"VICTOR BABEȘ" UNIVERSITY OF MEDICINE AND PHARMACY FROM TIMISOARA

FACULTY OF MEDICINE

Department IV: Biochemistry and Pharmacology

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PHD THESIS

BIOACTIVE COMPOUNDS, STRUCTURAL ANALYSIS AND STUDY OF ACTIVITY-STRUCTURE RELATIONSHIP

– A B S T R A C T –

Scientific Coordinator:

PROF. UNIV. DR. ȘIȘU EUGEN

T i m i ș o a r a
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This doctoral thesis falls within the modern directions of research in medicinal chemistry, harmoniously intertwining the concerns for theoretical problems with those regarding the exploitation of analytical techniques for investigating the structure of bio-conjugates and biomacromolecules. Thus, in this way are used both the facilities afforded by the performant programs of the *in silico* medicinal chemistry as well as the exceptional endowment with large-scale means of "omics" investigations. The work is structured according to the following plan:

General part includes general aspects related to:

1) Elements of bioinformatics
2) Glycomics
3) Lipidomics with a particular accent on literature data ilustrating the concern for a problem of great practical interest, analytical and theoretical – the cis-trans isomers of natural fatty acids
4) Mass spectrometry

Special part, that summs up the original contributions of the experimental approach, is comprised of two large sub-chapters:

A) Computational chemistry, in the organic structural analysis and the study of the structure-activity relationship, includes three directions of investigation pertaining to the *in silico* medicinal chemistry as follows:

I) QSAR study of a series of camptothecin derivatives
II) Method of identification of cis and trans configurations in bioactive lipids using quantum chemical calculations and QSFR
III) The use of semi-empirical quantum chemistry and of ab initio type methods for the creation of databases with fragmentation ions and the use of QSFR to identify the mass spectra of some isomeric diacetalized monosaccharides

B) The role and contribution of mass spectrometry in the analysis of compounds with biological significance that fall withing the directions of omics type research (glycomics in our case) and in turn includes three directions of investigation as follows:

I) Synthesis and analysis using MALDI-TOF of a maltodextrin functionalized at the reducing end
II) Unexpected behaviour in mass spectrometric analysis of trisilanolisobutyl-POS S, a key compound for the release of active medicinal ingredients
III) A preliminary study of the isolation, separation, purification and characterization by MALDI-TOF mass spectometry of the neutral polysaccharides from *Fomes fomentarius*

Thesis identification elements:
General part + Special part+ Concluzions + References = 121 pag.
Nr. of Tables (Thesis +Annexes)= 37
Nr. of Figures (Thesis +Annexes) = 58
Nr. published and attached papers = 6
Nr.of papers from references = 189

The general part begins with the presentation of some bioinformatics elements, this being the area of biology that focuses on the use of computer based methods to study the biological systems that could offer some precise predictions for the laboratory and clinical experiments.

The *in silico* medicinal chemstry methods include databases, quantitative structure-activity relations, pharmacophores generation, homology methods and other molecular modeling approaches, automatic learning, data extraction, network analysis instruments and instrumental analysis of data that use a computer. It covers the following areas of use on a large scale of equipments and computing software:

Chemoinformatics (also known as cheminformatics)
Molecular modeling and
Computational chemistry

The latter covers the following subfields: molecular modeling, similarity analysis, virtual screening, cluster analysis, de novo design and QSAR. In QSAR the aim is to corelate the molecular structure with the known biological properties and to build a statistical model. The advantage of such QSAR model is that it can be applied, with care and caution, to predict the action of molecules that have not been tested and not even synthesised !!. This allows that vast virtual libraries to be analysed and prioritised to enable the focus on those molecules with the highest chance of succeeding. Glycomics is presented in relation to its most ardent aspects, the major objective being the complete characterisation of the carbohydrate structure sets present in a cell or organism defined by the expresion and specificity of carbohydrate-modifying enzymes such as glycosyltransferases, glycosidases and transglycosidases. The chips with carbohydrates (glicoarrays) have recently appeared and are a powerful instrument for the study of carbohydrate binding proteins and their processing enzymes. The latter being strongly responsible for the co and posttranslational modifications, more than 50% of proteins being in fact glycoproteins.

