

## SCIENTIFIC REPORT

### Dendrimer-carbon nanostructure conjugates as drug delivery support

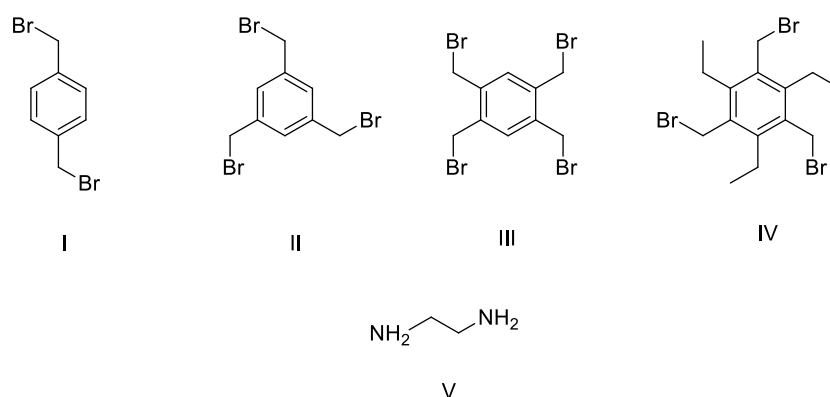
Phase 4 - period december 2013 – december 2014

#### Phase IV. Synthesis and physico-chemical characterization of dendrimer-carbon nanostructure-dendrimer complexes

**Activity:** *Synthesis and physico-chemical characterization of dendrimer-carbon nanostructure complexes- partial*

The objective of this phase is to obtain dendritic nanostructures (dendrimers of different generations) with potential use in the transport of biologically active materials at the cellular level, and their characterization in terms of physicochemical properties.

Two methods were used for the construction of dendrimer: convergence method and divergence method. For 1 generation dendrimer divergence method proved to be the best, and for highest generations the convergence method. This consisted in the construction of various generations dendron and their binding to the core by nucleophilic substitution reactions. Dendritic structures under construction have been used different types of aromatic and non-aromatic core having the opportunity to realize different ramifications of 2, 3 or 4 (Figure 1).



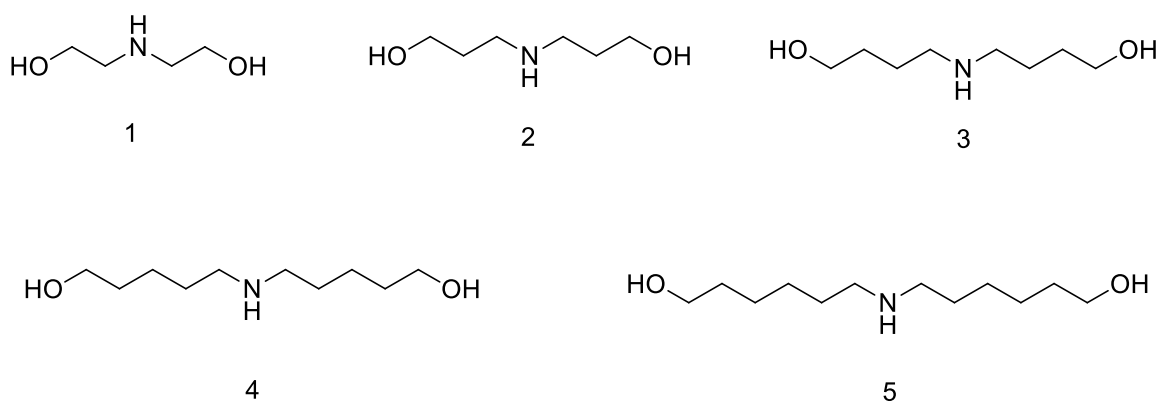
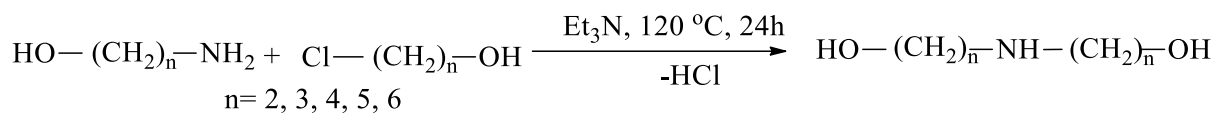
**Figure 1. Aromatic and non-aromatic dendrimer cores**

The obtaining of dendrons, 0 generation dendrimers and dendritic morpholines was achieved by completing several stages of synthesis:

1. Obtaining dialkanol amines
2. Obtaining dibromo derivatives
3. Obtaining 1 generation dendrons
4. Obtaining 0 generation dendrimers with aromatic core
5. Obtaining 0 generation dendrimers with non-aromatic core
6. Obtaining dendritic morpholines

## 1. Obtaining dialkanol amines

The first step in obtaining dendron was carrying amino diol derivatives. This stage was performed chemical synthesis of derivatives appropriately substituted aminoalcohol and hydroxides at the ends, so that the reaction product be a symmetrical dihydroxi amine (Scheme 1).



Scheme 1. Reaction for obtaining dialkanol amines.

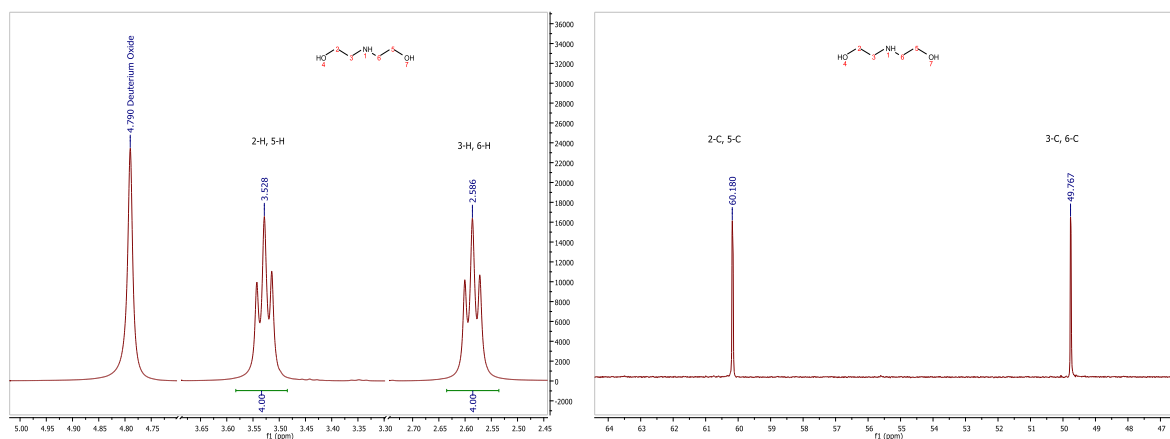
### *Procedure*

In a round bottom 25 ml with continuously stirring, mix in a part of 1: 9 = chloroalcanol: aminoalcanol. To the reaction mixture was added 3 parts of  $\text{Et}_3\text{N}$ . The reaction mixture was heated at  $120^\circ\text{C}$  for 24 hours. It is cooled and the excess of the amino alcohol and triethylamine are removed by vacuum distillation. Add a small amount of acetonitrile to dissolve the reaction mixture. The precipitated triethylamine hydrochloride was removed by filtration. Filtered solution was dry. The product obtained is in the form of viscous liquid and yellow-brown color. Product viscosity is dependent on the chain length, so for the short chain is less viscous, and for those with long-chain viscous. Tracking the reaction was performed by thin layer chromatography (TLC Silica gel 60 F<sub>254</sub>) products thus obtained were characterized by the following methods: MS (Agilent / Technologies 6320),  $^1\text{H}$  NMR (Bruker 600 MHz),  $^{13}\text{C}$ -NMR (Bruker 150 MHz) elemental analysis (Vario Micro Cube, CNH). The results are shown in Table 1.

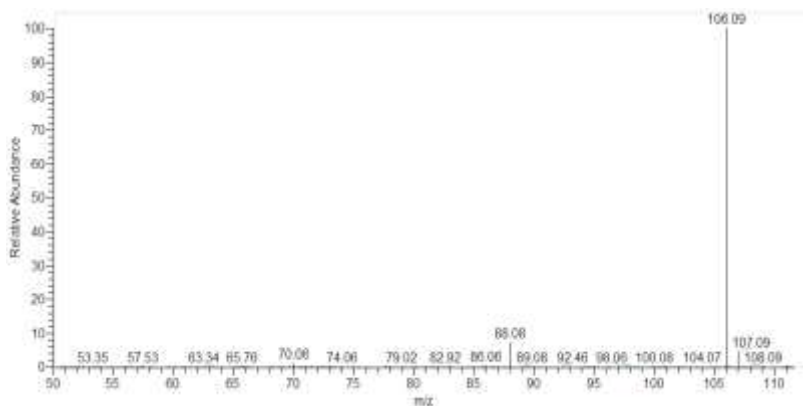
**Table 1. Analysis results.**

Dialkanol amine	Exact mass Obtained mass	Elemental analysis C:H:N:O (calc/obtained)	Yield %	Physical State, Color
<b>1</b>	105.14 105.09	45.70:10.55:13.32:30.44 45.15:10.98:12.99:30.88	92	Viscous, Light yellow-braun
<b>2</b>	133.04 133.12	54.11:11.35:10.52:24.03 53.69:11.55:11.08:23.68	90	Viscous, Light yellow-braun
<b>3</b>	161.24 161.15	59.59:11.88:8.69:19.85 58.69:12.21:8.09:21.01	91	Viscous, yellow-braun
<b>4</b>	189.30 189.18	63.45:12.25:7.40:16.90 64.26:12.66:7.50:15.58	89	Viscous, yellow-braun
<b>5</b>	217.20 217.04	66.31:12.52:6.44:14.72 66.22:12.32:6.14:15.32	90	Semi solid, Dark yellow-braun

Figure 2 present  $^1\text{H}$ -RMN and  $^{13}\text{C}$ -RMN spectra, and in figure 3 mass spectra for obtained diethanol amine.

