tetradeoxy-β-DL-erythro-hexopyranose (90)

EXPERIMENT

All melting points were recorded on a Fischer-Johns melting point apparatus and were uncorrected. Infrared spectra were taken on a Perkin Elmer Infracord 710 spectrometer, using sodium chloride cell with methylene chloride as a solvent. Mass spectra were recorded on a V.G.7070EHF mass spectrometer with a 70 FAB system. A Brucker AM-300 spectrometer was used to make all p.m.r. spectra and ^{13}C n.m.r. spectrum with $CDCl_3$ as a solvent and tetramethylsilane (TMS) as an internal reference except those presented in figs. 8A, 10B, 11B, which were made on a Varian EM-360 spectrometer. The anomers (**90**) and (**91**) were isolated on a HPLC instrument, Spectra-Physics 3000B, with a Modle 230 UV detector and silica column.

Preparative layer chromatograms were obtained on 20 X 20 cm (plate thickness: 0.2 cm) silica gel plates (E. Merck), which were activated at 120°C. UV light and iodine were used for visualization. Thin layer chromatograms were obtained on 0.2 mm silica gel 60 F_{254} plates. (E. Merck, Darmstadt, Germany). Column chromatography was performed with silica gel 60 (E. Merck Darmstadt, Germany). Absolute methanol was distilled from magnesium metal immediately prior to use. Tetrahydrofuran (THF) was freshly distilled from sodium metal. Solutions were concentrated under reduced presure using a rotary evaporator.

The 0.5 M potassium phosphate buffer solution (pH = 7) was used to prepare buffered plates for preparative layer chromatography.

The solvent systems used for the experiments are listed as follows:

Solvent A: chloroform-acetone, 9:1, v/v.

Solvent B: chloroform:acetone, 90:5, v/v.

Solvent C: methylene chloride:acetone, 10:0.3, v/v.

Solvent D: benzene:acetone:t-butanol, 10:0.5:0.5, v/v.

solvent E: benzene:ethylacetate, 2:1, v/v.

Solvent G: petroleum ether:acetone, 3:1, v/v.

Solvent H: chloroform:acetone 10:1, v/v.

Solvent I: chloroform.

Solvent J: benzene:acetone, 10:2, v/v.

Solvent K: methylene chloride.

Solvent L: ethyl acetate.

Solvent M: cyclohexane:acetone, 2.5:1, v/v.

EXPERIMENT

PART A: SYNTHSIS OF 1-DEOXY-N,N-BIS-DEMETHYL-PYRROMYCIN (58)

ISOLATION OF AKLAVINONE (55)

A pigmented compound was obtained from the ethyl acetate extracts of cultured and mycelial cakes of Streptomyces Galilaeus var. Siwenensis, (68). Further purification of this compound by preparative layer chromatography (solvent A) gave orange amorphous solids of which physical and spectroscopic properties were similar to aclacinomycin A reported by H. Umezawa et al., (76).

By acid hydrolysis of this amorphous solids (800 mg) in sulfuric acid (0.3 N, 50ml) at 85°C for 3 hr, a yellow precipitate was collected by filtration. This yellow precipitate was washed with water (20 ml, three times), and then collected by filtration. The yellow compound was recrystallized from acetone-ether, yielding 330 mg of aklavinone (55), as orange crystalline needles.

The physical and spectroscopic data of 55 is identical to the literature, (77-79).

m.p. = 169-173°C, Lit. m.p. = 171°C.

i.r. (cm^{-1}), Fig. 1A:

3550 (OH), 3475 (chelated phenolic OH), 1730 ($-COOCH_3$), 1675 (C=O), 1620 (chelated C=O).

p.m.r. (ppm), ($CDCl_3$), Fig. 1B-1:

1.10 (t, 3H, OCH_3), 1.50-1.70 (m, 2H, C_{13}-2H), 2.25 (broad d, 1H, H_{8a}), 2.60 (dd, 1H, H_{8e}), 3.50 (s, 1H, C_9-OH), 3.70 (s, 3H, OCH_3), 3.92 (s, 1H, C_7-OH), 4.13 (s, 1H, C_{10}-H), 5.35 (s, broad, 1H, H_{7e}), 7.30 (d, 1H, H_{11}), 7.70 (m, 3H, aromatic protons), 11.90 (s, 1H, 3-OH), 12.70 (s, 1H, 6-OH).

Double Irradiation p.m.r. Spectrum (ppm), ($CDCl_3$), Fig. 1B-2.

PREPARATION OF 1-CHLORO-4-O-(P-NITRO-BENZOYL)-3-N-TRIFLUOROACETYL DAUNOSAMINE (50)

A) To a solution of 3-N-trifluoroacetyl daunosamine (53) (530 mg) in methylene chloride (70 ml), was added p-nitrobenzyl chloride (1.35 g) and pyridine (1.5 ml) at room temperature. The solution was stirred magnetically at 40°C in an oil bath under anhydrous condition for a period of 15 hr.

The solution was concentrated under the reduced pressure and extracted with anhydrous ether (100 ml). After filtration of the solution, a white solid residue was collected and washed with anhydrous ether (100 ml). The combined ether solution was evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography over silica (Solvent L) to give in order of elution, p-Nitrobenzoic anhydride and the protected sugar 54 (1.1 g) (1,4-di-O-(p-nitrobenzoyl)-3-N-trifluoroacetyl-daunosamine). The yield was 80%. The product 54 was further recrystallized from chloroform as white crystals.

The melting point and spectruscopic data for 54:

m.p. = 195-197 °C, Lit. 197.0-198.5 °C, (53).

i.r. (cm^{-1}), Fig. 2A:

3450 (NH), 1750 (C=O), 1610 (aryl), 1540 (NO_2).

p.m.r. (ppm), ($CDCl_3$), Fig. 2B:

1.20 (d, 3H, CH_3), 2.20 (q, 1H, C_2-H_a), 2.35 (d, 1H, C_2-H_e), 4.10 (m, 1H, C_5-H_a), 4.55 (m, 1H, C_3-H_a), 5.45 (s, 1H, C_4-H_e), 6.15 (d, 1H, C_1-H_a), 6.65 (s, 1H, NH), 8.30 (m, 8H, aromatic protons)

B) To the stirred solution of 54 (210 mg) in methylene chloride (20 ml) in a flask in an ice bath, was passed anhydrous hydrogen chloride through a gas dispersion tube for 10 minutes. After being stirred for an additional 20 min, while the temperature remained unchange (0°C), the solution was filtered. The filtrate was evaporated to dryness under the reduced pressure to give a white foamy residue 50. This unstable foamy residue 50 was dissolved in methylene chloride (10 ml) and evaporated to dryness. This operation was repeated once more until the residue is free of hydrogen chloride. Compound 50, without further purification, was allowed to condense with aklavinone (55).

PREPARATION OF METHYL 4-O-(P-NITRO-BENZOYL)-3-N-TRIFLUORO- ACETYL-DAUNOSAMINE (46) AND THE TWO ANOMERS, 47 AND 48

To the stirred solution of methyl 3-N-trifluoroacetyl-daumosamine (45) (1.056 g) in methylene chloride (10ml) and pyridine (10ml) was added p-nitrobenzoyl chloride (966 ml) at 0°C. After being stirred at

0 °C for one hr, the solution was allowed to warm to 25 °C and stirred for additional 14 hr. Then the solution was evaporated under reduced pressure to gave a syrupy residue.

The residue was dissolved in chloroform (30 ml) and the solution was washed by a dilute sulfuric acid solution (3N, 10 ml, three times), and then a ice cold saturated sodium carbonate solution (10 ml, three times) and water (10 ml, three times). The organic layer was separated out, dried over magnesium sulfate (MgSO4) and then filtered. The filtrate was evaporated to dryness to afford **46** as a white amorphous solid (1.102 g), as a mixture of two anomeric isomers from ether. The yield was 96.4 %. m.p. = 70-74 C. Preparative layer chromatogrphy of **46** over silica gel (Solvent B) gave the two anomers, e.g. α-anomer **47** (Rf = 0.5) and β-glycoside **48** (Rf = 0.45), both as white amorphous solids from ether.

The spectroscopic data for **46**:

i. r. (cm^{-1}), Fig. 3A:

3420 (NH), 1730 ($CONHCF_3$), 1720 (C=O), 1530,1360 (NO_2), 1620 (phenyl nucleus), 1190 (C-O-C), 2850 (OCH_3).

The spectroscopic data for glycoside **47**:

p.m.r. (ppm), ($CDCl_3$), Fig. 3B:

1.20 (d, 3H, C_5-CH_3), 1.98-2.05 (m, 2H, C_2-H_2), 3.41 (s, 3H, C_5-OCH_3), 4.20 (dd, 1H, C_5-H), 4.68 (m, 1H, C_3-H), 4.93 (s, broad, 1H, Wh = 6 Hz C_1-H), 5.42 (s, broad, 1H, C_4-H), 6.46 (d, broad, 1H, NH), 8.28 (m, 4H, aromatic hydrogens).

Mass spectrum (M+/z), Fig. 3C:

375 ($M-OCH_3$), 304, 195, 150 ($O_2NC_6H_4CO$, 100%), 58.

The spectroscopic data for the glycoside 48:

p.m.r. (ppm), Fig. 4B:

1.28 (d, 3H, CH_3), 1.85 and 2.15 (two sets of doublet, 2H, CH_2), 3.58 (s, 3H, OCH_3), 3.87 (q, 1H, C_5-H), 4.70 (m, 1H, C_3H), 4.95 (s, 1H, C_4H), 5.45 (s, 1H, C_1H), 6.20 (d, 1H, NH), 8.30 (q, 4H, aromatic protons).

Mass spectrum (M^+/z), Fig. 4C:

375 ($M-OCH_3$), 304, 195, 150 ($O_2NC_6H_4CO$, 100%), 58.

PREPARATION OF 1-BROMO-4-O-(P-NITRO-BENZOYL)-3-N-TRIFLUOROACETYL-DAUNOSAMINE (49)

To the stirred solution of 46 (300 mg) in methylene chloride (30 ml) in a flask was bubbled anhydrous hydrogen bromide through a gas dispersion tube for 30 min, at 0°C. After that, the reaction solution was evaporated under reduced presure to remove methylene chloride, HBr and methanol evolved in reaction to dryness. The residue was dissolved in methylene chloride (30 ml), and the solution was evaporated to dryness. This operation was repeated once more until the residue 49 was free of hydrogen bromide. This compound, without further purification, was permitted directly to undergo glycosidation with aklavinone (55).

PREPARATION OF 7-O-4'-O-(P-NITRO-BENZOYL)-3'-N-(TRIFLUOROACETYL)-α-DAUNOSAMINYL-AKLAVINONE (56) AND THE GLYCOSID 57

Method A:

To the flask, containing crystalline aklavinone (55) (120 mg), the 1-chlorosugar 50 (100 mg) and freshly ground molecular sieve 3A (300 mg), was injected anhydrous methylene chloride (35 ml) through a rubber stopper followed by silver trifluoromethane sulfonate (80 mg) addition in anhydrous ether (10 ml) in the same manner. The solution was stirred at room temperature in darkness for 4 hr and then filtered. The filtrate was washed with 5 % NaCl solution (10 ml, two times). The organic layer was dried ($NaSO_4$) and filtered through a Butch funnel. The filtrate was evaporated to dryness under reduced pressure to give a orange foamy residue. The residue was separated by a column chromatography over silica (Solvent C) to give 56 as a major product and a small amount of 57. Compound 56 was slowly crystalised from methylene chloride and ether, yielding 90 mg of orange crystalline needles. m.p. = 174 - 177°C. The yield was 40 % after crystallization.

The spectroscopic data for 56:

i.r. (cm^{-1}), Fig. 5A:

3540 (OH), 3420 (NH), 1735 (broad and intense, $-COOCH_3$, $-C_6H_4-COO-$, $NHCOCF_3$), 1680 (C=O), 1630 (chelated C=O), 1620 (aromatic), 1535 (NO_2).

p.m.r. (ppm), Fig. 5B-1:

1.11 (t, 3H, J = 6.2 Hz, $C_{13}-CH_3$), 1.26 (d, 3H, J = 7.0 Hz, $C_{5'}-CH_3$), 1.54 and 1.79 (two sets of m, 2H, $C_{13}-H_2$), 2.10 (m, 2H, $C_{2'}$-HaHe), 2.34 (broad d, 1H, J_{8a8e} = 15 Hz, J_{8a7e} = 1 Hz, C_8-H_a), 2.62 (dd, 1H, J_{8a8e} = 15 Hz, J_{7e8e} = 5 Hz, C_8-H_e) 3.70 (s, 3H, $C_{10}-COOCH_3$), 4.20 (s, 1H, C_9-OH), 4.05 (s, 1H, $C_{10}-H_e$), 4.47

(m, 2H, $C_{3'}$-H_a and $C_{5'}$-Ha, $J_{C5'-H,CH3}$ = 7 Hz), 5.47 (broad s, 1H, $C_{4'}$-H_e), 5.64 (broad s, 1H, $C_{1'}$-H_e), 5.38 (broad d, 1H, C_7-H_e), 6.20 (broad doublet, 1H, NH), 7.73 (s, 1H, C_{11}-H), 7.33 (d, 1H, $J_{2,3}$ = 8 Hz, C_3-H), 7.85 (d, 1H, $J_{1,3}$= 1 Hz, C_1-H), 7.72 (t, 1H, C_2-H), 8.33 (m, 4H, four aromatic protons), 12.00 (s, 1H, C_4-OH), 12.78 (s, 1H, C_6-OH).

^{13}C n.m.r. spectrum (ppm), Fig. 5B-2:

125 (s, C_1), 126 (s, C_2), 121 (s, C_3), 157.5 (s, C_4), 192 (s, C_5), 157 (s, C_6), 67 (s, C_7), 32 (s, C_8), 73 (s, C_9), 47 (s, C_{10}), 122 (s, C_{11}), 181 (s, C_{12}), 35 (s, C_{13}), 14 (s, C_{14}), 165 (s, C_{15}), 53 (s, C_{16}), 134 (s, C_{17}), 131.5 (s, C_{18}), 133.5 (s, C_{19}), 131 (s, C_{20}), 143 (s, C_{21}), 135 (s, C_{22}), 101 (s, C'1), 30 (s, C'_2), 58 (s, C'_3), 72.5 (s, C'_4), 72 (s, C'_5), 17 (s, C'_6), 171 (s, C'_7), 115, 116, 117 and 118 (quartet, C'_8), 161.5 (s, C'_9), 161 (s, C''_1), 138 (s, C''), 132 (s, C''_3) and 152 (s, C''_4).

Mass spectrum (M+/z), (1):

M^+ = 786 (FAB mass-spectrum)

The spectroscopic data for **57**:

i.r. (cm^{-1}), (1):

3600, 3520 (OH), 3470, 3430 (NH_2), 3500 to 3100 (chelated OH), 1735 ($COOCH_3$), 1680 (C=O), 1635 (chelated C=O), 1610, 1580 (aromatic).

p.m.r. (ppm), Fig. 6B:

1.10 (t, 3H, C_{13}-CH_3), 1.51 (d, 3H, $C_{5'}$-CH_3), 1.52 and 1.55 (two sets of multiplet, 2H, C_{13}-H_2), 2.3 and 2.0 (two sets of multiplet, C'_2-H_2), 2.30 and 2.60 (two sets of multiplet, 2H, C_8-H_2), 3.70

(s, 3H, OCH_3), 3.9 (s, 1H, C_{10}-H), 4.2 (s, 1H, C_9-H), 4.5 (d, 1H, C'5-H), 4.7 (m, 1H, C'_3-H), 5.4 (dd, 1H, C'_1), 5.65 (d, 1H, C'_4-H), 5.75 (s, 1H, C_7), 7.35 (d, 1H, C_3-H), 7.6 (d, 1H, C_2-H), 7.75 (s, H, C_{11}-H), 7.9 (d, 1H, C^1-H), 8.5 (q, 4H, benzene protons), 11.86 (s, 1H, C_4-OH), 13.12 (s, 1H, C_6-OH).

Method B:

Aklavinone (50) (200 mg), silver trifluromethane sulfonate (380 mg) and finely powdered molecular sieve 3A (5 g) was placed in stirred anhydrous methylene chloride (100 ml). The solution was magnetically stirred at 40 - 45°C for 20 hr in darkness. Three one equivalent portions of freshly prepared 1-bromosugar 49 were added at 0, 5 and 10 hr interval and the additional silver trifluoromethane sulfonate (100 mg) was added at 10 hr interval. The reaction completed as judged by t.l.c. (Solvent D).

After being cooled to 0°C, the solution was filtered and the solid residue was washed with methylene chloride (15ml, 2 times). The filtrate and washing were combined, and evaporated to dryness to give a orange residue. Then the residue was dissolved in $CHCl_3$ (30 ml) followed by washing with 20 % KI solution (15 ml, two times) and water (10ml, two times). The organic layer was separated, dried over anhydrous sodium sulfate and filtered. The filtrate was evaporate under reduced pressure to dryness to give a orange residue. The preparative layer chromatography of the residue upon buffered silica gel (solvent E) afforded 56 as a major compound. Compound 56 was recrystallized from methylene chloride and ether, yielding 95 mg of

yellow crystalline needles. The yield was 26.9 % after recrystallization.

PREPARATION OF 1-DEOXY-N,N-BIS-DEMETHYL-PYRROMYCIN (10)

To a stirred solution of methanol (8 ml), saturated potassium carbonate solution (3 ml) and water (3 ml) was added **56** (90 mg) in tetrahydrofuran (8 ml). The mixture was stirred at room temperature for 15 hr and then cooled in an ice bath. The solution was adjusted to pH = 9 by dilute hydrochloric acid (0.5 %), and then exaustively extracted by chloroform (30 ml, three times). The combined extract was dried over anhydrous magnesium sulfate and filtered. After the filtrate being evaporated to dryness, a dark orange residue was obtained. The residue was subjected to preparative layer chromatography upon silica gel to give 1-deoxy-N,N-demethyl-pyrromycin (**10**) (25 mg) in 37.9% yield, as an amorphous solid after being crystalised from methylene chloride and ether.

The physical and spectroscopic data for **10**:

m. p = 132-143 C (decomposed).

i.r. (cm^{-1}), (Fig. 6A), (1):

3600, 3520 (OH), 3470, 3430 (NH_2), 3500-3100 (chelated OH), 1735 ($COOCH_3$), 1680 (C=O), 1635 (chelated C=O), 1610,1586 (aryl).

EXPERIMENT

PART B: PREPARATION OF METHYL 3-N-(BENZYL-CARBOX-AMIDO)-2,3,4,6-TETRADEOXY-DL-THREO-HEXOPYRANOSE (78) AND ITS 3-EPIMER (80)

PREPARATION OF 2,2-DIALLY-DIETHYLMALONATE (64)

To a stirred solution of acetone (6 litre), diethylmalonate (63) (607 ml, 4 moles) and powdered potassium carbonate (2800 g, 20 moles) was added allyl bromide (1384 ml, 16 moles) in one portion at room temperature. Then the reaction solution was heated to reflux with vigorously stirring over a period of 4 days.

During the reaction, a rubber balloon was made on the top of condenser and mercury seal was required so that a little pressure was created inside of the flask to enhance the reaction rate. After 4 days, the reaction was completed as judged by t.l.c., (Solvent G).

After being cooled to 0°C, the reaction solution was filtered to remove the potassium carbonate residue. The cake of potassium carbonate was washed with acetone (400 ml, three times). The filtrate and washing were combined and then evaporated to remove acetone to give a slight yellow liquid. After distillation of the liquid under reduced pressure at 87-89°C/1.5 mm, a colorless liquid 64 (912 g) was obtained in 95% yield.

The spectruscopic data for 64:

i. r. (cm^{-1}) Fig. 8A:

1720 (intense singlet, COOEt), 1640 (weak, C=C), 1200 (broad singlet, C-O-C).

p.m.r. (ppm), Fig. 8B:

1.32 (t, 6H, two CH_3), 2.55 (d, 4H, four allylic protons), 4.17 (q, 4H, two CH_2), 4.86-5.80 (m, 6H, six olefinic protons).

PREPARATION OF 2,2-DIALLY-MALONIC ACID (65)

2,2-diallyl-malonate (4 mol, 960 g) (64) was added to the stirred solution of methanol (2.4 L), water (4.3 L) and potassium hydroxide (16 mol, 896 g). The solution was heated to reflux with stirring for 15 hr. After being cooled to room temperature, the solution was carefully neutralized and adjusted to pH 2-3 by adding dropwise cooled concentrated HCl with efficient stirring at about 0 °C. Then the solution was extracted completely with methylene chloride (500 ml, three times). The extracts were combined and dried over anhydrous magnesium sulfate followed by filtration. The filtrate was evaporated under reduced presure to dryness to give 65 (669 g), in 91 % yield, as white crystals after being recrystallized from chloroform.

The spectruscopic data for 65:

m.p. = 126-128 °C

i.r. (cm^{-1}), Fig. 9A:

3550 (OH monomers), 3300-3000 (OH dimmers), 1760 (C=O monomers), 1710 (C=O dimmers), 1650 (C=C weak).

p.m.r. (ppm), Fig. 9B:

2.70 (d, 4H, four allylic protons), 5.16 (poorly resolved sextet, 2H, two H_{trans}), 5.20 (poorly resolved doublet, 2 H, two H_{cis}), 5.70 (m, 2H, two H_{gem}).

Mass spectra (M^+/z), Fig. 9C:

185 (M+1), 166($M-H_2O$), 125 [$M-(H_2O)-(CH_2=CHCH_2)$], 79 [$M+1-(H_2O)-(CO_2)-(CO)$].

PREPARATION OF 2-ALLYL-4-PENTENOIC ACID (66)

2,2-diallylmalonic acid (65) (2 mol, 280g) was placed into a solution of acetic acid (1.5 L), water (600 ml) and DMF (1.1 L). The mixture was stirred under reflux for 25 hr. The reaction process was monitored by t.l.c. (Solvent H). Then the reaction solution was exaustively extracted with chloroform (400 ml, three times). The extracts were combined and evaporated under reduced pressure to give a liquid. The liquid was distilled at 98-100°C/2.5 mm to give 66 (266 g) as a colourless liquid. The yield was 95 %.

The spectroscopic data for 66:

i.r. (cm^{-1}), Fig. 10A:

3500 (OH monomers), 3300-3000 (OH dimmers), 1742 (C=O monomers), 1702 (C=O dimmers), 1650 (mediate, C=C).

p.m.r. (ppm), Fig. 10B:

2.37 (broad d, 4H, four allylic protons), 4.96 (broad s, 2H, two H_{cis}), 5.20 (poorly resolved broad doublet, 2H, two olefinic H_{trans}), 5.83 (m, 2H, two olefinic H_{gem}), 11.5 (s, 1H, one carboxylic proton).

Mass spectrum (M^+/e), Fig. 10C:

M^+/z 140 (molecular ion), 95 (M-COOH, 14%), 99 [M-(CH_2-$CH=CH_2$)], 44 (18 %), 43 (100 %).

PREPARATION OF (R,S) 2-ALLYL-4-METHYL- γ -BUTYROLACTONE (67)

2-Allyl-4-pentenoic acid (66) (16 g) was added in one portion to a stirred solution of hexane (900 ml), p-toluenesulfonic acid monohydrate (16 g) and water (10 ml) in a 3 litre round-bottomed flask fixed with a 50 ml Black-stock water trap and a condenser.

The reaction solution was heated to reflux with stirring for 2 days. The reaction process was monitored by t.l.c., which revealed formation of compound 67, (Rf = 0.65), and high boiling point by-products, (Rf = 0.7), (Solvent N). After colling to room temperature, the solution was transferred to a separatory funnel. The organic hexane layer was separated from a small amount of solvated brown p-toluenesulfonic acid which could be used for recycle. The hexane solution was evaporated under reduced pressure to reduce the total volume of the solution to about 200 ml.

The remained solution was washed with an ice cold solution of sodium bicarbonate (50%, w/v), (30 ml, two times), and then ice cold water (30 ml, two times). The organic layer was separated from the aqueous solution, dried over anhydrous magnesium sulfate ($MgSO_4$) and filtered. The filtrate was evaporated to dryness to give a pale brown liquid. The distillation of the liquid under reduced pressure (87-90°C/2.6 mm) gave the colorless viscous liquid 67 (9 g) in 56.3% yield.

The spectroscopic data for 67:

i.r. (cm^{-1}), Fig. 11A:

1762 (very intense, COO- stretching), 1640 (mediate shoulder, C=C), 1180 (intense, CO-O-C bending).

p.m.r. (ppm), Fig. 11B:

1.38 (d, 3H, C_4-CH_3), 2.00-3.00 (m, 5H, C_3-CH_2, C_2-H and the two allylic protons), 4.50 (sixtet, 1H, C_4-H), 5.00 (s, broad, 1H, H_{cis}), 5.20 (broad doublet, 1H, H_{trans}), 5.50-6.00 (m, 1H, the olefinic H_{gem}).

Mass spectrum (M^+/e), Fig. 11C:

140 (molecular ion), 141 (M+1), 125 (M-CH_3), 96 (M-CO_2).

High resolution mass spectrum (M^+/z), Fig. 11D:

$C_8H_{12}O_2$: calculated 140.0837324, observed: 140.084244.

PREPARATION OF (R,S) 2-ALLYL-N-BENZYL-4-HYDROXY-ERYTHRO-PENTAN-AMIDE (75) AND (R,S) THREO DIASTEREOMER 77

Sodium hydride (9.6 g, 0.4 mol), which was washed with petroleum ether, was immediately placed into the stirred solution of absolute tetrahydrofuran (THF), (1.8 L), and benzylamine (37.4 g, 0.35 mol) at room temperature. After being stirred at room temperature for one hr, the solution was heated to reflux and stirred for additional 2 hr. During the reaction, nitrogen balloon was made at top of condenser to avoid any possibility of the oxidation of benzylamine by air.

To this solution, 2-allyl-4-hydroxy-γ-butyrolactone (67) (42 g, 0.30 mol) in 500 ml of tetrahydrofuran (THF) was added dropwise, over a period of one hr, with stirring under reflux. The additional

15 hrs. was permitted to complete the reaction. The reaction process was monitored by t.l.c.(Solvent A).

After being cooled to 0°C, hydrochloric acid (10 N) was added dropwise with efficient stirring to neutralize the solution to pH 7-6. The solution was evaporated under reduced pressure to remove THF solvent. To the remained solution, 100 ml of methylene chloride was added. The solution was washed with ice cold saturated sodium chloride solution (20 ml, two times). Then the organic layer was separated, dried over anhydrous sodium sulfate and filtered. The filtrate was evaporated under reduced pressure to dryness to give a pale brown syrup (45 g), which was subjected to column chromatography over silica, (Solvent I). Compound 75 was first eluted out and was a pale yellow syrup (15 g) after solvent evaporation. Compound 77 was collected at a latter fraction , after solvent evaporation and recrystallized from a solution of ether and chloroform (10:1 v/v), yielding 18 g of white crystals, m.p. = 69-70°C. The total yield of 75 and 77 was 44.5 % after column chromatography.

The spectroscopic data for 75:

i. r. (cm^{-1}) Fig. 12A

3580 (monomer OH stretching), 3430 (dimmer OH and NH stretching), 3320 (polymer OH and NH stretching), 1670 (CONH, broad), 1620 (C=C, weak shoulder), 1520 (broad, aromatic C=C and the amide II band).

p.m.r. (ppm), Fig. 12B:

1.19 (d, 3H, $-CH_3$, J =6.2 Hz), 1.57 and 1.80 (two sets of a double of triplets, 2H, two allylic protons), 1.97 (d, 1H, OH, J = 4.5 Hz),

2.20 (m, 1H, methine proton to carbonyl group, C_2-H), 2.45 (m, 2H, two methylene protons on C_3), 3.85 (m, 1H, C_4-H), 4.45 (dq, 2H, two benzylic protons, Jgem = 15 Hz, $J_{CH2,NH}$ = 6 Hz), 5.10 (s, broad, 1H, one olefinic H_{trans}), 5.03 (d, broad, 1H, one olefinic H_{cis}), 5.75 (m, 1H, one olefinic H_{gem}), 6.00 (s, broad, 1H, NH), 7.73 (m, 5H, five aromatic protons).

Mass spectra (M/e), Fig. 12C:

247 (molecular ion), 229 ($M-H_2O$), 202 [$M-(CH_3CH_2OH$], 189 (M-58, 30%), 149, 106 ($NHCH_2Ph$, 48%), 91 (CH_2-Ph, 100%).

The spectroscopic data for 77:

i.r. (cm^{-1}), Fig. 13A:

3580 (OH stretching, free H bonding), 3430 (dimmer OH and monomer NH stretching), 3320 (OH and NH stretching, intermolecular H bonding), 1670 (CONH, broad, intense), 1640 (shoulder, mediate, C=C stretching overlaps with CONH absorption), 1520 (aromatic C=C and amide II band).

p.m.r. (ppm), Fig. 13B:

1.19 (d, 3H, CH_3, J = 6.2 Hz), 1.60 and 1.85 (pentet and double of triplets, 2H, two allylic protons), 2.25 (m, 1H, one methine proton adjacent to carbonyl group, C_2-H), 2.35 (m, 2H, two methylene protons on C_3), 2.08 (s, broad, 1H, OH), 3.85 (m, 1H, C_4-H), 4,450 (dq, 2H, two benzylic protons), 5.03 (d, broad, 1H, one H_{cis}), 5.10 (s, broad, 1H, one H_{trans}), 5.75 (m, 1H, one H_{gem}), 6.00 (s, broad, 1H, NH), 7.73 (m, 5H, five aromatic protons).

Mass spectrum (M^+/e) Fig. 13C:

247 (M^+ molecular ion), 229 (M - H_2O), 202 (M - CH_3CHOH), 189 (M- $CH_3COH=CH_2$), 106 (NH-CH_2-Ph 50%), 91 (CH_2-Ph, 100%).

PREPARATION OF METHYL 3-N-(BENZYLAMINO-CARBONYL)-2,3,4,6-TETRADEOXY-DL-THREO-HEXOPYRANOSE (78) AND ANOMERS 82 AND 83

To the solution of (R,S) 2-allyl-N-benzyl-4-hydroxy-erythro-pentanamide (75) (1.5 g) in absolute methanol (150 ml) was bubbled dry ozone (O_3), through a gas dispersion tube, with a stirring at -20 to -25 C. The reaction time was permitted to be 17 minutes as judged by t.l.c.. During the interval of t.l.c., dry nitrogen gas was passed into the reaction solution instead of ozone (O_3) to stop the reaction temporarily. Then 3 ml of freshly distilled trimethyl phosphite [$P(OCH_3)_3$] was added into the solution. Then the solution was heated to reflux with a stirring for 16 hr. After cooling to room temperature, the solution was evaporated under reduced pressure to remove methanol to give a pale brown syrupy residue.

The residue was dissolved in methylene chloride (50 ml) followed by washing with the 20% (w/v), and then Na_2CO_3 solution, (20 ml, two times), and finally water (15 ml, two times). The organic layer was separated, dried over anhydrous magnesium sulfate, and filtered. After solvent evaporation to dryness under reduced pressure, a pale yellow syrupy residue was obtained. This residue was subjected to flash chromatography over silica (Solvent I) to give 78 (650 mg), in 74% yield as a mixture of α and β anomers, and a small amount of 79 (95 mg) after solvent evaporation. Compound 78 was collected at

earlier fraction and gave white amorphous solids after recrystallization from a solution of ether and petroleum (7:3 v/v), m.p. = 94-95°C. The 79 was eluted out at latter fraction and collected after solvent evaporation. The recrystallization of 79 from a solution of methylene chloride and ether (10:1, v/v) gave white needles. Compound 79 was converted to 78 almost quantitatively in absolute methanol at pH = 3.

Further preparative layer chromatography over silica gel (Solvent J) of 78 gave two anomers, 82 (Rf = 0.65) and 83 (Rf = 0.55). Both of them gave amorphous solids from a solution of ether and petroleum ether (5:1, v/v), and were unequivocally assigned to α-82 and β 83 glycosides respectively by analysis of their p.m.r. spectra. The melting point and spectroscopic data for α anomer 82:

m.p. = 75-80°C

i.r. (cm^{-1}), Fig. 14A:

3450 (OH stretching), 1670 (-CONH-), 1520 (aromatic skeleton),

p.m.r. (ppm), Fig. 14B:

1.19 (d, 3H, J = 6.3 Hz, CH_3), 1.55 (q, 1H, H_{4a}, $J_{4a,4e}$ = 12 Hz), 1.75 (broad doublet, 1H, H_{4e}, $J_{4e,4a}$ = 16 Hz), 1.85 (dd, 2H, H_{2a} and H_{2e}), 2.71 (m, 1H, W_H = 28 Hz, H_{3a}), 3.34 (s, 3H, C_1-OCH_3), 3.85 (m, 1H, C_5-H), 4.43 (d, 2H, two benzylic protons, J = 6 Hz), 4.80 (broad singlet, 1H, Wh = 6 Hz, C_1-H_e), 5.77 (s, broad, 1H, NH), 7.30 (m, 5H, five aromatic protons).

Mass spectrum (M^+/z), Fig. 14C:

263 (molecular ion), 248 (M-15), 232 (M-OCH_3), 160, 106 (NH-CH_2-Ph), 97, 91 (CH_2Ph, 100%).

The melting point and spectroscopic data for β anomer 83:

m.p. = 117-120 °C.

i.r. (cm^{-1}), Fig. 15A:

3450 (NH stretching), 1680 (-CONH-), 1520 (aromatic C = C stretching and amide II band), 1020, 1080 (C-O-C and C-N-C bending vibration).

p.m.r. (ppm), Fig. 15B:

1.28 (d, 3H, C_5-CH_3, J = 6.2 Hz), 1.45 (q, 1H, H_4a, $J_{4a,4e}$ = 12 Hz), 1.55 (q, 1H, H_4e, J_{4e4a} = 12 Hz), 1.80 (broad d, 1H, C_2-H_a, $J_{2a,2e}$ = 11 Hz), 2.00 (d, broad, 1H, C_2-H_e, $J_{2e,2a}$ = 11 Hz), 2.45 (m, 1H, C_3-H_a, Wh = 26 Hz), 3.50 (s, 3H, C_1-OCH_3), 3.55 (m, 1H, C_5-H, J = 6.2 Hz), 4.33 (dd, 1H, C_1-H_a, Wh = 9 Hz), 4.45 (d, 2H, two benzylic protons, J = 6 Hz), 5.70 (s, broad, 1H, NH), 7.25 (m, 5H, five aromatic protons).

Mass Spectra (M/z), Fig. 15C:

263 (molecular ion), 248 (M-Me), 231 (M-CH_3OH), 106 ($NHCH_2Ph$), 97, 91 (CH_2Ph, 100%).

High resolution mass spectrum (M^+/z), Fig. 15D:

$C_{14}H_{17}N_1O_2$: calculated 231.1259328, observed 231.1267090; $C_7H_8N_1$: calculated 106.0656760, observed: 106.0681000; C_7H_7: calculated 91.0547764, observed: 91.0543820.

The melting point and spectroscopic data for 79 (mixture of two anomers):

m.p = 155-157 °C

i.r. (cm^{-1}), Fig. 16A :

3570 (OH), 3450 (NH), 1680 (-CONH-), 1520 (benzine ring)

p.m.r. (ppm), Fig. 16B: (referable to Figs. 14B and 15B)

Mass Spectrum (M^+/e), Fig. 16C:

249 (molecular ion), 231 (M-18), 149, 106, 91 (100%, tropylium ion).

CONVERSION OF 3-N-(BENZYL-CARBOXAMIDO)-2,3,4,6-TETRA-DEOXY-DL-THREO-HEXOPYRANOSE (79) TO 78

To the stirred solution of 200 mg of 79 in absolute methanol (100 ml) was bubbled anhydrous hyrogen chloride gas until pH 3, through a gas dispersion tube, at 0 C. The solution was stirred under this condition for 3 hr. The usual work up gave a white amorphous crystal (203 mg), in 96 % yield. Its physical and spectroscopic properties are identical to those of 78.

PREPARATION OF METHYL 3-(N-BENZYL-CABOXAMIDO)-2,3,4,6-TETRADEOXY-DL-ERYTHRO-HEXOPYRANOSE (80) AND THE TWO ANOMERS 84 AND 85

To the solution of absolute methanol (100 ml) and 2-allyl-N-benzyl-

4-hydroxy-pentanamide (77) (1 g) was passed dry ozone through a gas dispersion tube with stirring at -20 to -25 C. After 15 minutes, the reaction was completed as judged by t.l.c. (Solvent A). During t.l.c., dry nitrogen gas was bubbled into the reaction solution to prevent further ozonolysis temporarily.