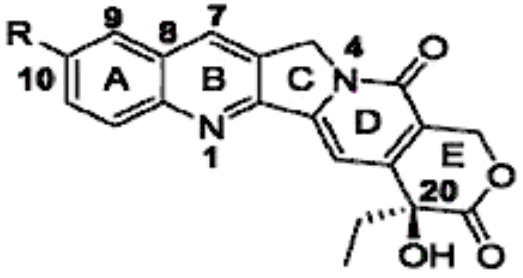
„Omics" represents a set of complex technologies used for the study of the roles, relations and activities of the different types of molecules that make up the cells of an organism. Mass spectrometry (MS) is an essential analytical technology on wich are developed and based „omics" areas as well as genomics, transcriptomics, proteomics and metabolomics (in the case of the latter with targted addressing: glycomics and lipidomics). This technique allows the identification of thousands of protein compounds or active biological compounds, metabolites in extremely low quantity form femto to atto molar.

The lipidomics part is treated more extensively regarding the detection by instrumental analysis of the corect ratio of cis vs. trans bond. The use of mass spectrometry in the study of artificial or natural glycans, concern that fall within glycomics is also widely ilustrated.

The special part contains two large subchapters, the first dedicated to

(A) Computational chemistry in the organic structural analysis and the study of structure-activity relationship and the second focused on (B) The role and contribution of mass spectrometry in the analysis of the structure of copounds with biological significance.

(A1) *QSAR study of a series of camptothecin derivatives*



Camptothecin (CTP) is a pentacyclic alkaloid isolated from the chinese tree "*Camptotheca acumunata"*. The design of some chemical modification on the A and B cycles in the positions 7 and 10 and the estimation of eficacy calls for the QSAR methods. Hansch has obtained the best QSAR model with the help of molecular descriptors that quantify the hydrophobicity of the π_X and π_Y substituents (hydrophobic parameter of the substituent). The molecular descriptors used in the research were supplied by the DRAGON program. Over 1000 molecular descriptors were tested and 37 were retained, the vast majority being of GETAWAY or 3D-MoRSE type. The best results were obtained in bi-parameter correlations with the indices: ***HATS, H* and *R*,** the second parameter being always the πY substituent constant. An ordering of the descriptors according to the decreasing value of the determination coeficient leads to the retention of the first 4 values for which the determiation coeficient is >0.9 and to the construction of the following correlation equations (7)-(10). The predicting ability of the 4 models was tested with the help of cross validation coeficients and the results were systematized in Table 2.4 [97].

$$A = log\,1/EC_{50} = -47{,}75(\pm 9.09)HATS7v - 1{,}46(\pm 0{,}15)\pi_Y + 10.19(\pm 0{,}89)$$
$$(n = 14, r^2 = 0{,}916, q^2 = 0{,}864, F = 59{,}69, s = 0.176) \quad (7)$$

$$A = log\,1/EC_{50} = -5{,}72(\pm 1{,}20)H1v - 1{,}33(\pm 0{,}16)\pi_Y + 12.49(\pm 1.46)$$
$$(n = 14, r^2 = 0{,}904, q^2 = 0{,}855, F = 51{,}66, s = 0{,}188) \quad (8)$$

$$A = log\,1/EC_{50} = -8{,}18(\pm 1{,}74)R2p - 1{,}33(\pm 0{,}16)\pi_Y + 12{,}64(\pm 1.52)$$
$$(n = 14, r^2 = 0{,}902, q^2 = 0{,}852, F = 50{,}36, s = 0.190) \quad (9)$$

$$A = log\,1/EC_{50} = -4{,}60(\pm 0{,}97)H1p - 1{,}30(\pm 0{,}16)\pi_Y + 11{,}50(\pm 1.26)$$
$$(n = 14, r^2 = 0{,}903, q^2 = 0{,}850, F = 51{,}16, s = 0.188) \quad (10)$$

Table 2.4. The results obtained following the testing of prediction ability of the 4 QSAR models (7) – (10) through internal validation according to the algorithms implemented in the MobyDigs program