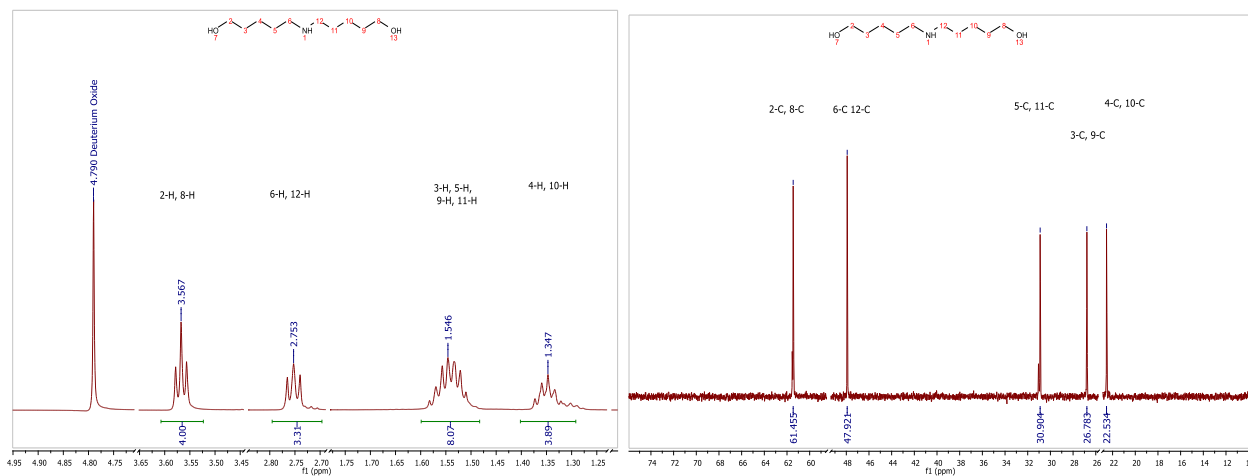


**Figure 2.  $^1\text{H}$ -RMN (600 MHz, 298 K,  $\text{D}_2\text{O}$ ) and  $^{13}\text{C}$ -RMN (150 MHz, 298 K,  $\text{D}_2\text{O}$ ) for diethanol amine**

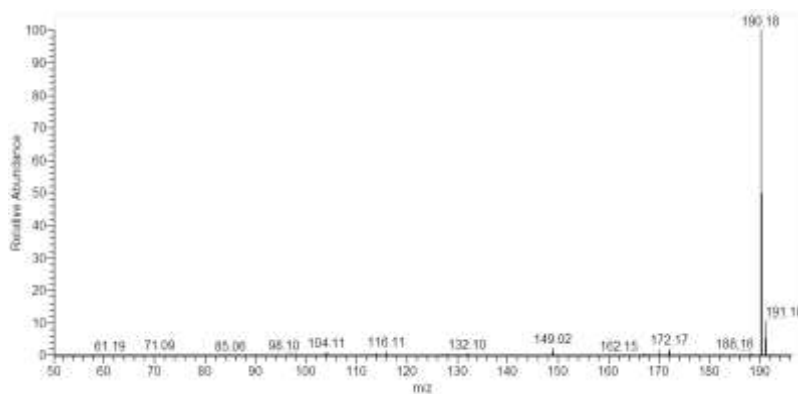


**Figure 2. Mass spectrum for diethanol amine**

Figure 4 present  $^1\text{H}$ -RMN and  $^{13}\text{C}$ -RMN spectra, and figure 5 mass spectrum, for dipropanol amine.



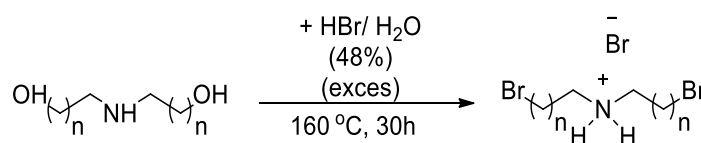
**Figure 4.  $^1\text{H}$ -RMN (600 MHz, 298 K,  $\text{D}_2\text{O}$ ) and  $^{13}\text{C}$ -RMN (150 MHz, 298 K,  $\text{D}_2\text{O}$ ) for dipropanol amine**



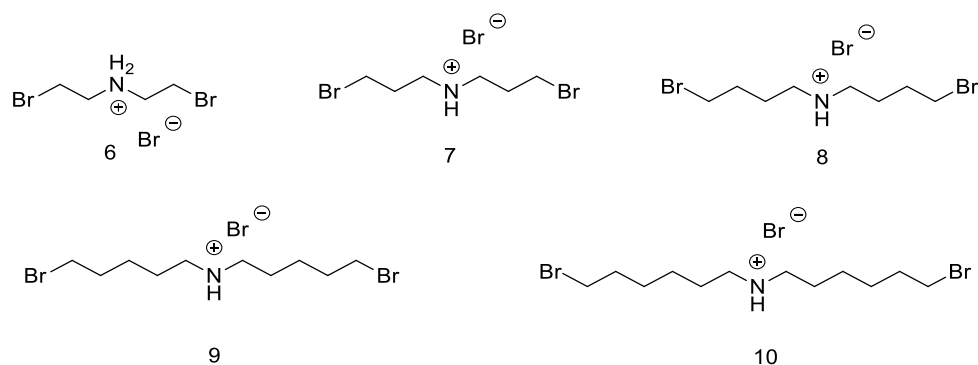
**Figure 5. Mass spectrum for dipropanol amine**

## 2. Obtaining dibromo derivatives (dibromo amine)

In order to achieve the dendrons has choose nucleophilic substitution reaction of bromine atoms with the nitrogen atom of the corresponding dialkanol amines. To achieve this step is necessary substitution of the OH groups with Br atoms (see Figure 2).



n=1 DEA  
n=2 DPrA  
n=3 DBuA  
n=4 DPeA  
n=5 DHxA



**Scheme 2. Reaction for obtaining bromine saults.**

## Procedure

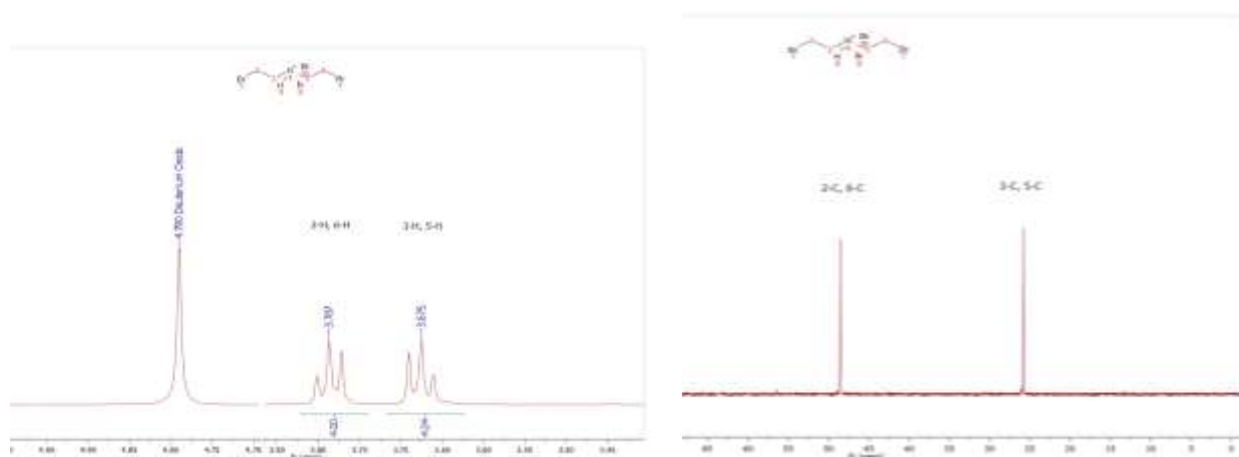
In a round bottom flask of 100 ml equipped with a Dean-Stark trap, was added at cool, with continued stirring, 50 ml 48% HBr and 0.06moli dialkanol amine. The reaction was heated in an oil bath at 160<sup>0</sup>C for 16h. At the end of reaction the unreacted HBr is distilled in a vacuum. The reaction was dissolved in ethanol with activated charcoal and filter. Added to dryness. Purify with diethyl ether, giving a white crystalline compound.

To obtain the dibromo derivatives, were the following steps. Dissolve the salt of dibromo compound in water and add calcium carbonate to mildly basic pH. Tracking the reaction was performed by thin layer chromatography (TLC Silica gel 60 F<sub>254</sub>) products thus obtained were characterized by the following methods: MS (Agilent / Technologies 6320), <sup>1</sup>H NMR (Bruker 600 MHz), <sup>13</sup>C-NMR (Bruker 150 MHz ) elemental analysis (Vario Micro Cube, CNH). The results are shown in Table 2.