The 2 ml of trimethyl phosphite [$P(OCH_3)_3$] was added into the reaction solution. The solution was heated to reflux with a stirring

for additional 15 hr, and then evaporated under reduced pressure to remove methanol. The residue was dissolved in methylene chloride (50 ml) followed by washing with 20% sodium carbonate solution (20 ml, two times) and water (15 ml, two times). The organic solution was separated and dried over anhydrous magnesium sulfate followed by filtration. The filtrate was evaporated to dryness under reduced pressure to give a pale yellow syrup. The flash collum chromatography (Solvent K) of this syrupy gave **80** (520 mg) in 70% yield, and as white amorphous solids from solution of ether and petroleum (7:3, v/v), m.p. = 57 - 59 °C.

Further preparative layer chromatography of **80** over silica gel (Solvent J) gave two anomers, **84** (Rf = 0.65) and **85** (Rf = 0.5) and both as white amorphous solids from a solution of ether and petroleum ether (v/v 5:1) with m.p. = 57-58 °C and m.p. = 55-56 °C respectively. They were unambiguously assigned to β-**84** and α **85** glycosides respectively by analysis of their p.m.r. spectra. The melting point and spectroscopic data for β anomer **84**:

m.p. = 57-58 °C

i.r. (cm^{-1}), Fig. 17A:

3450 (NH), 1670 (-CONH-, stretching), 1520 (aromatic C=C bending and the amide II band), 1050 (-NH-C- bending).

p.m.r. (ppm), Fig. 17B:

1.21 (d, 3H, J = 6.2 Hz, C_5-CH_3), 1.45 (m, 1H, C_4-H_a), 1.80 (broad doublet, 1H, C_4-He, $J_{4e,4a}$ = 14 Hz), 1.56 (m, 1H, C_2-H_a), 1.90 (broad d, 1H, C_2H_e, J_{HeHa} = 12 Hz), 2.72 (m, 1H, C_3-H_e, Wh = 13.5 Hz), 3.43 (s, 3H, C_1-OCH_3), 3.98 (m, 1H, C_5-H), 4.37 (d,

2H, benzylic protons, J = 5.4 Hz), 4.76 (dd, 1H, Wh = 8.5 Hz, C_1-H_a), 6.20 (s, broad, 1H, NH), 7.30 (m, 5H, aromatic hydrogens).

Mass Spectra (M^+/z)), Fig. 17C:

91 (tropylium ion), 97 (27%), 106 ($NHCH_2Ph$), 157 (100%), 162 (47%), 231 ($M-CH_3OH$), 262 (M-1), 263 (molecular ion).

High resolution Mass spectrum (M^+/z), Fig. 17D:

$C_{15}H_{21}O_3N_1$: calculated observed: 263.1527710.

The melting point and spectroscopic data for α anomer **85**:

m.p. = 55 - 56°C.

i.r. (cm^{-1}), Fig. 18A:

3450 (free hydrogen bonding NH stretching), 3310 (intramolecular hydrogen bonding NH stretching), 1660 (-CONH-), 1560 (benzine ring skeleton and amide II), 1520, 1050 (-NH-C-).

p.m.r. (ppm), Fig. 18B:

1.14 (d, 3H, C_5-CH_3, J = 6.2 Hz), 1.55 (m, 1H, C_4-H_a), 2.05 (broad doublet, 1H, C_4-H_e), 1.97 (poor t, 2H, C_2-H_a and C_2-H_e), 2.73 (m, 1H, C_3-H_e, Wh = 11 Hz), 3.18 (s, 3H, C_1-OCH_3), 3.80 (m, 1H, C_5-H), 4.40 (d, 2H, two benzylic protons), 4.70 (d, 1H, C_1-H_a, Wh = 7.5 Hz), 7.30 (m, 5H, five aromatic protons), 7.85 (s, broad, 1H, NH).

Mass Spectra (M/z), Fig. 18C:

91 (100%, tropylium ion), 97 (97%), 106 ($NHCH_2Ph$), 162, 231 (M-MeOH).

High resolution Mass spectrum (M^+/z), Fig. 18D:

$C_{14}H_{17}O_2N_1$: calculated 231.1259328; observed 231.1255950.

PREPARATION OF METHYL 3-(N-BENZYLAMINO-METHYL)-2,3,4,6-TETRADEOXY-DL-THREO-HEXOPYRANOSE (86) AND THE TWO ANOMERS 88 AND 89

To the solution of anhydrous tetrahydrofuran (THF) (50 ml) and lithium aluminium hydride (80 mg) was added 86 (100 mg) at 0°C. After being stirred for 20 min at 0°C, the reaction solution was heated to 50°C and stirred for additional 15 hr. The solution was cooled to room temperature followed by adding water (30 ml), and then stirred at room temperature for 40 min. The solution was filtered to remove some aluminium hydroxide residue evolved in the reaction. The filtrate was evaporate to reduce the total volume of the solution to about 30 ml. Then it was extracted with methylene chloride (20 ml, two times) exaustively. The combined extract was dried over anhydrous magnesium sulfate, filtered and evaporated to dryness to give a syrupy residue. The flash collum chromatography of the residue over silica gave 86 (70 mg), as a colourless syrup. The yield was 74%. The further preparative layer chromatography of 86 upon silica gel (Solvent M) gave two anomers, 88 (Rf = 0.26) and 89 (Rf = 0.17), which are assigned to α and β anomer respectively. The spectroscopic data for α anomer 88:

i.r. (cm^{-1}), Fig. 19A:

2850 ($-OCH_3$), 1130 (C-O-C), 1060.

p.m.r. (ppm), Fig. 19B:

1.16 (d, 3H, $-CH_3$), 1.75 (m, 5H, $C_4-H_aH_e$, $C_2-H_aH_e$ and C_3-H_a), 2.10 (broad s, 1H, NH), 2.45 (t. 2H, $C_{H2}-N-$), 3.34 (s, 3H, $-OCH_3$),

3.76 (s, 2H, two benzylic protons), 3.85 (m, 1H, C_5-H_a), 4.75 (d, 1H, C_1-H_e), 7.34 (m, 5H, five aromatic protons).

Mass spectrum (M^+/z), Fig. 19C:

250 (M+1), 234 (M-CH_3), 218 (M-OCH_3), 120 (CH_2NHCH_2Ph), 91 (CH_2Ph, 100%).

The spectroscopic data for β isomer **89**:

i.r. (cm^{-1}), Fig. 20A:

2850 (-OCH_3), 1390, 1130, 1080 (-C-O-C-), 1000.

p.m.r. (ppm), Fig. 20B:

1.24 (d, 3H, -CH_3), 1.75 (m, 6H, C_2-H_aH_e, C_4-H_aH_e, C_3-H_a and NH), 2.55 (d, 2H, two methylene protons, C-CH_2-N-), 3.48 (s, 3H, -OCH_3), 3.55 (m, 1H, C_5-H_a), 3.79 (s, 2H, two benzylic protons-CH_2-Ph), 4.35 (dd, 1H, C_1-H_a), 7.35 (m, 5H, five aromatic protons).

Mass spectrum (M^+/z), Fig. 20C:

249 (molecular ion), 248 (M-H), 234 (M-CH_3), 120 (CH_2NHCH_2Ph), 91 (tropylium ion, 100 %).

High resolution Mass spectrum (M^+/z), Fig. 20D:

$C_{15}H_{23}O_2N_1$: calculated 249.1728840, observed, 249.1675870;

$C_{14}H_{20}O_2N_1$: calculated 234.1494084, observed 234.1481020;

$C_8H_{10}N_1$: calculated 120.0813264, observed 120.0804290.

PREPARATION OF METHYL 3-N-(BENZYLAMINO-METHYL)-2,3,4,6-TETRADEOXY-DL-ERYTHRO-HEXOPYRANOSE (87) AND THE TWO ANOMER 90 AND 91

Compound **80** (150 mg) was placed into stirred the solution of tetrahydrofurane (50 ml) and lithium aluminium hydride (80 mg) at 0 °C. After being stirred for 20 min at 0°C, the solution was heated to 50°C and stirred for 3hr. Then the solution was cooled to room temperature and stirred for additional 15 hr. The solution was cooled to 0°C followed by water addition (30 ml) and stirred for 30 min, then filtered. The filtrate was evaporated under reduced pressure to reduce total volume to about 40 ml and was extracted with methylene chloride (20 ml, two times). The extract was dried over anhydrous magnesium sulfate, filtered and evaporated to dryness to give a colourless syrupy residue. The flash chromatography of the syrupy residue over silica gave **87** (92 mg), as a colourless syrup, in 64.7 %. Preparative layer chromatography of **87** over silica gel gave **90** (Rf = 0.3) and **91** (Rf = 0.25), as a pair of anomers, with β and α glycosidic bond respectively.

The spectroscopic data for β isomer **90**:

i.r. (cm^{-1}), Fig. 21A:

3050 (weak shoulder, aromatic C-H), 2850 ($-OCH_3$), 1050 (tense, -NH-C-), 1000.

p.m.r. (ppm), Fig. 21B:

1.25 (d, 3H, $-CH_3$, J = 6 Hz), 1.50 to 1.70 (m, 5H, $C_4-H_aH_e$, $C_2-H_aH_e$ and NH), 2.15 (m, 1H, C_3-H_e), 2.65 (d, 2H, CH_2-N), 3.44 (s, 3H, CH_3O), 3.81 (s, 2H, two benzylic hydrogens), 3.75 (m, 1H, C_4-H), 4.50 (dd, 1H, C_1-H_a, Wh = 7.5 Hz), 7.30 (m, 5H, aromatic protons).

Mass spectrum (M^+/z), Fig. 21C:

249 (molecular ion), 234 (M-CH_3), 218 (M-OCH_3), 120 (CH_2NH-CH_2Ph), 106 ($NHCH_2Ph$), 91 (CH_2Ph, 100%)

High resolution mass spectrum, (M^+/z), Fig. 21D:

$C_{15}H_{23}N_1O_2$: calculated 249.1728840, observed 249.1718140;

$C_{14}H_{20}N_1O_2$: calculated 234.1494084, observed, 234.1515660;

$C_{14}H_{20}N_1O_1$: calculated 218.1544934, observed, 218.1544340;

$C_7H_8N_1$: calculated 106.0629970, observed 106.0650330;

$C_8H_{10}N_1$: calculated 120.0796360, observed 120.0796360;

C_7H_7: calculated 91.0547764, observed 91.0530090.

The spectroscopic data for αanomer **91**:

i.r. (cm^{-1}), Fig. 22A:

3050 (weak shoulder, benzene), 2850 (-OCH_3), 1120, 1050 (C-O-C).

p.m.r. (ppm), Fig. 22B:

1.15 (d, 3H, -CH_3), 1.45-1.85 (m, 5H, C_4-H_aH_e, C_2-H_aH_e and NH), 1.95 (m, 1H, NH), 2.80 (t, 2H, -CH_2-N-), 3.31 (s, 3H, -OCH_3), 3.80 (s, 2H, two benzylic protons), 3.95 (m, 1H, C_5-H_a), 4.65 (broad s, 1H, C_1-H_e), 7.30 (m, 5H, five aromatic hydrogens).

Mass spectrum (M^+/z), Fig. 22C:

249 (molecular ion), 234 (M-CH_3), 218 (M-OCH_3), 120 (CH_2NH-CH_2Ph), 91 (CH_2Ph, 100%).

SPECTRA

All melting points were recorded on a Fischer-Johns melting point apparatus and were uncorrected. Infrared spectra were taken on a Perkin Elmer Infracord 710 spectrometer, using sodium chloride cell with methylene chloride as a solvent. Mass spectra were recorded on a V.G.7070EHF mass spectrometer with a 70 FAB system. A Brucker AM-300 spectrometer was used to make all p.m.r. spectra and ^{13}C n.m.r. spectrum with $CDCl_3$ as a solvent and tetramethylsilane (TMS) as an internal reference except those presented in figs. 8A, 10B, 11B, which were made on a Varian EM-360 spectrometer.

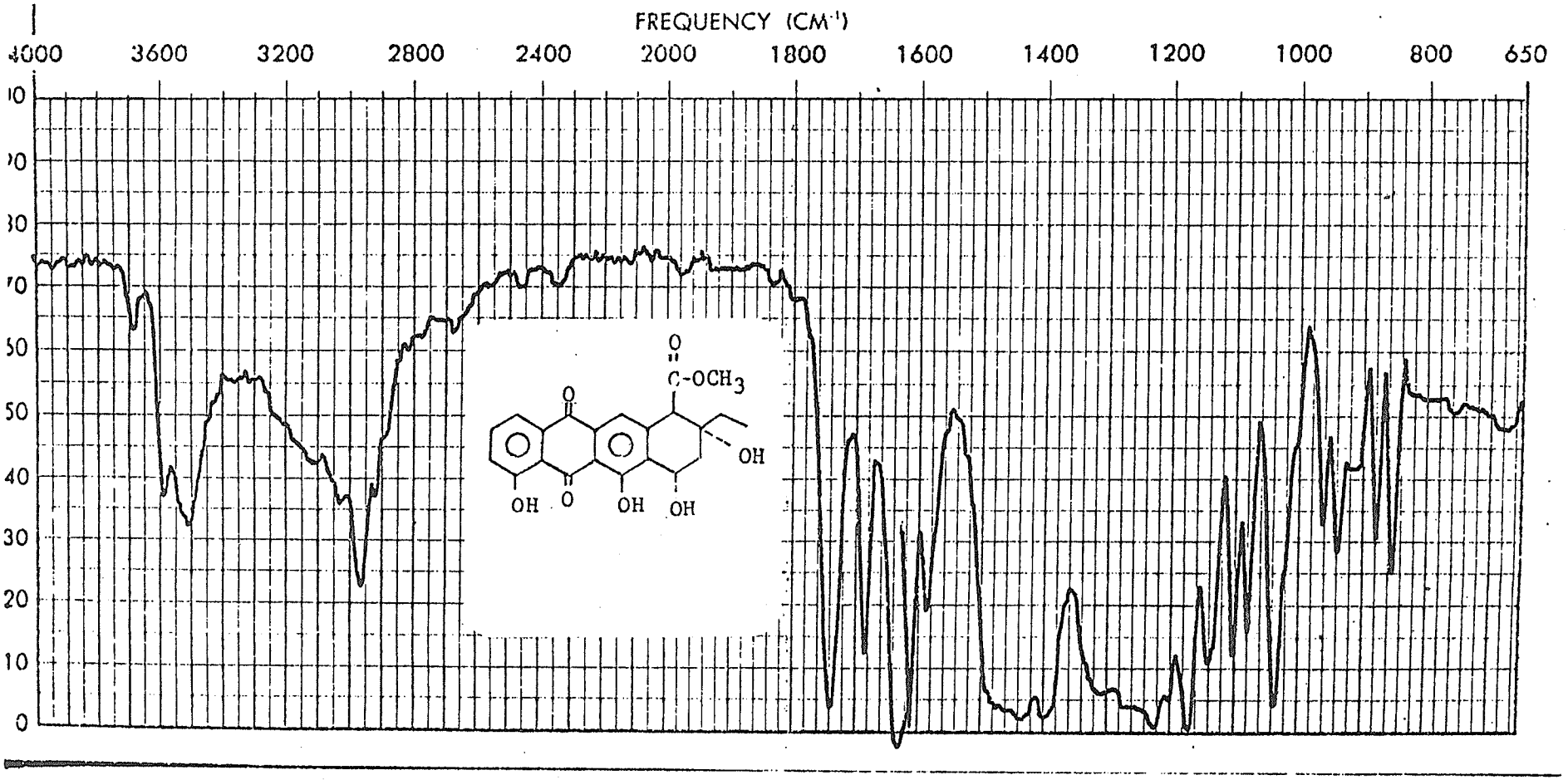


Fig. 1A I. r. Spectrum of Aklavinone (55)

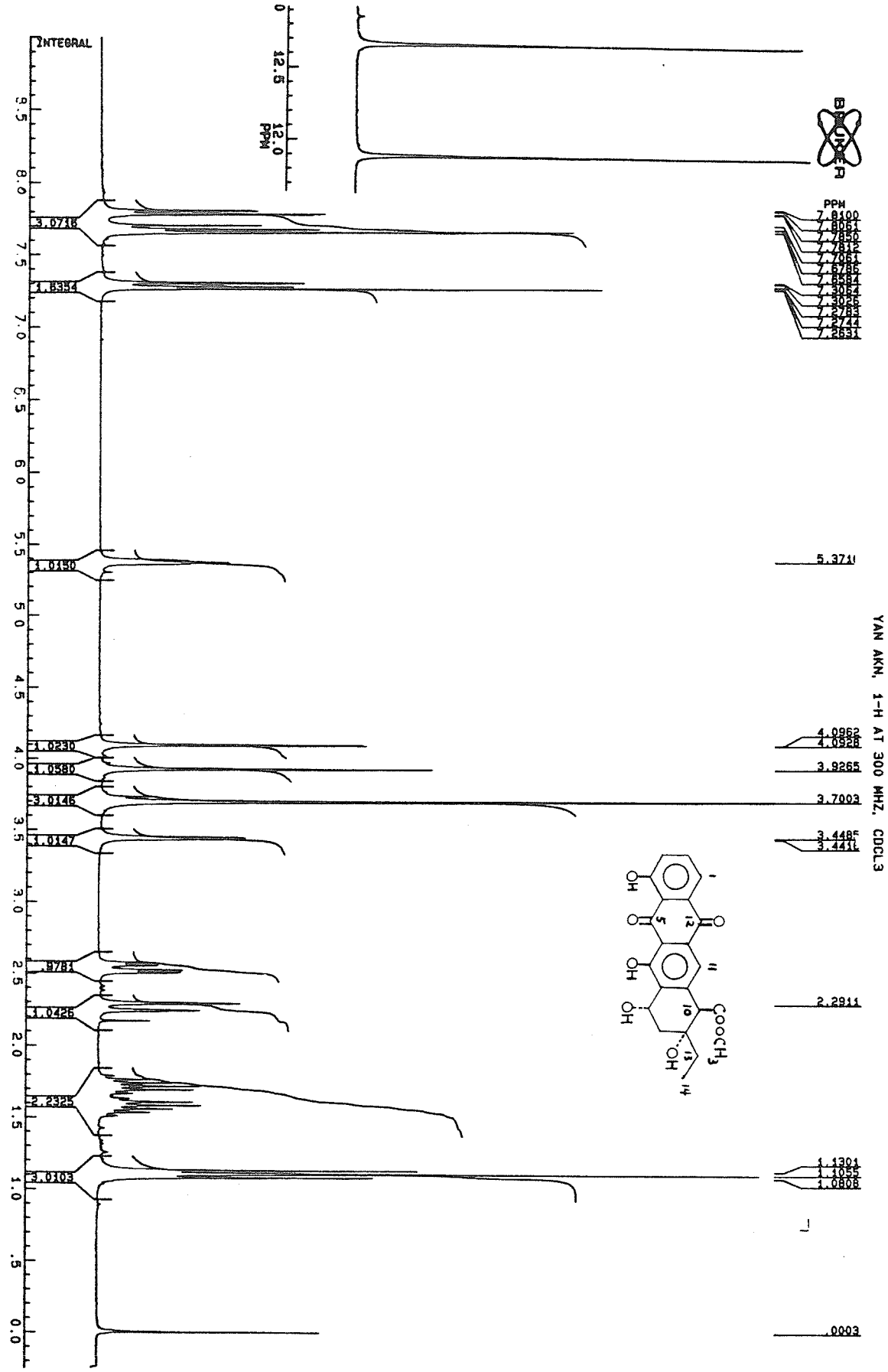
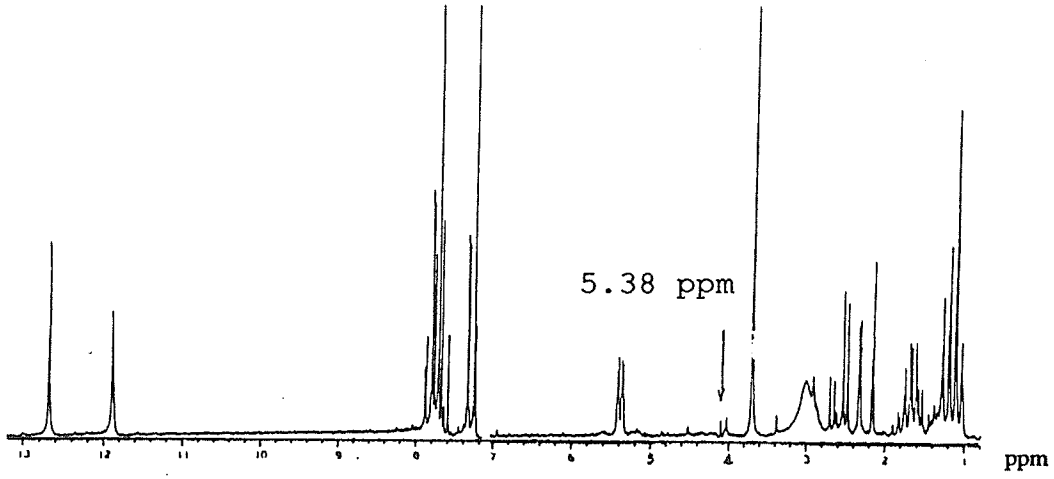


Fig. 1B-1 P. m. r. Spectrum of Aklavinone (55)

a)



b)

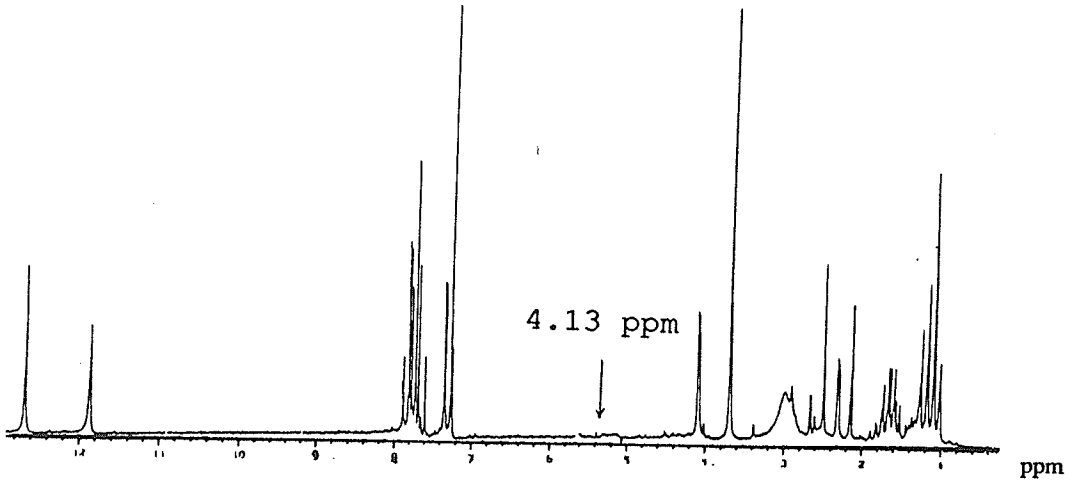


Fig. 1B-2 The Double Irradiation P. m. r. Spectrum of Aklavinone (55):a) The Double Irradiation Applied on the H_7-H, at δ = 5.38 ppm; b) The Double Irradiation Applied on the H_{10}-H, at δ = 4.13 ppm.

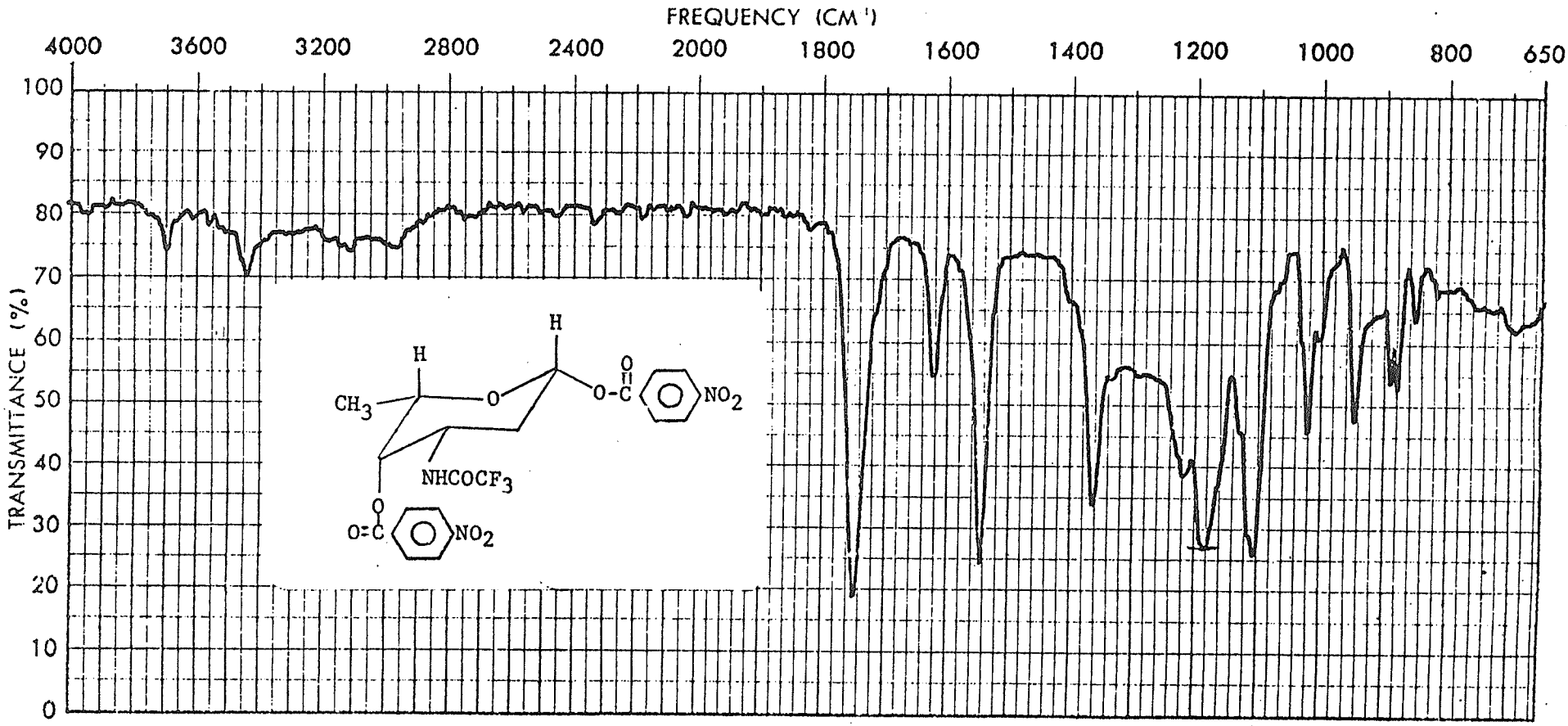


Fig. 2A I. r. Spectrum of 1,4-Di-O-(p-nitrobenzoyl)-3-N-trifluoroacetyl-daunosamine (54)

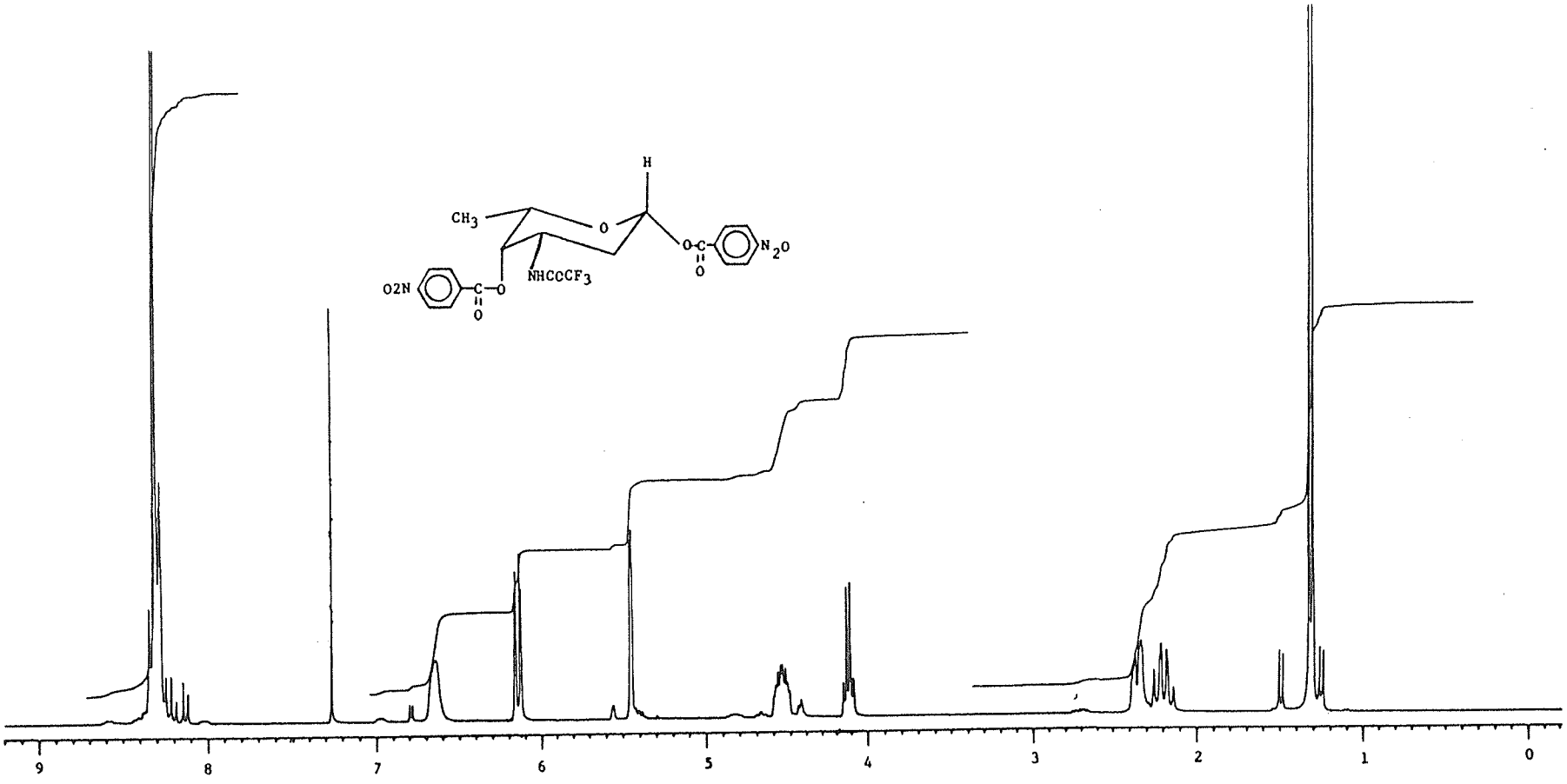


Fig. 2B P. m. r. Spectrum of 1,4-Di-O-(p-Nitrobenzoyl)-3-N-trifluoroacetyl-daunosamine (54)

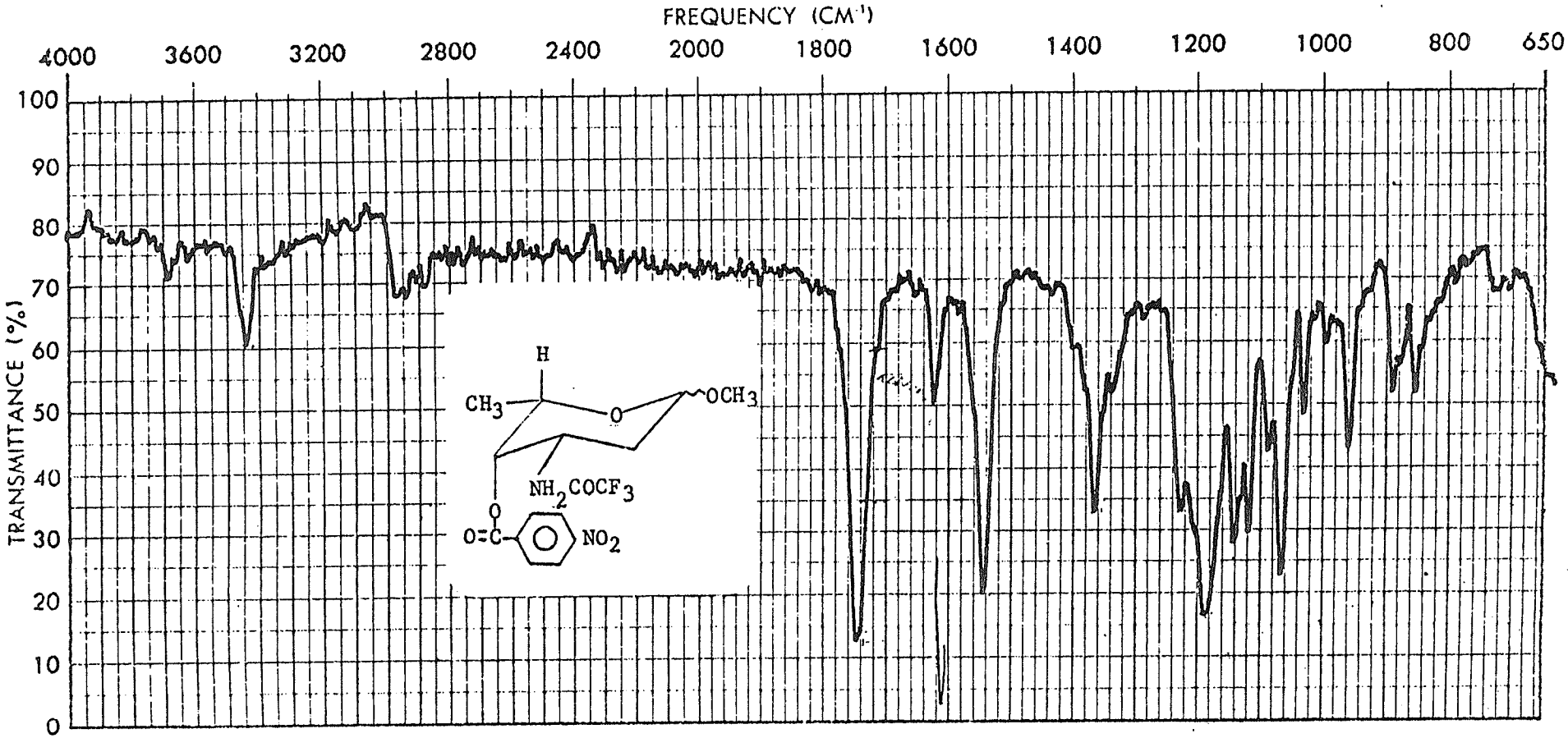


Fig. 3A I.r. Spectrum of Methyl 4-O-(p-nitrobenzoyl)-3-N-trifluoroacetyl-daunosamine (46)

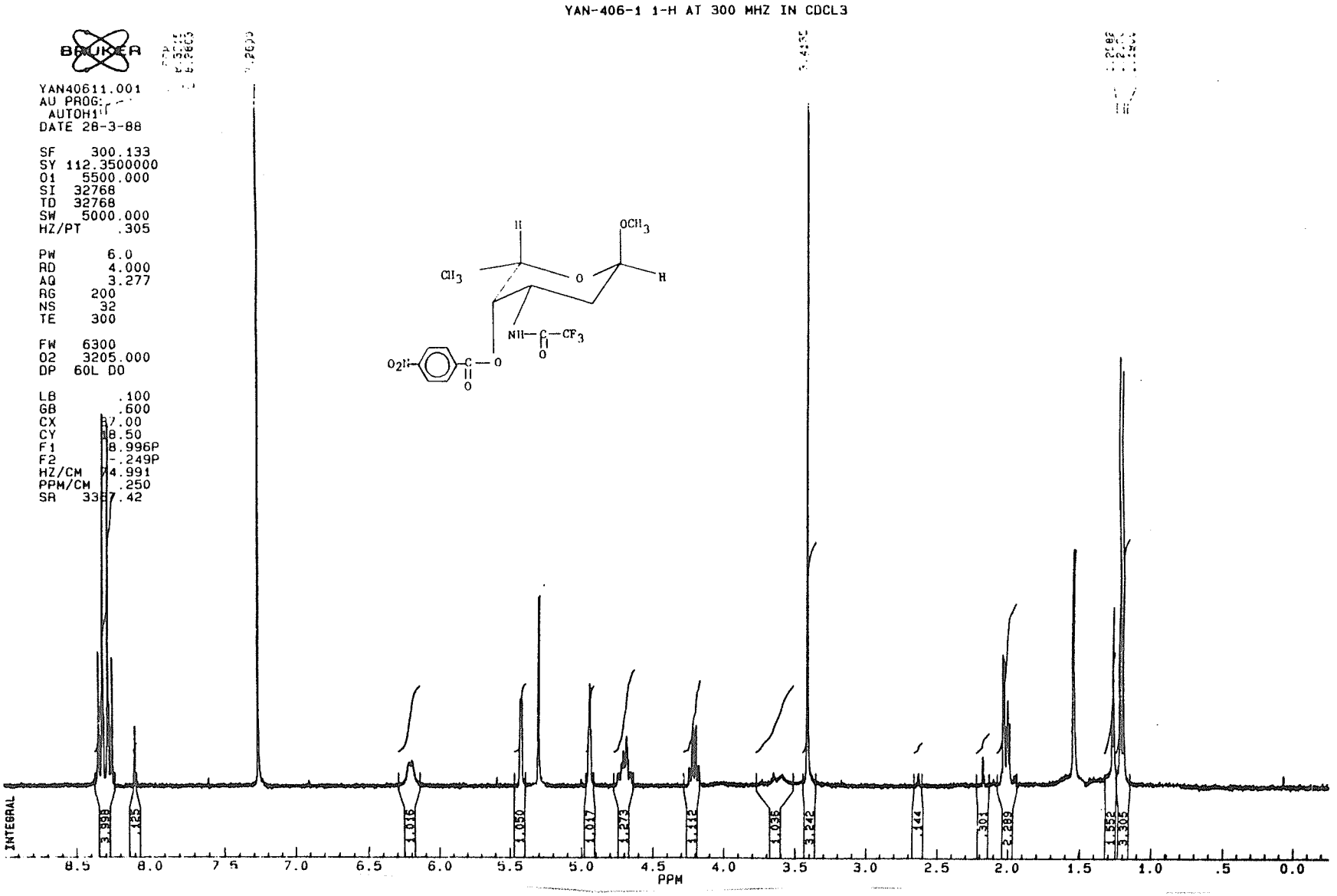


Fig. 3B P.m.r. Spectrum of Methyl 4-O-(p-nitrobenzoyl)-3-N-trifluoroacetyl-α-daunosamine (47)