Modelul QSAR	q^2_{boot}	r^2_{Y-s}	q^2_{Y-s}	$r^2_{adj}(EV)$	SDEP	SDEC
2	**0,763**	**0.057**	**-0.537**	**0,900**	**0.198**	**0.156**
3	**0,789**	**0.155**	**-0.373**	**0,886**	**0.204**	**0.166**
4	**0,829**	**0.144**	**-0.396**	**0,884**	**0.207**	**0.168**
5	**0,710**	**0.274**	**-0.177**	**0,885**	**0.207**	**0.167**

2) The new QSAR model strenghten the significance of the π_Y descriptor and its influence on the biological activity of binding to topo I from the cleavable complex with DNA

3) Instead of the π_X descriptor that quantifies the hydrophobic effect of the X substituent, the predictor variables used in our QSAR (HATS7v H1v, R2p and H1p) models, are proposed that lead to the (7) – (10) equations whose prediction coeficients (leave one out) have the greatest values (0.864-0.850).

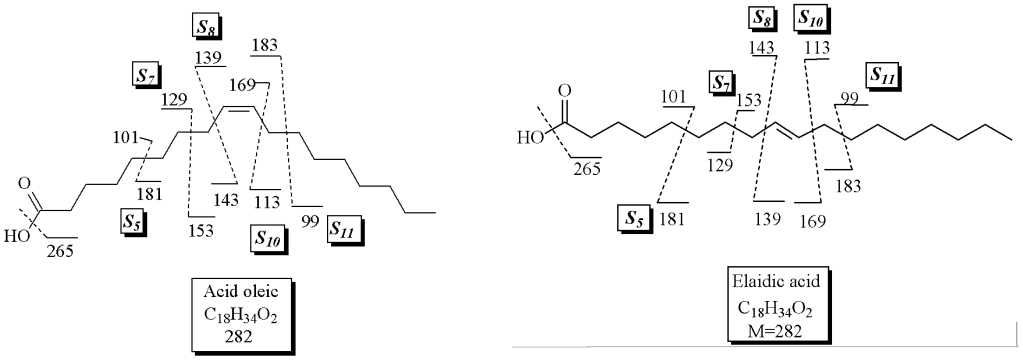
4) The correlation capacity and predictive ability of the proposed 4 QSAR models (eq. 7 – 10) was statistically validated. Finally, it can be concluded that vdW volume and the polarizability of CTP analogues are important factors that intervene during the biological

interaction CTP derivative and topo I from the cleavable (reversible) with the DNA of cancer cells;

5) The correlations between experimental biological activity (taken from the literature) and molecular descriptors was done with the help of MobyDigs program. Following the carried out investigations, models of a higher statistical quality that of the model developed by Verma and Hansch [97] were proposed.

(**A2**) *Method of indentifying cis and trans configurations in bioactive lipids using mass-energy profiles.*

European Food Safety Authority (EFSA) showed that the risk of death due to heart diseases increases by 20-30% if over 2% of the total daily diet is provided by the *trans* fats. Considering the most trivial case of oleic acid (and its elaidic isomer) massively present in edible animal and vegetable fats, we realise the the **NIST Chemistry WebBook SRD 69** shows the EI-MS spectra with quasi identical fragmentation patterns and with a high grade of similarity !!!



Starting from this observation, we raised the problem if the relativity of the intensity of some primary ions selected from the spectrum, contains decodable structural information regarding C=C configuration and tried to answer by approaching the QSFR technique. In this approach, the quantum chemically calculated mass-energy fragmentation profiles are compared with the coresponding mass-intensity profile obtained from the experimental spectrum of the analyte. The true structure is considered to be the one that gives the highest matching score !!! Because there are no unified entalpy databases, these were calculated using the RM1 quatum semiempirical method using RHF operators for molecules or ions and UHF for radicals or radical ions, accesible through the HyperChem programs package. The fragmentation entalpy ($\Delta_f H_{frag}$) of the molecule to obtain each of these ions was calculated using the RM1 semiempirical method and the equation:

$\Delta fHfrag = \Delta fH(Ii+) + \Sigma\Delta fH(Fi) - \Delta fH(M)$

Where: $\Delta_f H(I_i+)$ is the formation entalpy of the respective primery ion, $\Sigma\Delta_f H(F_i)$ is the sum of the formation entalpies of fragments which rezult collaterally with the primary ion and $\Delta_f H(M)$ is the molar formation entalpy of the candidate structure. The matching score (P) of the candidate structures profile with the experimental profile was calculated with MS Excel using the following equation wher the liniar correlation coeficient R appears:

$$P\ (\%) = 100(1-R)/2$$

Table 2.7 shows the intensities of the primary ions from the spectra of chemical standards (columns E-M), the resulting matching scores for the oleic acid (row 17) and for the elaidic acid (row 18).