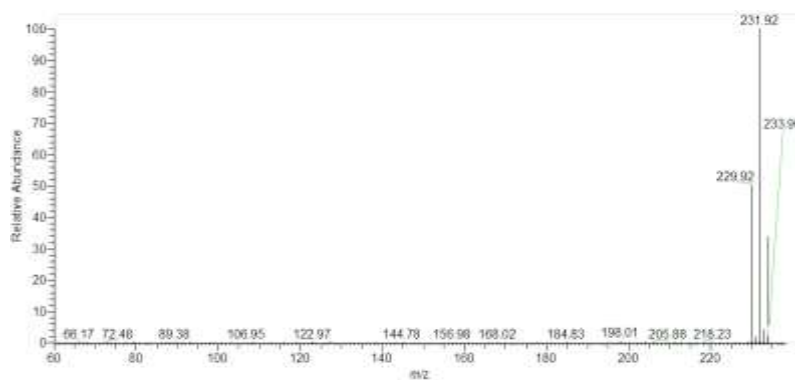
**Table 2. Analysis results.**

Compound	Exact mass Obtained mass	Elemental analysis C:H:N:O (calc/obtained)	Yield %	Physical State, Color
<b>6</b>	230.93	15.41:3.23:76.87:4.49	84	Crystalline white
	230.92	16.25:3.41:75.88:4.46		
<b>7</b>	258.98	21.20:4.15:70.53:4.12	82	Crystalline white
	258.95	20.88:4.26:71.29:3.57		
<b>8</b>	285.98	33.36:6.30:55.48:4.86	86	Crystalline white
	285.95	33.02:6.48:54.99:5.51		
<b>9</b>	315.09	30.33:5.60:60.53:3.54	86	Crystalline Yellow-white
	315.01	29.46:5.12:60.01:5.41		
<b>10</b>	342.04	33.99:6.18:56.53:3.30	82	Crystalline Yellow-white
	342.01	32.54:6.11:57.98:3.37		

Figure 6 present  $^1\text{H}$ -RMN and  $^{13}\text{C}$ -RMN spectra, and figura 7 mass spectrum masa, for bisz(2-bromoethyl)-amine bromide.

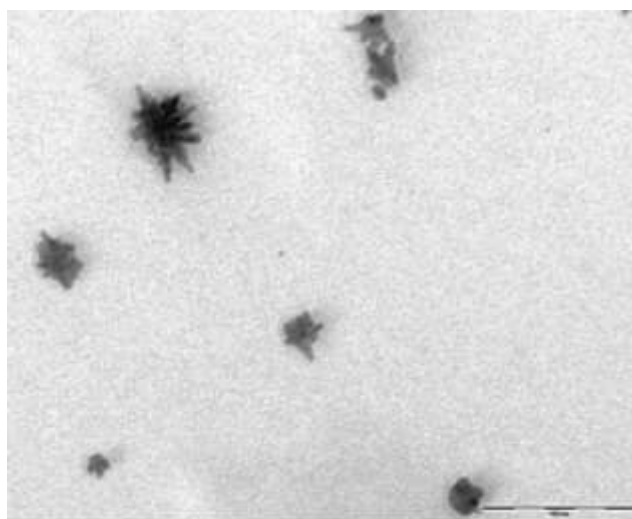


**Figure 6.  $^1\text{H}$ -RMN (600 MHz, 298 K,  $\text{D}_2\text{O}$ ) and  $^{13}\text{C}$ -RMN (150 MHz, 298 K,  $\text{D}_2\text{O}$ ) spectra for bisz(2-bromoethyl)-amine bromide.**



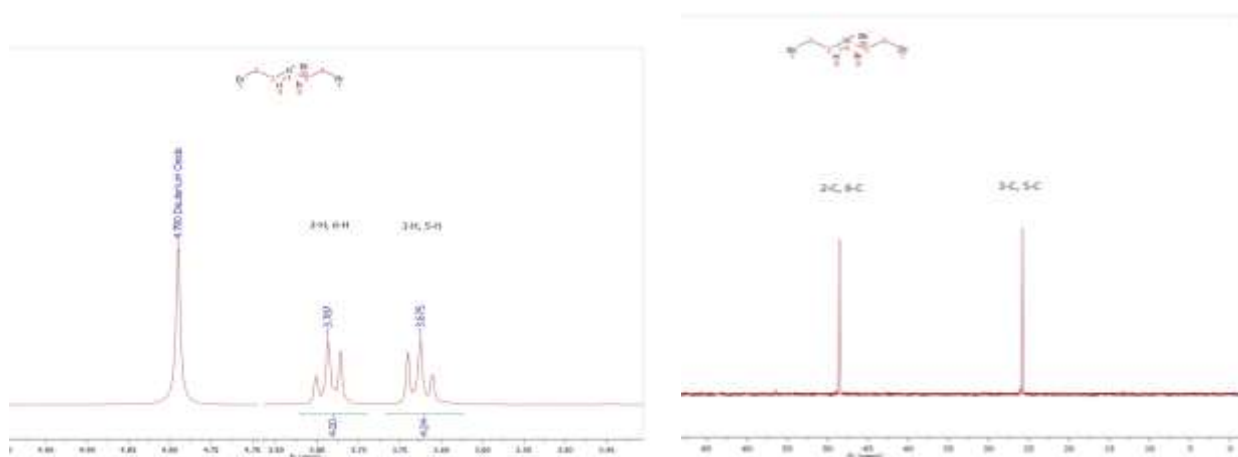
**Figure 7. Mass spectra for bisz(2-bromoethyl)-amine bromide.**

Tem image analysis of the bromide salts show the appearance of star-shaped crystals. Figure 8 present TEM image for bisz(2-bromoethyl)-amine bromide.

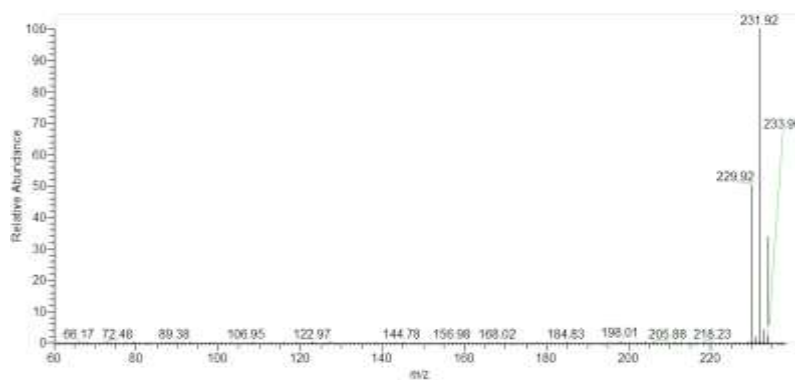


**Figure 8. TEM micrograph for bisz(2-bromoethyl)-amine bromide.**

Figure 9 present  $^1\text{H}$ -RMN and  $^{13}\text{C}$ -RMN spectra, and figura 10 mass spectra, for bisz(2-bromopropil)-amine bromide.

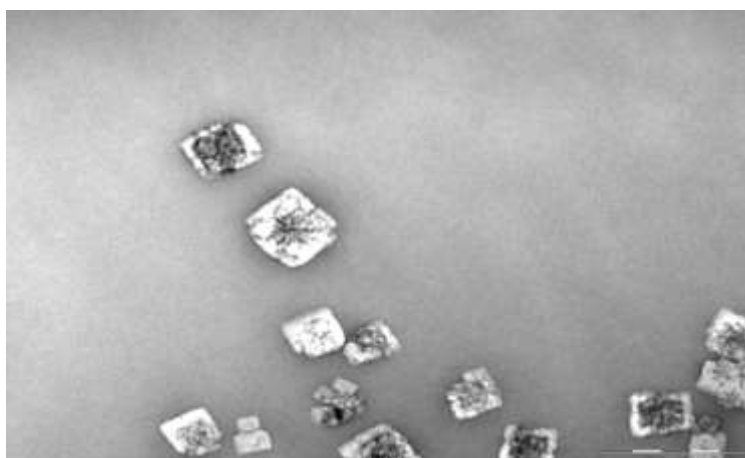


**Figure 9.  $^1\text{H}$ -RMN (600 MHz, 298 K,  $\text{D}_2\text{O}$ ) and  $^{13}\text{C}$ -RMN (150 MHz, 298 K,  $\text{D}_2\text{O}$ ) spectrum for bisz(2-bromopropil)-amine bromide**



**Figure 10. Mass spectra for bisz(2-bromopropil)-amine bromide.**

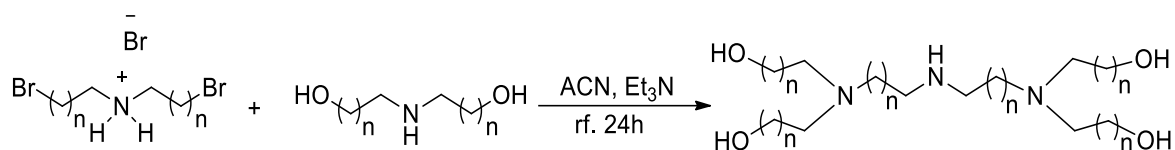
Figure 11 present TEM image for bisz(2-bromoethyl)-amine bromide. Noted the appearance of the cubic crystal.