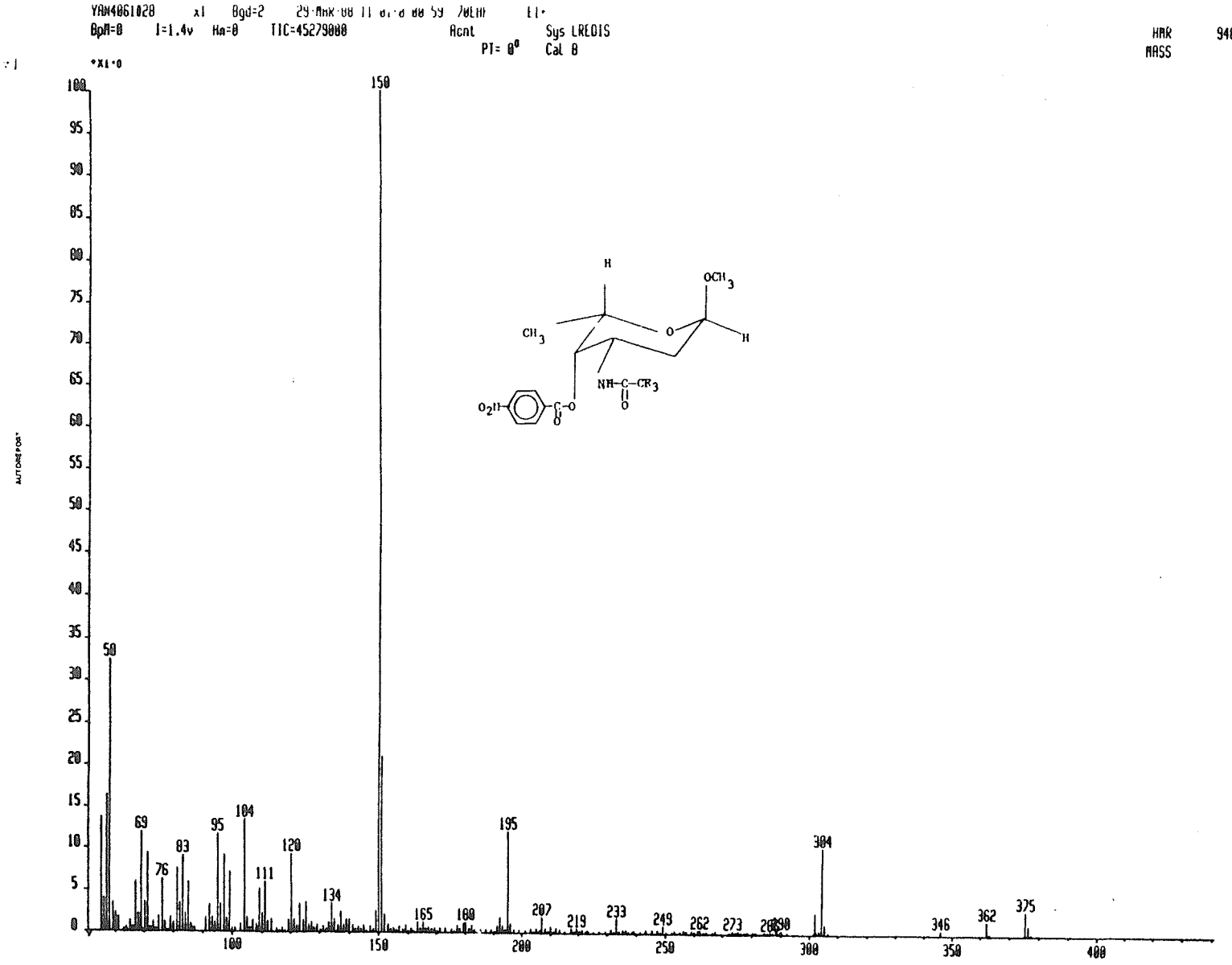


Fig. 3C Mass Spectrum of Methyl 4-O-(p-nitrobenzoyl)-3-N-trifluoroacetyl-α-daunosamine (47)

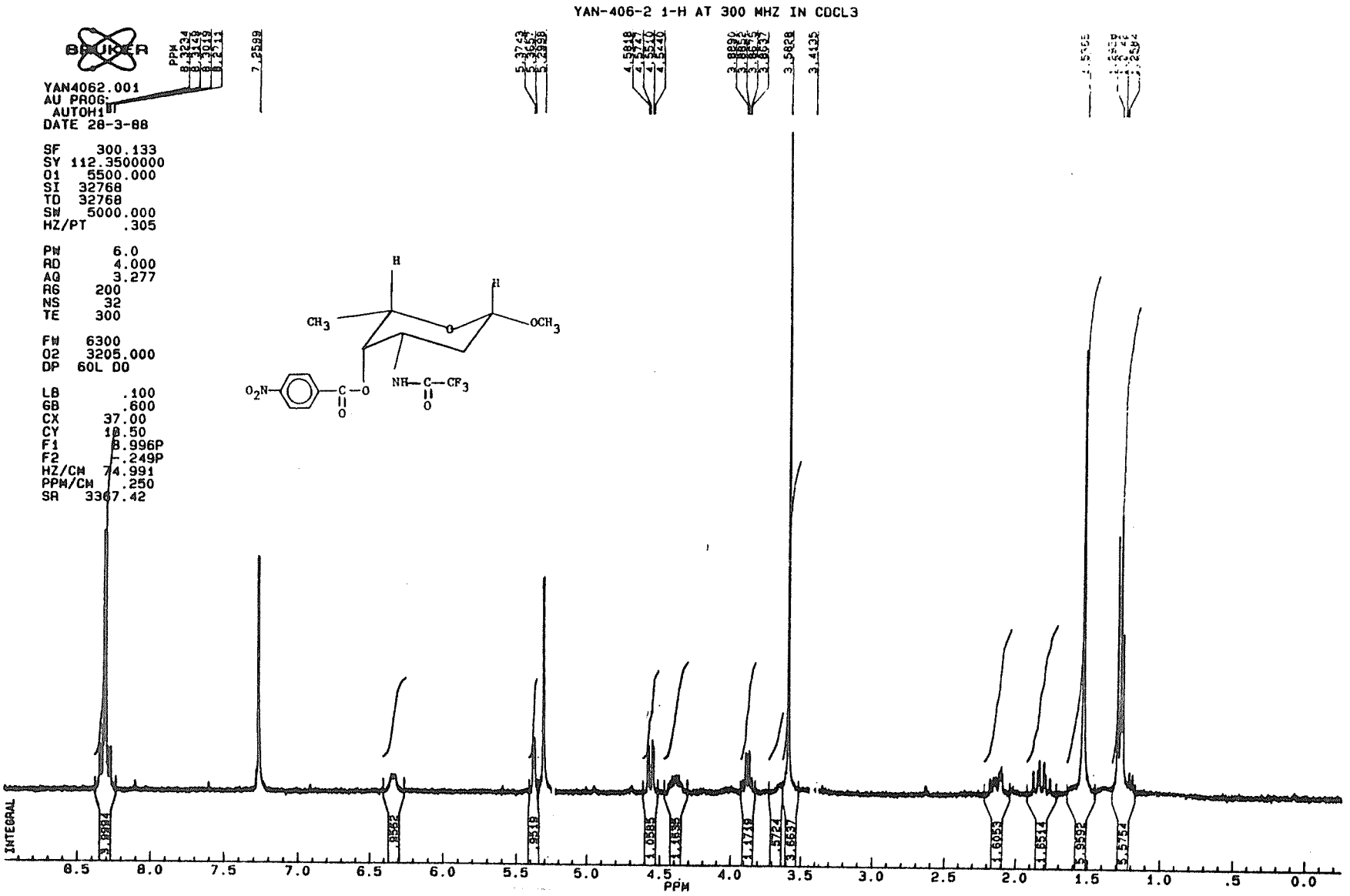


Fig. 4B P.m.r. Spectrum of Methyl 4-O-(p-nitrobenzoyl)-3-N-trifluoroacetyl-β-daunosamine (48)

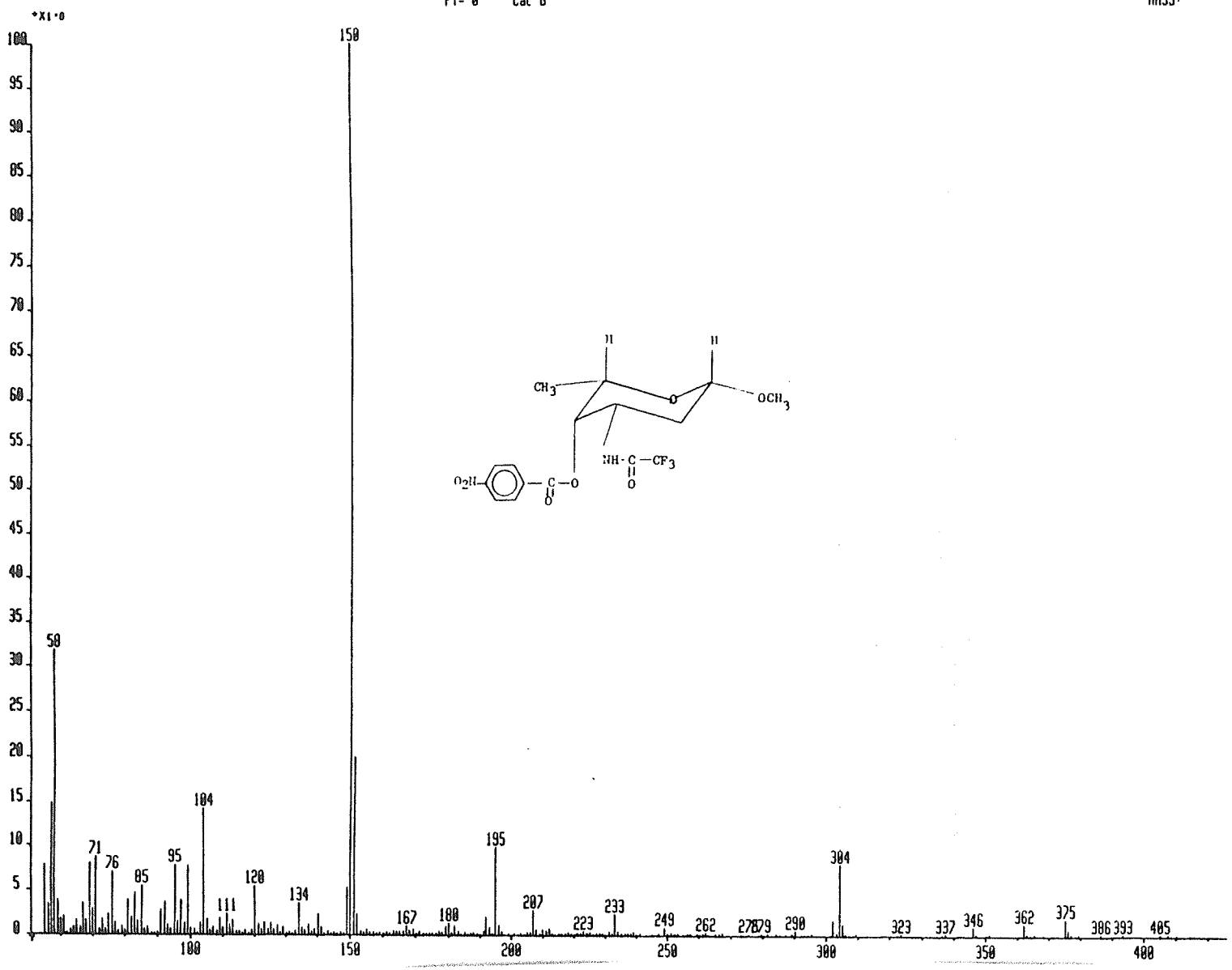


Fig. 4C Mass Spectrum of Methyl 4-O-(p-nitrobenzoyl)-3-N-trifluoroacetyl-β-daunosamine (48)

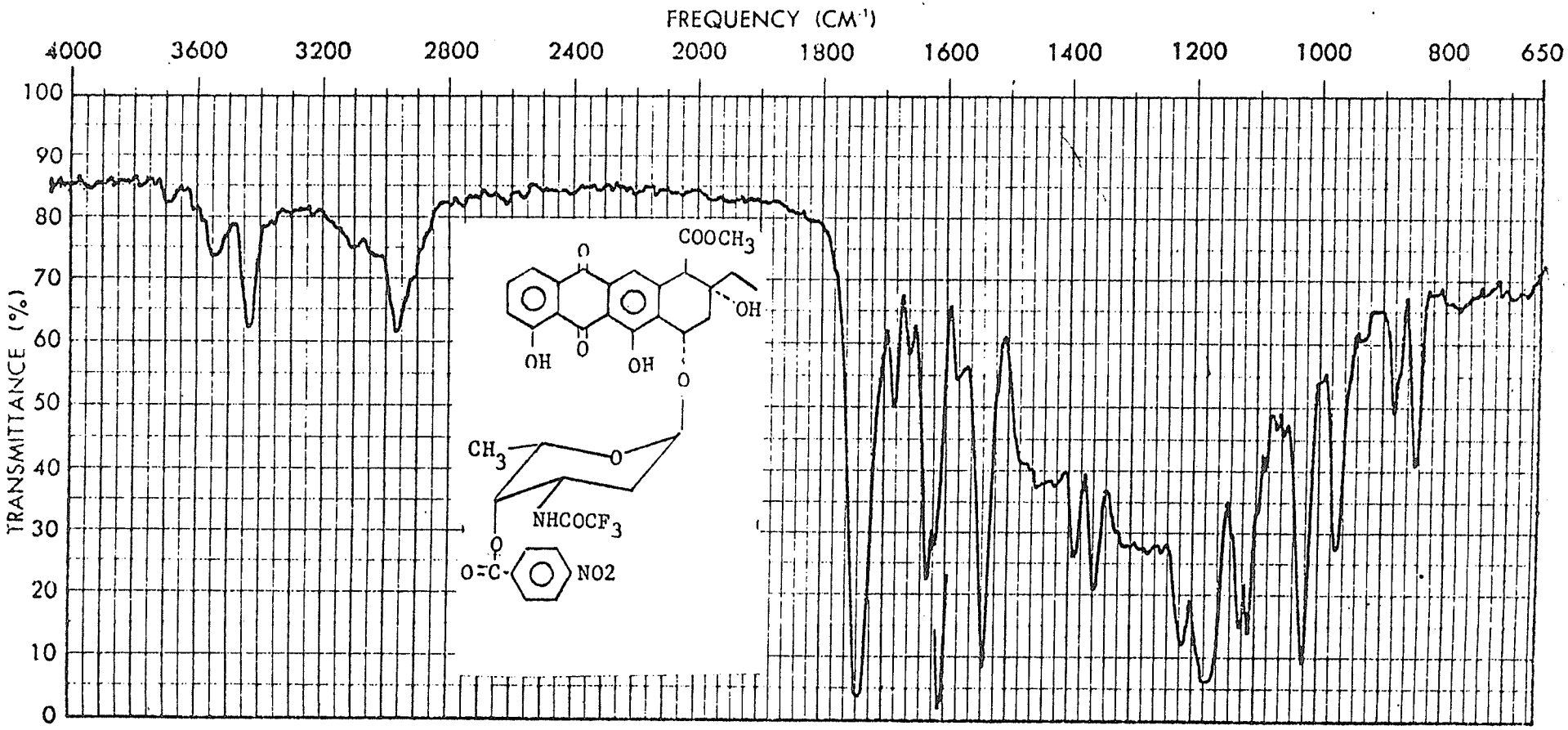


Fig. 5A I. r. Spectrum of 7-O-[3'-N-Trifluoroacetyl-4'-O-(p-nitrobenzoyl)-α-daunosaminyl]-aklavinone (56)

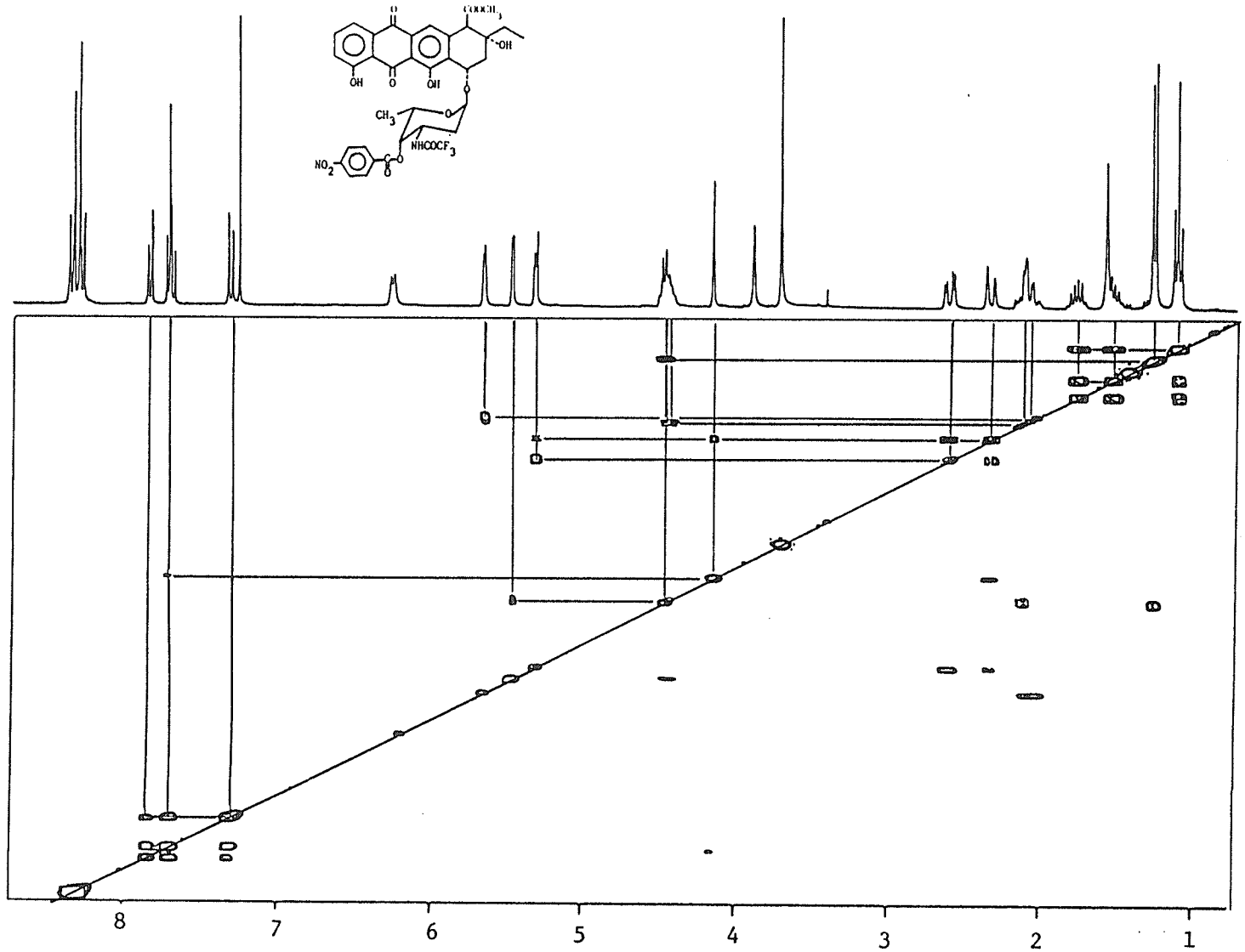


Fig. 5B-1 Two-Dimensional P. m. r. Spectrum of 7-O-[3'-N-Trifluoroacetyl-4'-O-(p-nitrobenzoyl)-α-daunosaminyl]-aklavinone (56)

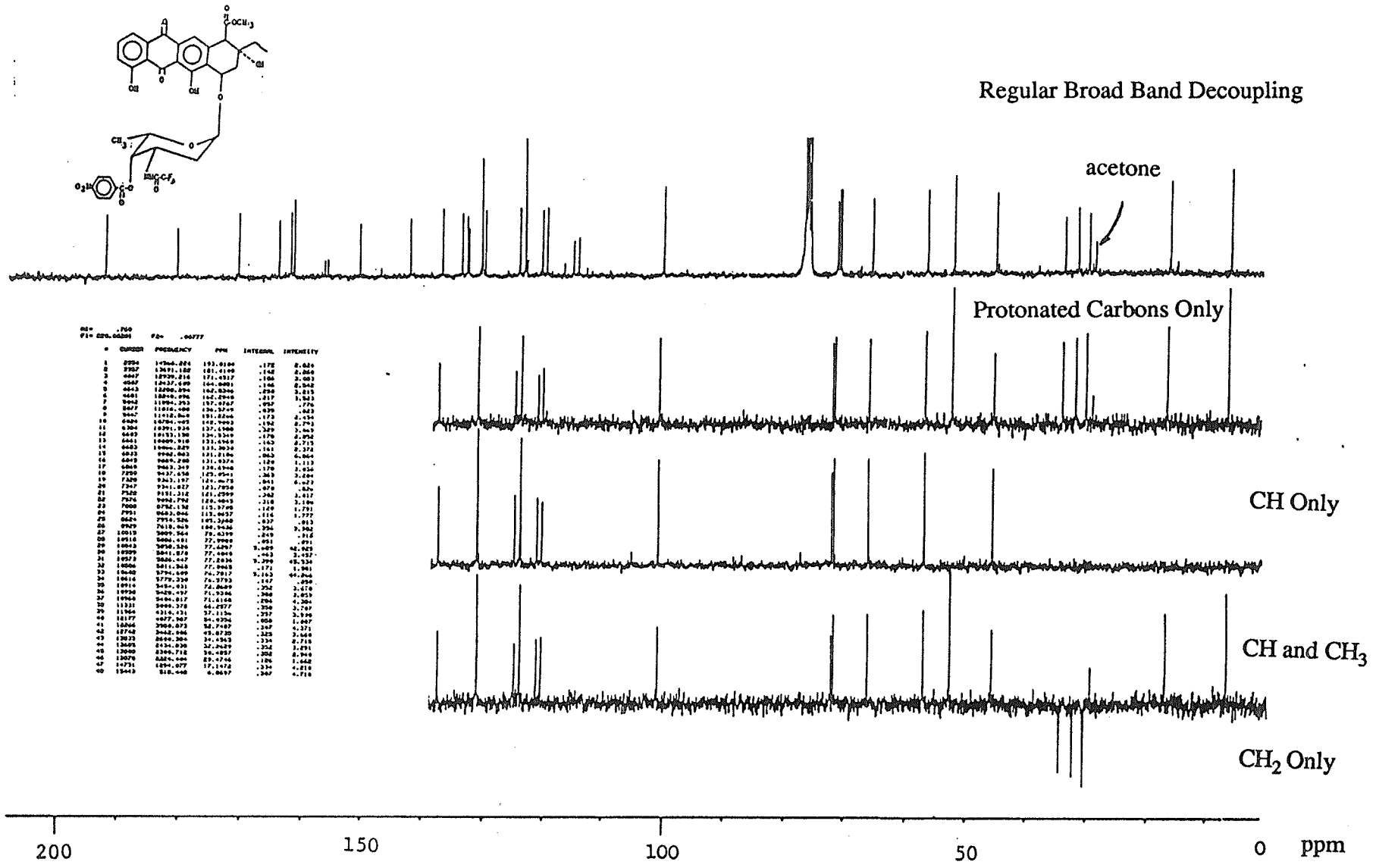


Fig. 5B-2 ^{13}C N. m. r. Spectrum of 7-O-[3'-N-Trifluoroacetyl-4'-O-(p-nitrobenzoyl)-α-daunosaminyl]-aklavinone (56)

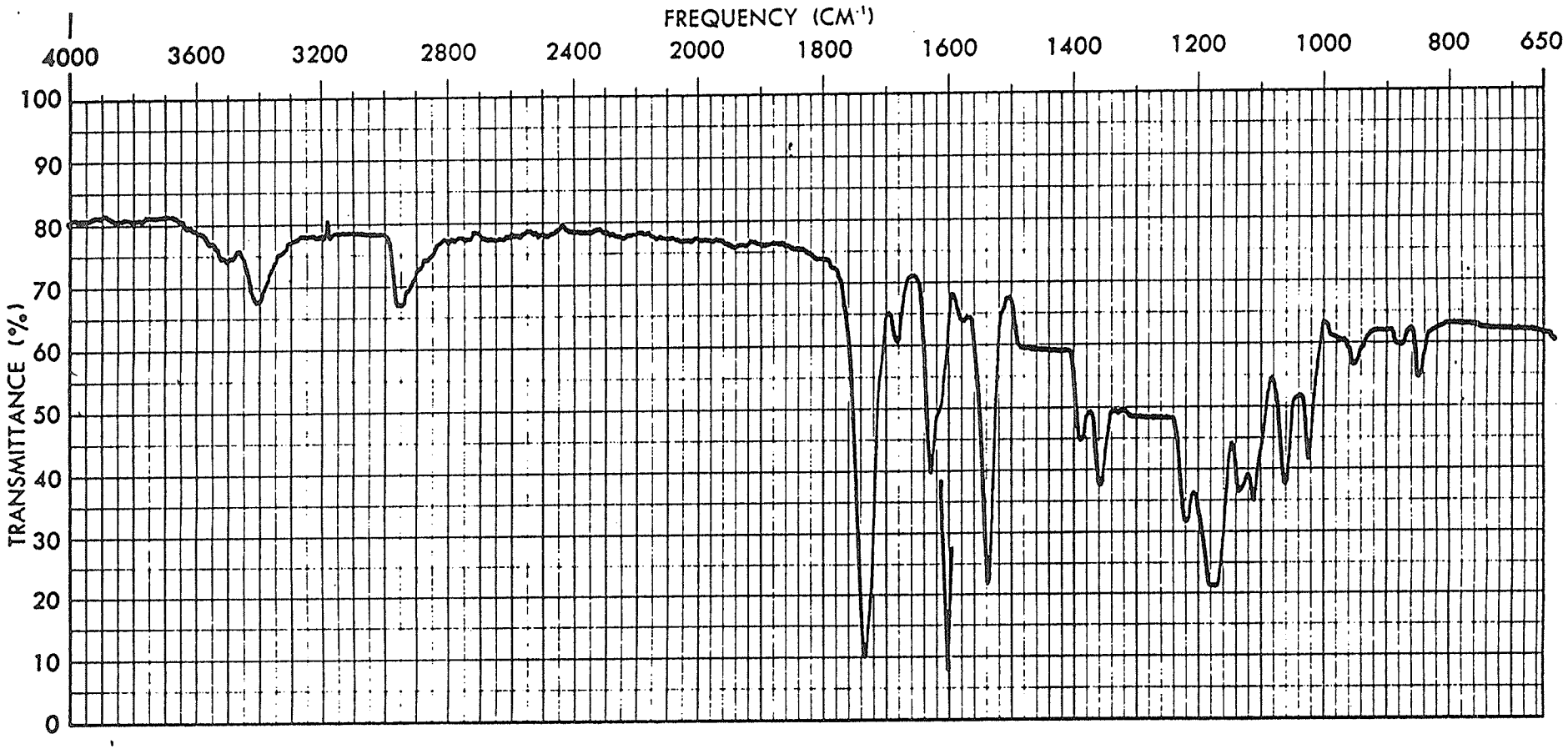


Fig. 6A I. r. Spectrum of 7-O-[3'-N-Trifluoroacetyl-4'-O-(p-nitrobenzoyl)-β-daunosaminyl]-aklavinone (57)

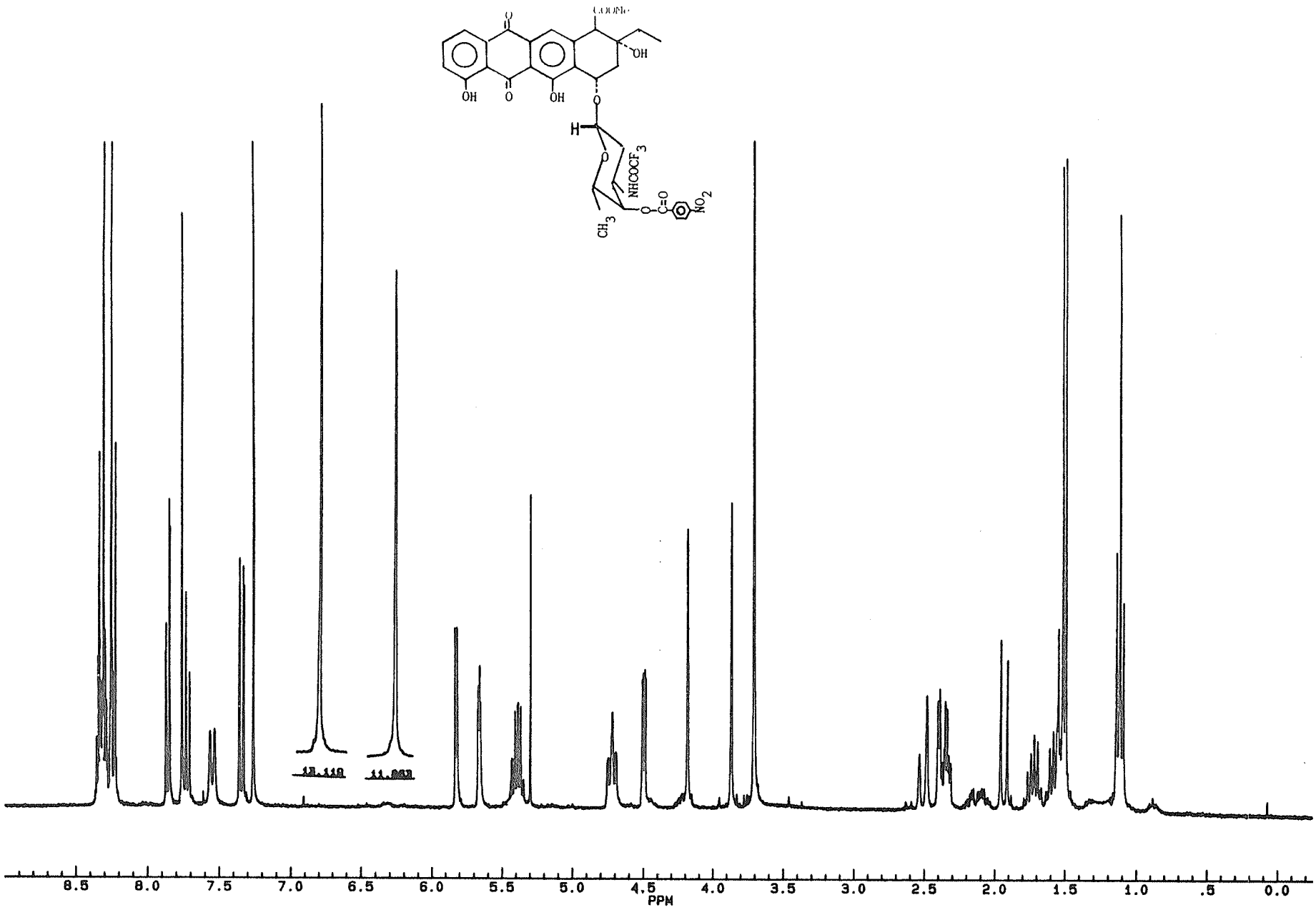


Fig. 6B P. m. r. Spectrum of 7-O-[3'-N-Trifluoroacetyl-4'-O-(p-nitrobenzoyl)-β-daunosaminyl]-aklavinone (57)

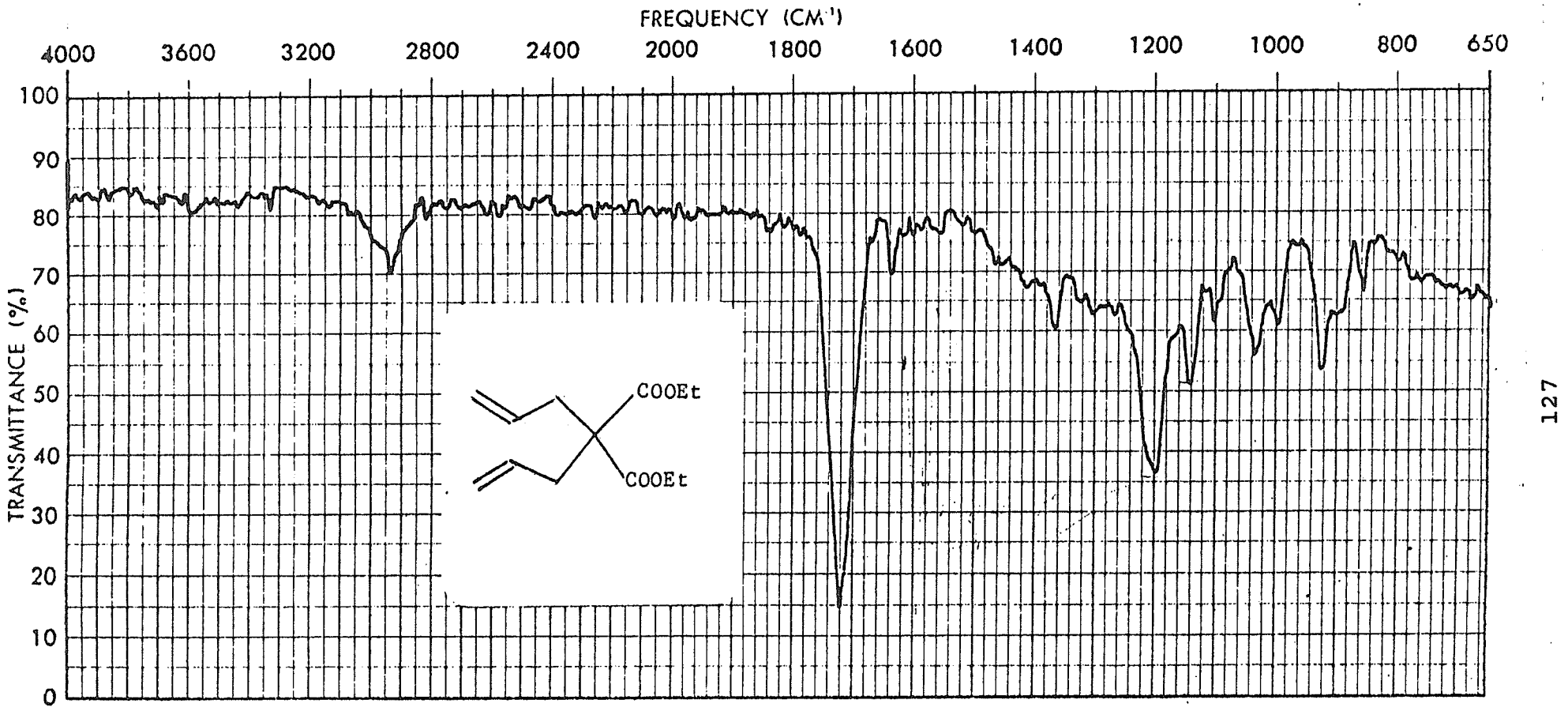


Fig. 8A **I. r. Spectrum of 2, 2-Diallyl-ethylmalonate (64)**

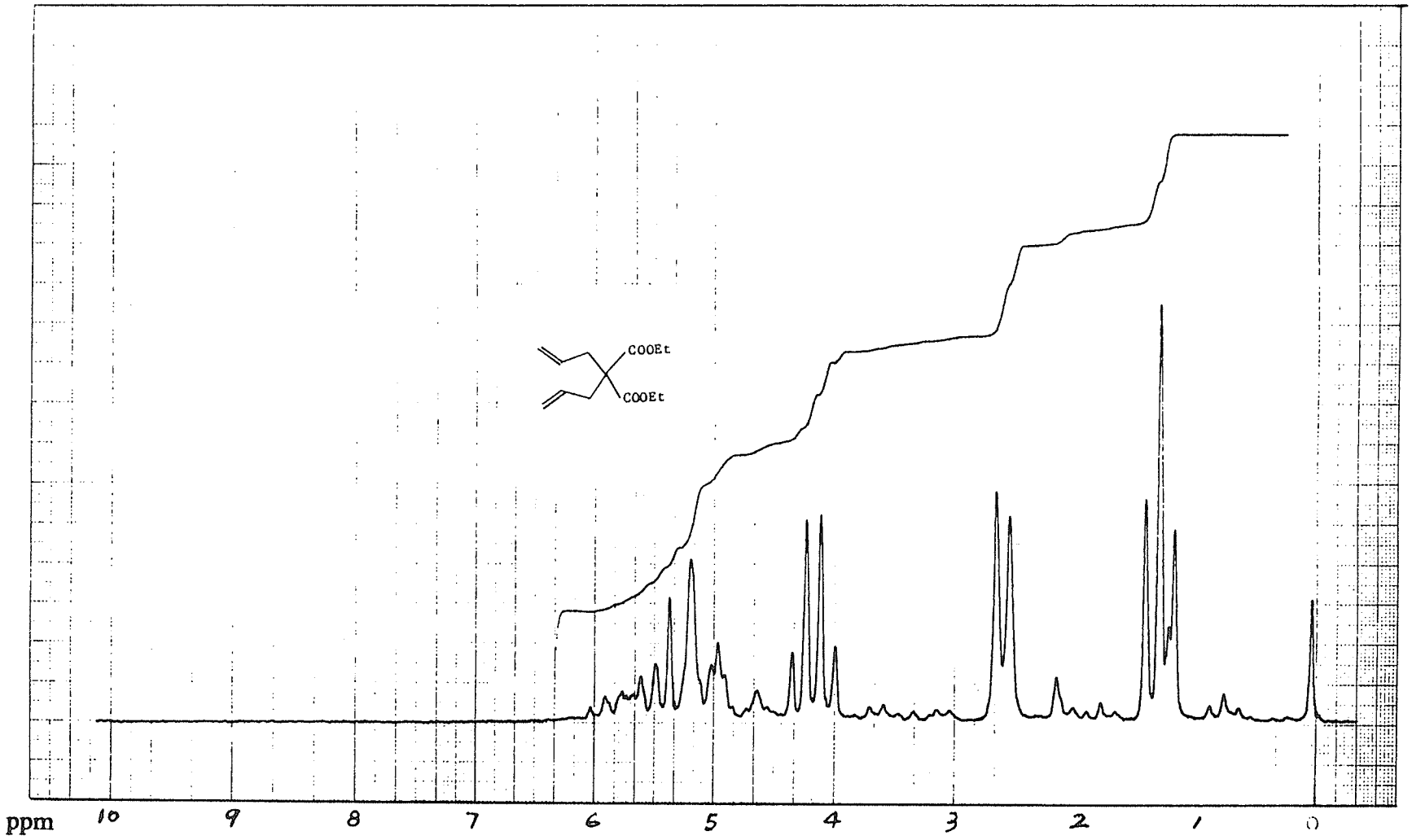


Fig. 8B P. m. r. Spectrum of 2, 2-Diallyl-ethylmalonate (64)