Table 2.7. MS Excel page for the calculation of the matching score of mass-energy profiles for oleic and elaidic acids with the standard mass spectra

E17 =100*(1-CORREL(E4:E16,$C4:$C16))/2

	$\Delta_f H_{frag}$ (kcal/mol)			Curenti ionici (unitati arbitrare)								
	Acid elaidic	Acid oleic	MS ions	#133071: 9-Octadecenoic acid (Z)-(CAS) $$ Oleic acid	#133072: 9-Octadecenoic acid (Z)-(CAS) $$ Oleic acid	#133073: 9-Octadecenoic acid (Z)-(CAS) $$ Oleic acid	#133074: 9-Octadecenoic acid (Z)-(CAS) $$ Oleic acid	#133075: 9-Octadecenoic acid (Z)-(CAS) $$ Oleic acid	#133076: 9-Octadecenoic acid (Z)-(CAS) $$ Oleic acid	#133077: 9-Octadecenoic acid (Z)-(CAS) $$ Oleic acid	#133078: 9-Octadecenoic acid (Z)-(CAS) $$ Oleic acid	#133080: 9-Octadecenoic acid (Z)-(CAS) $$ Oleic acid
4	244.8	245.4	**99**	800	590	268						340
5	259	258.8	**101**	900	870	357						430
6	270.4	270.5	**113**	500	410							210
7	246.6	247.2	**129**	2900	1190				1140			370
8	269.6	268.3	**139**	300	400	268	490					230
9	271.7	271.9	**143**	700	210							100
10	232.2	234.3	**153**	200	200		240					140
11	270.4	270.5	**169**		70							60
12	254.3	254.3	**181**	100	50							50
13	233	235.2	**183**		20							30
14	229.3	229.2	**264**		410	1339	1120	300	420	864	1900	300
15	264.7	264.7	**265**		100	268	220	100	82	169	372	100
16	199.8	201.5	**282**		50	268	160	40	60	62	500	50
17	**SCOR (%) Acid oleic →**			**59.5**	**44.4**	**63.9**	**49.4**	**42.5**	**36.8**	**47.4**	**57.3**	**45.4**
18	**SCOR (%) Acid elaidic →**			59.3	43.9	63.2	48.2	41.6	**36.8**	46.5	56.4	44.7
19	Amprente masa-energie											
20						Spectre de masa						

The matching scores are higher in the case of oleic acid (highlighted in green in Table 2.7) for 8 of the 9 standards of oleic acid.In conclusion:

1)The relativity of the primary ions intensities from the mass spectra of fatty acids contains structural information regarding *cis – trans* configuration of C=C bond from the fatty acids.

2) The mass-energy fragmentation profiles calculated by semiempirical methods (RM1) can serve to decode this structural information generated by the diferent configuration of the C=C bond [102].

(**A3**) *Fragmentation energy-mass profiles calculated for a series of acetalized monosaccharides for the purpose of their identification by mass spectrometry*

The QSFR method was successfully applied for the discrimination of 10 di-isopropylidene stereoisomers: DAG(1), DAM(2), DAGal(3), DAF(4), DAS(5), DAAlo(6), DAF_spirane(7), DAM-beta(9), DAGal_furane(8), DAS-spirane(10), going through the following stages: a) establishing the candidate structures and calculation of mass-energy profiles for them; b) recording the mass spectrum of di-O-isopropylidene derivative standard; c) optimisation of the geometry of the anaysed structures by: molecular mechanics (MM+), semiempirical calculation (RM1 and PM7) and of *ab initio* (DFT) type; d) calculation of fragmentation entalpies (ΔfH) (**RM1,PM7,DFT**) and Gibbs fragmentation energies (ΔfG)(**DFT**); e) method validation by correctly establishing the structure of the standard. Each mass-energy experimental profile of the standard will be compared with the ten calculated profiles. The maximum match should be obtained for the true structure if the calculated energy corectly describes the fragmentation. The calculation were performed only for **primary ions**. The formation of these ions occurs with a minimum energy consumption because they result by breaking of **a single bond** and possibly the ellimination of a small molecule (Table 2.8).