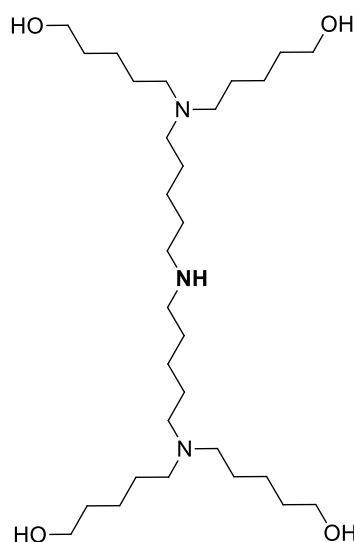
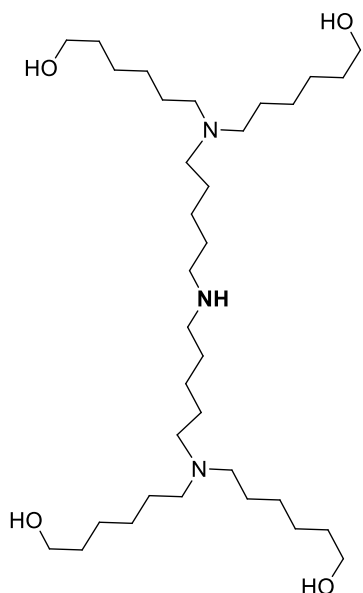
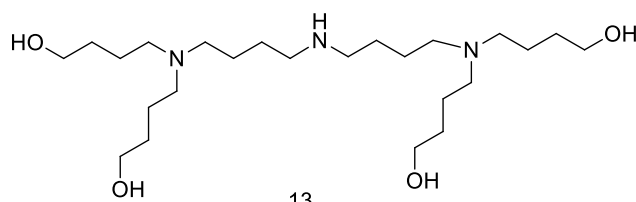
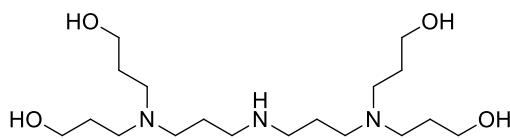
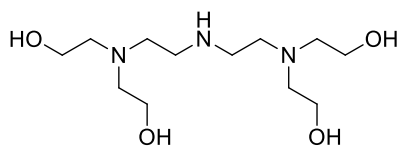
**Figure 11. TEM micrograph for bisz(2-bromoethyl)-amine bromide.**

### 3. Obtaining 1 generation dendrons

1 generation dendrons was obtained by synthesis of salts of bromine derivatives and corresponding dialcohols dibromide to an increase symmetrical generation. Figure 3 shows the coupling reaction.



n=1 DEA  
n=2 DPrA  
n=3 DBuA  
n=4 DPeA  
n=5 DHxA



**Scheme 3. Reaction for obtaining 1 generation dendrons.**



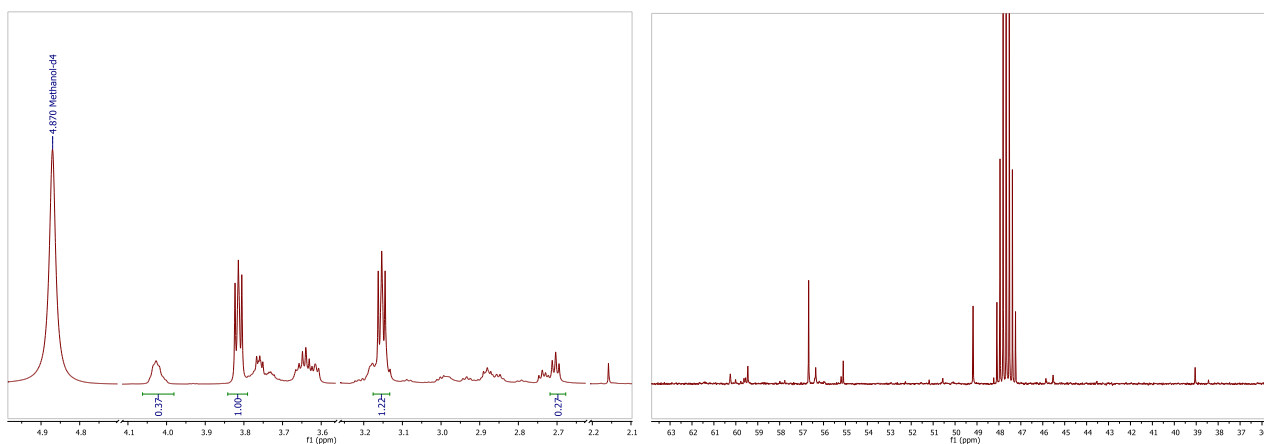
## Procedure

In a round bottom flask of 50 ml under continuous stirring was dissolved in 25ml acetonitrile an dibromo derivative: dialkanol amine = 1: 3. Add 5 parts triethylamine. The mixture was heated at reflux for 24 hours. The reaction mixture was cooled, the solvent was removed by distillation. Dialkanol amine excess was removed by distilling under vacuum at a high temperature. To the resulting mixture was added triethylamine and filtered. The product obtained is concentrated in vacuo to give a yellow-orange liquid. Tracking the reaction was performed by thin layer chromatography (TLC Silica gel 60 F<sub>254</sub>) products thus obtained were characterized by the following methods: MS (Agilent / Technologies 6320), <sup>1</sup>H NMR (Bruker 600 MHz), <sup>13</sup>C-NMR (Bruker 150 MHz ) elemental analysis (Vario Micro Cube, CNH). The results are shown in Table 3.

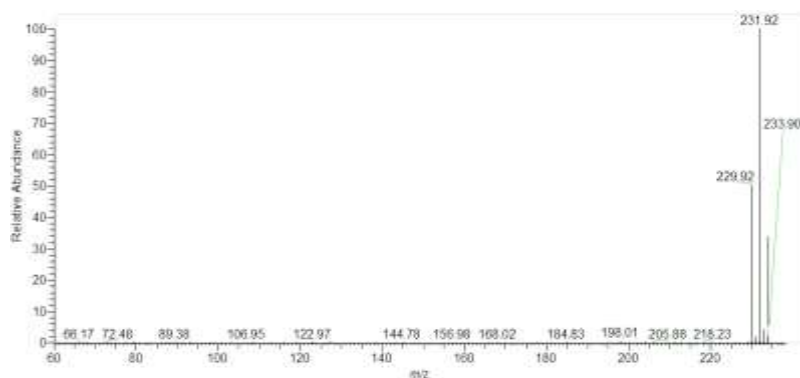
**Table 3. Analysis results.**

Compound	Exact mass Obtained mass	Elemental analysis C:H:N:O (calc/obtained)	Yield %	Physical State, Color
<b>11</b>	279.80	51.59:10.46:15.04:22.91	59	Liquid
	279.22	50.22:10.88:14.69:24.21		Yellow
<b>12</b>	363.54	59.47:11.37:11.56:17.60	60	Liquid
	363.32	59.22:11.89:11.65:17.24		Orange
<b>13</b>	447.40	64.39:11.93:9.39:14.29	58	Viscous liquid
	447.15	64.01:11.87:9.85:14.27		Orange

Figure 12 present <sup>1</sup>H-RMN and <sup>13</sup>C-RMN spectrum, and figure 13 mass spectra, for 1 generation diethanolamine dendron.

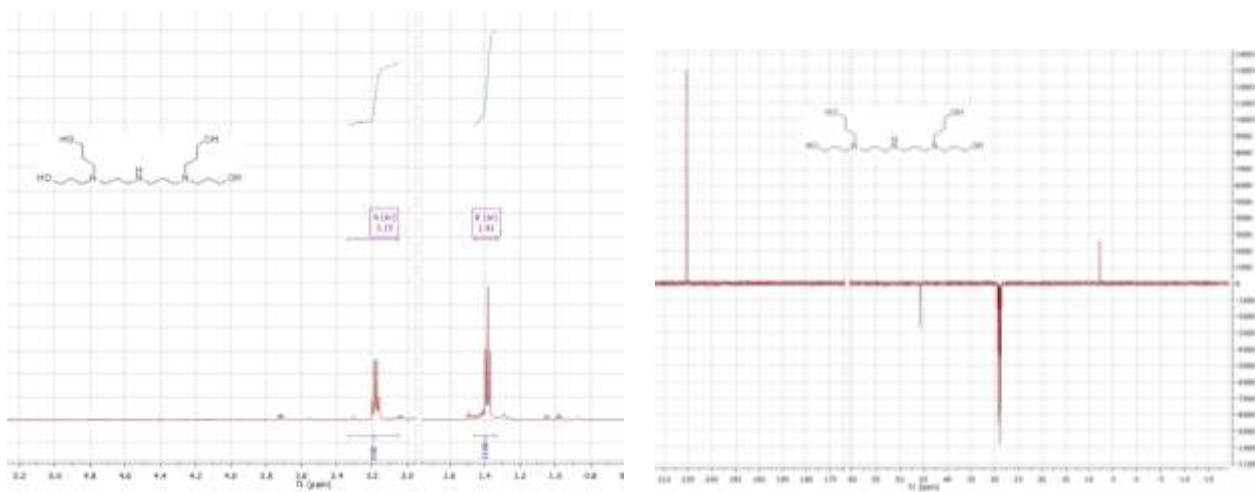


**Figure 12. <sup>1</sup>H-RMN (600 MHz, 298 K, MeOD) and <sup>13</sup>C-RMN (150 MHz, 298 K, MeOD) for 1 generation diethanolamine dendron.**

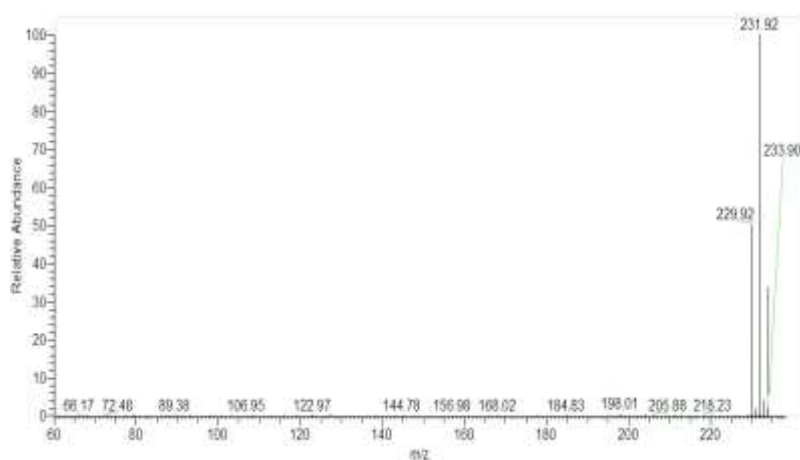


**Figure 13. Mass spectra for 1 generation diethanolamine dendron.**

Figure 14 present  $^1\text{H}$ -RMN and  $^{13}\text{C}$ -RMN spectra and figura 15 mass spectra, for 1 generation dipropanolamine dendron.