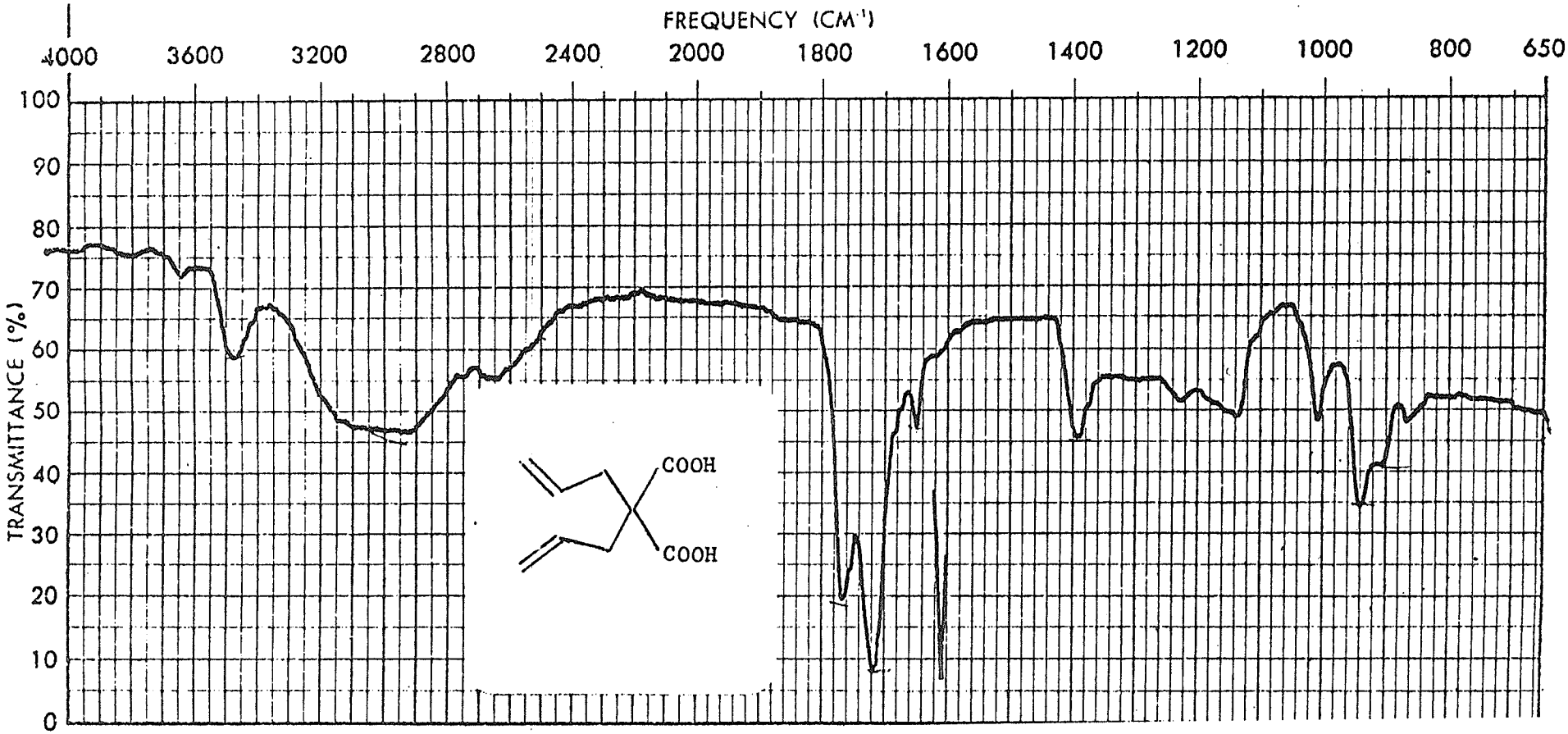


Fig. 9A **I. r. Spectrum of 2, 2-Diallyl-malonic acid (65)**

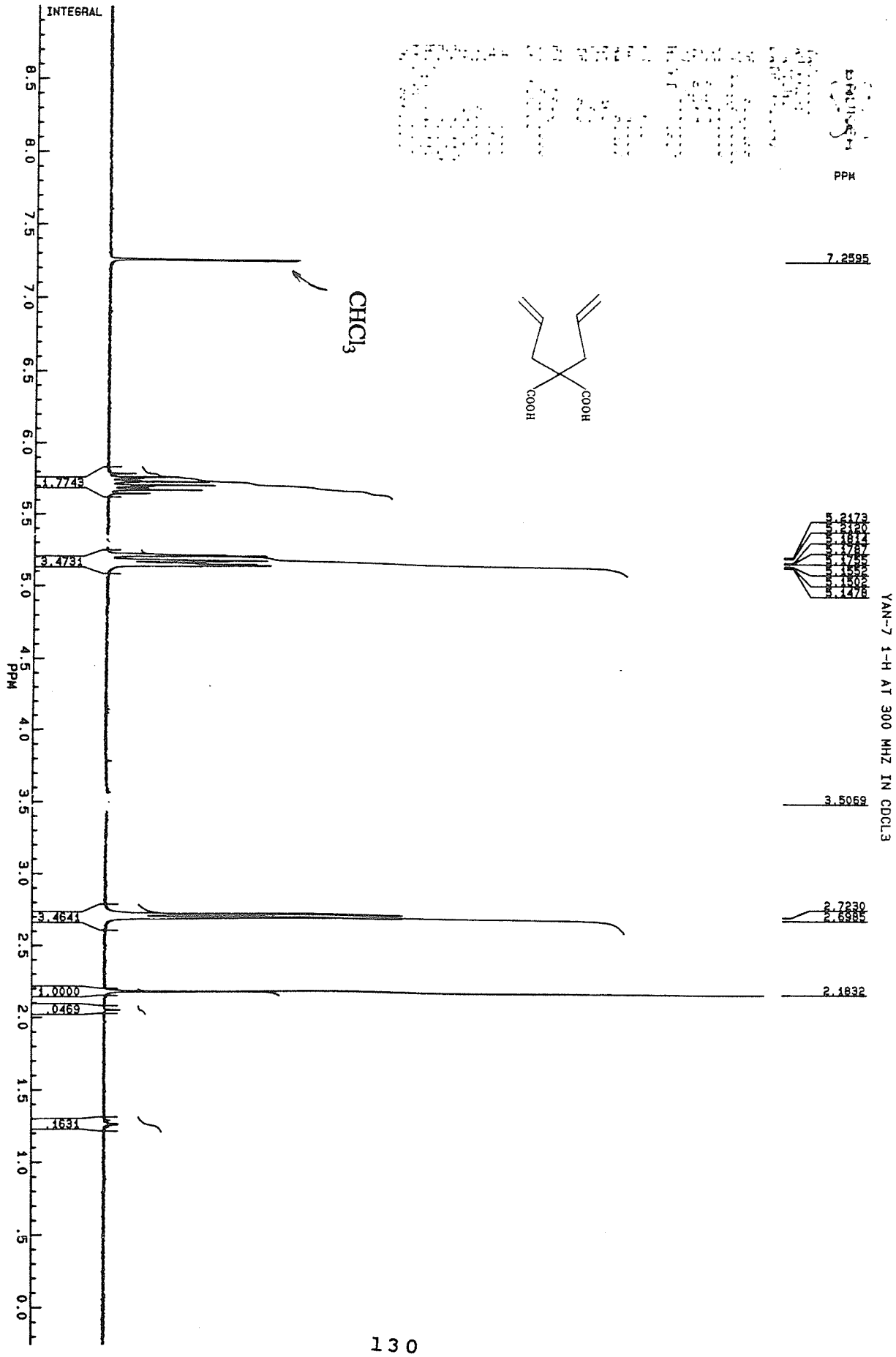


Fig. 9B P. m. r. Spectrum of 2, 2-Diallyl-malonic acid (65)

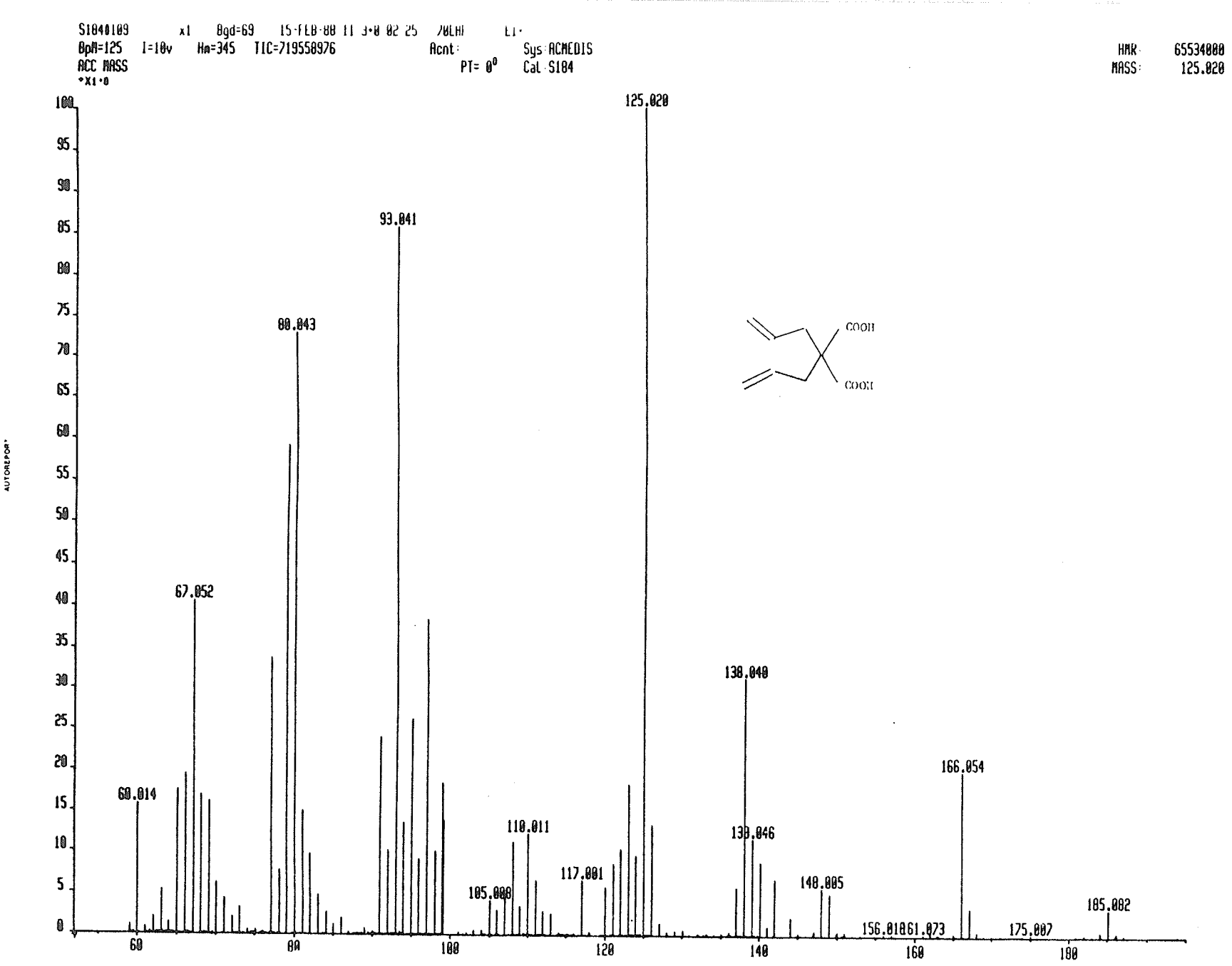


Fig. 9C **Mass Spectrum of 2, 2-Diallyl-malonic acid (65)**

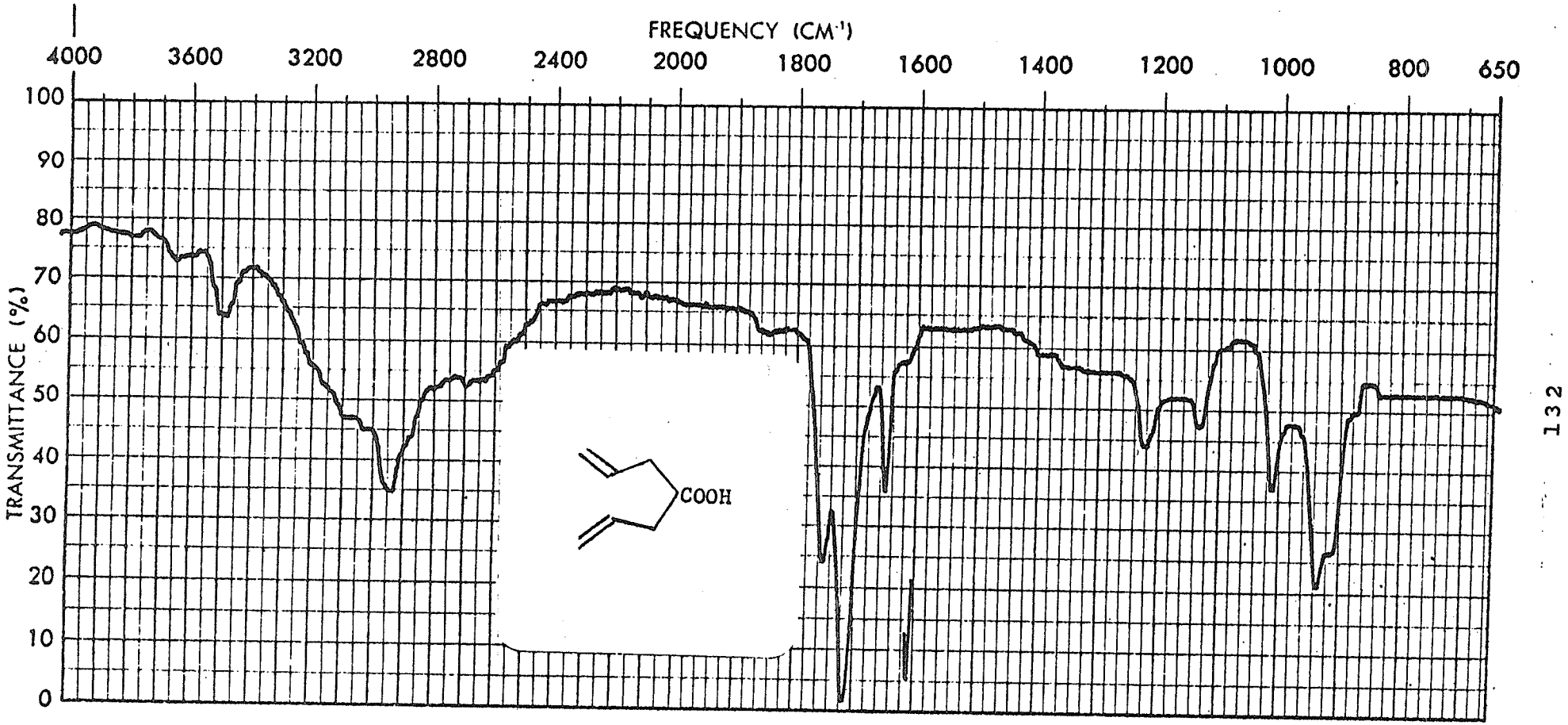


Fig. 10A I. r. Spectrum of 2-Allyl-4-pentenoic acid (66)

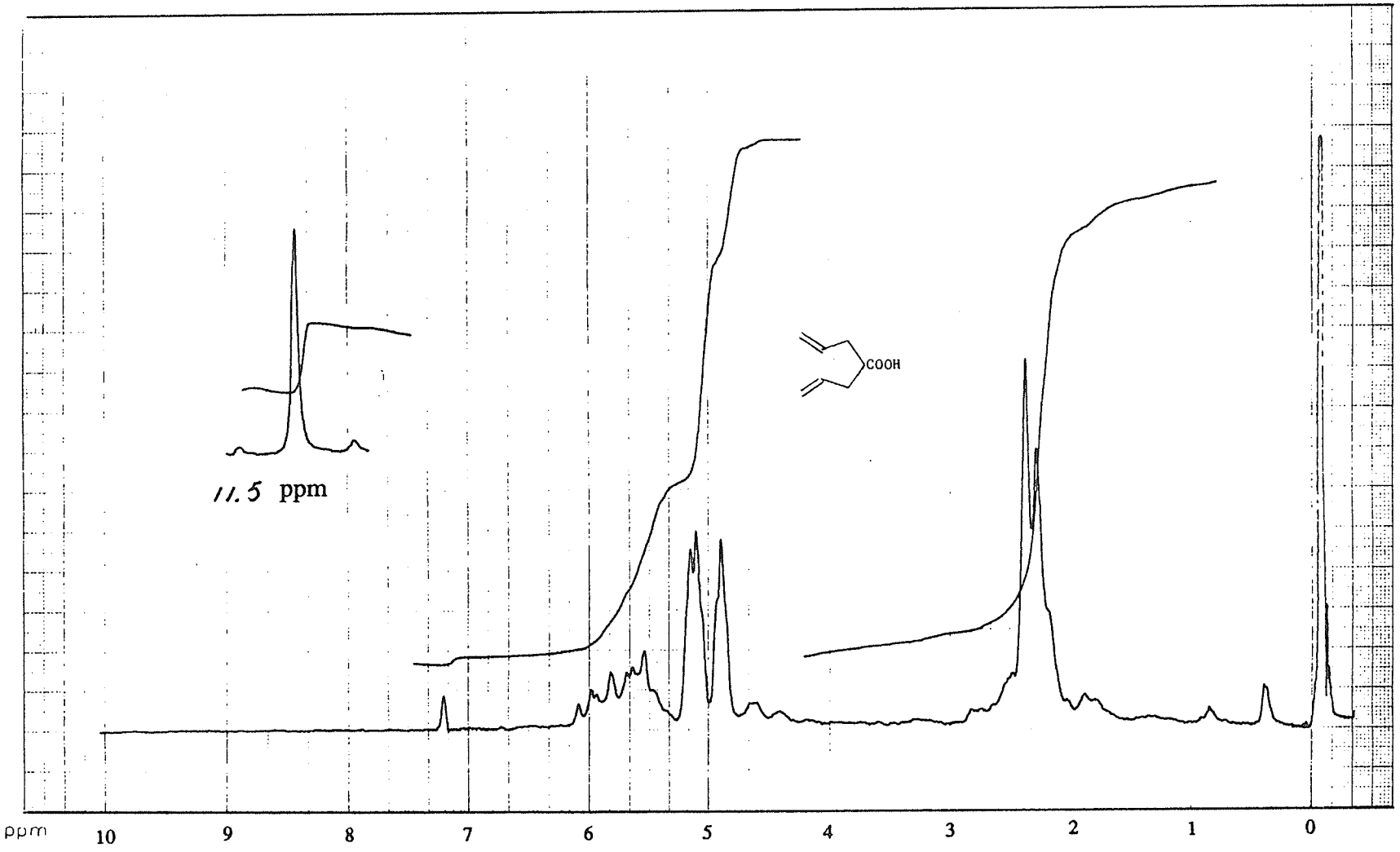


Fig. 10B P. m. r. Spectrum of 2-Allyl-4-pentenoic acid (66)

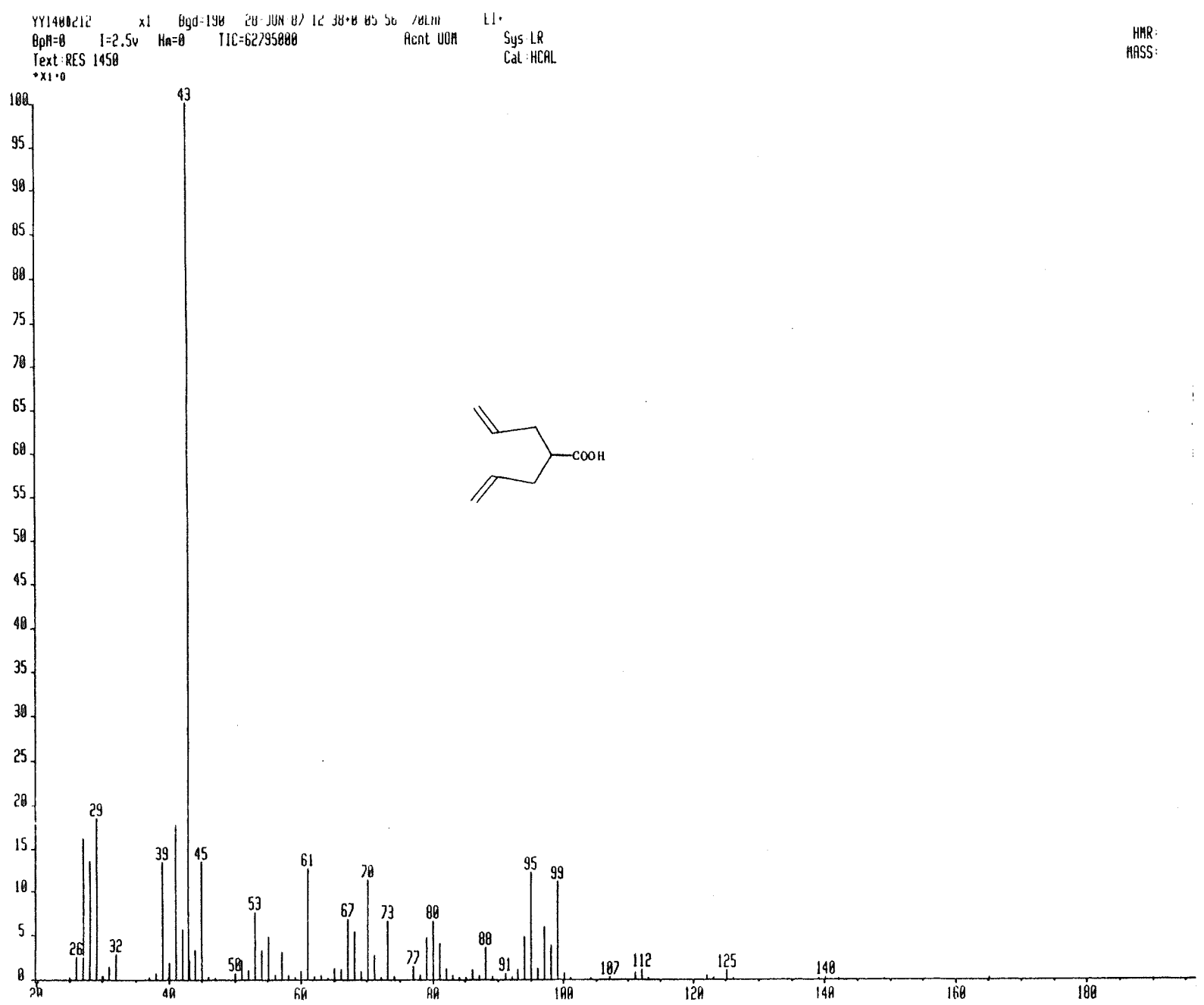


Fig. 10C **Mass Spectrum of 2-Allyl-4-pentenoic acid (66)**

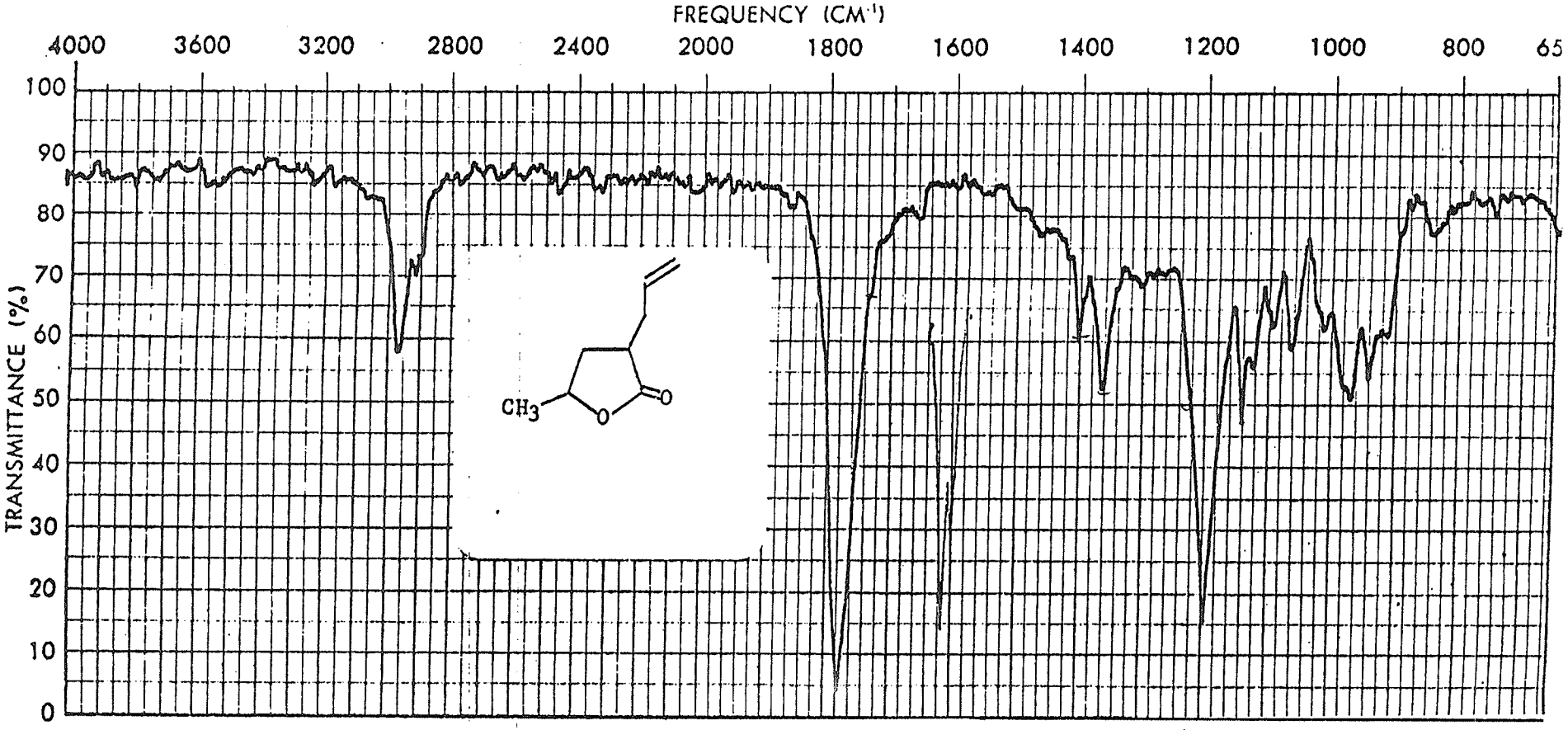


Fig. 11A I. r. Spectrum of 2-Allyl-4-methyl-γ-butyrolactone (67)

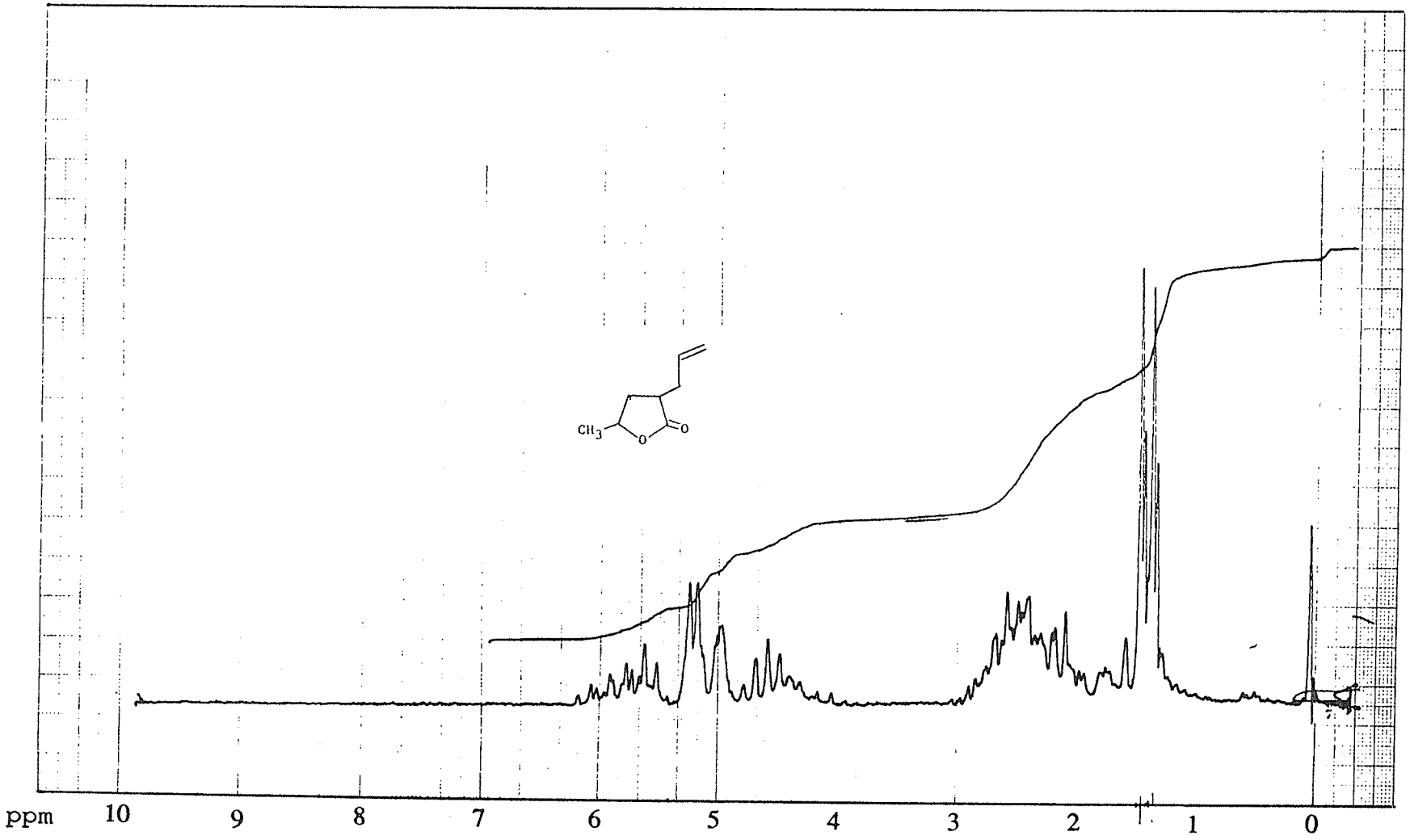


Fig. 11B P. m. r. Spectrum of 2-Allyl-4-methyl-γ-butyrolactone (67)

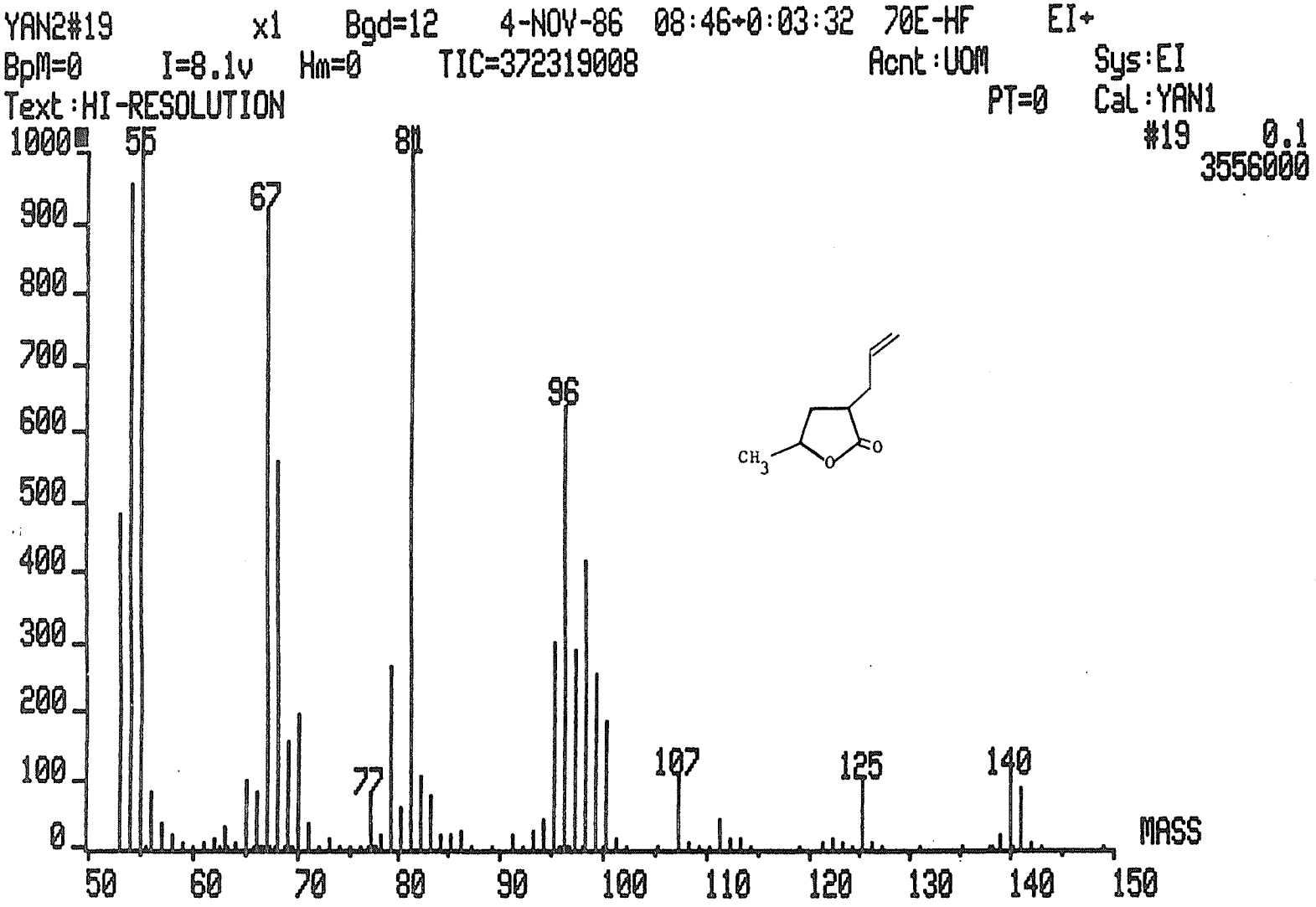
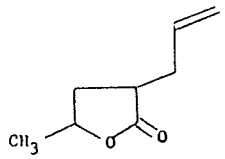