Table 2.8. For the mass-energy profiles of 1-10 isomers, seven ions were considered that are formed by the loss of some molecules and radicals from the molecular ion

Ion	Fragmentation
m/z 245	[M-CH3]+
m/z 229	[M-CH2OH]+
m/z 187	[M-acetone-CH3]+
m/z 171	[M - acetone - CH2OH]+
m/z 159	[M- dimethyl dioxolane]
m/z 127	[M – 2*acetone - OH]+
m/z 101	[Dimethyl dioxolane ring]

For the fragmentation of molecule M, the fragmentation entalpies (ΔfHfrag) were calculated with the relation:

$$\Delta fHfrag = \Delta fH\,(Ii+) + \Sigma\Delta fH\,(Fi) - \Delta fH\,(M)\ (1)\ \text{for}\ \Delta fGfrag\ (2)$$

Where: $\Sigma\Delta fH$ (F*i*) is the sum of the formation entalpies of the fragments lost by the molecule, ΔfH (I*i*+) is the formation entalpy of the resulting primary ion and ΔfH (M) is the molecular formation entalpy of the candidate structure. Similarly ΔfGfrag is evaluated.

The quantum calculation of Gibbs entalpies and energies used three methods: **RM1,PM7, DFT** both for ΔH and ΔG. The following databases are obtained (for ΔH si ΔG):

Table 2.10. Δ_fH (kcal/mol) database calculated with RM1 and Ec. (1)
Table 2.11. Δ_fH (kcal/mol) database calculated with PM7 and Ec. (1)
Table 2.12. Δ_fH (kcal/mol) database calculated with DFT (B3LYP/6-31G) and Ec. (1)
Table 2.13. Δ_fG (kcal/mol) database calculated with DFT (B3LYP/6-31G) and Ec. (2)

The validation of identification accuracy of some di-O-isopropylidene monosaccharides isomers was done usind the DAS (diacetone-l-sorbose). We used the fragmentation entalpies (ΔfH) and Gibbs energies (ΔfG) as the fragmentation energy descriptors and the equation for the calculation of the profiles matching score:

$$P\ (\%) = 100(1-R)/2$$

The optimization was done according to the three quantum chemical calculations methods: RM1, PM7, DFTΔH and DFTΔG. The mass spectrum of the DAS standard at 70 eV was previously determined [116]. The calculation of the matching score was performed using MS Excel. Only the three common primary ions (m/z 245, m/z 187 and m/z 127) of the candidate structures **1-10** were used. The values of the fragmentation energies were extracted from the calculated database shown above. The true structure indicated by the method is the one that corresponds to the maximum score. Thus it was calculated:

Table 2.22. Excel page for obtaining the mass-energy matching scores quantum chemically calculated with the RM1 method
Table 2.23. Excel page for obtaining the mass-energy matching scores quantum chemically calculated with the PM7 method
Table 2.24. Excel page for obtaining the mass-energy matching scores quantum chemically calculated with the DFT method (for Δ_fH)
Table 2.25. Excel page for obtaining the mass-energy matching scores quantum chemically calculated with the RM1 method (for Δ_fG)

In the end the 4 lists were combined obtaining the list of probabilities resulting from the matching of the quantum calculated mass-energy profiles for the ten candidate structures, with the experimental profile of the analyte (DAS standard (5))

Table 2.26. Lists of probabilities resulted from the matching of quantum calculated mass-energy profiles for the ten candidate structures with the experimental profile of the analyte (the DAS standard having the structure (5)). The corect results are highlighted in green. The false positive result is highlighted in red.