**Figure 14.  $^1\text{H}$ -RMN (600 MHz, 298 K,  $(\text{CD}_3)_2\text{CO}$  and  $^{13}\text{C}$ -RMN (150 MHz, 298 K,  $(\text{CD}_3)_2\text{CO}$ ) for 1 generation dipropanolamine dendron.**

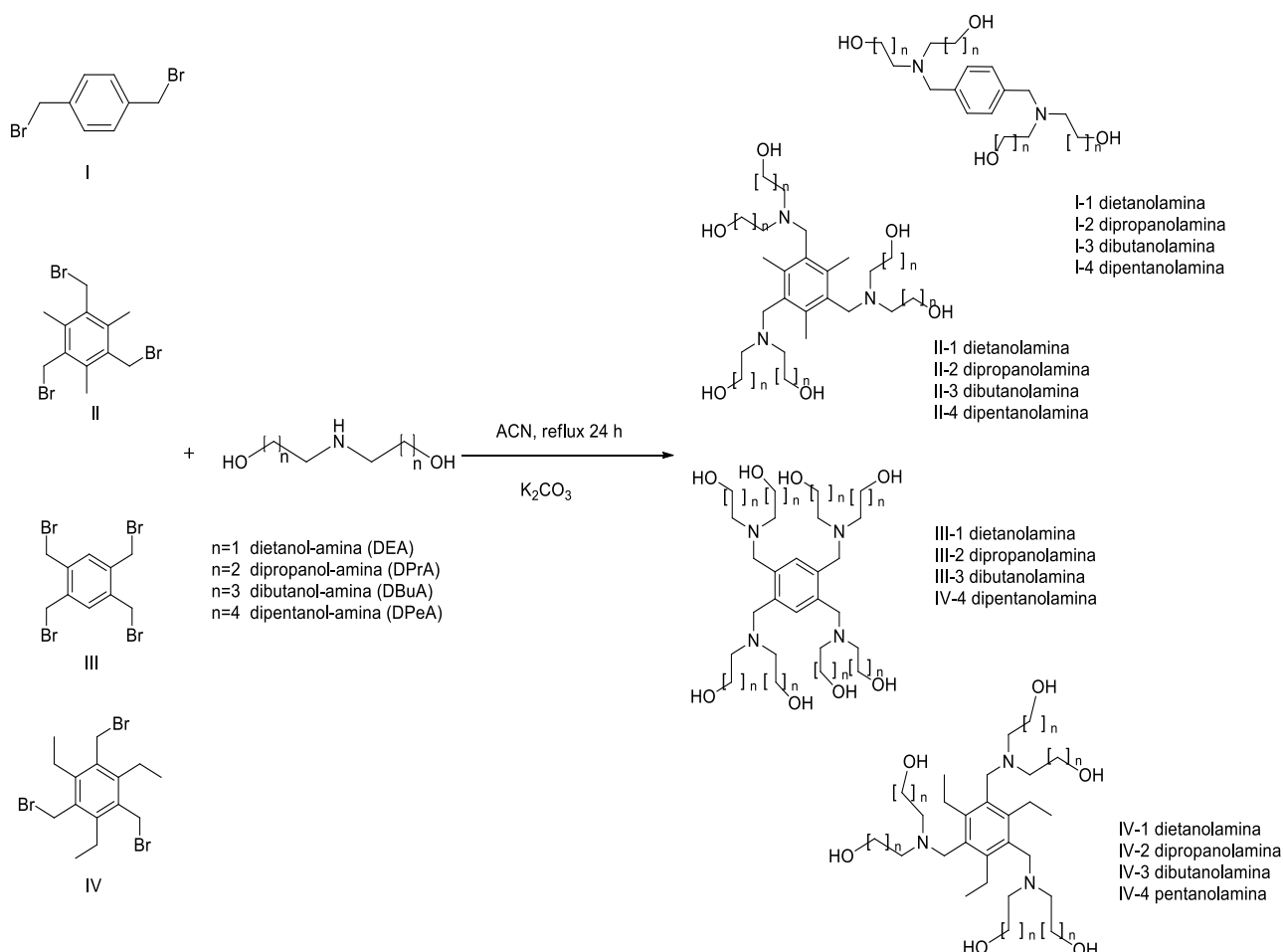


**Figure 15. Mass spectra for 1 generation dipropanolamine dendron.**

#### 4. Obtaining aromatic core 0 generation dendrimer

0 generation dendrimer was obtained through substitution of the Br atoms on an aromatic ring or non-aromatic with previously obtained dendrons. There were used four aromatic rings di, tri and tetrabromo substituted and diols of different lengths.

The method is based on the nucleophilic substitution of the bromine atom by N atom of the synthesized dendrons. Scheme 4 and 5 show the reactions of obtaining 0 generation dendron with aromatic or non-aromatic core.



**Scheme 4. Reaction for obtaining aromatic core 0 generation dendrimers.**

#### Procedure

In a round bottom flask of 50 ml is dissolved under continuous stirring in approximately 30 ml of acetonitrile, an aromatic ring, in a proportion of 1: 2 for the disubstituted core, 1: 3 for tri-substituted ring and 1: 4 for the tetra core. Add 1 part of potassium carbonate. The reaction mixture was heated at 85<sup>0</sup>C for 24 hours. After the reaction mixture is cooled, KBr was filtered. Wash 3x with 20ml methanol. Hot dialkanol amine in excess was distilled under vacuum. Tracking the reaction was performed by thin layer chromatography (TLC Silica gel 60 F<sub>254</sub>) products thus obtained were characterized by the following methods: MS (Agilent / Technologies 6320), <sup>1</sup>H NMR

(Bruker 600 MHz),  $^{13}\text{C}$ -NMR (Bruker 150 MHz ) elemental analysis (Vario Micro Cube, CNH).

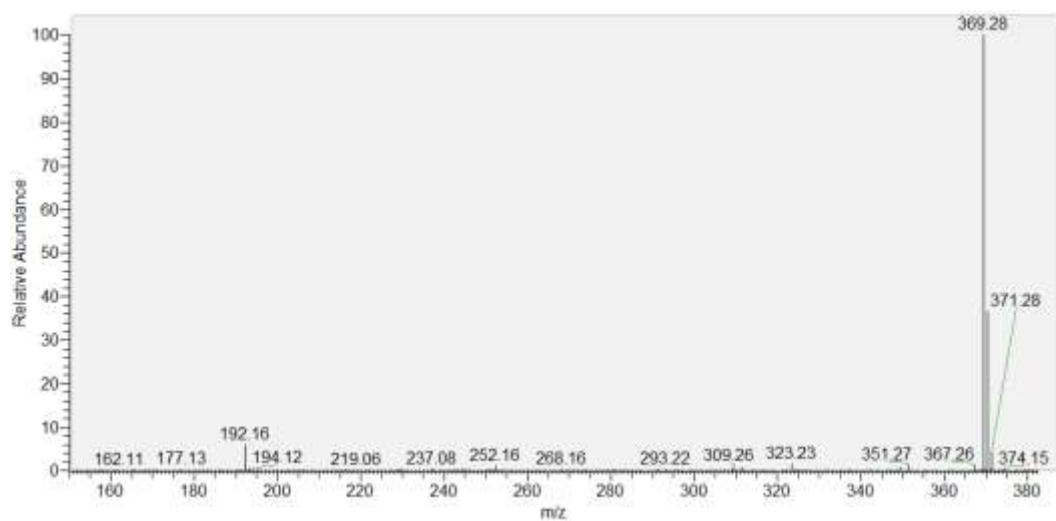
The results are shown in Table 4.