Fig. 11C **Mass Spectrum of 2-Allyl-4-methyl-γ-butyrolactone (67)**

YAN3#17 x1 Bgd=1 4-NOV-86 08:58+0:03:13 70E-HF EI+
BpM=81 I=9.0v Hm=231 TIC=402916992 Acnt:UOM Sys:EI
Text:HI-RESOLUTION PT=0 Cal:YAN3

M/E	C 12	H	O	MMU	DBE	OBS.MASS
140	8	12	2	-0.5	3.0	140.0842440



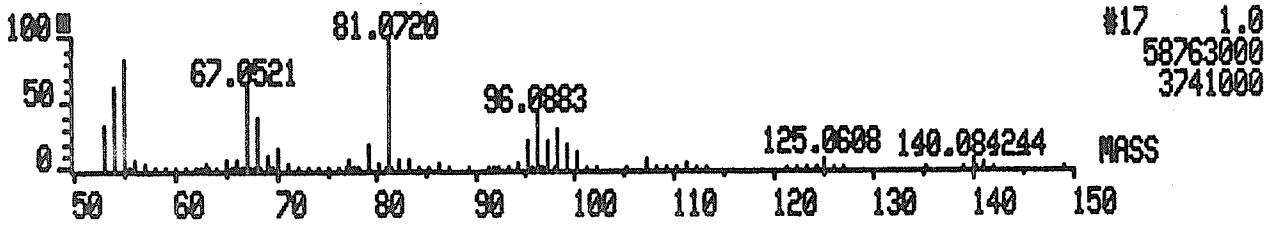


Fig. 11D High Resolution Mass Spectrum of 2-Allyl-4-methyl-γ-butyrolactone (67)

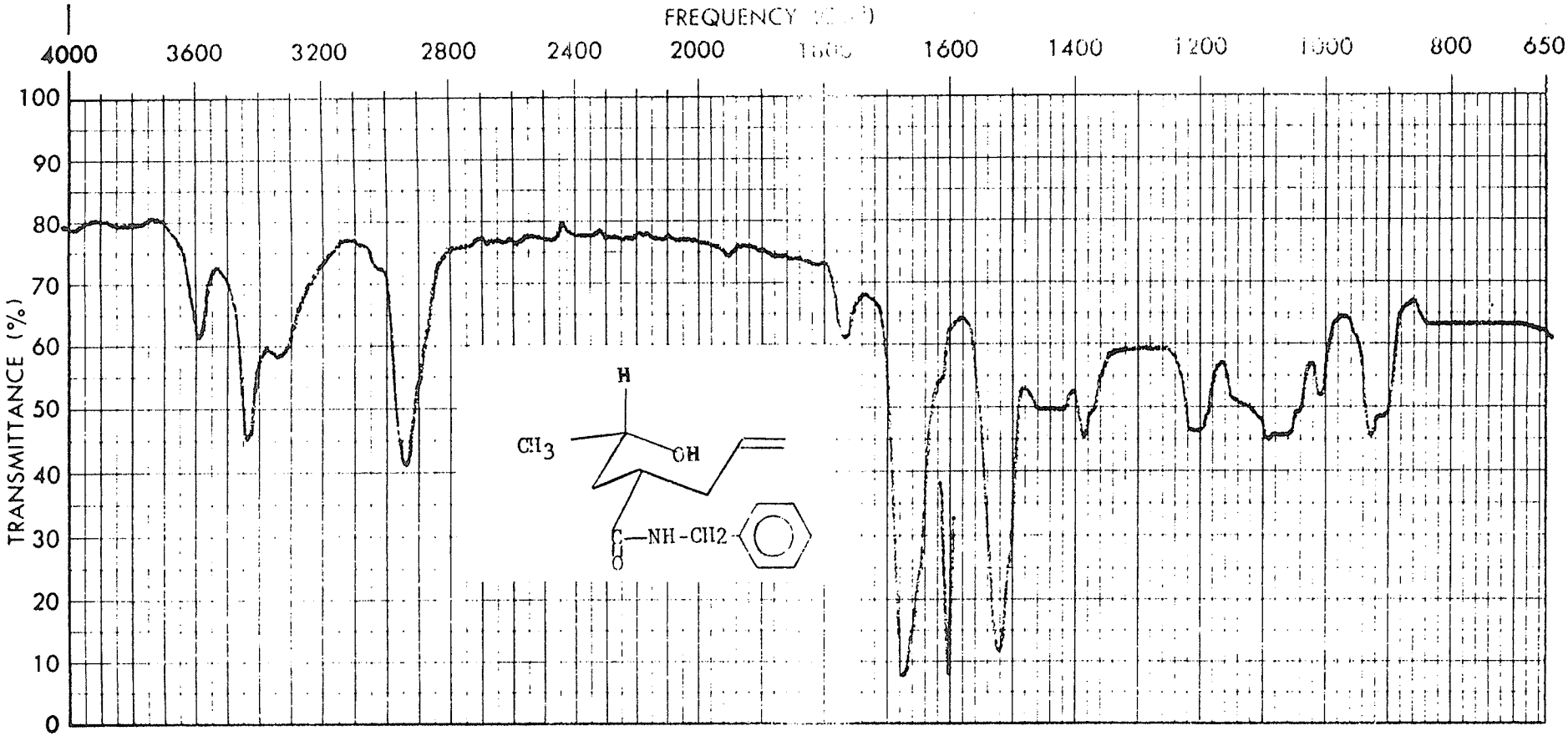
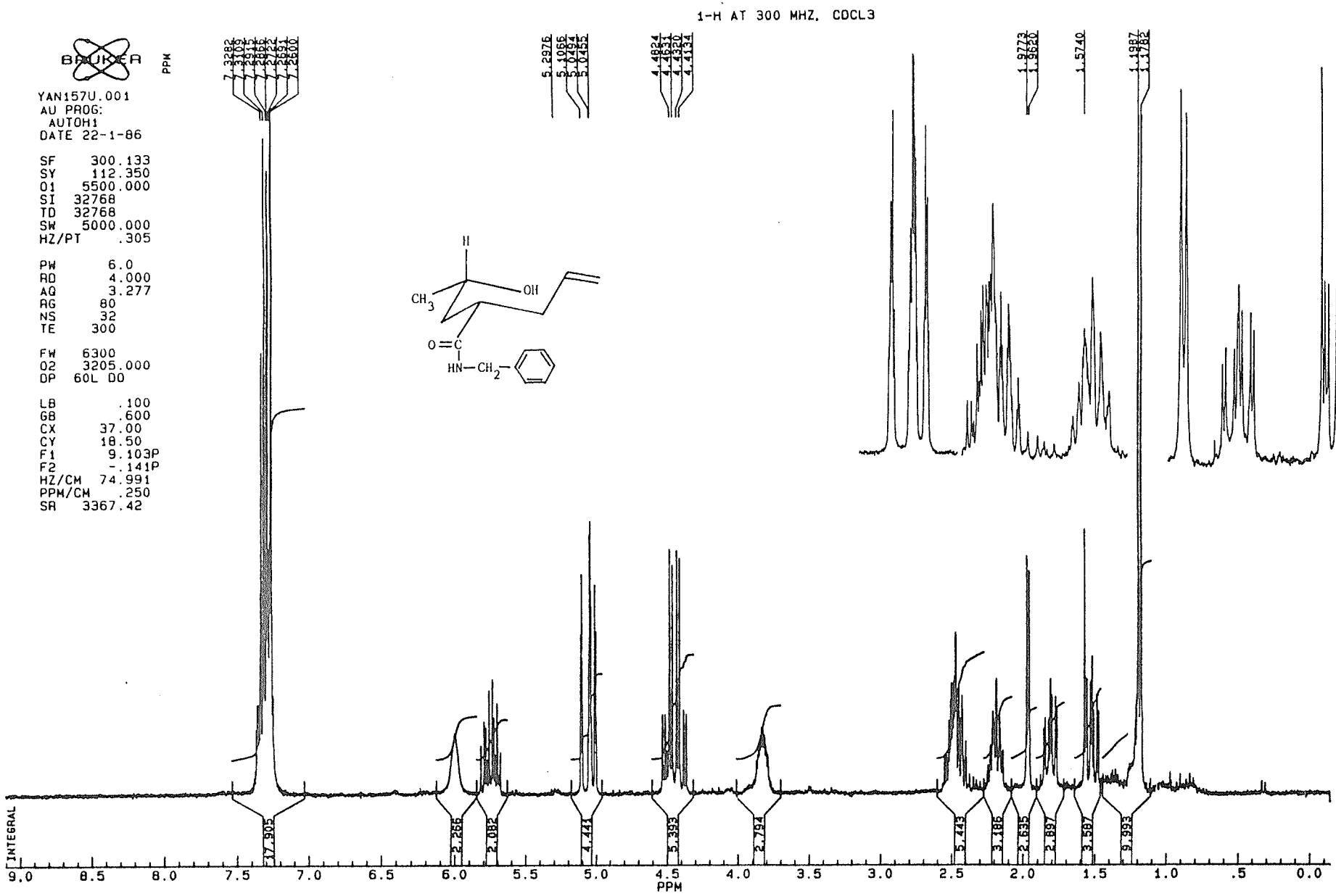


Fig. 12A I. r. Spectrum of (R,S) 2-Allyl-4-hydroxy-N-benzyl-erythro-pentanamide (75)



140

Fig. 12B **P. m. r. Spectrum of (R,S) 2-Allyl-4-hydroxy-N-benzyl-erythro-pentanamide (75)**

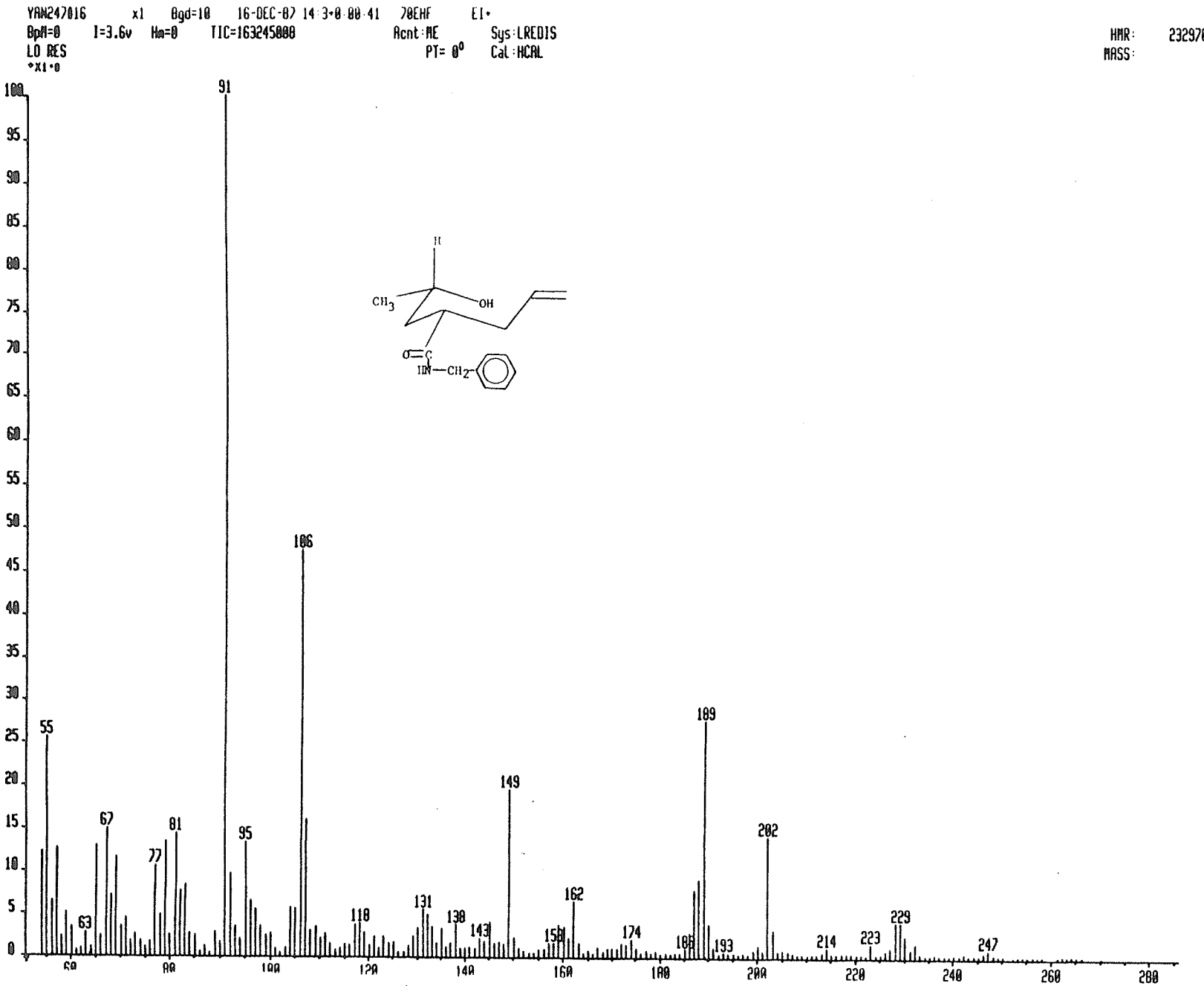


Fig. 12C Mass Spectrum of (R,S) 2-Allyl-4-hydroxy-N-benzyl-erythro-pentanamide (75)

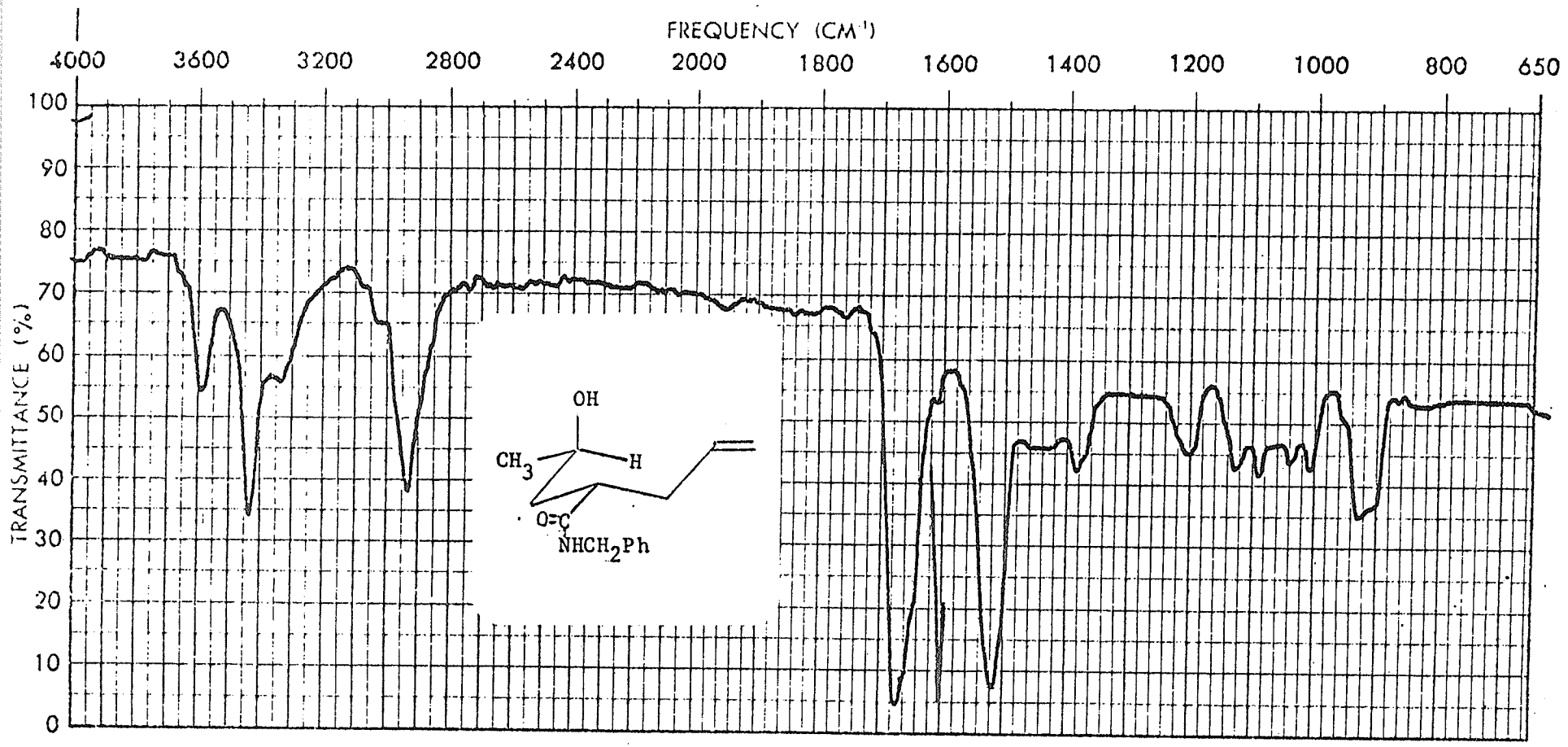


Fig. 13A I. r. Spectrum of (R,S) 2-Allyl-4-hydroxy-N-benzyl-threo-pentanamide (77)

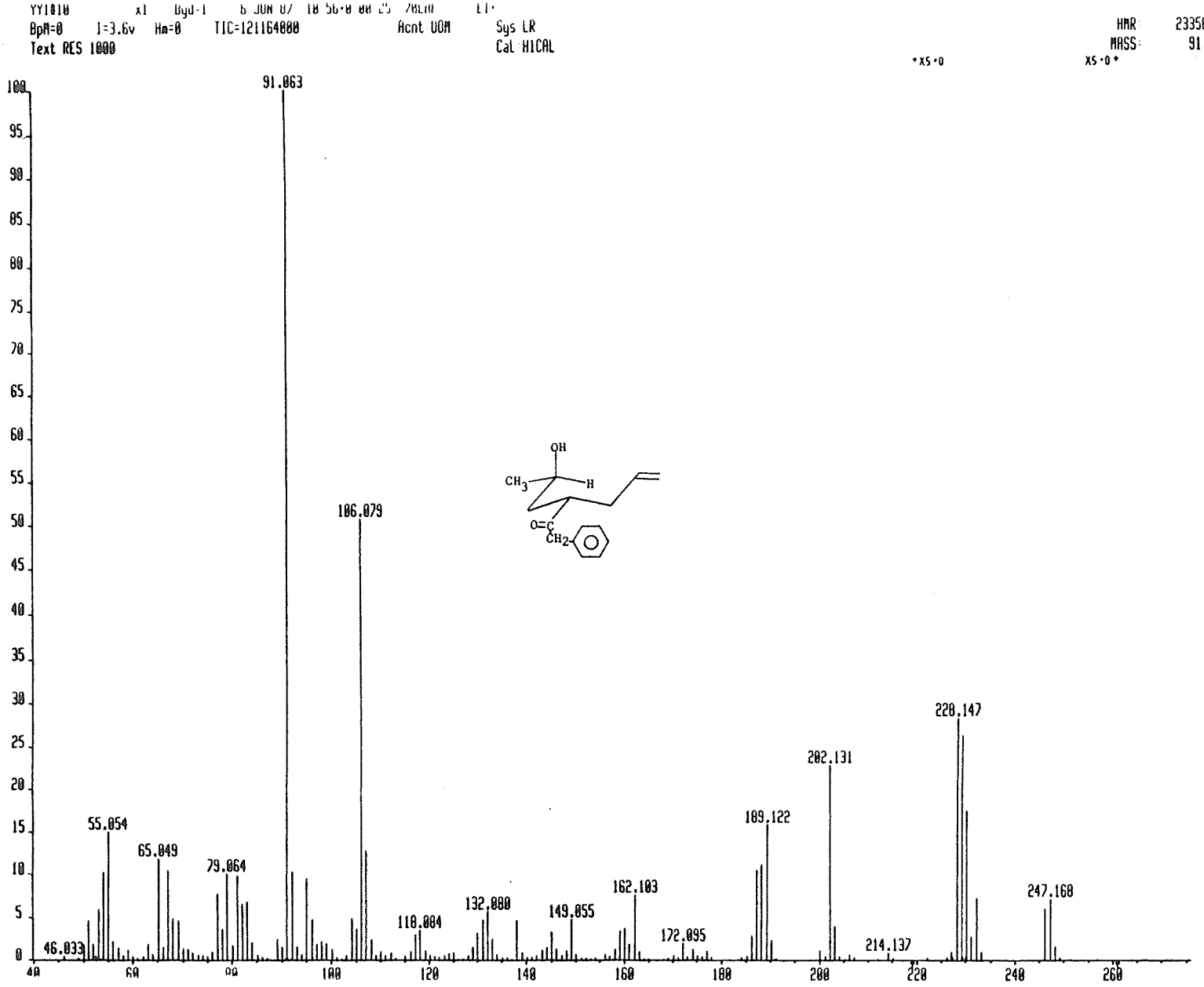


Fig. 13C **Mass Spectrum of (R,S) 2-Allyl-4-hydroxy-N-benzyl-threo-pentanamide (77)**

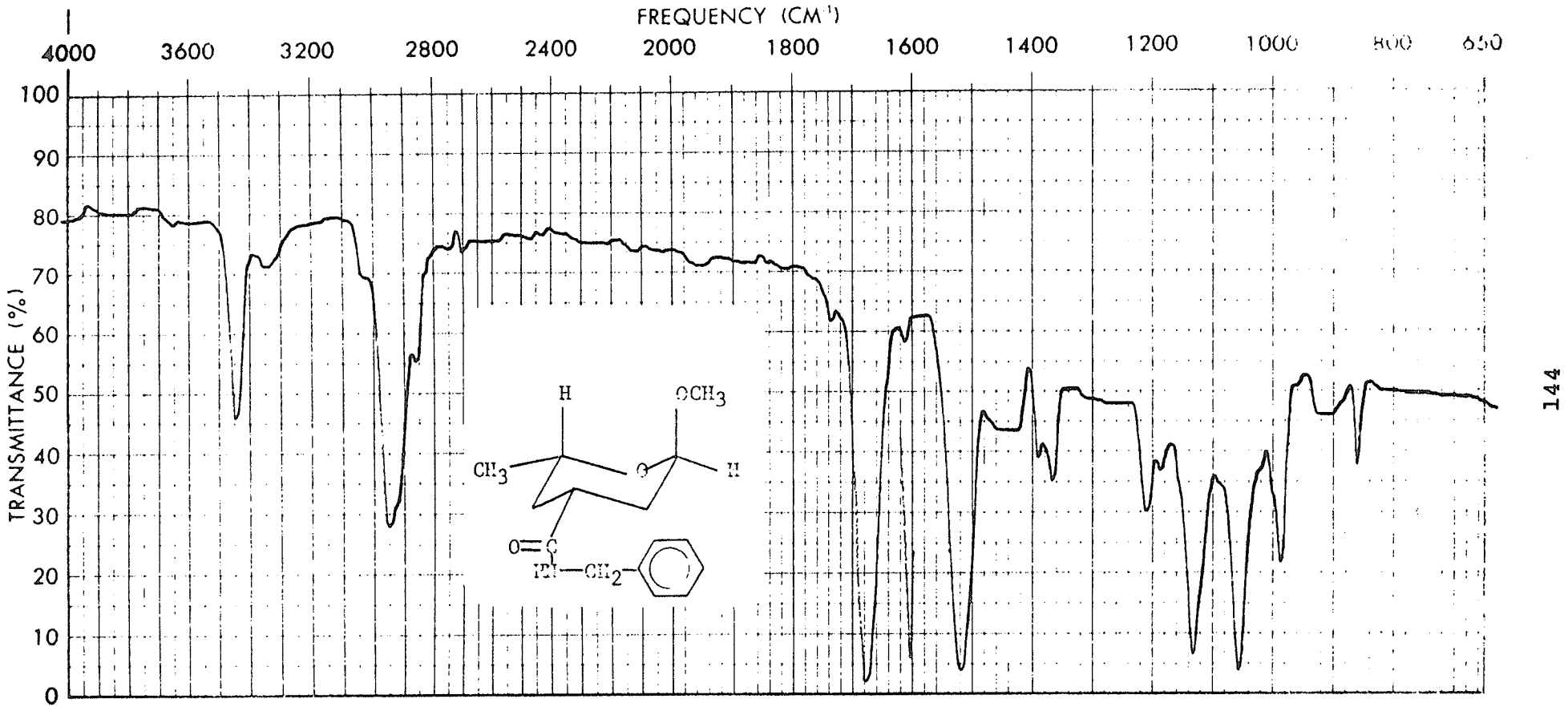


Fig. 14A I.r. Spectrum of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-α-DL-threo-hexopyranose (82)

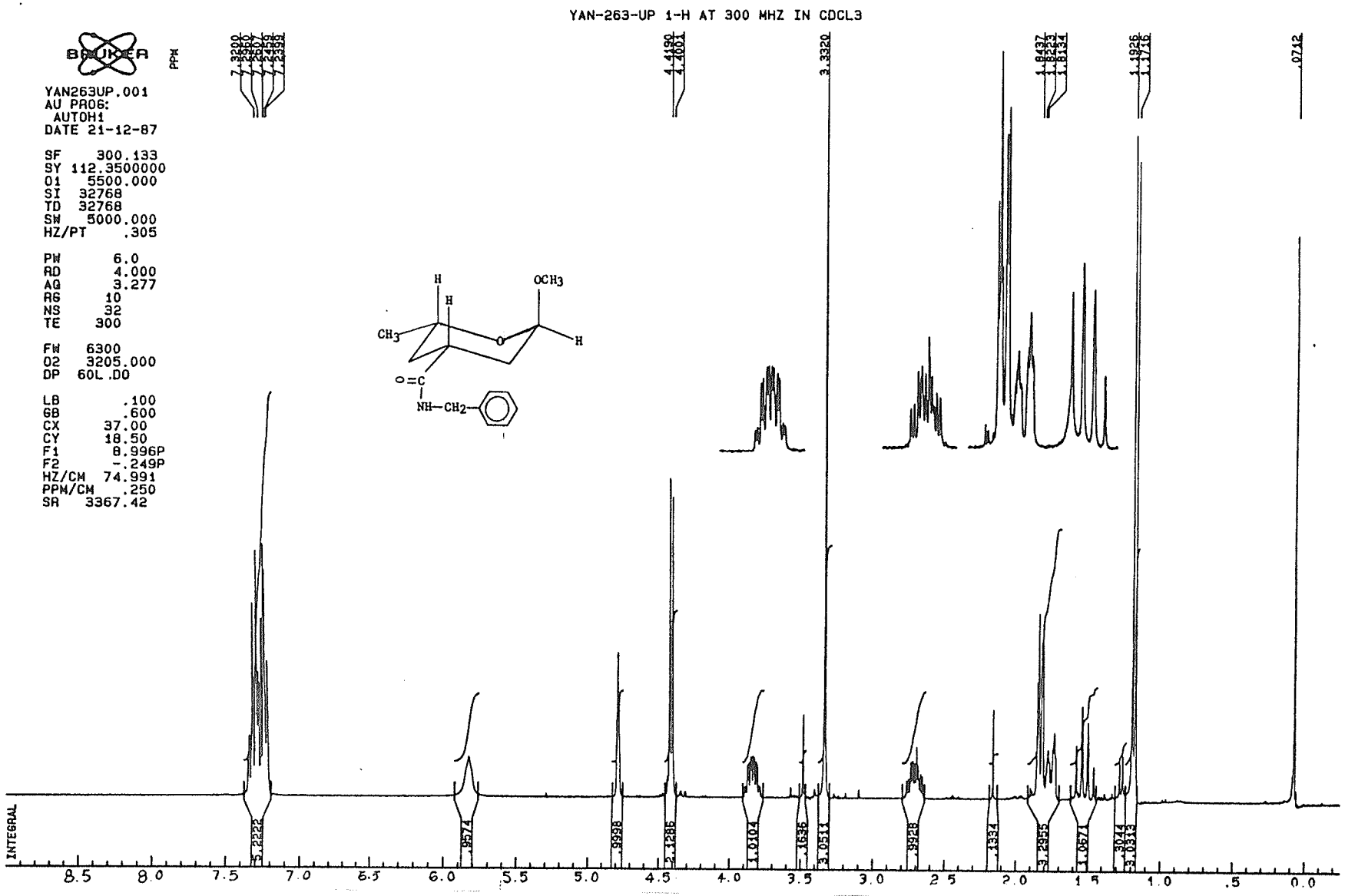


Fig. 14B P.m.r. Spectrum of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-α-DL-threo-hexopyranose (82)

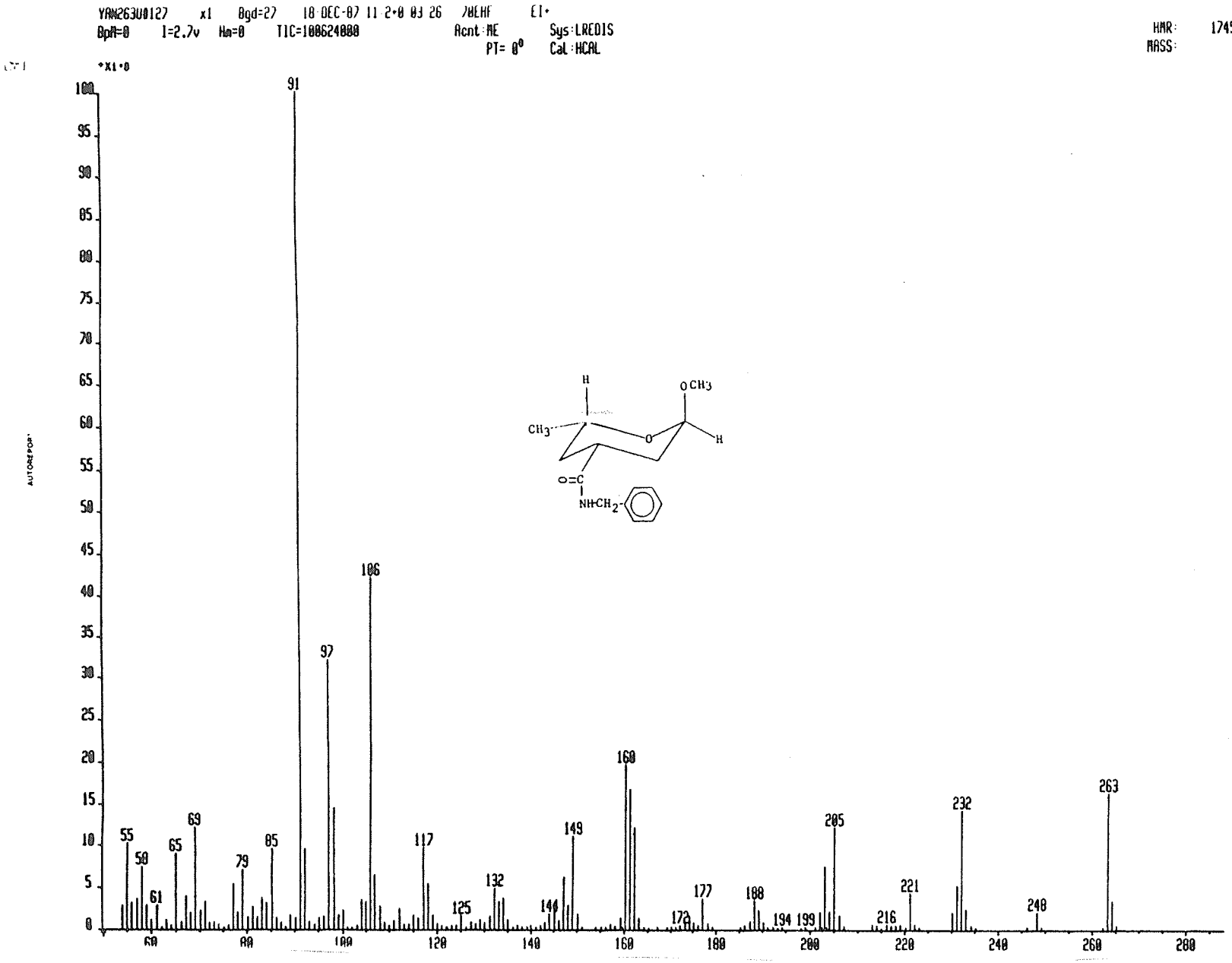


Fig. 14C Mass Spectrum of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-α-DL-threo-hexopyranose (82)

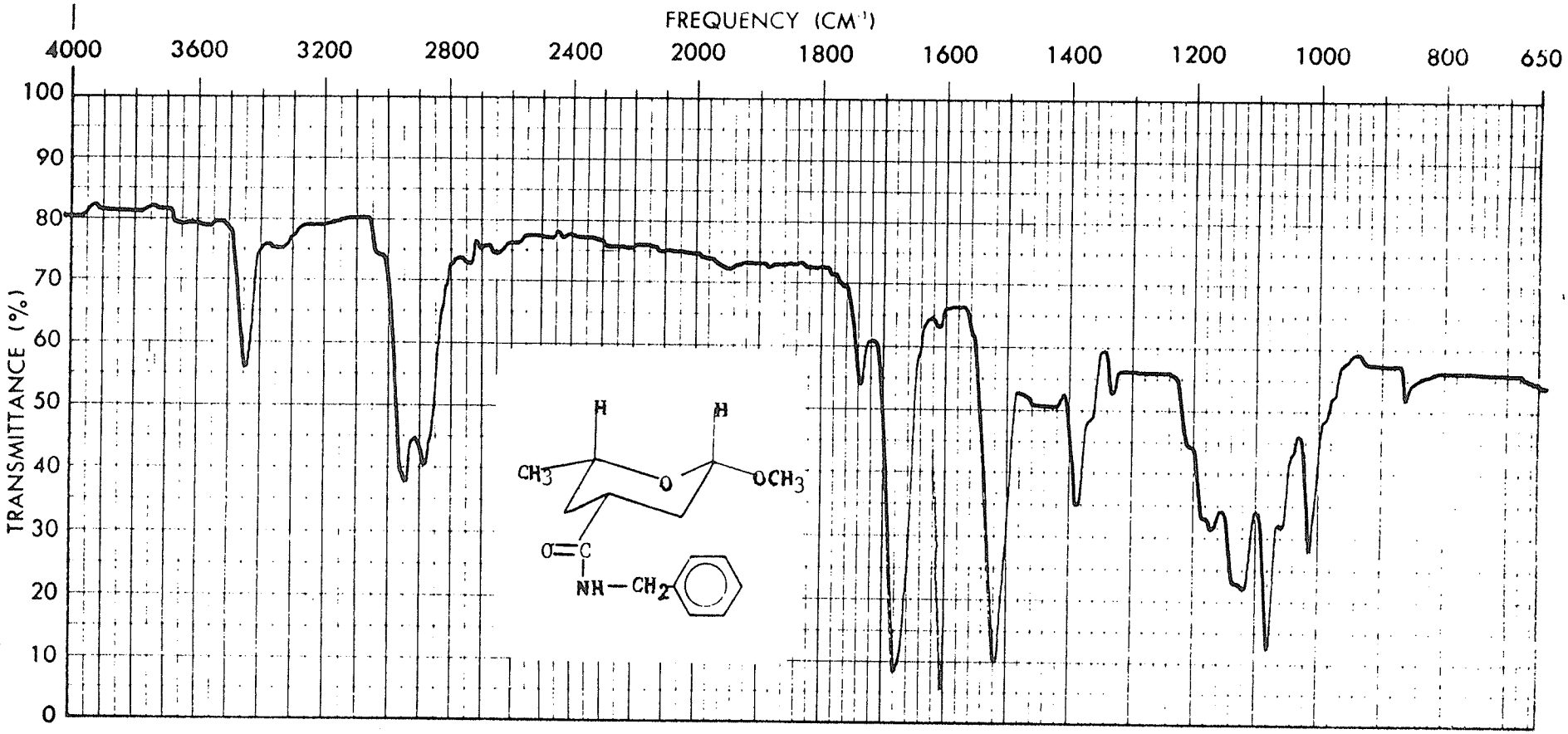


Fig. 15A I.r. Spectrum of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-β-DL-threo-hexopyranose (83)