Metoda →	RM1		PM7		DFT $\Delta_f H$		DFT $\Delta_f G$	
Locul in clasament ↓	Structura	P(%)	Structura	P(%)	Structura	P(%)	Structura	P(%)
1	**5**	**92,1**	**7**	**92,2**	**5**	**94,8**	**5**	**95,4**
2	**10**	89,9	**8**	91,7	**10**	91,0	**9**	92,6
3	**2**	83,4	**5**	86,7	**9**	89,4	**10**	92,5
4	**7**	82,5	**10**	85,7	**2**	87,7	**6**	91,3
5	**3**	82,5	**9**	85,4	**6**	85,5	**2**	91,3
6	**6**	82,3	**2**	82,5	**7**	82,4	**7**	87,0
7	**4**	82,1	**1**	80,9	**8**	80,4	**8**	86,0
8	**1**	80,7	**4**	80,2	**4**	79,7	**4**	85,1
9	**9**	80,7	**6**	79,9	**3**	76,7	**3**	84,3
10	**8**	79,4	**3**	78,4	**1**	76,4	**1**	83,7
Interval P(%)		12,6		13,8		18,4		11,7
Diferența locurile 1-2 (%)		**2,2**		**0,5**		**3,8**		**2,8**

The accuracy (P value of the true structure) increases in the following order: RM1 < DFTDH < DFTDG.

The selectivity (P difference between places 1 and 2) increases in the following order: RM1 < DFTDG < DFTDH.

Three primary ions with their intensities, present in all ten possible energy profiles **were enough** to differentiate the DAS structure from the other nine candidate structures [83].

In conclusion:

The experimental study conducted in this thesis demonstrates that the mass-energy profiles obtained by quantum calculations can highlight modifications of the fragmentation intensity due to the different configuration of the asymetric carbon atom or of the double bond configuration from the chemical structure allowing the following conclusions:

1) the databases containing the mass-energy profiles can be utilised for the optimisation of energy descriptors and of the quantum calculation that ensures the correct identification of isomers and also being reusable.

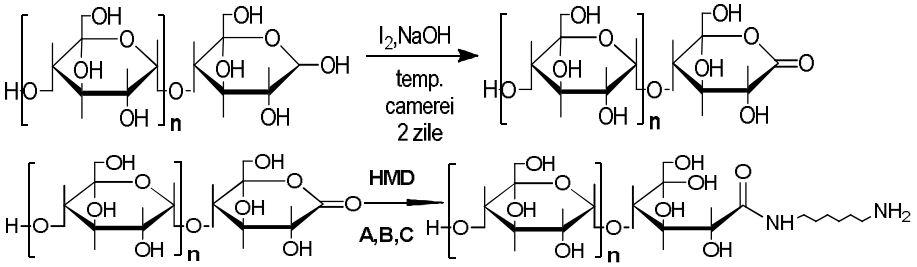
2) is using data from the mass spectrum that the discrimination of isomeric chemical structures cannot use: common ions from the spectra of candidate structure and of analyte;

3) does not require dedicated hardware only the usual one (computers + QC programs); the mass spectra that are useful for the analysis are the ones obtained from the separation units of the analytical system: chromatography, electrophoresis, ion mobility, mass separator;

4) can be optimized and validated with standard mass spectra from libraries because the method decodes analytical informations that are not instrument dependent [83].

(**B1**) *The synthesys and analysis using MALDI-TOF mass spectrometry of a maltodextrin functionalized at the reducing end*

Amphiphilic block copolymers of a A-B type form a variety of structures consisting of self assembled aggregates which in the right solvents solvate preferentially one of the blocks. Starting from a medium molecular mass glycan, this was modified at the reducing end by oxidation obtaining the coresponding lactone that by aminolysis with HMD leads to an aminoglycan according to the reaction sequence below:



Both the lactone (Dextrinlactone 10) and the aminoglycan (Dexamid 10) were characterized by IR spectroscopy and MALDI-TOF mass spectrometry. In the end are presented thermal analysis experiments performed on the glycan, lactone and aminoglycan with the purpose of demonstrating the formation of the aminoglycan without resorting to spectroscopic arguments [144]. In conclusion:

1.In the process of obtaining Dextrinlactone 10 and Dexamid 10, the working protocols were modified leading to economical syntheses without affecting the yield.

2. Dextrinlactone 10 was structurally characterized by MALDI-TOF spectrometry thus establishing the measurement conditions and proceeding to the complete assignment of all the peaks in the spectrum. This is the first MS analysis dedicated to a lactone and communicated in the literature. It was found that the fingerprint ions that characterize the product are of: (a) high intensity - monosodium ions $[Glc\text{-}Ln + Na]^+$ (b) medium intensity – monopotassium ions $[Glc\text{-}Ln + K]^+$ (c) low intensity the monoprotonated ones that in general are presented under the multiple dehydrated form $[Glc\text{-}Ln + H]^+$- 3,4,5H2O.