**Table 4. Products analysis results.**

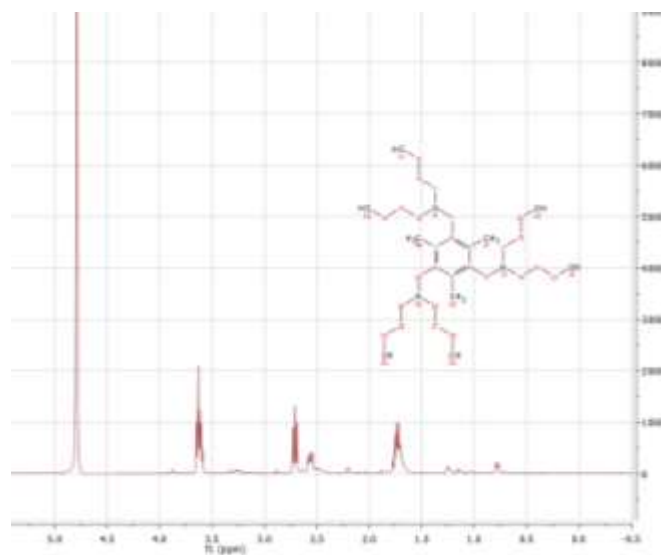
<b>Compound</b>	<b>Exact mass Obtained mass</b>	<b>Elemental analysis C:H:N:O (calc/obtained)</b>	<b>Yield %</b>	<b>Physical State, Color</b>
<b>I-1</b>	312.20	61.51:9.03:8.97:20.49	72	Liquid
	312.21	61.68:8.96:8.88:20.48		Yellow
<b>I-2</b>	368.51	65.19:9.85:7.60:17.37	69	Liquid
	368.28	64.81:9.88:7.23:18.08		Orange
<b>I-3</b>	424.62	67.89:10.44:6.60:15.07	69	Viscous liquid
	424.34	66.15:10.26:6.58:17.01		Orange
<b>I-4</b>	480.72	69.96:10.90:5.83:13.31	71	Viscous liquid
	480.40	69.55:10.84:5.89:13.72		Orange
<b>II-1</b>	471.63	61.12:9.62:8.91:20.35	83	Liquid
	471.34	60.78:9.22:8.98:21.02		Yellow
<b>II-2</b>	555.42	64.83:10.34:7.56:17.27	81	Liquid
	555.43	64.26:11.02:7.24:17.48		Yellow-orange
<b>III-1</b>	546.36	57.12:9.22:10.25:23.41	78	Liquid
	546.29	56.82:9.35:10.19:23.64		Yellow
<b>III-2</b>	658.49	61.98:10.10:8.50:19.43	72	Liquid
	658.28	61.55:10.01:8.99:19.45		Orange
<b>IV-1</b>	457.32	60.37:9.47:9.18:20.98	89	Liquid
	457.39	60.03:9.56:9.11:21.30		Dark yellow
<b>IV-2</b>	541.41	64.29:10.23:7.76:17.72	86	Viscous liquid
	541.29	64.06:10.26:7.58:18.10		Yellow-orange

Figures 16-24 present  $^1\text{H}$ -RMN,  $^{13}\text{C}$ -RMN, and mass spectra, respectively for obtained compounds.

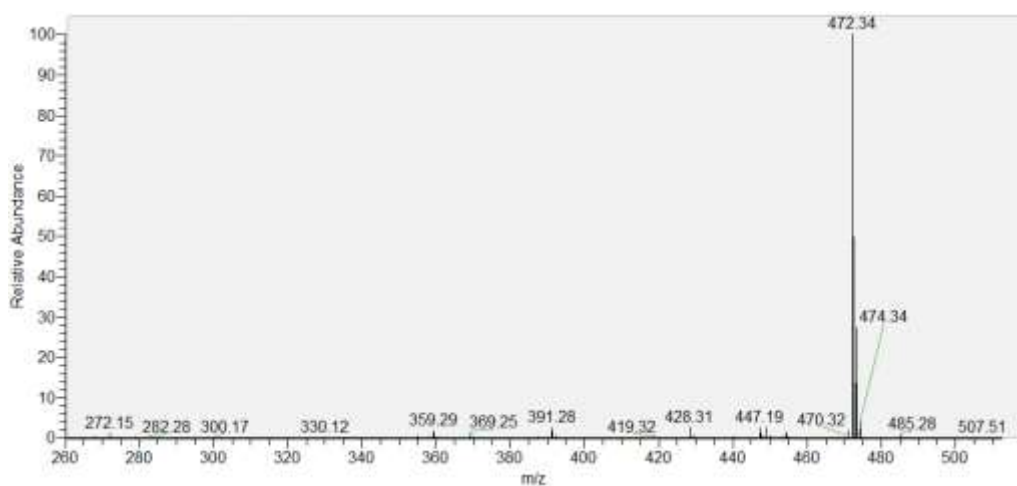




**Figure 19. Mass spectra for compound I-2.**



**Figure 20. <sup>1</sup>H-RMN (600 MHz, 298 K, D<sub>2</sub>O) spectra for compound II-1.**



**Figure 21. Mass spectra for compound II-1.**

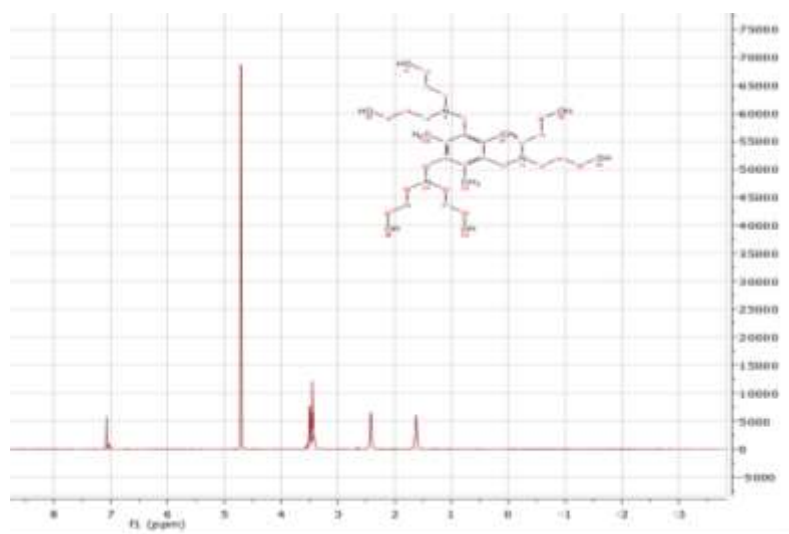


Figure 22.  $^1\text{H}$ -RMN (600 MHz, 298 K,  $\text{D}_2\text{O}$ ) spectra for compound II-2.

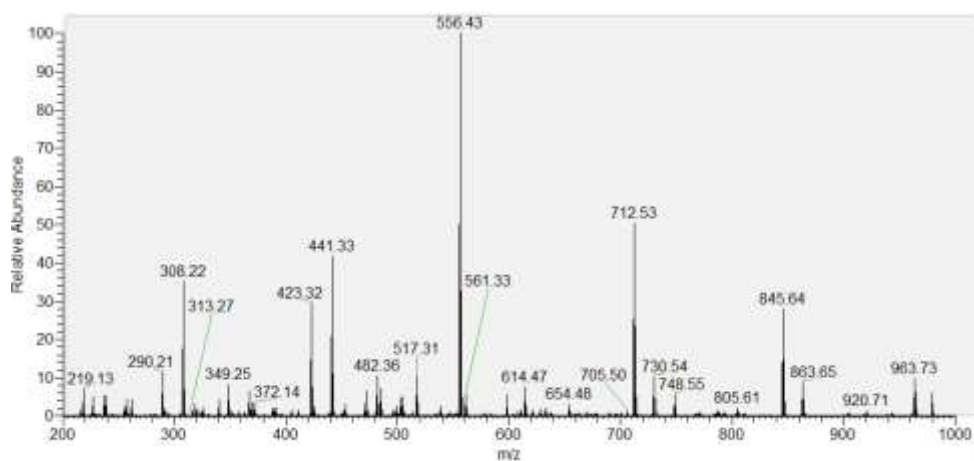


Figure 23. Mass spectra for compound II-2

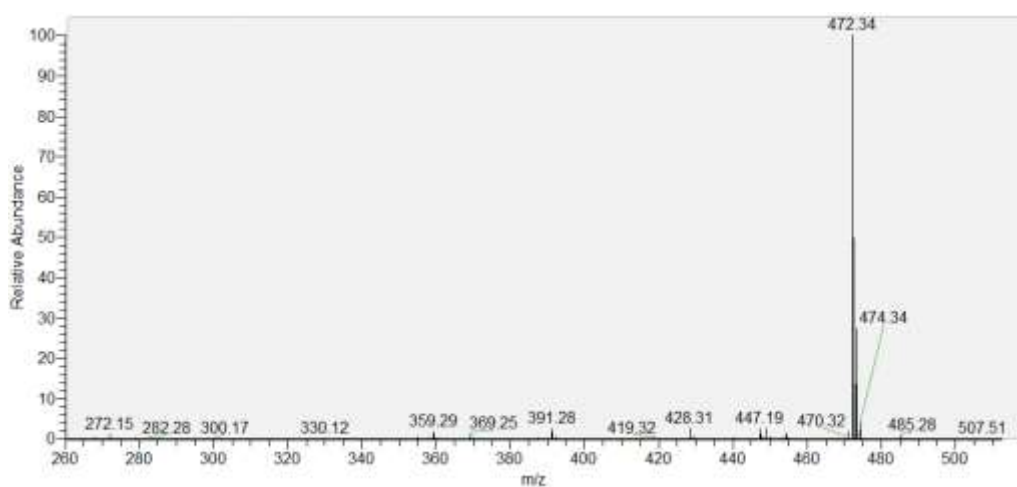
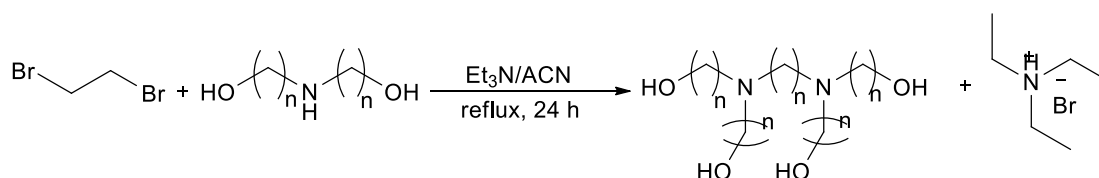


Figure 24. Mass spectra for compound IV-1.