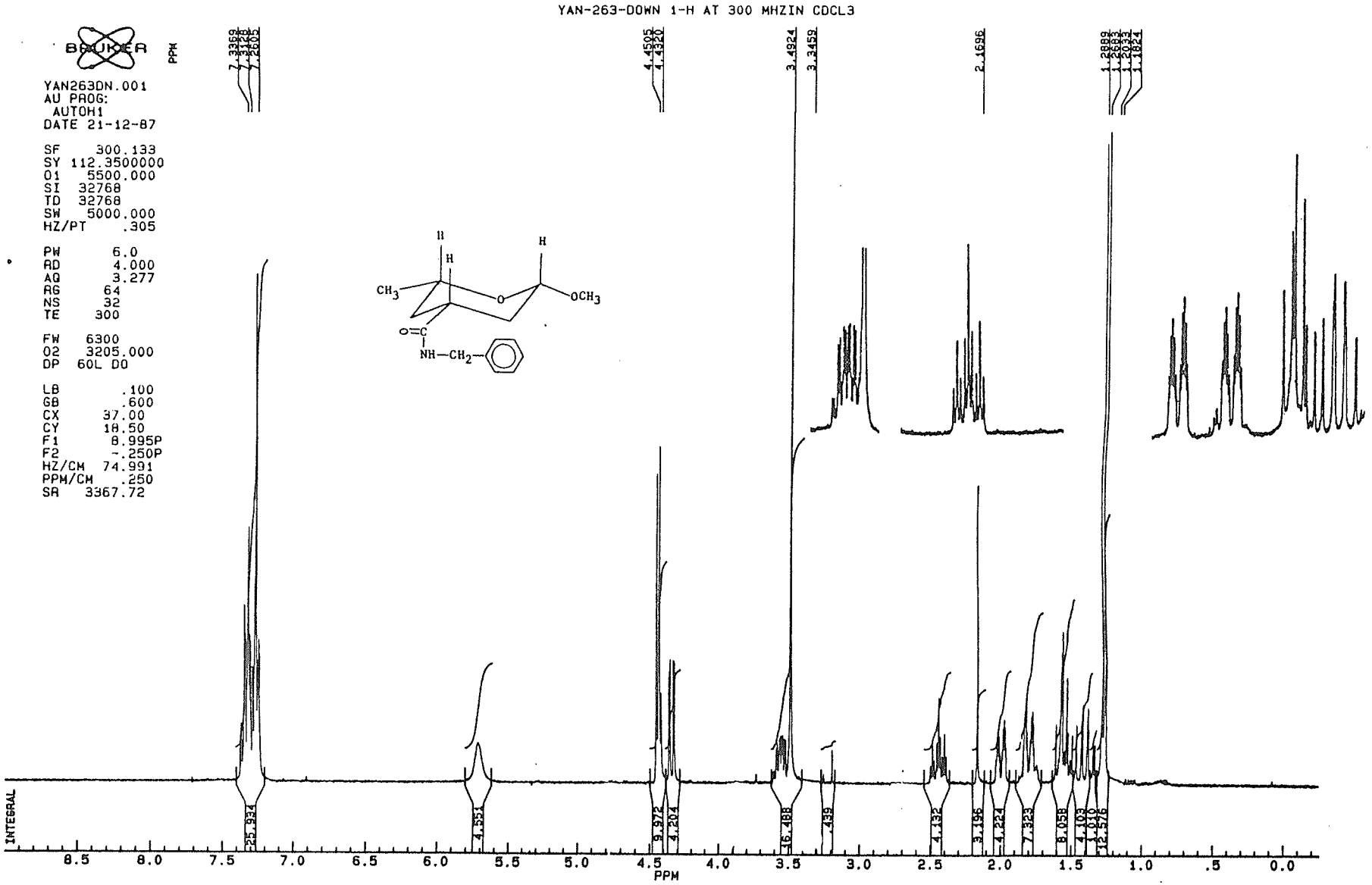


Fig. 15B P.m.r. Spectrum of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-β-DL-threo-hexopyranose (83)

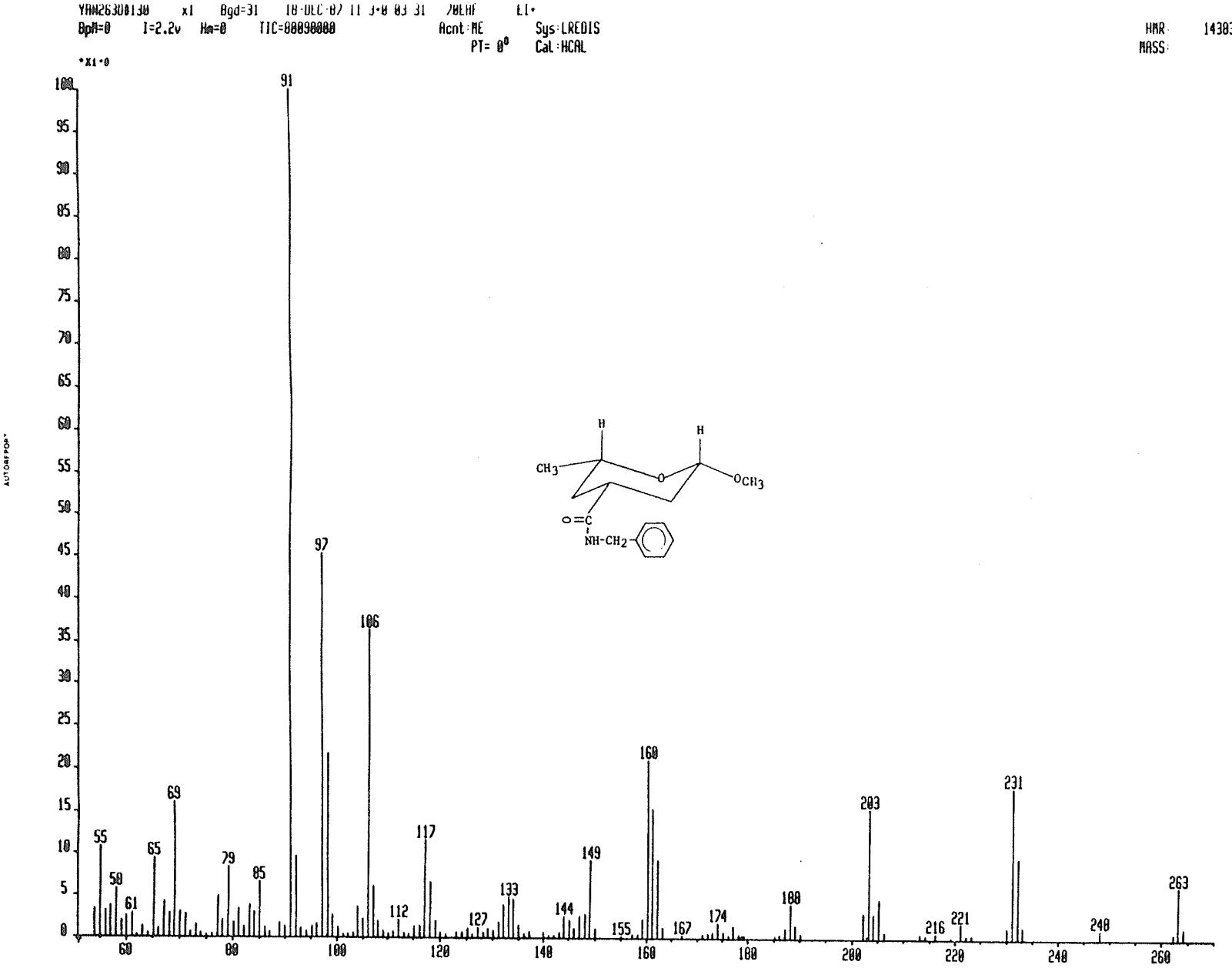


Fig. 15C Mass Spectrum of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-β-DL-threo-hexopyranose (83)

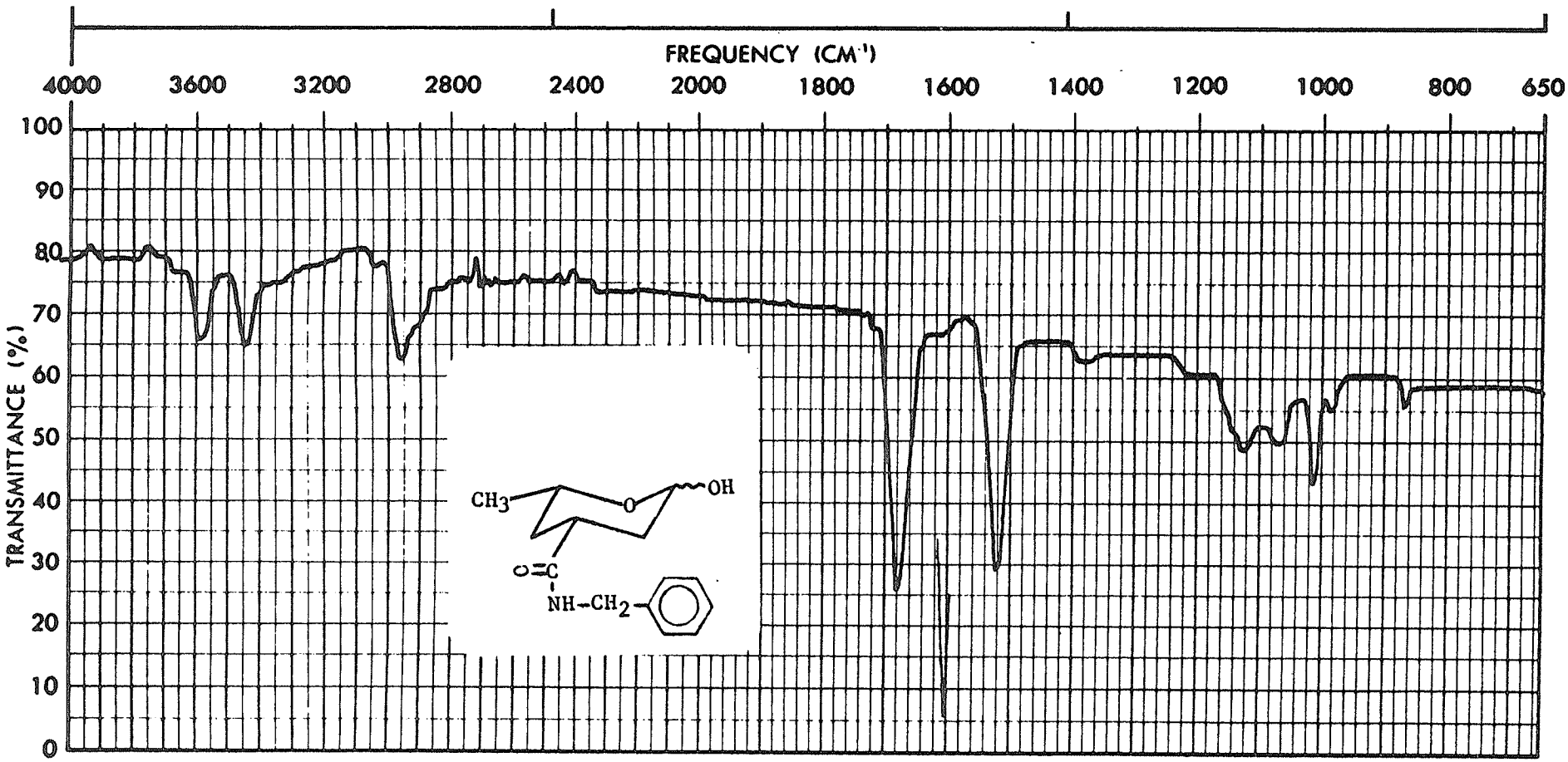


Fig. 16A I.r. Spectrum of 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-DL-threo-hexopyranose (79)

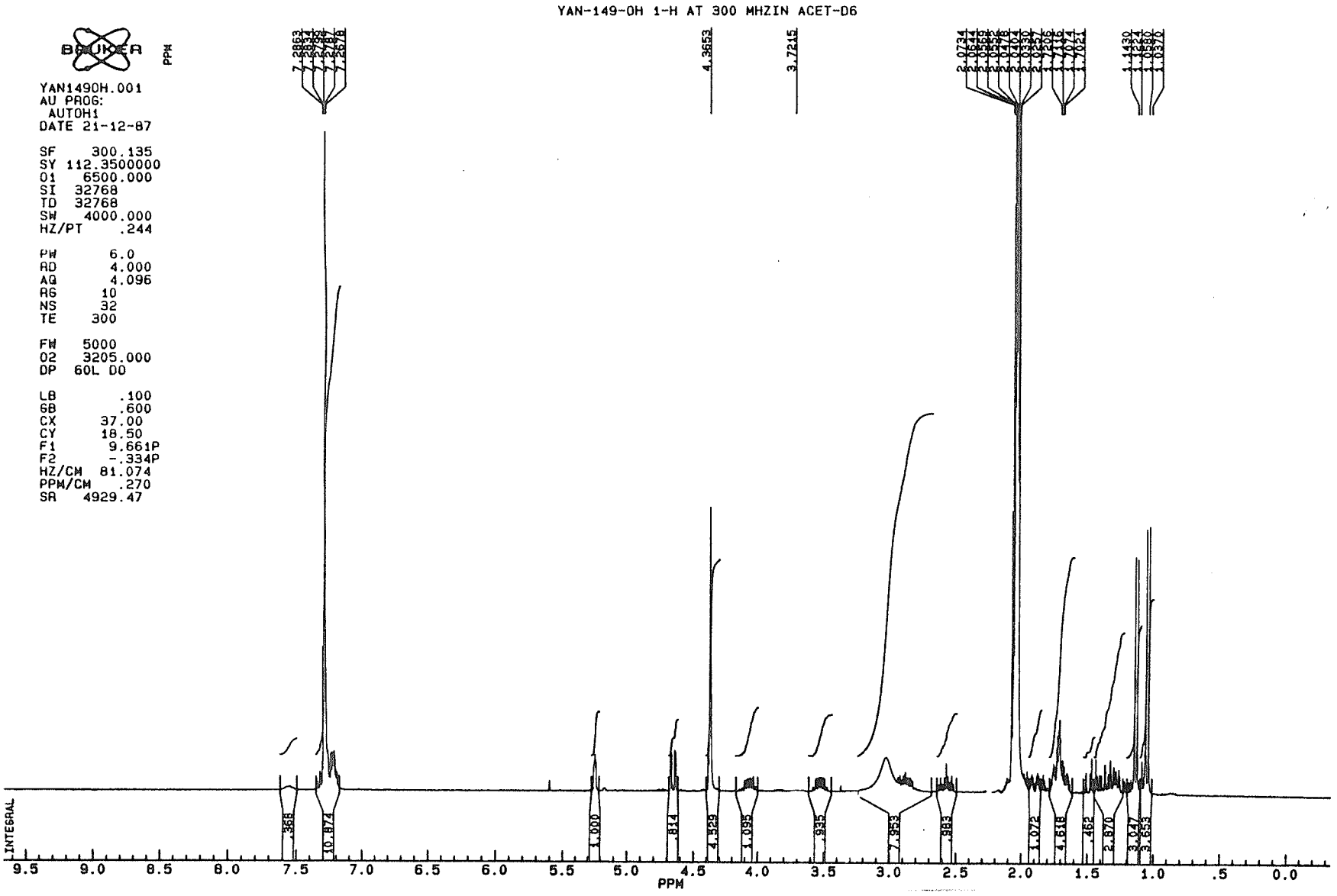


Fig. 16B P.m.r. Spectrum of 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-DL-threo-hexopyranose (79)

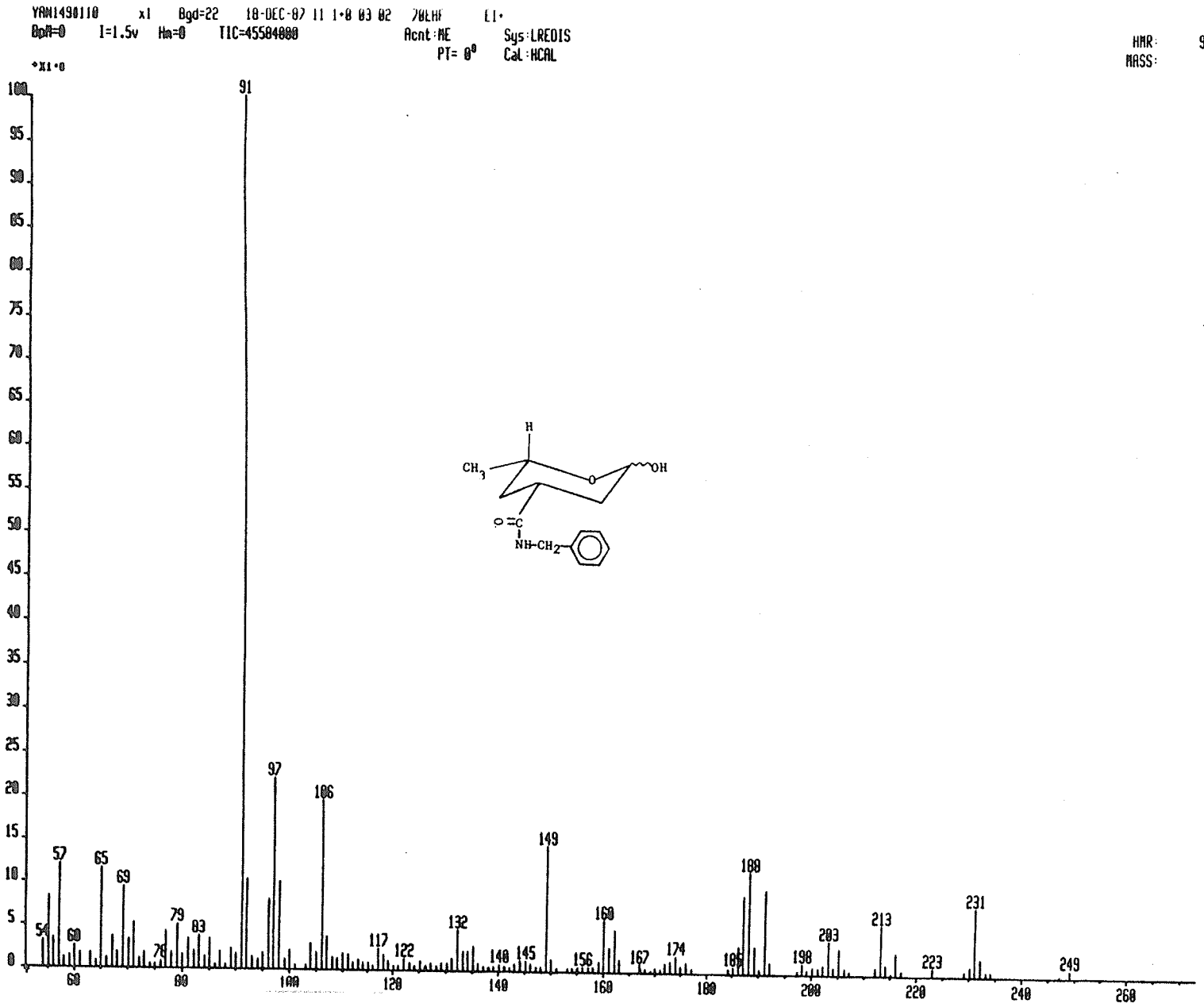


Fig. 16C Mass Spectrum of 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-DL-threo-hexopyranose (79)

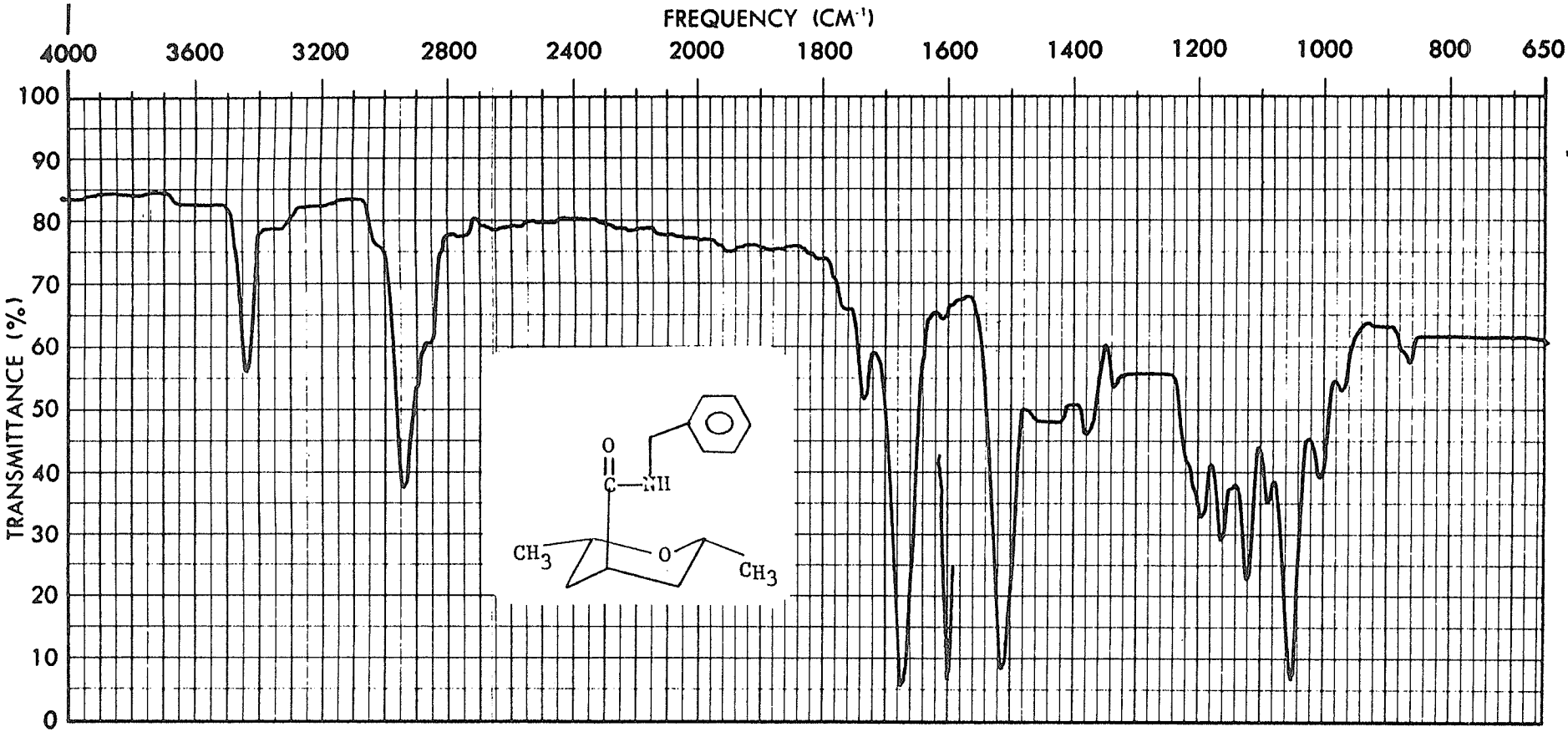


Fig. 17A I.r. Spectrum of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-β-DL-erythro-hexopyranose (84)

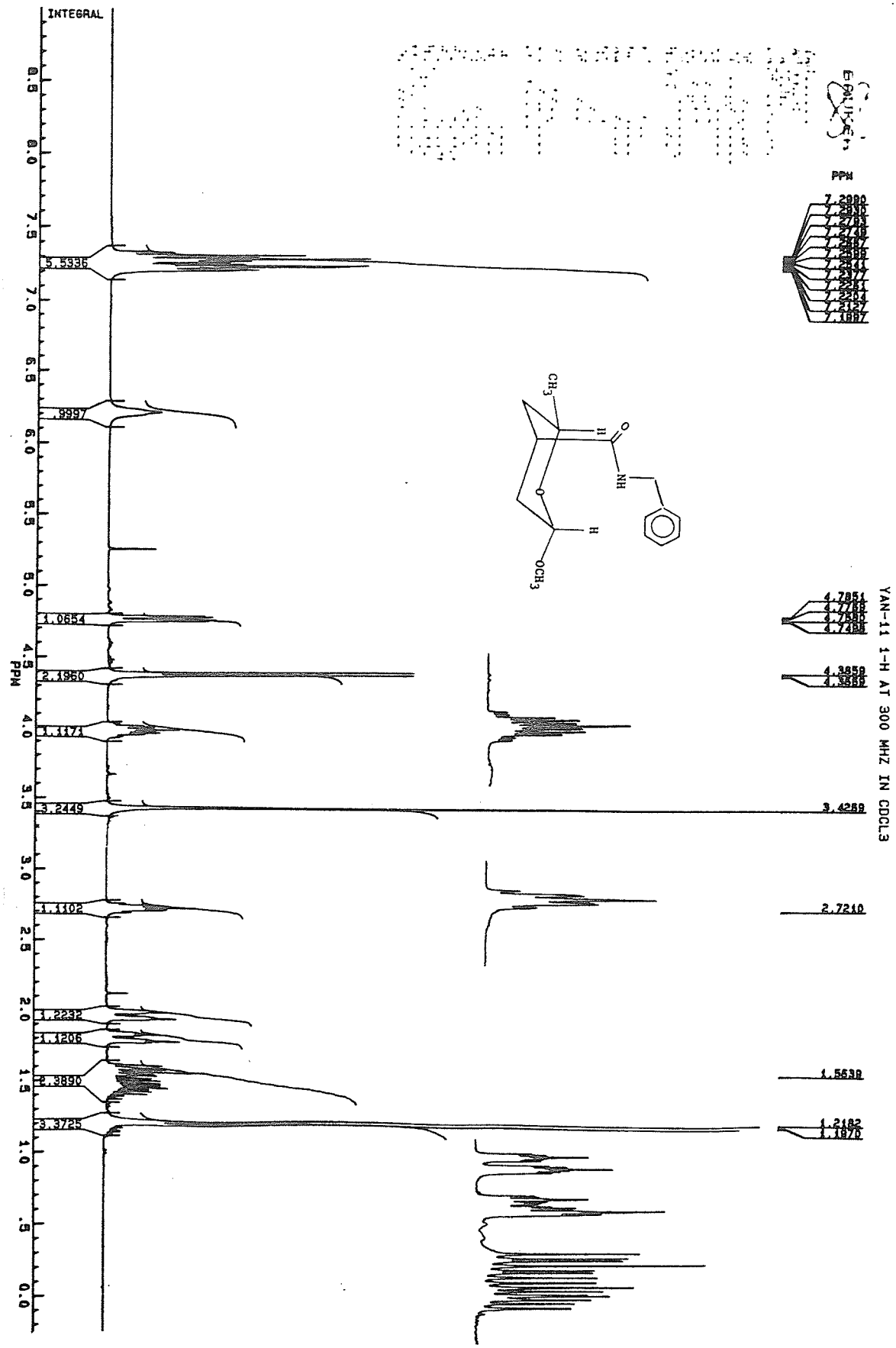


Fig. 17B P.m.r. Spectrum of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-β-DL-erythro-hexopyranose (84)

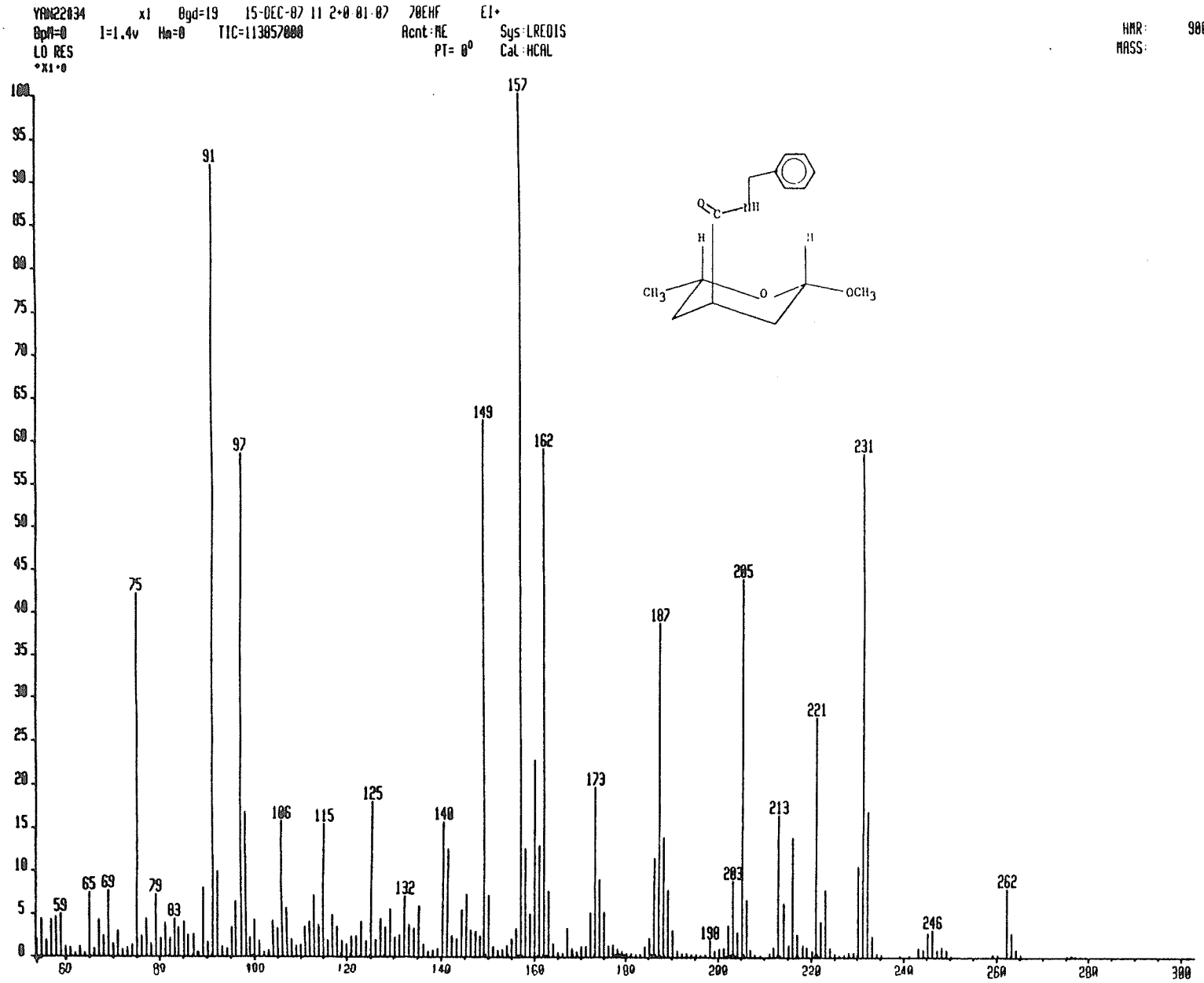


Fig. 17C Spectrum of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-β-Dl-erythro-hexopyranose (84)

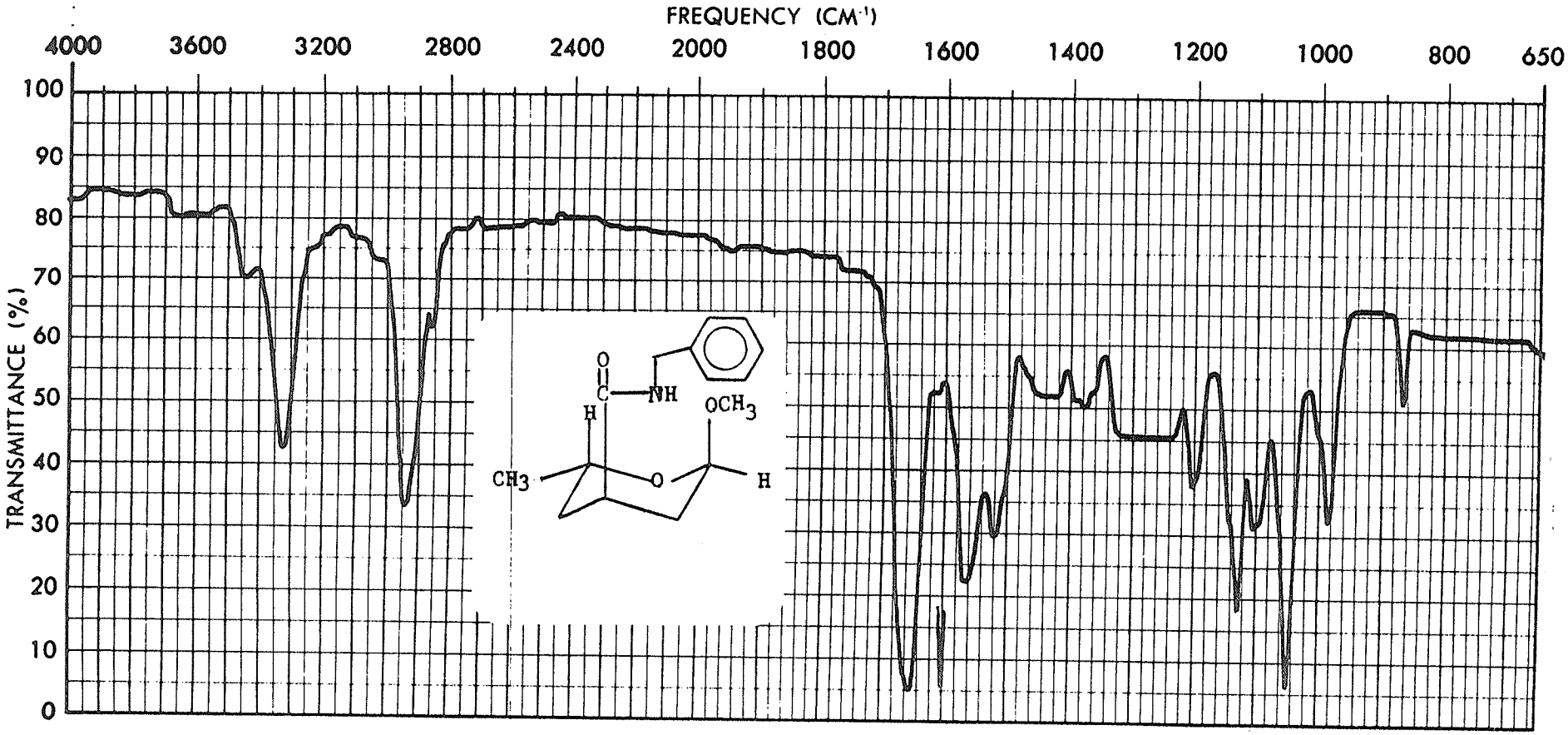


Fig. 18A I.r. Spectrum of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-α-DL-erythro-hexopyranose (85)

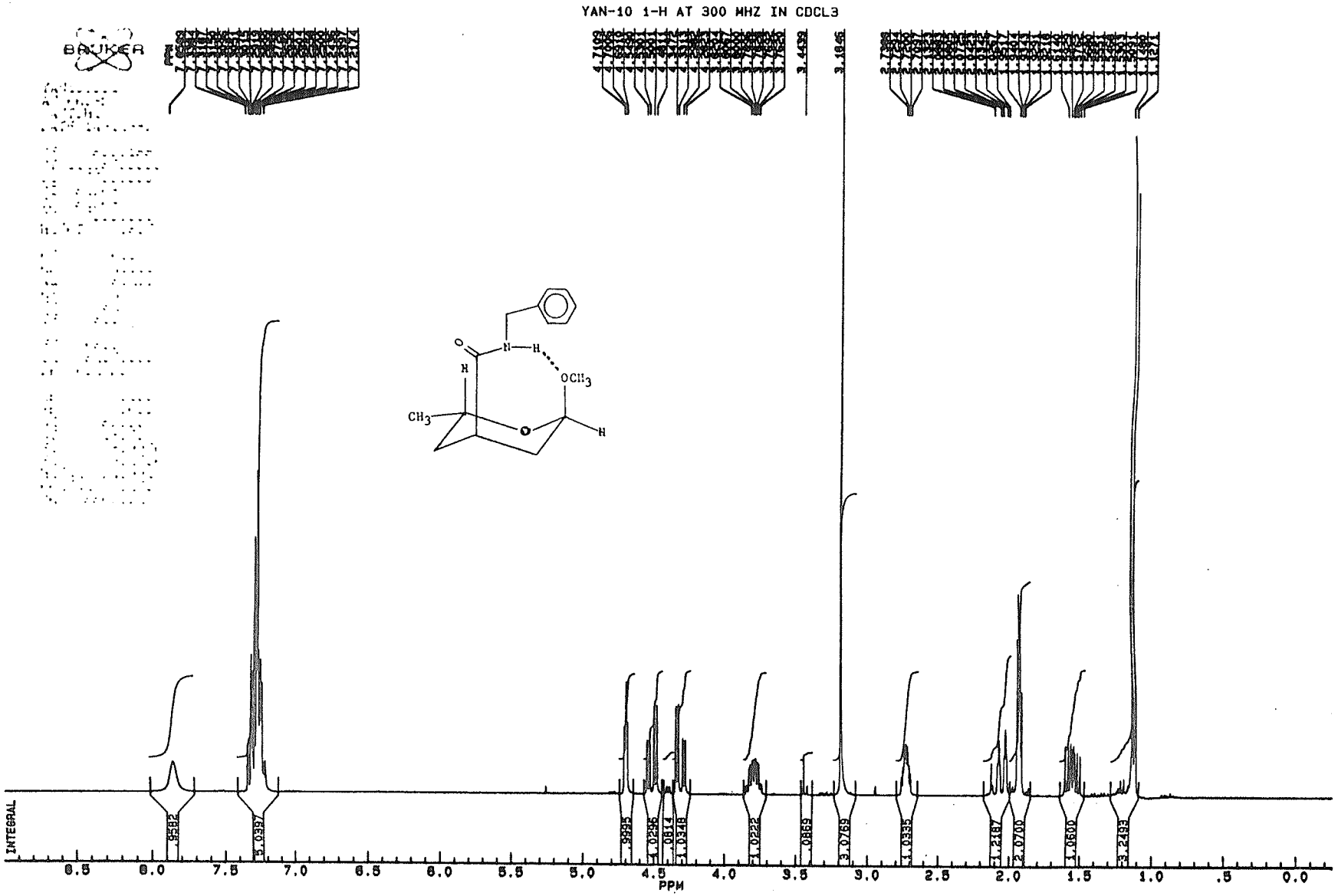


Fig. 18B P.m.r. Spectrum of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-α-DL-erythro-hexopyranose (85)

