

FRAGMENTATION OF PAMAM DENDRIMERS IN METHANOL

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Abstract. The decomposition of ethylenediamine core poly(amidoamine) (PAMAM) dendrimers (generation –0.5) was investigated by gas chromatography. The decomposition of PAMAM dendrimer G–0.5 into triester **2** is caused by the *retro*-Michael reaction. Equilibrium between the Michael and *retro*-Michael reactions is shifted towards the *retro*-Michael reaction at an elevated temperature. Similar decomposition is possible in the case of higher PAMAM dendrimer generations.

Key words: PAMAM dendrimer, *retro*-Michael reaction.

INTRODUCTION

Poly(amidoamine) (PAMAM) dendrimer (where the initiator core is ammonia or ethylenediamine (EDA)) molecules are considered to be relatively stable structures and they are usually dissolved and used in methanol solutions [1]. However, in everyday practice it has been noticed that the properties of these solutions change considerably on prolonged storage. The problem is particularly acute for molecular geneticists, who use PAMAM dendrimers for example for transporting DNA into the cell. In order to elucidate the chemical background of these changes we investigated the stability of a model compound – N,N,N,N-tetra-2-methoxycarbonylethylethylenediamine (generation G–0.5 of the EDA core PAMAM dendrimer) – in methanol. This choice was made on the basis of the following considerations: the dendrimeric compound G–0.5 can be easily purified by column chromatography (CC), this compound and all its decomposition products can be analysed by gas chromatography (GC), the results

of experiments can be generalized and used to predict the stability of the higher generations of PAMAM dendrimers.

EXPERIMENTAL

Synthesis of samples

N,N,N,N-tetra-2-methoxycarbonylethylethylenediamine (tetraester) was synthesized according to [2] and purified using CC before the fragmentation experiment. The purified product (98.9 % by GC) contained 0.5% N,N,N-tri-2-methoxycarbonylethylethylenediamine (triester), which was verified by the ^{13}C NMR spectrum.

General method of experiments

The 5% solution of G-0.5 in methanol was divided into four flasks. These were kept at -15 , 4 , $22-25^\circ\text{C}$ (room temperature), and 50°C , respectively. After certain intervals samples from these solutions were analysed by GC. The identity of tetraester and triester was verified by NMR analysis of separately prepared and purified tetraester and triester samples (see the corresponding ^{13}C and ^1H chemical shifts in Table 1).

Table 1. ^{13}C and ^1H NMR chemical shifts of EDA core based PAMAM dendrimer G-0.5 (tetraester **1**) and its decomposition product (triester **2**) in CDCl_3

Group	No. of carbons	^{13}C	^1H
Tetraester			
$-\text{C}^*\text{H}_2\text{N}<$	2	52.17	2.47
NC^*H_2	4	49.68	2.74
$\text{C}^*\text{H}_2\text{CO}$	4	32.54	2.42
C^*OO	4	172.89	–
COOC^*H_3	4	51.46	3.65
Triester			
$-\text{C}^*\text{H}_2\text{N}<$	1	53.50	2.55
$-\text{C}^*\text{H}_2\text{NH}-$	1	47.08	2.65, 1.80(NH)
NC^*H_2	2	49.27	2.76
NC^*H_2	1	45.14	2.89
$\text{C}^*\text{H}_2\text{CO}$	2	32.46	2.45
$\text{C}^*\text{H}_2\text{CO}$	1	34.69	2.53
C^*OO	2	172.99	–
C^*OO	1	173.10	–
COOC^*H_3	2	51.56	3.68
COOC^*H_3	1	51.56	3.69

Gas chromatography

A Shimadzu GC-14B gas chromatograph equipped with a flame ionization detector and a low-polarity fused silica capillary column Chrompack CP-Sil 5CB, $25\text{ m} \times 0.25\text{ mm} \times 0.12\text{ }\mu\text{m}$, coated with dimethylpolysiloxane, was used

for GC analyses, carrier gas helium 2 mL/min. The temperatures of the injector and detector were 200 and 250 °C, respectively. The following temperature program was used: 130 °C (2 min), 12 °C/min, 250 °C (2 min).

NMR analysis

An AMX500 MHz Bruker instrument was used for high field (11.7 T) ^1H and ^{13}C NMR spectroscopy. The samples were dissolved and measured in CDCl_3 solution. The corresponding ^{13}C and ^1H chemical shifts are presented in Table 1.