## 5. Obtaining non-aromatic core 0 generation dendrimers

Obtaining 0 generation dendrimer with aromatic ring was based on the same principle as for dendrimers with aromatic ring. Scheme 5 shows the reaction of obtaining them.



V-1 n=2 dietanol-amina  
 V-2 n=3 dipropanol-amina  
 V-3 n=4 dibutanol-amina  
 V-4 n=5 dipentanol-amina  
 V-5 n=6 dihexanolamina

**Scheme 5. Reaction for obtaining non-aromatic core 0 generation dendrimers.**

### Procedure

In a round bottom flask of 50 ml is dissolved under continuous stirring in approximately 30 ml of acetonitrile, and the corresponding dibromo dialkanol amine derivative, in a proportion of 1: 2. Added 1 part of potassium carbonate. The reaction mixture was heated at 85<sup>0</sup>C for 24 hours. After the reaction mixture is cooled, filtered KBr. Wash 3x with 20ml methanol. Hot dialkanol amine in excess was distilled under vacuum. Tracking the reaction was performed by thin layer chromatography (TLC Silica gel 60 F<sub>254</sub>) products thus obtained were characterized by the following methods: MS (Agilent / Technologies 6320), <sup>1</sup>H NMR (Bruker 600 MHz), <sup>13</sup>C-NMR (Bruker 150 MHz ) elemental analysis (Vario Micro Cube, CNH). The results are shown in Table 4.

**Table 4. Analysis results for reaction products.**

Compound	Exact mass Obtained mass	Elemental analysis C:H:N:O (calc/obtained)	Yield %	Physical State, Color
V-1	236.31	50.83:10.24:11.85:27.08	88	Liquid
	236.18	50.44:9.87:11.99:27.70		Light yellow-braun
V-2	292.41	57.50:11.03:9.58:21.89	89	Liquid
	292.24	57.62:10.89:9.46:22.03		Light –braun
V-3	348.52	62.03:11.57:8.04:18.36	86	Liquid braun
	348.46	61.86:11.62:8.11:18.41		
V-4	404.36	65.30:11.96:6.92:15.82	81	Liquid braun
	404.29	65.22:11.99:6.85:15.94		



Figure 25 present  $^1\text{H}$ -RMN and  $^{13}\text{C}$ -RMN spectrum, and figura 26 mass spectra, for compound V-1.

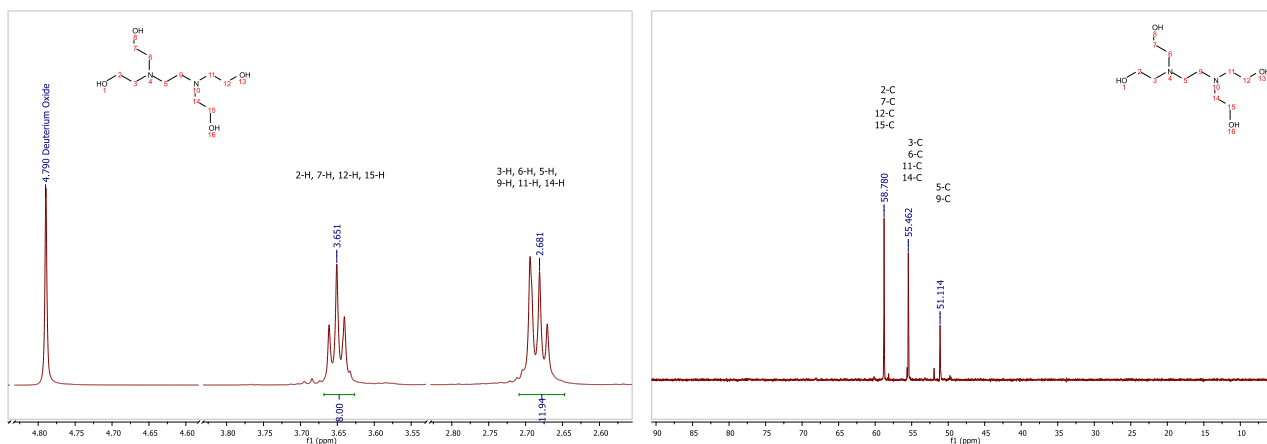


Figure 25.  $^1\text{H}$ -RMN (600 MHz, 298 K,  $\text{D}_2\text{O}$ ) and  $^{13}\text{C}$ -RMN (150 MHz, 298 K,  $\text{D}_2\text{O}$ ) spectrum for compound V-1.

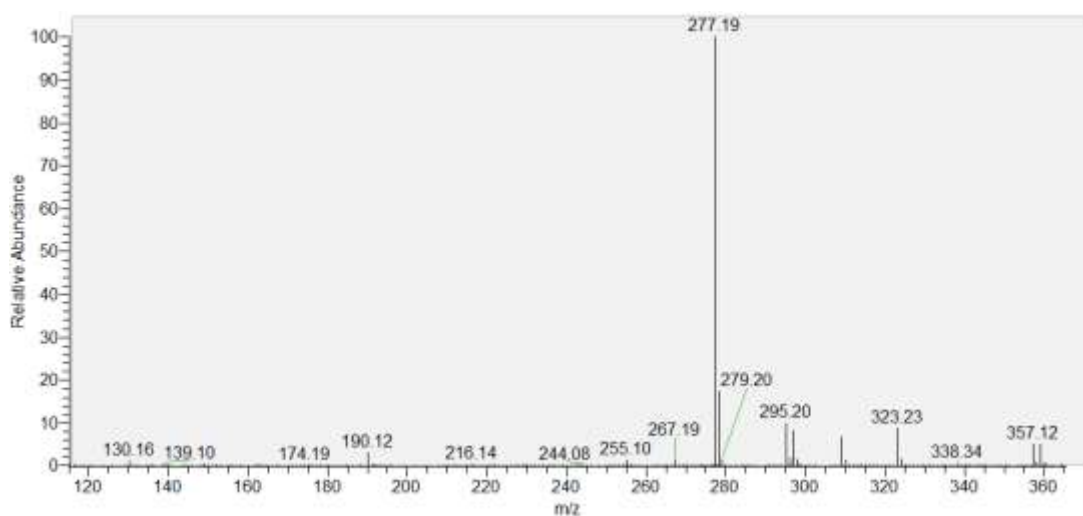
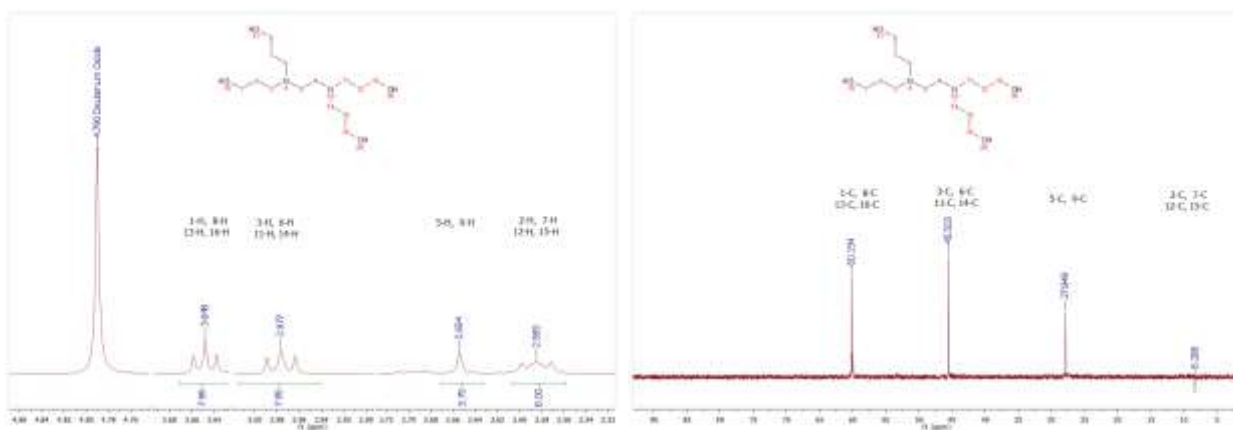
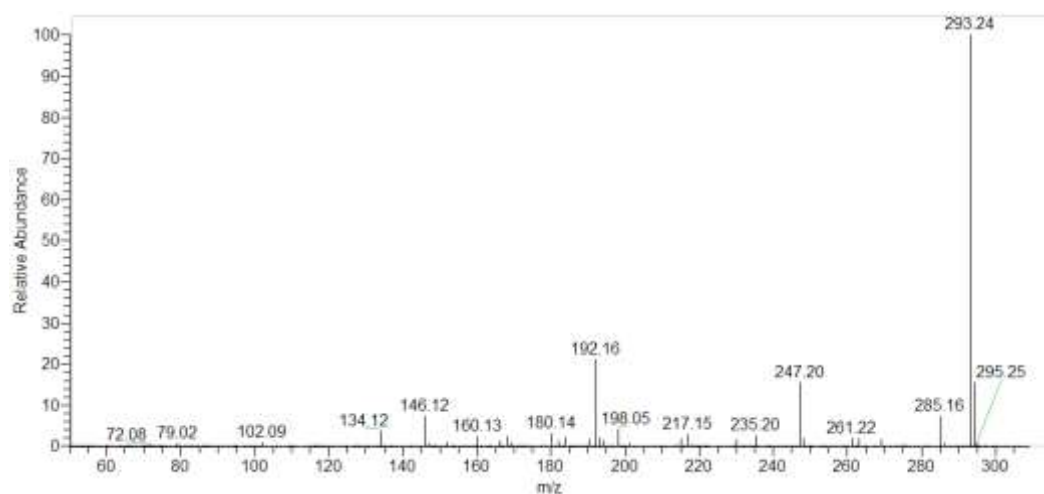


Figure 26. Mass spectra for compound V-1.

