ARTIFICIAL MEMBRANES BY THE SELF-ASSEMBLY OF PYRIDINIUM SALTS WITH A FREE AMINO GROUP DERIVED FROM PYRYLIUM SALTS WITH LONG ALKYL SUBSTITUENTS[**]

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In this paper we describe the preparation of a new synthetic membrane formed by the self-assembly of a pyridinium salts **1a-d** with N-triethyl-triamino and two long alkyl substituents (undecyl, palmitoyl, stearoyl) in the 2,6-(α) positions. Experimental protocol to obtain the new synthetic membranes and their properties (membrane stability – phase separation, optical microscopy, dynamic light scattering) are also described. The reaction of this membranes with fluorescein is also reported.

INTRODUCTION

In previous papers we described the preparation of new artificial membranes by the self-assembly of a pyridinium salts with long alkyl substituents in α or γ positions, having either ethyleneamino-terminal group,[1] or with various alkyl or phenyl substituents at nitrogen atom.[2,3] Experimental protocol and various conditions to obtain new synthetic membranes were also presented.[1-3]

In this work, the syntheses are extended to others pyridinium salts having long alkyl substituents and the application of this procedure for manufacturing synthetic bilayer membranes is discussed.

Cationic amphiphiles derived from pyridinium salts have been recently investigated in connection with DNA trafficking into living cells.[4]

The syntetic bilayer membranes form single and multiwalled vesicles. The trapping efficiency of the water-soluble substances in the bilayer matrix depends on molecular structure and aggregate's morphology.[5]

Synthetic vesicles have attracted attention because they have been very useful in the understanding the nature of biomembranes and in developing new techniques of biomimetics.[6]

It has been found that the N-type substituents of amphiphile affects the bilayer membrane stability and sorption ability of the different organic markers.

So, hydrophobic organic compounds such as sodium picrate, bromophenolblue and fluorescamine are bound predominantly to the lipophilic quaternary ammonium ions. The experiments show that hydrophilic substances (*e.g.* L-alanine and L-serine aminoacids) are not permeable across the

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hydrophobic bilayer membrane.[7] However, complexation of acrylic polymers[7] or ferromagnetic polymer markers[8] with the bilayer may be achieved by incorporation of polymer side chains into the bilayer matrix.

The main objective of this study was to develop synthetic bilayer membranes derived from N-triethyl-triaminopyridium perchlorates **1a** – **d** having together two saturated long alkyl substituents and one polar group with a free terminal amino group and to determine the effect of concentration of amphiphiles on the membrane's properties and stability.

The results suggest that the bilayer assembly with a free amino group derived from the pyridinium salts with different long alkyl substituents may be capable to bond both organic (*e.g.* fluorescamine, [9-11] riboflavin and substituted fluorescein as fluorescence marker[12]) and inorganic compounds.

The alkyl chain is highly oriented in bilayer membranes. This makes interesing to explore the effect of an additional long substituent with amino group at nitrogen atom which increase the affinity for different compounds in the molecular devices.

Moreover, the incorporation of organic marker as fluresceine into hydrophilic synthetic membrane films allows the modification of their barrier properties, improving its potential commercial application as drugs and ion transport.

RESULTS AND DISCUSSION

Materials and Methods

The synthesis of pyridinium salts **1** has been described in detail elsewhere.[13] The synthetic procedure consists in the well studied conversion,[14-16] of pyrylium salts into pyridinium salts by the reaction with primary amines. Using this method more 2,4,6-trisubstituted / 2,3,4,6-tetrasubstituted pyrylium and pyridinium salts having either one or two linear α/γ-alkyl side chains were obtained in the last years in our laboratory. [1-3, 8, 13, 17-25]

Table 1 presents the various amphiphiles which are studied in this paper:

All new compounds were fully characterized by ^{1}H- and ^{13}C-NMR (at 400 MHz for protons and at 100 MHz for carbons), IR and UV spectra and by elemental analysis.