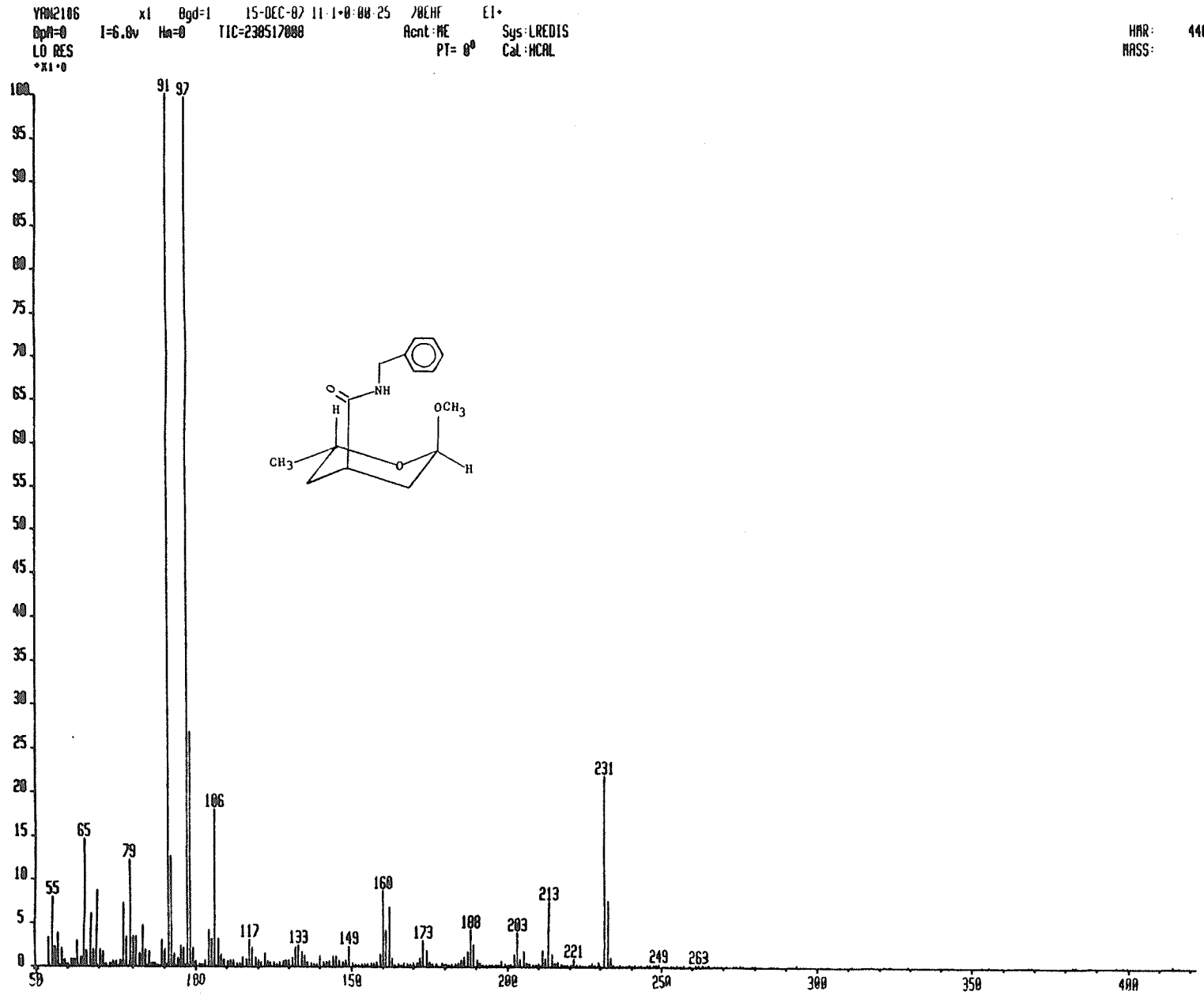
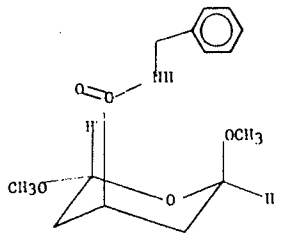


Fig. 18C Mass Spectrum of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-α-DL-erythro-hexopyranose (85)

```
YAND#26        x1    Bgd=25    5-JAN-87  16:0+0:02:37   70EHF      EI+
BpM=69    I=10v    Hm=499   TIC=411628992              Acnt:          Sys:ACMEDIS
ACC MASS                                                   PT= 0°    Cal:YAND
   M/E   C   H  O  N    MMU    DBE     OBS.MASS
         12
-------------------------------------------------------------------------------
   231  14  17  2  1    0.8    7.0   231.1251070
        9  17  4  3   -3.2    3.0
```



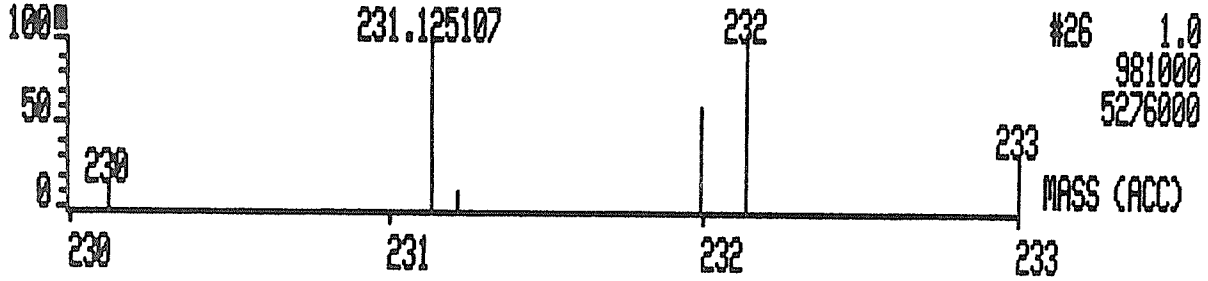


Fig. 18D High Resolution Mass Spectrum of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-α-DL-erythro-hexopyranose (85)

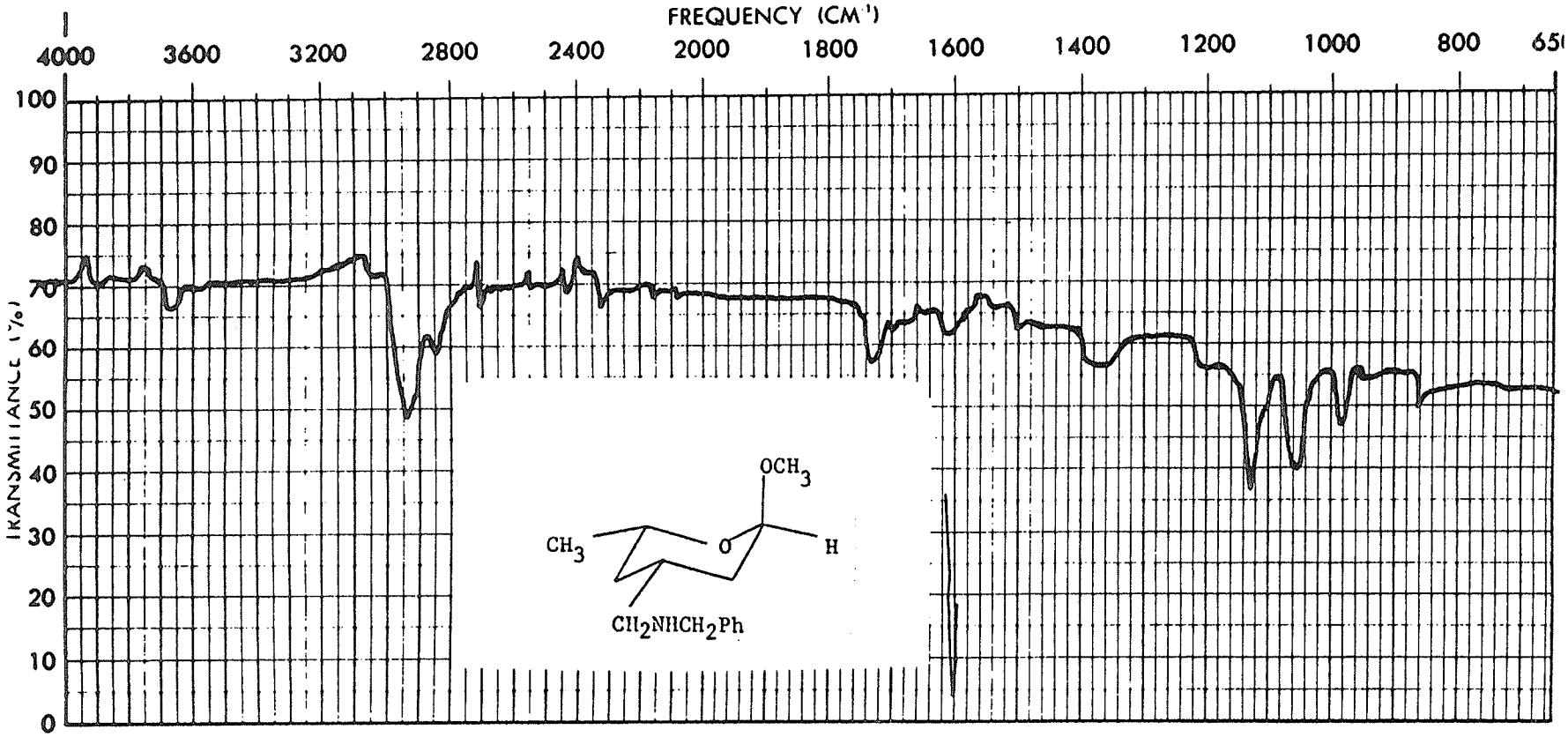


Fig. 19A I.r. Spectrum of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-α-DL-threo-hexopyranose (88)

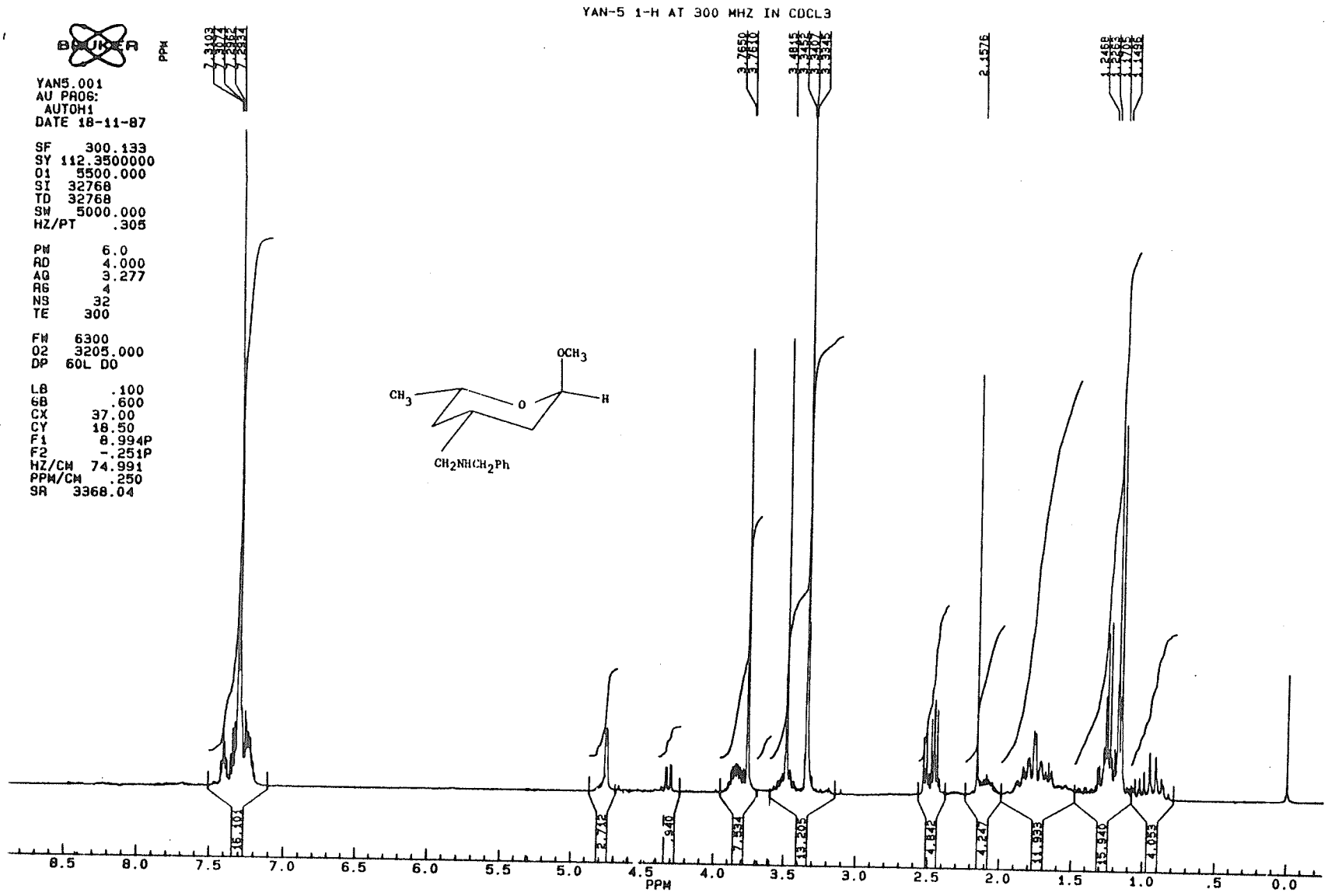


Fig. 19B P.m.r. Spectrum of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-α-DL-threo-hexopyranose (88)

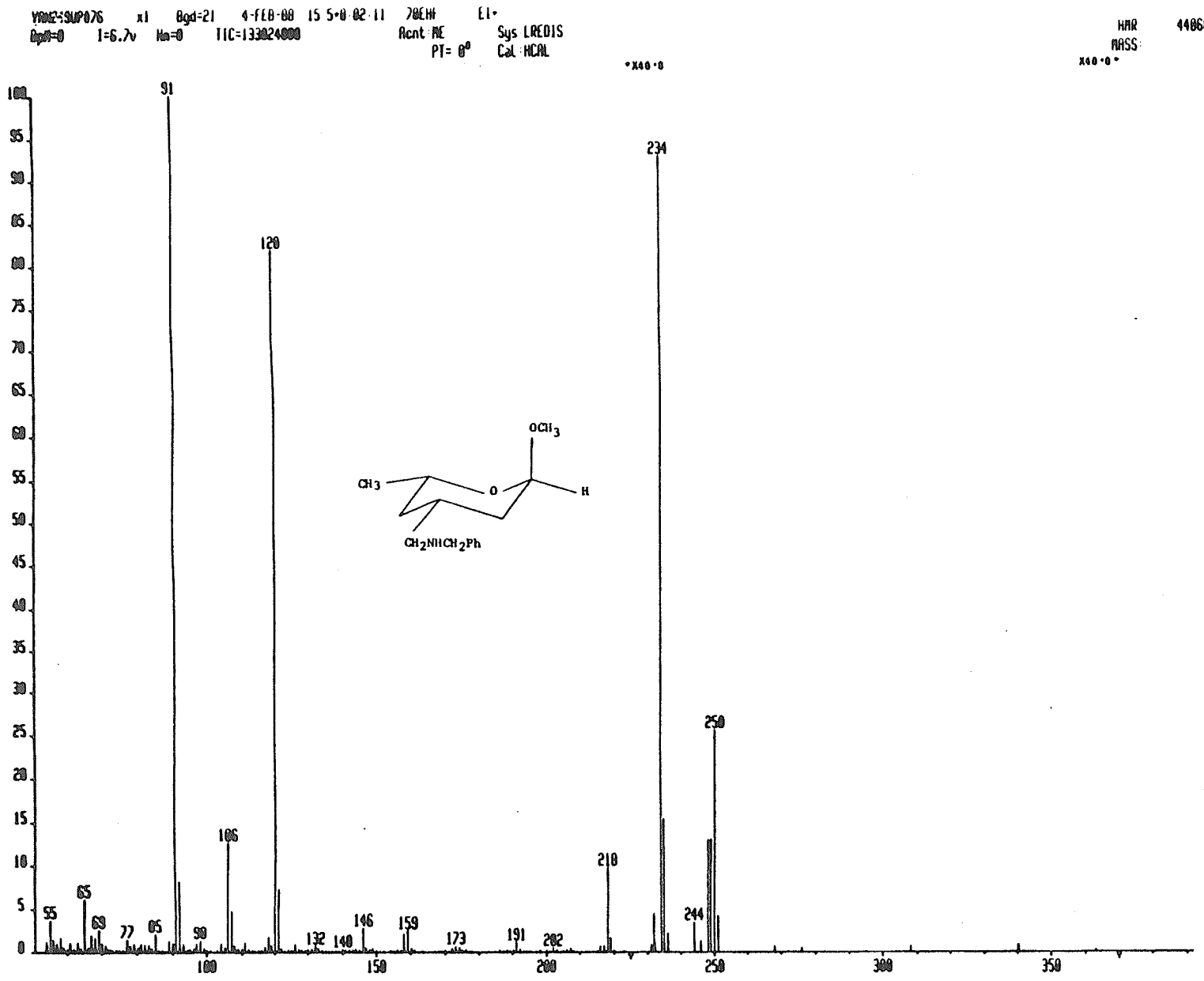


Fig. 19C Mass Spectrum of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-α-DL-threo-hexopyranose (88)

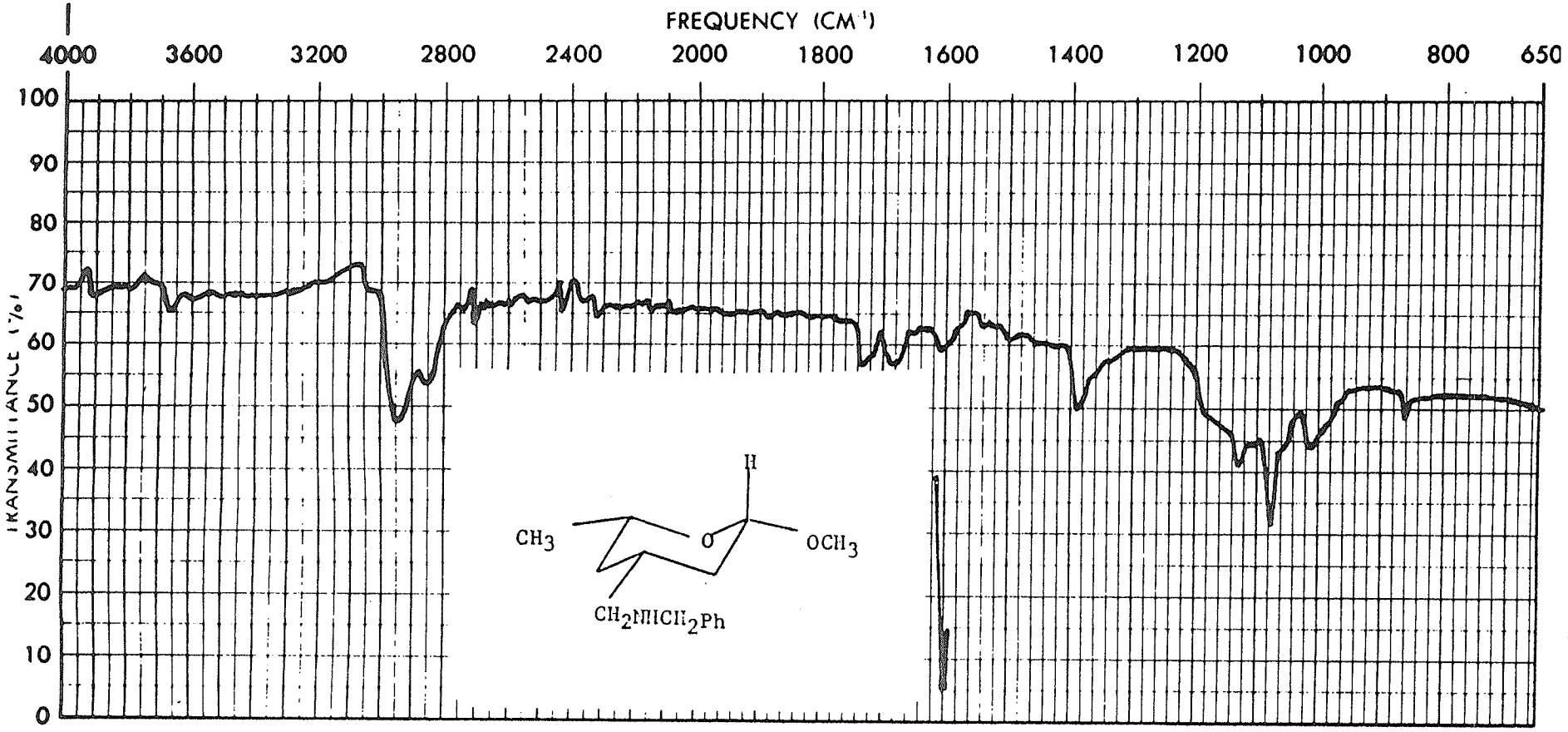


Fig.20A I.r. Spectrum of Methyl 3-N-(benzylamino-methl)-2,3,4,6-tetradeoxy-β-DL-threo-hexopyranose (89)

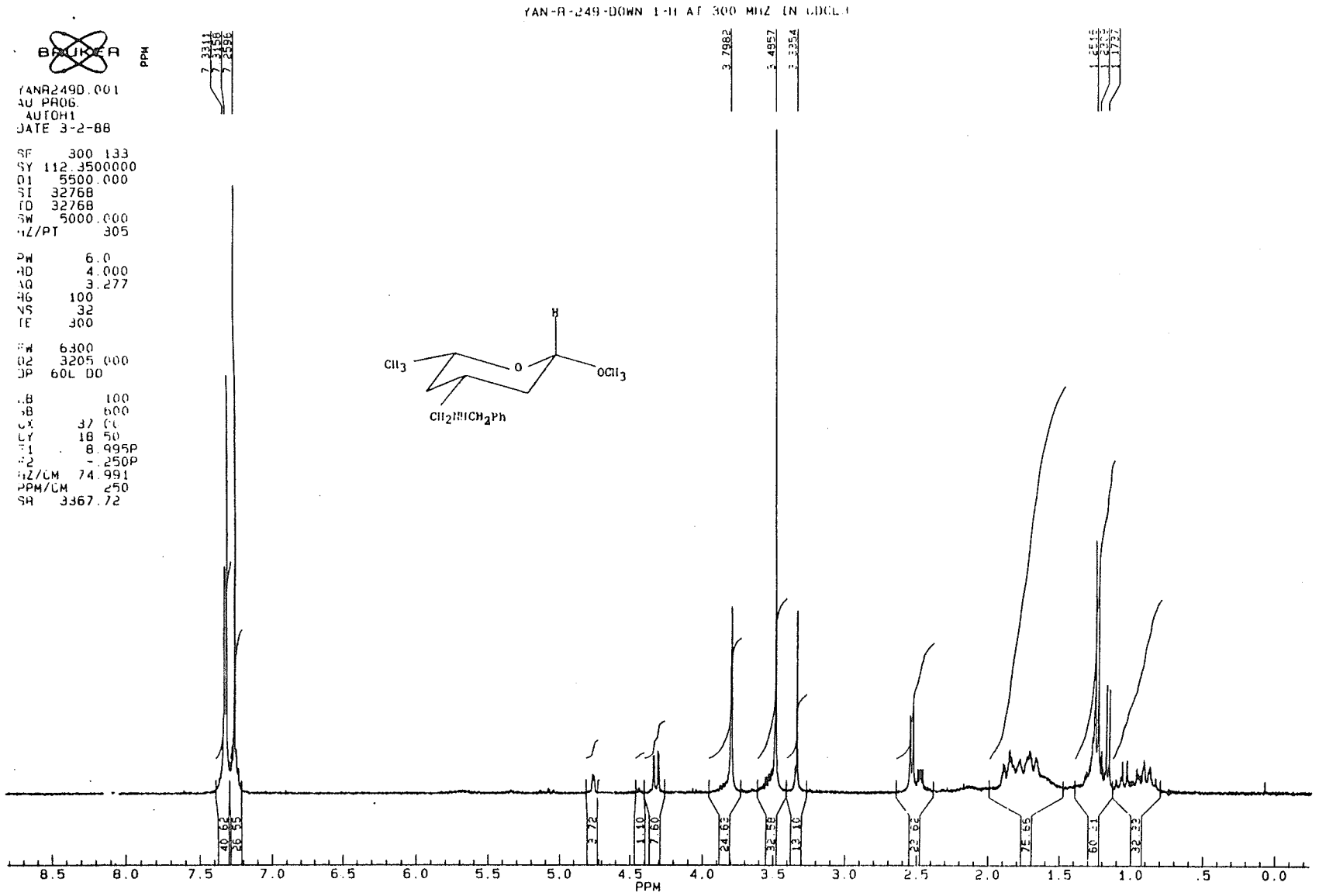


Fig. 20B P.m.r. Spectrum of Methyl 3-N-(benzylamino-methyl)-2,3,4,6-tetradeoxy-β-DL-threo-hexopyranose (89)

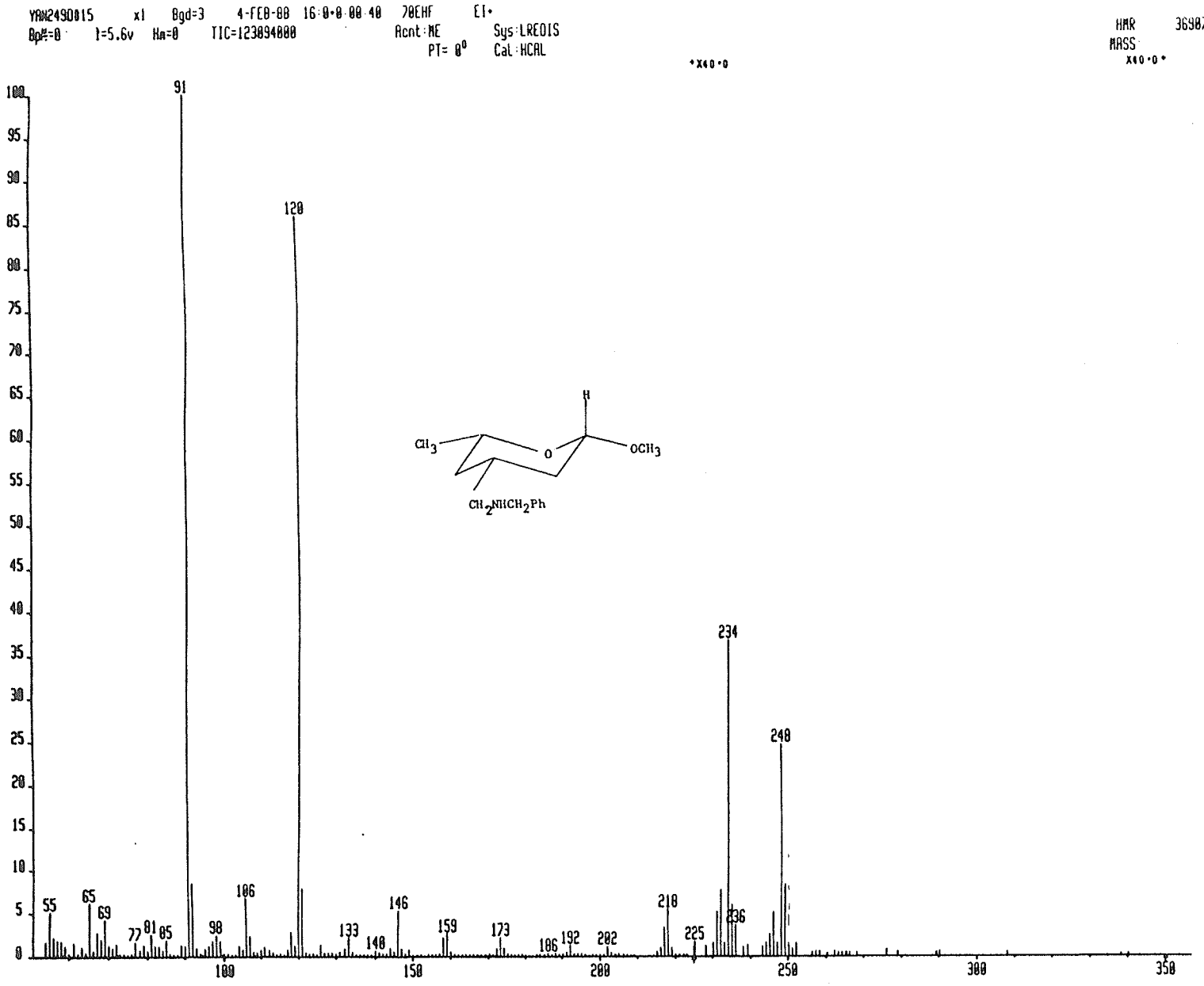


Fig. 20C Mass Spectrum of Methyl 3-N-(benzylamino-methyl)-2,3,4,6-tetradeoxy-β-DL-threo-hexopyranose (89)

Fig. 20D High Resolution Mass Spectrum of Methyl 3-N-(benzylamino-methyl)-2,3,4,6-tetradeoxy-β-DL-threo-hexopyranose (89)

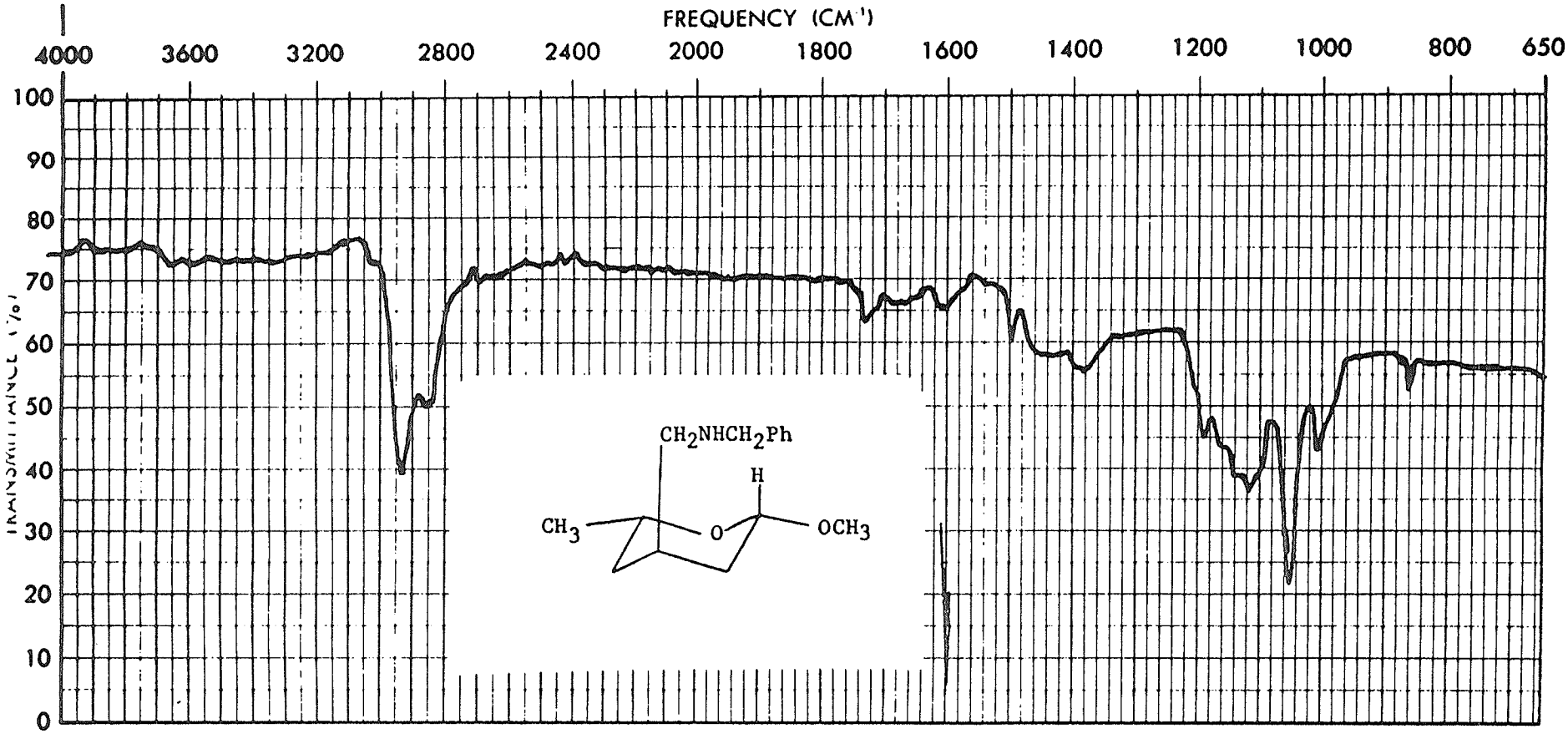


Fig. 21A I.r. Spectrum of Methyl 3-N-(benzylamino-methy)-2,3,4,6-tetradeoxy-β-DL-erythro-hexopyranose (90)

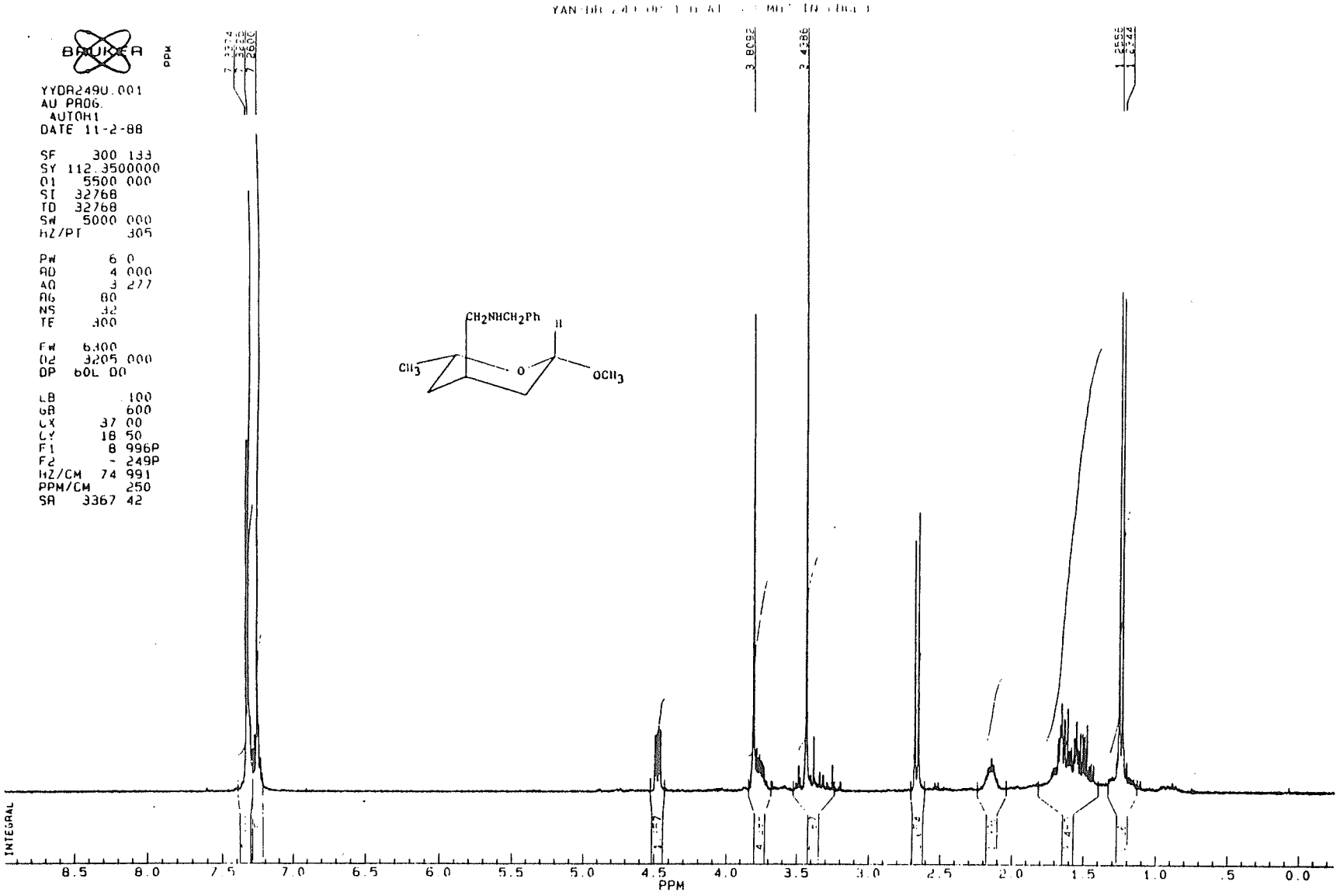


Fig. 21B **P.m.r. Spectrum of Methyl 3-N-(benzylamino-methy)-2,3,4,6-tetradeoxy-β-DL-erythro-hexopyranose (90)**