RESULTS AND DISCUSSION

A tetraester/triester equilibrium existed almost at all investigated temperatures (the chromatograms A, B, C, and D in Fig. 1 characterize equilibrium between tetra- and triester at 50 °C). However, at lower temperatures the equilibrium was shifted considerably towards the tetraester. The results of the changes in the

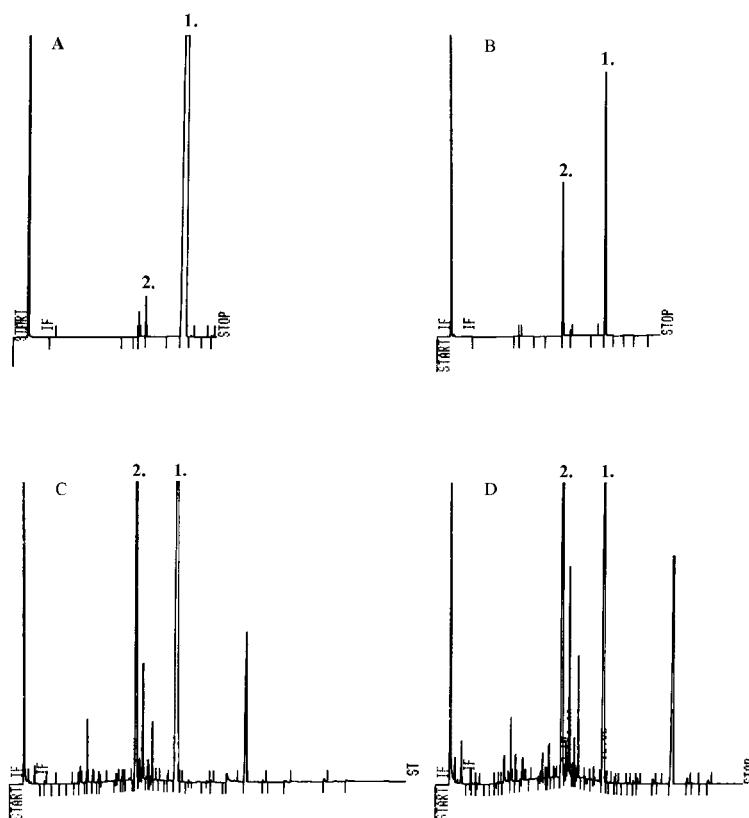


Fig. 1. Gas chromatograms of the decomposition of tetraester (1) into triester (2) at 50 °C during different reaction times: A – 0 h (1 – 98.9%, 2 – 0.5%); B – 24.5 h (1 – 74.1%, 2 – 25.1%); C – 71.5 h (1 – 43%, 2 – 39.6%); D – 167.5 h (1 – 28.3%, 2 – 32.6%).

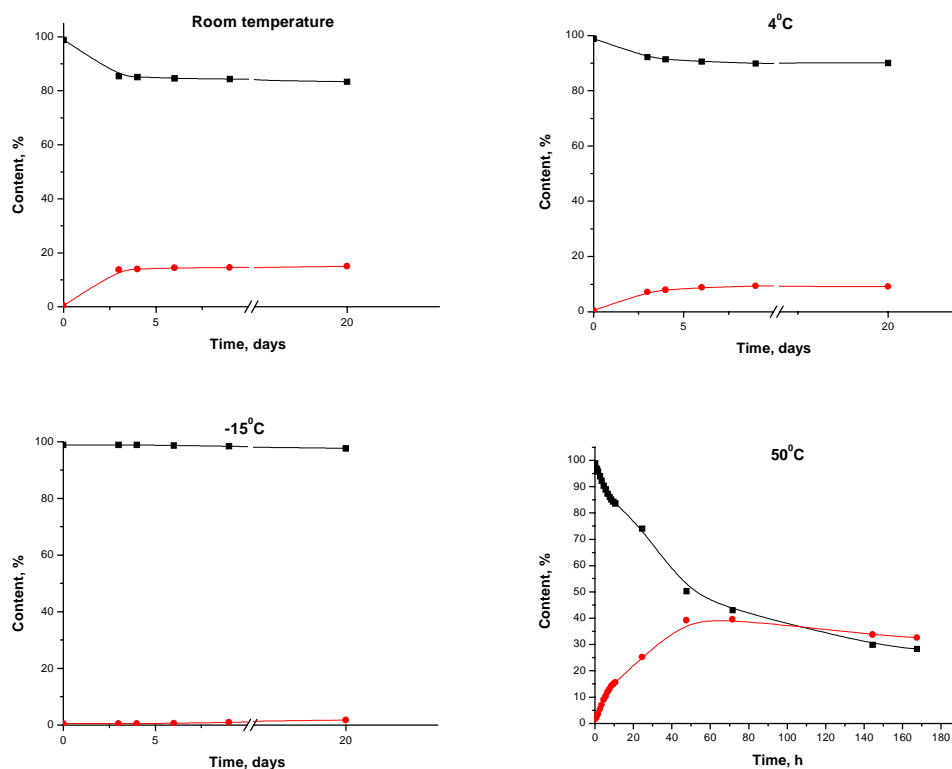


Fig. 2. Decomposition of PAMAM dendrimer G-0.5 (as 5% methanol solution) at different temperatures: ■ the content of tetraester (area % on gas chromatogram), ● the content of triester (area % on gas chromatogram).

tetraester methanol solution on standing at different temperatures are presented in Fig. 2. At 50°C also irreversible decomposition of the compounds was observed (additional peaks in Fig. 1C and D, Fig. 2).