Table 1

The alkylpyridinium amphiphiles synthesized
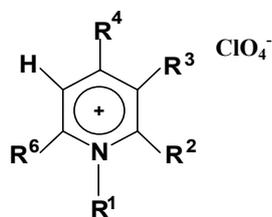
R^1: -(CH$_2$ CH$_2$ NH)$_2$- CH$_2$ CH$_2$ NH$_2$

Amph.	R^2	R^3	R^4	R^6
1a	C$_{11}$H$_{23}$	H	CH$_3$	CH$_3$
1b	C$_{11}$H$_{23}$	H	CH$_3$	C$_{11}$H$_{23}$
1c	C$_{15}$H$_{31}$	H	CH$_3$	C$_{15}$H$_{31}$
1d	C$_{17}$H$_{35}$	H	CH$_3$	C$_{17}$H$_{35}$

Membranes derived from pyrilium salts **1a-1c** were prepared using a Langford Sonomatic, T 175 bath-type sonicator, a similar procedure to that described previously was employed. [1-3]

In order to learn to control the self-assembly process these membranes have been studied by optical microscopy (OM), phase separation (membrane stability), dynamic light scattering (DLS) and differential scanning calorimetry (DSC).[26]

Membrane Stability

All compounds foamed intesively when sonicated and formed either emulsions or microdispersions. These were stable for variable times depending on the structure of the amphiphile. The phases separated either as an upper oily layer or as precipitates. The time interval from sonication until phase separation occured and the aspect of the membranes are presented in table 2.

Table 2

Aspect of the membranes after sonication and phase separation times

Amph.	CA[a], mM	Aspect	Time, days	Observations
1a	1.97	emulsion	90	separates an upper oily layer
1b	1.87	emulsion	80	separates an oily layer
1c	1..93	emulsion	76	separates an oily layer
1d	1.95	emulsion	77	separates gel-like fluffs which agglomerates in time

[a] Concentration of the amphiphile after sonication.

In previous papers[1-3] we presented the relationships between cationic amphiphiles and their aggregates and the influence of chemical structures on the membrane stability.

When the amphiphiles have a short alkyl (methyl) or alkylamino (ethylene-amino) at the N-substituent, the corresponding membrane has a tendency to agglomerate as precipitates or microdispersion, but the time interval is relatively great. Higher stabilities were obtained for all membranes derived from N -triethyltriamino-pyridinium salts **1a-d**.

The higher stability was obtained in the case of **1a** which has two long substituen in the vicinal positions. The third alkyl side chain decreases the phase separation times but not significantly as in N-methyl case. For all amphiphiles, the time interval from sonication until phase separation was over 60 days.

Optical Microscopy (OM)

The influence of chemical structures on the aggregate morphologies which was observed by OM is presented in Table 3 and Figs. 1a-f.

Fig. 1a and 1b present the photographs obtained after sonication of pyridinium salt **1a** having one undecyl side chain in α-position and after dilution of the sample. In this case, the primary structure was vesicles and ordered structures by the self-assembly of vesicles were obtained.

All 2,6-disubstituted pyridinium salts **1b-d** give complex structure with variable morphologies. In this case, after dilution of the samples (Fig. 1d and 1f) variable primary structures were observed. For medium chains (2,6-diundecyl) the primary structures were vesicles (Fig. 1c). In contrast, long 2,6-dipalmitoyl chains in α-positions (**1c**) generated complex structures formed out of vesicles and globules or twisted globules and vesicles (Fig. 1 e, f).

These experimental data suggests the forming of different intra- and intermolecular hydrogen bonds. The fact is relevant for the DLS experiments which were carried out at concentrations in the range 0.001-0.01 mM.

Fig. 1h present the photograph obtained after sonication of pyridinium salt **1a** and after reaction with fluorescein. In this case, complex structures were obtained.

Table 3

Agregate morphologies of pyridinium salts amphiphiles

Amph.	Aggregates	Figures
1a	Vesicles	1a
1a[a]	Parallel chains of vesicles	1b
1b	Vesicles	1c
1b[a]	Complex structures, globules and vesicles, globular assembly.	1d
1c	Complex structures, globules and vesicles	1e
1c[a]	Complex structures, globules and vesicles, complex assembly.	1f
1d	Complex structures, globules and vesicles, twisted globules and vesicles	1g
1a[b]	Complex structures	1h