Figures 27-28 present  $^1\text{H}$ -RMN,  $^{13}\text{C}$ -RMN and mass spectra, respectively, for compound V-2.



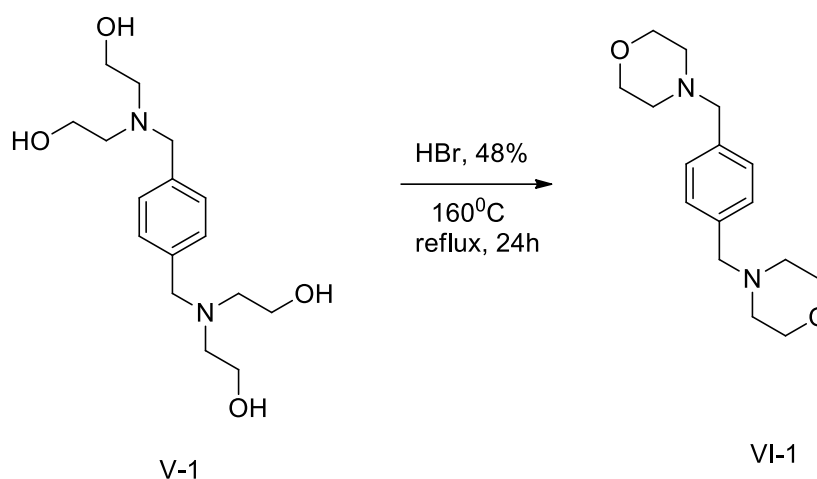
**Figure 27.**  $^1\text{H}$ -RMN (600 MHz, 298 K,  $\text{D}_2\text{O}$ ) and  $^{13}\text{C}$ -RMN (150 MHz, 298 K,  $\text{D}_2\text{O}$ ) spectrum for compound V-2.



**Figure 28.** Mass spectra for compound V-2.

## 6. Obtaining dendritic morpholines

When the 0 generation dendron generation has tried bromination coming from diethanolamides synthesis of amine with  $\text{HBr}$ , bromination has occurred. Instead of NMR and MS analysis found that there was a cyclization reaction with elimination of water. This was achieved by a relatively simple reaction for obtaining dendritic morpholines (Scheme 6).



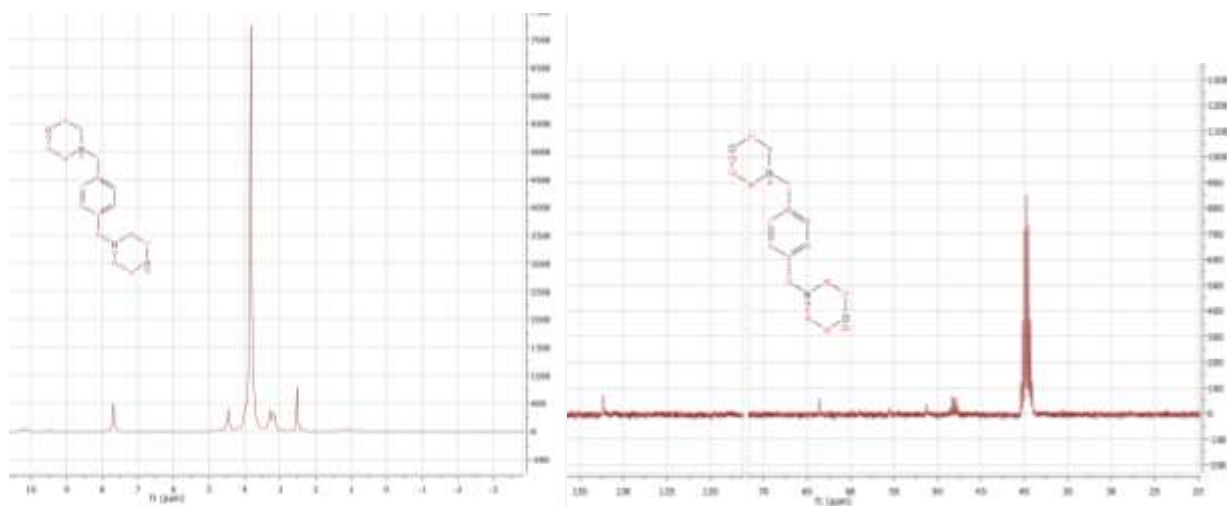
**Scheme 6. Obtaining reaction for morpholine derivatives.**

### Procedure

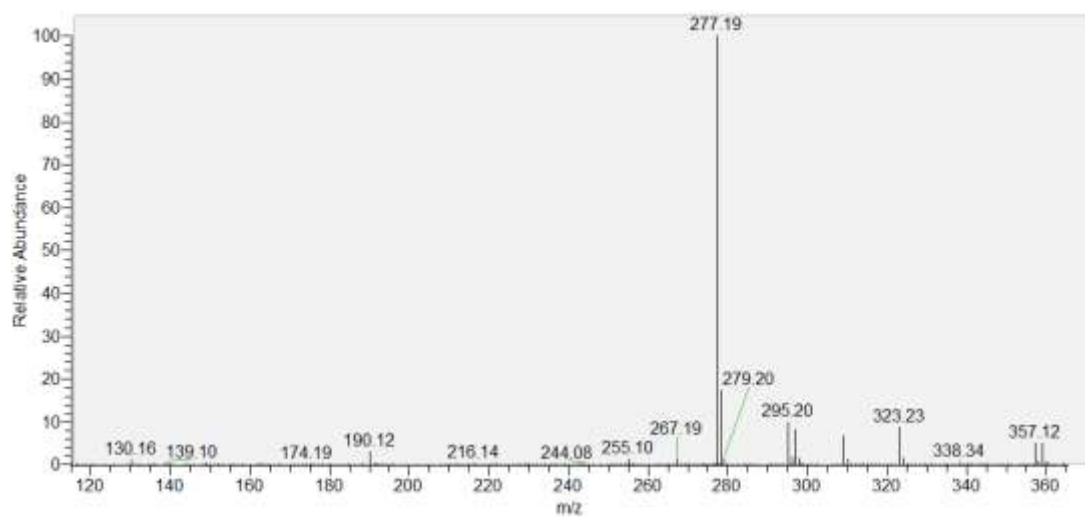
In a round bottom flask of 50 ml equipped with a Dean-Stark trap, was added, with continued stirring, 30 ml 48% HBr and 0.03moli of compound V-1. The reaction was heated in an oil bath at 160<sup>0</sup>C for 24 hours. At the end of reaction unreacted HBr is distilled in a vacuum. The reaction was dissolved in ethanol with activated charcoal and filter. Added to dryness. Purify with diethyl ether, giving a white crystalline compound. Tracking the reaction was performed by thin layer chromatography (TLC Silica gel 60 F<sub>254</sub>) products thus obtained were characterized by the following methods: MS (Agilent / Technologies 6320), <sup>1</sup>H NMR (Bruker 600 MHz), <sup>13</sup>C-NMR (Bruker 150 MHz ) elemental analysis (Vario Micro Cube, CNH). The results are shown in Table 2.

Compound	Exact mass Obtained mass	Elemental analysis C:H:N:O (calc/obtained)	Yield %	Physical State, Color
VI-1	276.18 276.19	69.53:8.75:10.14:11.58 69.46:8.85:10.06:11.63	91	Liquid Light yellow- braun

Figures 29-30 present <sup>1</sup>H-RMN, <sup>13</sup>C-RMN and mass spectrum, respectively for compound VI-1.



**Figure 29.**  $^1\text{H}$ -RMN spectrum (600 MHz, 298 K,  $\text{D}_2\text{O}$ ) and  $^{13}\text{C}$ -RMN (150 MHz, 298 K,  $\text{D}_2\text{O}$ ) compound V-1.



**Figure 30.** Mass spectrum for compound VI-1.

The results of this phase have materialized in these ISI and international conferences:

1. Szeffler B, Diudea MV (2014) Strain in Platonic fullerenes. *Structural Chemistry* 25(1) 319-325. DOI: **10.1007/s11224-013-0244-y** (IF=1.900)
2. Harsa AM, Harsa TE, Bolboaca SD, Diudea MV (2014) QSAR in flavonoids by similarity cluster prediction. *Current Computer-Aided Drug Design* 10(2) 115-128. DOI: **10.2174/1573409910666140410104542** (IF=1.942)
3. Diudea MV, Bende A, Nagy CL (2014) Carbon multi-shell cages. *Physical Chemistry Chemical Physics* 16(11) 5260-5269. DOI: **10.1039/c3cp55309d** (IF=4.198)
4. Füstös ME, Sipos TA, Diudea MV, Katona G (2014) Synthesis of novel aromatic core zero generation dendrimers. A new approach for phenyl-dimorpholine preparation. *Croat. Chem. Acta*, DOI: **submitted**
5. Nagy K, Nagy CL, Katona G, Diudea MV (2014) Theoretical study of opened [4,4] armchair carbon nanotube Y-junctions. *JCF-Fruhjahrssymposium* 2014. March 26-29, Jena, Germany. WEB: <http://www.fjs.uni-jena.de/posters-2/330-p139.html>
6. Sipos TA, Füstös ME, Katona G (2014) Synthesis and Characterization of Zero Generation Dendrimers Containing Aromatic Core. *XX. International Conference on Chemistry* 2014. November 6-9, Cluj-Napoca, Romania. WEB: [www.emte.ro](http://www.emte.ro)