The formation of the triester from tetraester occurs most probably via a *retro*-Michael reaction (Fig. 3) [3,4]. The obtained curves of the conversion of tetraester **1** into triester **2** can be explained assuming that there exists an equilibrium between the Michael reaction and the *retro*-Michael reaction in the methanol solution of the dendrimeric compound.

The observed triester amount was considerable even at 4°C. It was only at -15°C that the extent of the *retro*-Michael reaction was inessential. At every investigated temperature an equilibrium between the tetraester and triester (Michael and *retro*-Michael reactions) was achieved (tetraester/triester ratio after 20 days: at 50°C 29/33 (165 h); at room temperature 83/15; at 4°C 90/9; at -15°C 98/2). Note that side reactions occurred at all temperatures used, with products showing up in the gas chromatogram; at 50°C, the rate of the formation of side products increased noticeably after heating overnight (Fig. 1).

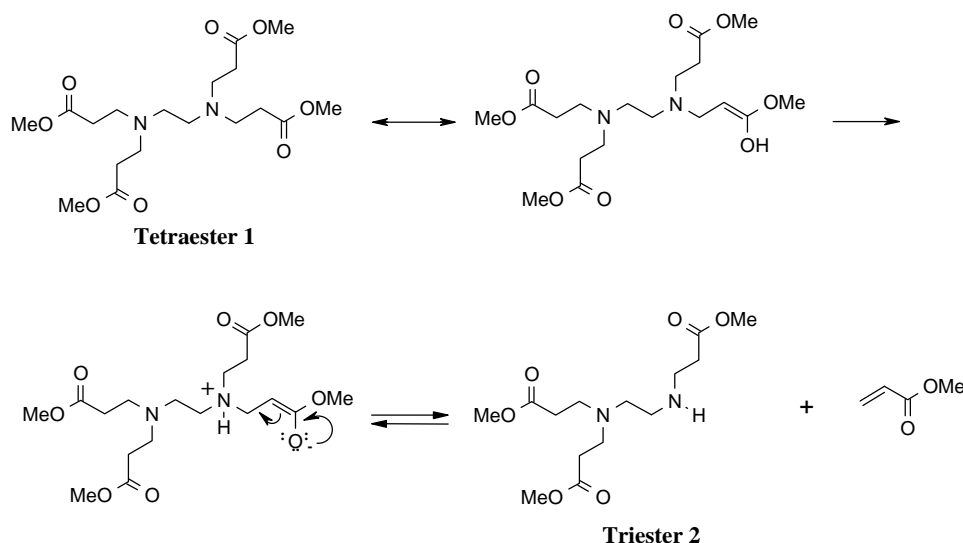


Fig. 3. The decomposition of tetraester to triester (*retro*-Michael reaction).

The higher generations of PAMAM dendrimers cannot be analysed by GC; however, an assumption can be made that they undergo similar *retro*-Michael decomposition reactions. This hints to the possible reasons of the changes of the properties (e.g. “transport activity”) of PAMAM dendrimers on prolonged storage in methanol. This might also be the reason why we obtained a mixture of tetraester/triester 98.9/0.5 in CC purification of the tetraester sample (see Experimental). The possibility of the *retro*-Michael reaction should be also considered in the preparation of regular PAMAM dendrimers as one of the sources of PAMAM dendrimer “structural errors” giving rise to the same asymmetrical dendrimeric side product as those obtained from an incomplete Michael addition (triester **2** in the case of PAMAM generation –0.5).

CONCLUSIONS

In the methanol solution of EDA-core (PAMAM) dendrimer G–0.5 an equilibrium exists between the Michael and *retro*-Michael reactions, resulting in the formation of triester **2**. An increase in temperature shifts the equilibrium towards the *retro*-Michael reaction. Very likely, similar decomposition takes place also in the case of PAMAM dendrimers of higher generations.

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POLÜAMIDOAMIINI DENDRIMEERI METANOOLI LAHUSES LAGUNEMISE UURIMINE

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Etüleendiamiintuumaga polüamidoamiini (PAMAM) dendrimeeri põlvkonna G–0.5 (tetraester **1**) lagunemine triestriks **2** on tingitud *retro*-Michaeli reaktsioonist. Tasakaal Michaeli ja *retro*-Michaeli reaktsiooni vahel nihkub temperatuuri tõustes *retro*-Michaeli reaktsiooni suunas. Tõenäoliselt leiab analoogne lagunemine aset ka kõrgemate PAMAM dendrimeeride põlvkondade korral.