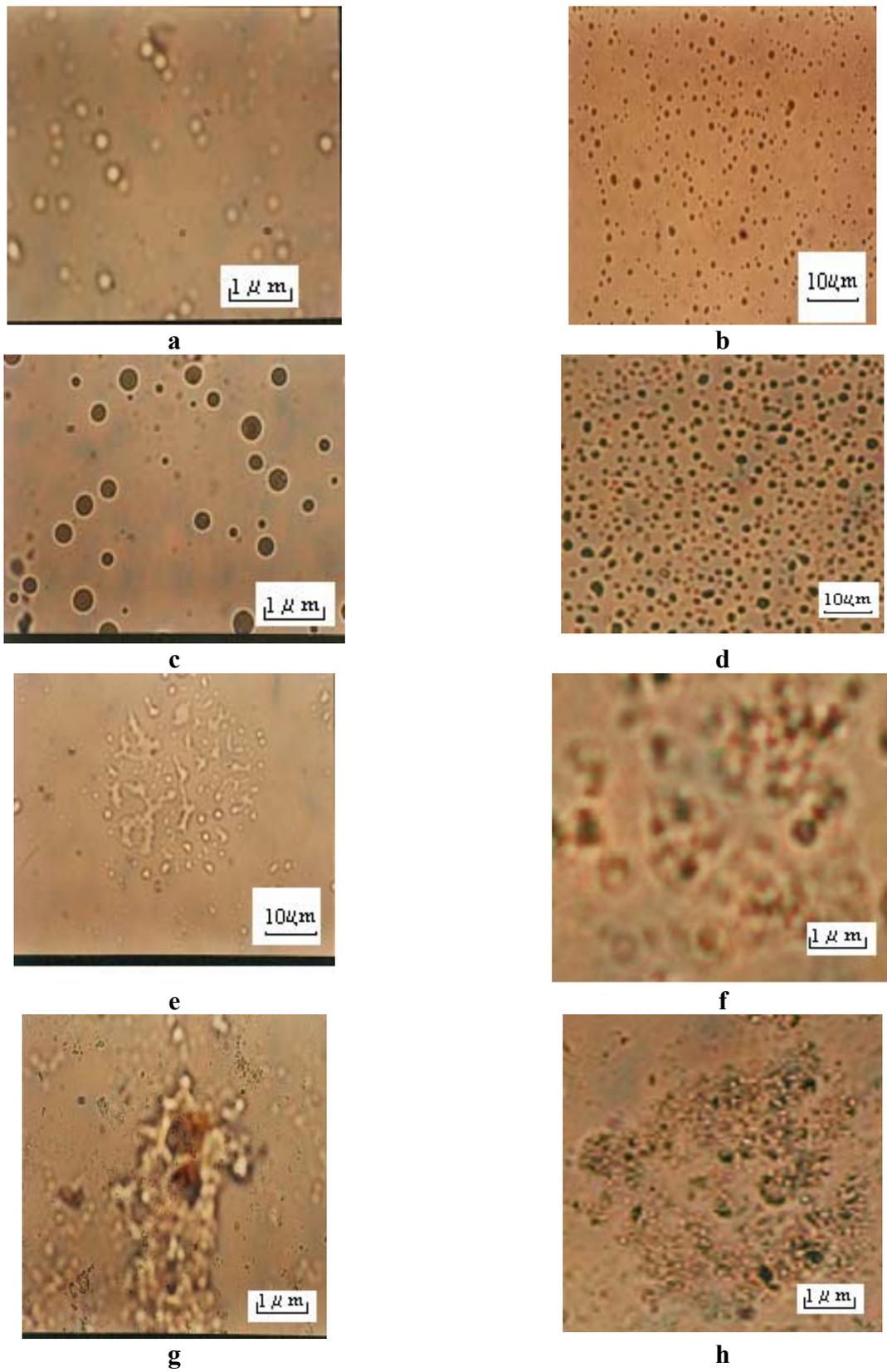[a]: dilluted; [b]: after reaction with fluorescein.

Fig. 1 – The OM photographs for membranes derived from:
a: 1a; b: 1a dilluted; **c: 1b; d: 1b** dilluted; **e: 1c; f: 1c** dilluted; **g: 1d; h: 1a** after reaction with fluorescein.

Reaction with Fluorescein

The fluorescence marker has been used to label amino phospholipids in liposome and encapsulation of this marker (derived fluorescein or riboflavin) was also described.[9-12]

In all cases the fluorescence intensity is a direct measure of the amount of the reacted amine or the permeation property of the surfactant vesicle.