3. The first analysis by MALDI-TOF-MS a glycan with an amide bond (Dexamid 10) that shows the exhaustive interpretation of the spectrum. The characteristic is present at appreciable intensities of the monosodium ions, flanked by the monopotassium and monoprotonated ions (the latter at small intensities and only under the double and triple dehydrated form).

4.Thermal analysis was used for the first time to demonstrate the formation of the aminoglycan, DEXAMID 10 by comparing the thermograms (TG, DTA, DTG) of the obtained product with the thermograms of the reactants and their physical blend.

(**B2**)*Unexpected behaviour in mass spectrometric analysis of trisilanolisobutyl-POSS, a key compound for the release of active medicinal ingredients*

During recent progress in nanomedicine, POSS derivatives have proved their usefulness due to their high degree of biocompatibility when used in grafts, advanced DDS, biological sensors etc. During derivatization trials of trisilanolisobutyl POSS (none of them successful), the analysis of the reactants and reaction products by (+) ESI-OT-MS lead to a fragmentation experiment in multiple stages by CID. There are only three mentions in the literature ilustrating a MS1-MS10 multiple fragmentation sequence. The analysis of trisilanolisobutyl POSS by (+) ESI-OT-MS is a premiere in the organosilicon compounds class occupying the 4th place in the all-time classification of the MS tandem analysis.

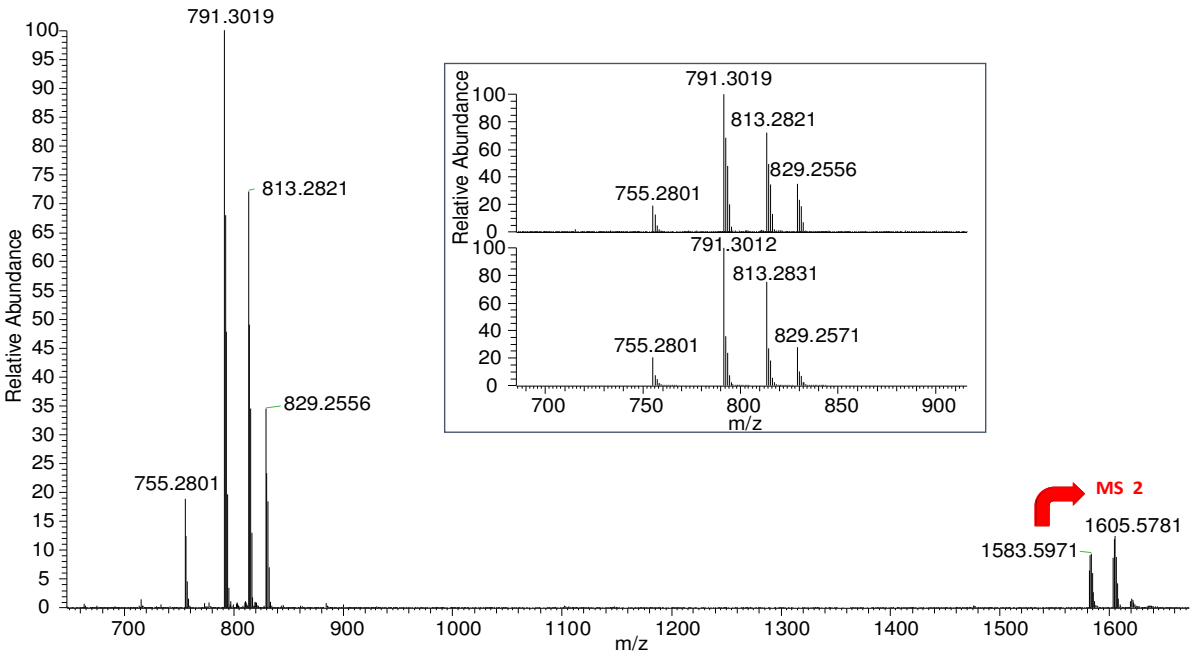


Figure 3.14.A (+)ESI-OT-HRMS spectrum of trisilanolisobutyl-POSS ($Si_7O_{12}C_{28}H_{66}$). The inset shows the pseudomolecular ions $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ region – experimental (above) and calculated (below).