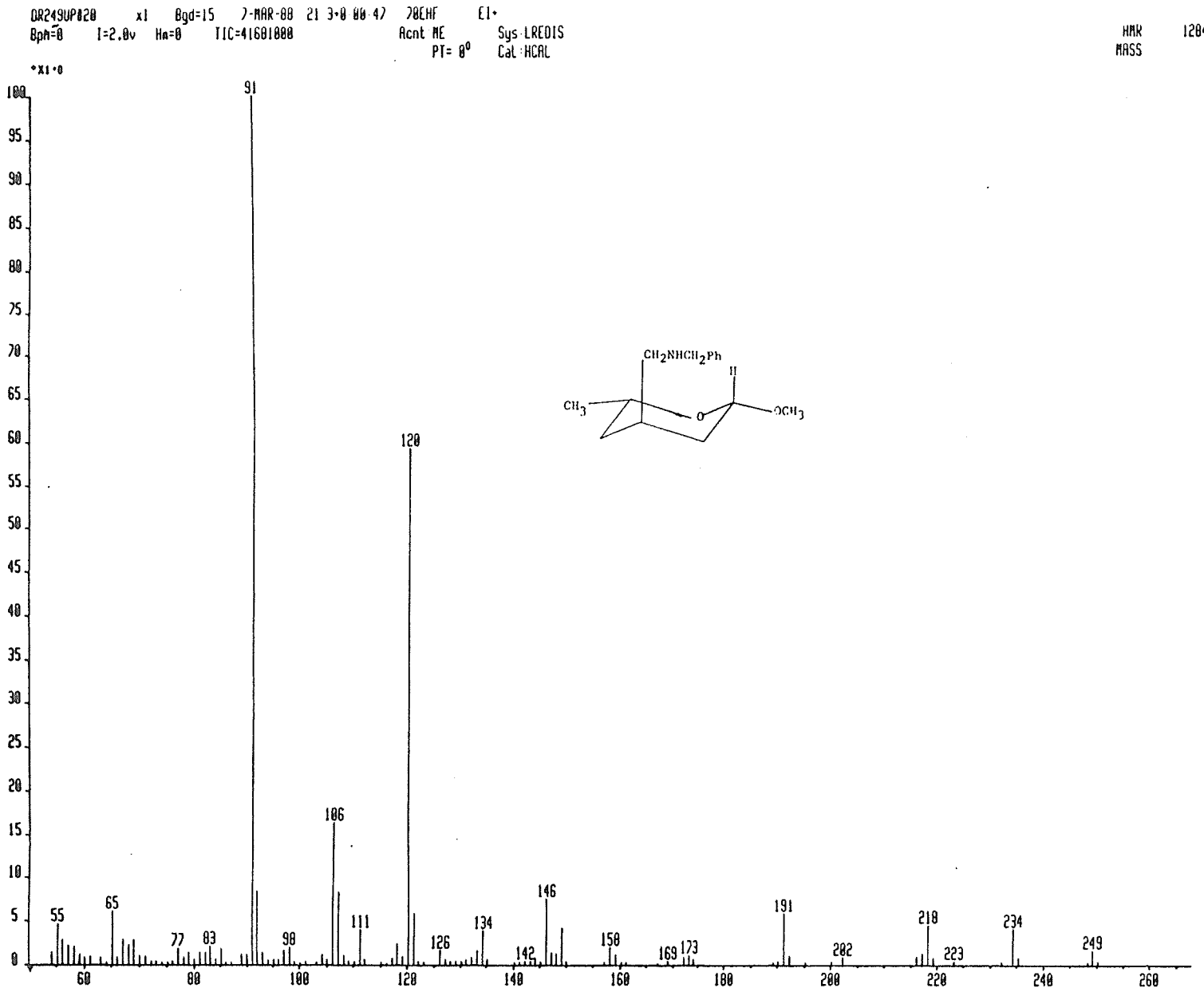
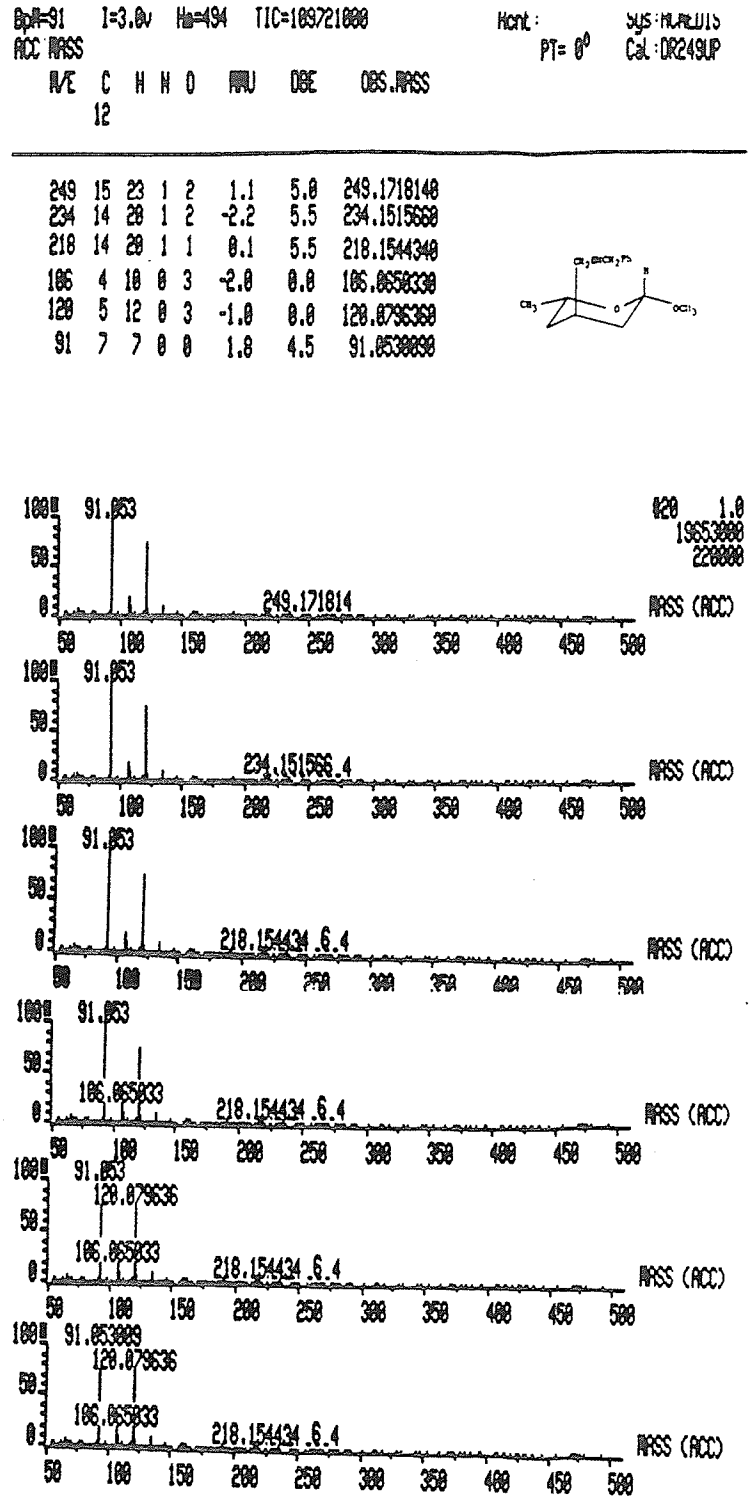


Fig. 21C Mass Spectrum of Methyl 3-N-(benzyl-carboxamido)-2,3,4,6-tetradeoxy-β-DL-erythro-hexopyranose (90)

Fig. 22D High Resolution Mass Spectrum of 3-N-(benzylamino-methyl)-2,3,4,6-tetradeoxy- β -DL-erythro-hexopyranose (90)



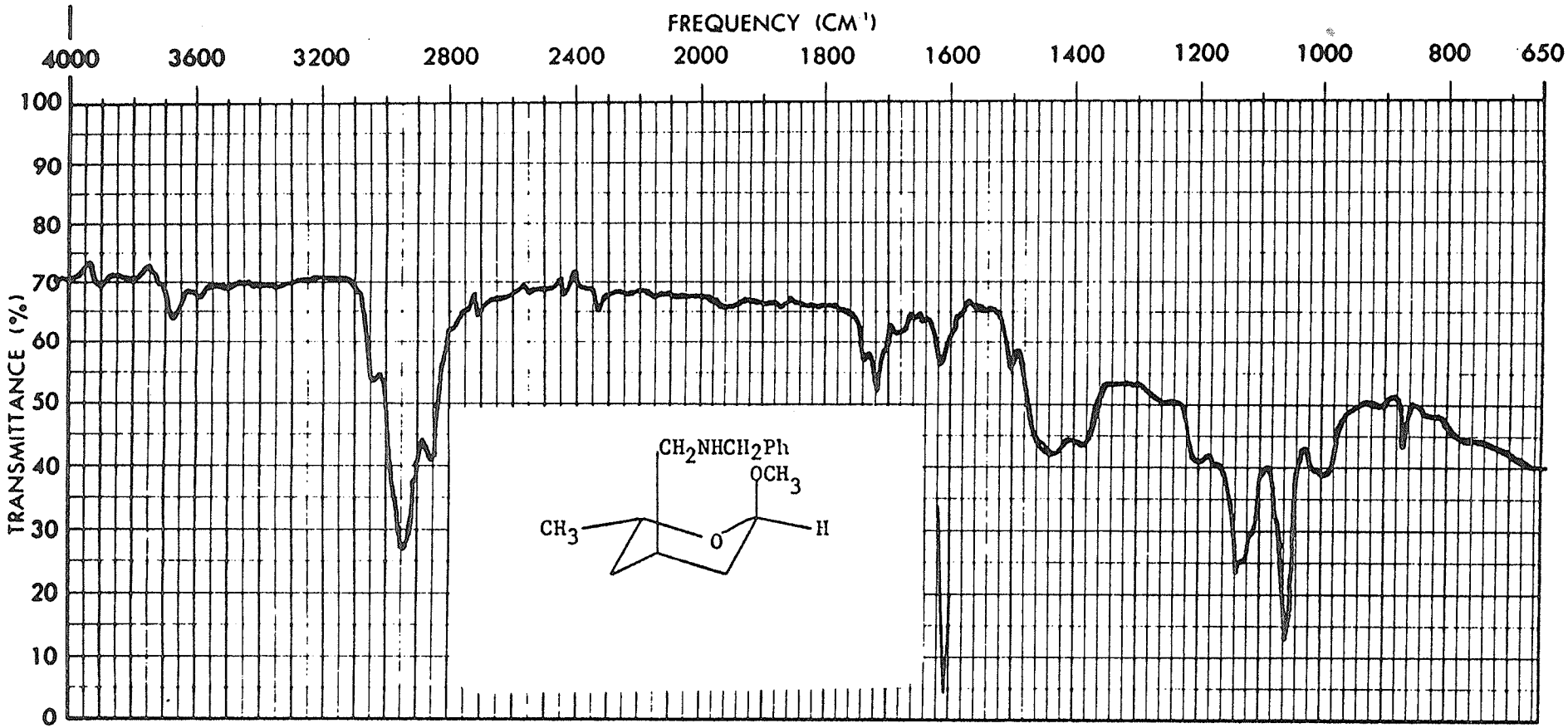


Fig. 22A I.r. Spectrum of 3-N-(benzylamino-methyl)-2,3,4,6-tetradeoxy-α-DL-erythro-hexopyranose (90)

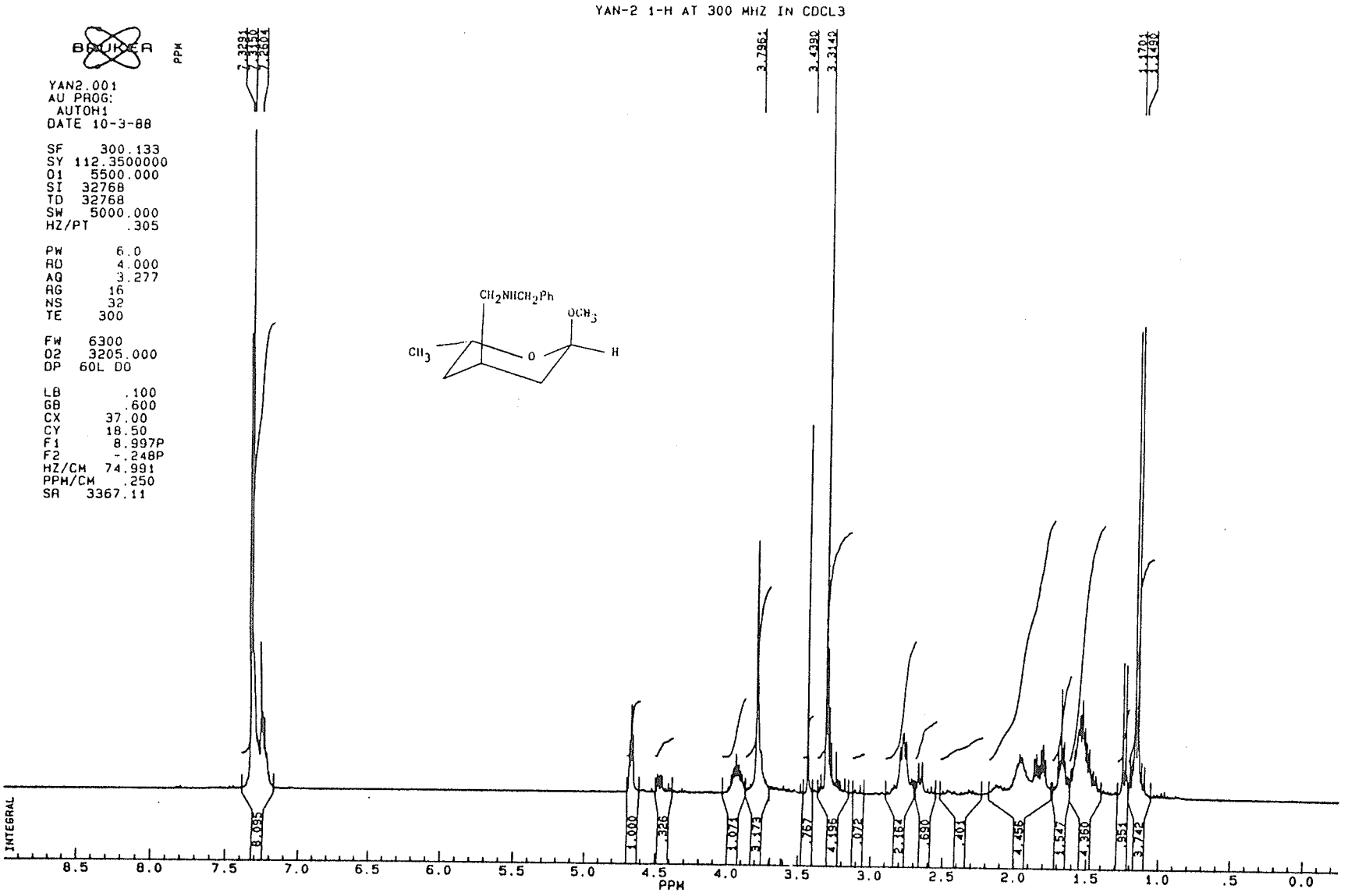


Fig. 22B P.m.r. Spectrum of 3-N-(benzylamino-methyl)-2,3,4,6-tetradeoxy-α-DL-erythro-hexopyranose (90)

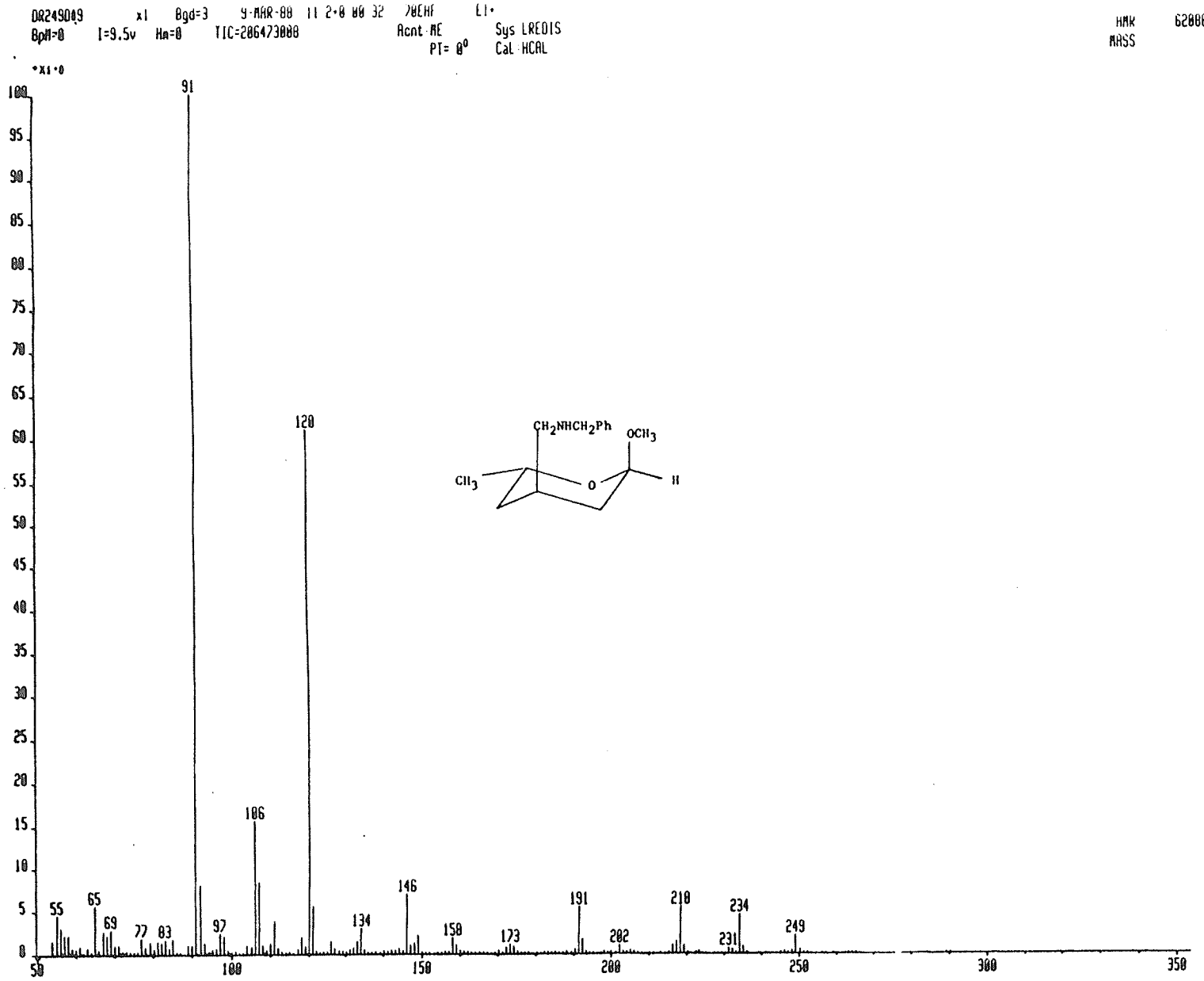


Fig. 22C Mass Spectrum of 3-N-(benzylamino-methyl)-2,3,4,6-tetradeoxy-α-DL-erythro-hexopyranose (90)

REFERENCE

(1) C.M. Wong; Yulin Yan and H-Y.P. Lam; Kangshengsu (Chinese Journal of Antibiotics), 11 (5): 398-409, (1986).

(2) a) F. Arcamone; A. Di. Marco; M. Gaettani and T. Scotti. G.; Microbiol, 83: 9 (1961).

b) H. Brockmann; "Pyrromycines and pyrromicinones. Rhodomicines and Rhodomycinones", Sostanze Natural, 33-51 (1961), Acead. Nazl. Lincei, Rome.

(3) H. Brockmann; Fortschr; Chem. Organ. Naturst., 21: 121, (1963).

(4) a) G. Cassinelli and P. Orezzi; G. Microbiol. 11: 167, (1963).

b) M. Dubost; P. Ganter; R. Maral; L. Ninet; S. Pinnert; J. Prend'Homme and G. H. Werner, C. R. Acad. Sci., 257: 1813, (1963).

c) F.Arcamone; "Doxorubicin anticancer antibiotics", Academic press., (1981).

(5) F. Arcamine; G. Cassinelli; G. Famtirri; A. Grein; P. Orezzi; C. Pol and C. Spalla; Biotechnol. Bioeng., 11: 1101, (1969).

(6) T. Oki; Y. Matsuzawa; A. Yoshimoto; K. Numata; I. Kitamura; S. Hori; A. Takamatsu; H. Umezawa; M. Ishizaka; H. Naganawa; H. Suda; M. Hamada and T. Takeuchi; J. Antibiotics, 28: 830, (1975).

(7) Pettit, G. R.; Dreerle, R. H.; Brown, P.; Brazhnikova, M. C. and Gause, G. F.; J. Am. Chem. Soc., 97 (25): 7387-7388, (1975).

(8) Lunel, J. and Preud'homme, J.; Ger. Offen. 1,911,240, (cl. Co7c, A 61k), 02, Oct., 1969, (C. A., 72: 20603p, (1970).

(9) a) R. H. Blum and S. K. Carter; ANN. Intern. Med., 80: 249, (1974).

b) E. A. Lefrak; J. Pitha; S. Rosenheim and J. A. Gottlieb; Cancer, 32: 302 (1973).

c) L. Lenaz and J. A. Pafe; Cancer Treat. Rev., 3: 111 (1976).

(10) Mcguire, W. P.; Cancer Treat. Rep. 62, 855-863, (1978).

(11) W. J. Pigram; W. Fuller and L. D. Hamilton; Nature New Bio., 235(5): 17, (1972).

(12) H. W. Moore; Science, 197: 527, (1977).

(13) a) T. R. Tritton and G. Yee; Science, 217: 248, (1982).

b) E. Goormaghtigh and J. M. Ruysschaert, Biochemica, Biophys. Acta., 271-282, 1984.

(14) Andrew, H-J. Wong; Giovanni Ughetto; Gary J. Quigley; and Alexander Rich; Biochemistry, 26, 1152-1163, (1987).

(15) F. Arcamone; G. Cassinelli; G. Francesch; R. Mondelli; P. Orezzi and S. Penco; Gazz. Chim. Ztal., 100: 49, (1979).

(16) Casazza, A. M.; Cancer Treat. Rep., 63, 835-844, (1979).

(17) a) Bachmann, E. and Zbinden, G.; Toxicol. Lett., 3, 29-34, (1979).

b) Gosalvez, M.; Blanco, M.; Hunter, J.; Miko, M.; and Chance, B.; Eur. J. Cancer, 10, 657-674, (1974).

c) Goormaghtigh, E.; Pollakis, G.; and Ruysschaert; J. M.; Biochem. Pharmacol., 32, 889-893, (1983).

d) Carlo Bianchi; Anna Bagnato; Marco G. Paggi; and Aristide Floridi; Experimental and Molecular Pathology, 46, 123-135, (1987).

(18) a) N. R. Bachur; S. L Gordon and M. V. Gee; Mol. Pharmacol., 13: 901, (1977).

b) C. Winterborn; FEBS Lett., 136: 89, (1981).

d) P. H. Hochstem. Biochem. Biophys. Res., 77: 797, (1977).

e) N. R. Bachur; Cancer Res., 38: 1945, (1978).

(19) J. Westendorf; H. Marquardt; and H. Marquardt; Cancer Research, 44, 5599-5604, (1984).

(20) Henry, D. W.; Cancer Treat. Rep., 63, 845-854, (1979).

(21) a) L. Tong; D. W. Henry and E. M. Acton; J. Med. Chem., 22: 36, (1979).

b) J. W. Lown; H-H. Chen et al.; Biochem. Pharmacol., 27: 1, (1979).

(22) C. M. Wong; W. Haque; H-Y Lam; K. Marat; E. Bock and A. Q. Mi; Can. J. Chem., 61, 1788, (1983).

(23) K. Krohb; H. H. Ostermeyer and K. Tolkien; Chem. Ber., 112: 3453, (1979).

(24) S. Neidle and G. Taylor; Biochem. Biophys. Acta, 479: 450, (1977).

(25) a) F. Acarmone; L. Bernardi; B. Patelli; P. Giardino; A.Di. Marco; A. M. Casazza; C. Soranzo and G. Pratesi; Experientia, 34: 1255, (1978).

b) A. Di. Marco; A. M. Casazza et al.; Cancer Chemother. Pharmacol., 1: 249, (1978).

(26) Boeckman, R R JR; Sum F-W.; J. Am. Chem. Soc., 104: (17), 4605-4610, (1982).

(27) Kende As; Rizzi J. P.; Tetra. Lett., 22: (19), 1779-1782, (1981).

(28) Contalone P. N; Pizzolato, G.; J. Am. Chem. Soc., 103: (14), 4251-4254, (1981).

(29) Tsung-tee Li and Yu lin Wu; J. Am. Chem. Soc., 103 (23), (1981).

(30) M. J. Broadhurst; C. H. Hassalli; J. Chem. Soc., "Perkin Trans I", 9: 2227-2238, (1982).

(31) S. Penco; A. M. Casazza, et al; Cancer Treatment Reports, 67(7-8): 665-673, (1983).

(32) S. Penco; F. Angelucci; F. Arcamone; M. Ballabio; G. Barchielli; G. Franceschi; G. Franchi; A. Suarato; and E. Vanotti; J. Org. Chem., 48(3): 405-406, (1983).

(33) C. M. Wong; Qi-Qiao Mi; Jiali Ren; W. Haque and K. Marat; Tetrahedron, 40(22): 4789-4795, (1984).

(34) F. M. Hanster and Dipakranjan Mal; J. Am. Chem. Soc., 106: 1098-1104, (1984).

(35) J. F. Honek; Michael L Mancini and Bernard Bellean; Synthetic Communications, 13(12): 977-983, (1983).

(36) Z. Abmed and M. P. Cava; Tetrahedron letters, 22(52): 5339-5242, (1981).

(37) C. M. Wong; D. Popien; R. Schwenk; and J. TeRaa; Can. J. Chem., 49, 2712, (1971).

(38) Wong, C. M.; Gordon, P. M.; Chen, A. G.; Lam, H. Y. P.; Can. J. Chem., 65(6): 1375-1379, (1987).

(39) a) F. Zunino; R. A. Gambtta; A. Dimarco; and A. Zaccarra; Biochem. Biophys. Acta, 277:489, (1972).

b) Gabbay, E. J.; Grier, D.; Fingerle, R. E. Reimer; R., Lery, R, Pearce, S. W.; and Wilson, W. D.; Biochemistry, 15: 2062-2070, (1976).

(40) a) A. Di. Marco; A. M. Casazza; R. Gambetta; R. Supino; and F.Zunino; Cancer Res., 36: 1962, (1976).

b) E. F. Fuchs; D. Horton; W. Weckerle and B. Winter; J.Antibiot., 32: 233, (1979).

(41) a) F. Arcamone; S. Penco; S. Redaclli; S. Hanessian; J. Med.Chem.,19(12): 1424-1425, (1976).

b) F. Arcamone; sergio penco; A. Vigevani; S. Redaelli; G. Franchi; A. D. Marco; A. M. Casazza; T. Dasdia; F. Formell; A. Necco; and C. Soranzo; J. Med. Chem., 3: 70, 44, (1975).

(42) a) F. Arcamone; S. Penco; S. Redaelli et al.; J. Med. Chem., 19: 1424, (1976).

b) F. Arcamone; Ade. Med. Oncol. Res. Edac., 5: 21 (1979).

(43) W. R. Bachur; Cancer Res., 38: 1954 (1978).

(44) G.Zbinden; M. Pfister and C. Holderegger; Toxicol. Lett., 1: 267, (1978).

(45) a) M. Ogawa et al.; Cancer Treat. Rep., 63: 931, (1979).

b) H. Umezawa et al.; J. Antibiot., 1978.

(46) E. F. Fuchs; D. Horton; W. Wecherle and B. Winter; J. Antibiot., 32: 203, (1979).

(47) C. M. Wong; T-L Ho and W. P. Niemczura; Can. J. Chem., 53: 3144, (1975).

(48) H-K Hung; H-Y Lam; W. Niemczuza; M-C Wang and C. M. Wong; Can. J. Chem.; 56: 638-644, (1978).

(49) a) Dyong, I.; Weimann, R.; Angew. Chem. Int. Ed. Engl. 17: 682, (1978).

b) Hauser, F. M.; Rhee, R. P.; J. Org. Chem. 46: 227, (1981).

c) Deshong, P.; Leginus, J. M.; J. Am. Chem. Soc., 105: 1686, (1983).

(50) Dyong, I.; Wiemann, R.; Chem. Ber. 113, 2666, (1980).

(51) Marsh, J. P.; Mosher, C. W.; Acton, E. M.; Goodman, L.; J. Chem. Commun., 973, (1967).

(52) D. Horton and W. Weckerle; Carbohy. Res. 44, 227-240, (1975).

(53) Fronza, G.; Fuganti, C.; Grasselli, P.; J. Chem. Soc., Chem. Commun., 442, (1980).

(54) a) Fuganti, C.; Grasselli, P.; Fantoni, P.; Tetrahedron Lett., 4017, (1981).

b) D. Horton and W. Weckerle; Carbohy. Res., 44, 227-240, (1975).

(55) J. P. Marsh; Jr. C. W. Mosher; E. M. Acton; and L. Goodman; Chem. Commun., 973-975, (1967).

(56) a) F.Acarmone; "Adriamycin-antitumor antibiotics", Academic press., 196-255 (1981).

b) A. Martin; "Mary pairs and claude Monneret." J. Chem. Soc. Chem. Commun., 6, 305-306, (1983).

c) A. Warm and P. Vogel; J. Org. Chem., 51: 1751, (1980).

d) H. H. Baer and H. R. Hanna; Can. J. Chem., 58: 1751, (1980).

e) Hassan S. Elkhadem; David L. Swartz et al.; Carbohydrate Research, 58: 230-234, (1977).

(57) P. M. Wovkulich; and M. R. Uskokovic; J. Am. Chem. Soc., 103: 3956, (1981).

(58) a) F. M. Hauser; R. P. Rhee; and S. R. Ellenberger; J. Org. Chem., 49, 2236-2240, (1984).

b) Servi, Stefano; J. Org. Chem., 50 (26), 5865-5867, (1985).

(59) F. M. Hauser and S. R. Ellenberger; J. Org. Chem., 51, 50-57, (1986).

(60) A. Warm and P. Vogel; J. Org. Chem. 51, 5348-5358, (1986).

(61) E. M. Acton; A. N. Fujjwara; D. W. Henry; J. Med. Chem., 17(6): 659-666, (1974).

(62) a) F. Arcamone; A. Bargiotti; A. D. Marco, S. Penco; Ger. Offen., 2,618,822 (cl. Co7H 15/24), 11 Nov., 1976, (C. A., 86: 140416, 1977).

b) F. Arcamone; S. Penco; S. Redaelli et al.; J. Med. Chem., 19: 1424, (1976).

c) H. Tanaka; T. Yoshioka; Y. Shimauchi; A. Yoshimoto; T. Ishikura; H. Naganana; T. Takeuchi and H. Umezawa; Tetrahedron Lett., 25(31): 3355-3358, (1984).

(63) B. A. Pearman; J. M. McNamara; I. Hasan; S. Hatakeyama; H. Sekizaki; and Y. Kishi; J. Am. Chem. Soc., 123(14): 4248-4251, (1981).

(64) Oki. T; Yoshimoto. A; Matsuzawa. Y; Takeuchi. T; Umezawa; H.; J. Antibiot., 33: 1331, (1980).

(65) a) G. Cassinelli; F. D. Matteo; S. Forenza; M. C. Ripamonti;G. Rivola; F. Arcamone; A. D. Marco; A. M. Casazza; C. Soranzo and G. Pratesi; J. Antibiot., 33: 1468, (1980).

b) A. C. Dickinson et al.; Biochem. and Biophys. Res., 125: 584 (1984).

c) F. Arcamone; Adv. Med. Oncol. Res. Educ. 521 (1979).

d) G. Mathe; M. Bayssas; J. Gouvcia et al.; Cancer Chemother. Pharmacol., 1: 259, (1978).

(66) Du Rong Sheng et al; Kangshengsu (Chinese Journal of Antibiotics), 2: 108, (1982).

(67) T. J. Gordon and C. M. Jackman; W. D. Ollis; and I. O. Sutherland; Tetrahedron Letters, 8: 28-34, (1960).

(68) a) K. Eckardt; D. Tresselt and J. Tax; Tetrahedron, 30: 3787-3791, (1974).

b) D.Tresselt; K.Eckardt and J.Tax; Tetrahedron, 31: 613-617, (1975).

(69) P. Deslongchamps; "Stereoelectronic Effects in Organic Chemistry", (Organic Chemistry Series, vol. 1, Editor: J. E. Baldwin), page 5, 720, (1983).

(70) T. H. Smith; A. W. Fujiware; W. W. Lee; Helen Y. Wu and D. W. Henry; J. Org. Chem., 42: (23), 3653-3660, (1977).

(71) a) T. Oki; Y. Matsuawa; A. Yoshimoto; K. Numata; I. Kitamura; S. Hori; A. Takamatsu; H. Umezawa; M. Ishizuka; H. Naganawa; H. Suda; M. Hamada; and T. Takenchi; J.Antibiot., 28: 830, (1975).

b) T. Oki; I. Kitamura; A. Yoshimoto; Y. Matsuzawa; N. Shibamoto; T. Ogasanra; T. Inui; A. Takamatsu; T. Takeuchi; T. Masuda; M. Hamada; H. suda; M. Ishizuka; T. Sawa; and H. Umezawa; J. Antibiot., 32: 791 (1979).

(72) F. Arcamone and S. Penco; Cancer Chemother. Rep., 6: part 3, 123, (1975).

(73) M. Dejter-Juszynski and H. M. Flowers; Carbohydr. Res., 28: 61, (1973).

(74) M. Dejter-Juszynski and H. M. Flowers; Carbohydr. Res., 23: 41, (1972).

(75) E. M. Acton; A. N. Fajiware; and D. W. Henry; J. Med. Chem., 17: 659, (1974).

(76) F. Arcamone; G. Cassinelli; G. Franceschi; P. Orezzi; R. Mondelli; Tetrahedron Lett., 30: 3353, (1968).

(77) a) R. G. Pearson and J. Songstad; J. Am. Chem. Soc., 89:8, 1827-1836, (1967).

b) R. G. Pearson; J. Chem. Education. 45(9), 581-587, (1968).

c) S. Ahrland; Chemical Physics Letters, 2 (5) 303-306, (1968).

d) S. Salille; Angew. Chem. Internat. Edit. 6(11), 365-376, (1967).

(78) G.Kotowyzcz; T. Schaefer; and E. Bock; Can. J. Chem., 42, 3541, (1964).

(79) J. A. Pople; Mol. Phys., 1: 168, (1958).

(80) a)Eberhard Breitmaier and Gerhard Baiier; ^{13}C NMR Spectroscopy: A Working Manual with Exercise, pp 56, 315, (1984).

b) E. Breitmaier and W. Voelter; ^{13}C NMR Spectroscopy: Methods and Application, Chapter 4, Chapter 5, 223-234, (1974).

(81) K. Nakanishi; "Infrared Absorption Spectroscopy", Namkodo Company Limited, page 43, (1962).

(82) S. Mizushima; J. Chem. Phys., 21: 815, (1953).

(83) a) M. Bodanszky and M. A. Ondetti; Peptide Synthesis, Interscience, New York, 1966.

b) Jerry March; Advanced Organic Chemistry, Third Edition, p. 1987-188, 669, (1985).

(84) J. B. Lambert; H. F. Shurvell; L. Verbit; R. G. Cooks; and G. H. Stout; Organic Structural Analysis, (Collier Macmillan Publishers), page, 41-43, (1976).

(85) S. W. Tobey; J. Org. Chem., 34, 1281, (1969).

(86) S. J. Angyal, V. a. Pickles and R. Ahluwalia; Carbohydrate Res., 3, 300, (1967).

(87) a) A. Gaudemer; "Determination of configurations by NMR Spectroscopy", Stereochemistry: Fundamental and Methods, Vol. 1, (Edited by Henri B. Kagan), page 102, (1977).

b) J. F. Stoddart; " Stereochemistry of Carbohydrates", page 129-136, (1971).

(88) A. Gaudemer; "Determination of configurations by NMR Spectroscopy", Stereochemistry: Fundamental and Methods, Vol. 1, (Edited by Henri B. Kagan), p. 118-121, (1977).

(89) a) Alan Bassindale; The THird Dimension In Organic Chemistry, Chapter 14, (1984).

b) A. Gaudemer; "Determination of configurations by NMR Spectroscopy", Stereochemistry: Fundamental and Methods, Vol. 1, (Edited by Henri B. Kagan), p. 118-121, (1977).

(90) K. Umezawa et al; Cancer Res. 38, 1782, (1978).