Fig. 2 shows the reaction of fluorescein with amine-aggregates derived from the pyridinium salts **1a**–**d** with different long alkyl substituents.
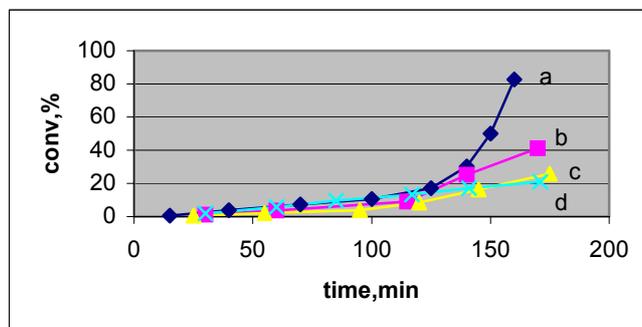

Fig. 2 – Reaction with fluorescein.

In all cases the fluorescein reaction time was less 200 minutes.

For the synthetic membrane derived from **1a** (having one undecyl side chain in α-position), when the single-walled vesicles was obtained, the fluorescein reacted almost quantitatively.

For membranes derived from **1b-d** having a new second long chain the secondary process by penetration of fluorescein into the inner-surface of vesicles was not possible. In this case, fluorescein reacted only with the amine groups located at the outer surface of the double–walled vesicles.

Thus, a new long α-alkyl side chain for membrane derived from **1b** lead to drastic decrease of fluorescein conversion and the reaction ceases at 40%. In this case amino group should exist in the both manner, into the inner and at the outer surface of the double-walled vesicles.

The membranes derived from **1c** and **1d** present a great difference between the leghts of the N-substituent and the 2,6-α-alkyl side chains. In this case, the amino group exist majoritary in the inner surface of the double-walled vesicles and the reaction ceases to produced at 20% conversion.

Dynamic Light Scattering (DLS)

The formation of aggregates was observed by DLS measurements.

To obtain the particles size and size distribution in aqueous solution DLS measurements were performed on a commercially laser light scattering spectrometer (NICOMP 370). It was expected that if no self-assembly occur then and the diameter of the sample (at room temperature) after irradiation will be equal to that measured before irradiation.

In such situation on DLS diagrams only one peak in the region of 200 nm was obtained. On the other hand, if the self-assembly process is successful, one peak in the region of higher diameters should be detected. All the samples in which two or more distribution peaks will be find should be interpreted as the intermediate states of process.

Just after 1 min the formation of aggregates was observed. Finally after cca 30 min. the diameters of the aggregates remained unchanged upon further irradiation. However, on final stage of self-assembly, bimodal distribution of particles was obtained.

Table 4 presents the size of aggregates, distributions and the final time for membranes derived from **1a-d**. According with the Gaussian distribution, at this time, the system was stable.

The formation of aggregates with considerably dimensions was obtained for **1a** and put in evidence by Nicomp Analysis Distribution (NAD). For **1b-1d** the size of aggregates increases and different distributions were obtained (Table 4).

Long-lasting stability of membranes was also investigated. Over two months hydrodynamic radius of the samples was measured three times. After two month from preparation, no precipitation was noticed. The obtained DLS results were in each case relatively identical.

Table 4

DLS results for **1a-1d** aqueous solutions at 20 °C

Cpd.	CA M×10³	Conc. DLS M×10⁵	Time min / sec	Aggregate	
				d, nm	%
1a	3.20	1.59	29/10	267.9	18.0
				2567.8	82.0
1b	3.20	1.61	24/39	97.8	38.0
				998.8	62.0
1c	3. 19	1.60	22/23	95.4	44.0
				850.9	56.0
1d	3.20	1.61	22/42	98.9	43.1
				830.4	56.1

EXPERIMENTAL PART

The NMR spectra have been recorded on a BRUKER AVANCE DRX 400 instrument, equipped with a 5 mm inverse detection multinuclear probe head and field gradients on the z axis, operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C nuclei. The COSY45, HMQC and HMBC spectra have been recorded with standard Bruker parameters in the versions employing pulsed field gradients. All spectra have been recorded in deuterated chloroform, and the chemical shifts have been reported as δ values referenced to TMS as internal standard. Infrared spectra were run on a BRUKER VERTEX 70 instrument equipped with a Golden Gate diamond ATR. The UV-VIS spectra were recorded with a SPECORD instrument in 1 cm cuvettes. Melting points were measured in open capillary tubes (for the low melting compounds) or on a hot-stage melting points apparatus (equipped with a polarizator to check for nematic properties). The OM was performed with a microscope NIKON AFX-DX equipped with a digital camera. The dynamic light scattering (DLS) experiments were carried out using a NICOMP 370 equipment.