Figure 3.14.A shows the MS^1 spectrum of trisilanolisobutyl-POSS ($Si_7O_{12}C_{28}H_{66}$), the dimer monoprotonated ion (pseudomolecular) being isolated and fragmentd through CID to obtain the MS^2 spectrum. In the carefully established working conditions, the fragmentation experiment was conducted 8 more times. All spectra are presented in detail and the peak asignment is complete. In conclusion:

1.In the MS^1-MS^{10} experiments, the asignment of the structure of the fragmentation ions lead, depending on the signal intensity, to 3 major ion series that have as a starting point the doubly dehydrated molecular ion. It seems that that innitial experiment created a suficiently stable structure to be able to observe later the step by step loss of a residue of: a) i-butene (ions of higher intensity) b) of a one or more residues of propene and i-butene (ions of medium intensity) c) of two isobutyl radicals followed by the loss of a propene and isobutene molecule.

2.For the generation of propene molecules (that are eliminated by producing medium intensity fragmentation ions) from the isobutyl radicals, two McLafferty transposition [161] alternatives were proposed.

3. Regardless of the working conditions that have been tried, no loss of isobutene or propene from the molecular or pseudomolecular ions was observed before the precursor ion is doubly dehydrated. No monodehydrated ions were observed.

4.In the end the complete surgical „decarbonization” (Figure 3.15) of the open cage trisilanolisobutyl POSS was achieved [161].

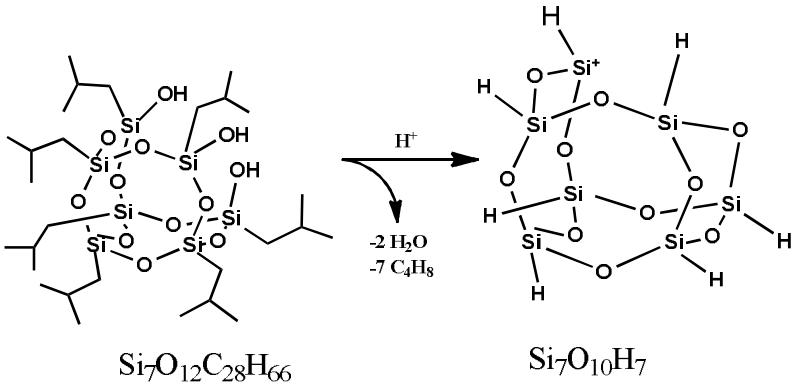


Figure 3.15. Step by step "surgical" removal of the hydrocarbonate radicals from trisilanolisobutyl POSS by fragmentation in multiple stages.

(**B3**) *Investigation of the neutral polysaccharider from Fomes fomentarius: a prelimianary study*

The MALDI-TOF technique has allowed remarkable progress in the past 20 years regarding the widening of the measurement range as well as numerous improvements by extending the matrices, ionization promoters both in positive and negative ion analysis modes. In the following are presented the results obtained during the extraction, isolation, purification and structure determination of the neutral polysaccharides from the fruiting body of *Fomes fomentarius,* harvested from the spontaneous flora of Valcea county (Romania) using a „top-down" type experiment and MALDI-TOF spectrometry.

The frozen fruit preserved under liquid nitrogen was powdered and degreased by extracting repeatedly the lipophilic and colored contaminants. The resulting residue was extracted with water to separate the polisaccharide fraction. The combined and isolated aqueous extract was fractionated on a Sepharose Fast Flow column in two fractions, one containing neutral polysaccharides (NP) and the other one acidic polysaccharides (AP) [166,188]. The neutral polysaccharide fractions were then analysed by MALDI-TOF-MS. The absence of a mass loss of 146 amu suggests that the polysaccharide does not contain fucose as it is known in literature. Therefore, a neutral fraction was subjected to total acid hydrolysis followed by TLC identification, with standards, of the hydrolyzate composition. The identification highlights the existence of manose and galactose and the absence of fucose [166, 188]. The photodensitometry of the analytical plates of the 3 hydrolyzate fractions suggests the existence of a Gal-Man heteropolymer unknown in literature which probably has the formula $[(Gal)_5(Man)_2]_n$.

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