General procedure for the preparation of membranes

Given amounts (1-4 mM) of suspending pyridinium salts **1a-1d** were ultrasonically irradiated in distilled water. In all cases, pyridinium salts with long alkyl substituents were sonicated without anionic surfactants.

Sonication was performed in a cleaning ultrasonic bath (Langford Sonomatic, T 175) having the frequency of 33 kHz and 100 watts electrical power, using a 50 ml Erlenmeyer flask with a bottom area of 20 cm^2, containing water-pyridinium salts suspension, the total power being in the range of 0.5 watts/cm^2. The temperature of cleaning bath was kept constant at 25°C. The sonication times in the ultrasonic cleaning bath were 60 min for all compounds. The obtained membranes which were either emulsions or microdispersions were stored in closed vessels until analysis.

Reaction with fluorescein

An amine vesicle solution (5 mL) was mixed with a given amount of fluorescein in acetone (1%), at 20 °C. The reaction mixture was maintained with stirring at room temperature for 20-40 min. A 200 μL aliquot was added to 1mL ethanol and the fluorescence intensity at 460 nm was determined.

The fluorescein reaction was finished when excess of fluorescein was observed. For the last aliquot, for all experiments, the modification of fluorescence intensity was not observed after 24 hours.

Dynamic light scattering (DLS)

The dynamic light scattering (DLS) experiments were carried out using a NICOMP 370 equipment. The scattering data were processed with two types of distributions: a Gaussian or a multimodal distribution referred to as Nicomp Analysis Distribution (NAD).

REFERENCES

1. M. V. Bogățian, C. Deleanu, D. Simion (Nuță), M. Maganu, A. C. Corbu, F. Chiraleu and G. Bogățian, Rev. *Roum. Chim*., **2006**, *51*, 273.

2. M. V. Bogățian, M. Vinatoru, G. Bogățian, G. Mihai and T. S. Balaban, Rev. *Roum.Chim.,* **2004**, *49,* 819.

3. M. V. Bogățian, M. Vinatoru, G. Bogățian, C. Deleanu, V. Cimpeanu, F. Chiraleu, G. Mihai and T. S. Balaban, *Romanian International Conference on Chemistry and Chemical Engineering – RICCCE IV***,** Ed. Printech Bucharest, Romania, Proceedings –vol. 4, p. 137-144, 22-24 Sept. 2005**.**

4. A. Roosjen, J. Smisterova, C, Driessen, T. Andersg, A. Wagenaar, R. Huest, D. Hoekstra and J. B. F. N. Engberts, *Eur. J. Org. Chem.*, **2002**, 1271; D. A. P. Meekel, A. Wagenaar, J. Smisterova, J. E. Kroez, P. Haadsna, B. Basgroof, M. C. Stuart, A.Brisson, M. J. H. Ruiters, D. Hoekestra and J. B. F. N. Engberts, *Eur. J. Org. Chem.*, **2000,** 665.

5. T. Kunitake , "Synthetic Bilayer Membranes", Ed. Wiley, New-York, **1991**.

6. P. Yager and P. E. Schoen, Mol. Cryst. Liq. Cryst., **1984**, 106c, 371; T. Shimizu and M. Hato, *Biochim. Biophys. Acta*, **1993**, *1147,* 50; H. Kunwahara, M. Hamada, Y. Ishiksuro, and T. Kunitake, *J. Amer. Chem. Soc.,* **1993**, *115*, 3002; H.Kunwahara, M. Hamada, Y. Ishikowa and T. Kunitake, *J. Amer. Chem. Soc.,* **1993**, *115*, 3002; T. Kunitake and T. Shinkata, *J. Amer. Chem. Soc.*, **1983**, *105*, 6070.

7. T. Kunitake , "Modern Trends of Colloid Science in Chemistry and Biology", Int. Symp. on Colloid & Surface Science, Interlaken, Switerland, Conferene Procededings, Eicke H. F. , Birkhauser verlag Basel, 1985, 34.

8. M. Bogățian, Ph. D. Thesis, Roumanian Academy, Institute of Organic Chemistry "Costin D. Nenitzescu", 1998.

9. G. M. Funk, C. E. Hunt, D. E. Epps and P. K. Braun, *J. of Lipid Research*, **1986**, *27*, 792.

10. H. C. Lee and J. G. Forte, *Biochim. Biophys. Acta.*, **1979**, *554*, 375.

11. T. Kunitake, Y. Okahata and S. Yasunami, *J. Amer. Chem. Soc.,* **1982**, *104*, 5547.

12. Y. C. Chung and H. J. Lee, *Bull. Korean Chem. Soc.*, **1999**, *20*, 1.

13. M. V. Bogățian, M. Vinatoru, G. Bogățian, D. Simion, F. Chiraleu, V. Campeanu and C. Deeanu, *Rev. Roum.Chim.*, **2007**, *52*, 899.

14. A. T. Balaban and C. D. Nenitescu, *Justus Liebigs Ann. Chem.,* **1959**, 625, 66; A. T. Balaban and C. D. Nenitescu, *Justus Liebigs Ann. Chem.*, **1959**, *625*, 74; P. F. G. Praill and A. L. Whitear, *Proc. Chem. Soc*., **1959**, 312.

15. A. T. Balaban, A. Dinculescu, G. N. Dorofeenko, G. W. Fischer, A. V. Kobilik, V. V. Mezheritskii and W. Scroth, "Pyrylium Salts. Syntheses, Reactions and Physical Properties", Adv. Heterocyclic Chem., Suppl., A. R. Katritzky, Ed. Academic Pess, New-York, 1984, vol. 2.

16. T. S. Balaban and A. T. Balaban "Pyrylium Salts" in Science of Synthesis – Houben-Weyl, Methods of Molecular Transformations, Volume 14, E. J. Thomas, Ed., Thieme, Stuttgart, 2003**,** Chapter 14.1, p. 11-200.

17. M. V. Bogățian, A.Hartopeanu, A. Nicolescu, F. Chiraleu, M. Maganu, M. Vinatoru, D. Simion and C. Deleanu, *Rev. Roum. Chim.*, submitted to press.

18. M. V. Bogățian, G. Bogățian, S. Udrea, A. C. Corbu, F. Chiraleu, M. Maganu, and C. Deleanu, *Rev. Roum. Chim.*, **2006,** *51,* 291.

19. M. V. Bogățian, V. Cimpeanu, C. Deleanu, A. C. Corbu, G. Bogatian and T. S. Balaban, *ARKIVOC*, **2005**, (x), 272-284   (http://www.arkat-usa.org/ark/journal/2005/I10_Balaban/1275/AB-1275B.pdf).

20. M. V. Bogățian, C. Deleanu, S. Udrea, F. Chiraaleu,M. Plaveti, M. G. Danila, G. Bogățian and T. S. Balaban*, Rev. Roum.Chim*., **2003**, *48,* 717.

21. M. V. Bogățian, V. Campeanu, S. Serban, G. Mihai and T. S. Balaban*,* Rev. *Roum.Chim***., 2001**, *46,* 115.

22. M. V. Bogățian, G. Mihai, M. Plăveți, F. Chiraleu, M. Maganu, V. Bădescu and T. S. Balaban, *Rev. Roum.Chim*., **1998**, *43*, 315.

23. M. V. Bogățian, G. Mihai, M. Plăveți, F. Chiraleu, C. Deleanu, V. Bădescu and T. S. Balaban, *Rev Roum. Chim*., **1996**, *41*, 979.

24. M. V. Bogățian, C. Deleanu, G. Mihai and T. S. Balaban, *Z. Naturforsch*., **1992**, 47b, 1011.

25. T. S. Balaban , M. V. Bogățian, M. Plăveti, V. Bădescu and G. Mihai*, Rev Roum. Chim.*, **1991**, *36,* 229.

26. This work will be reported elsewhere.