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Compositional analysis of nitrile terminated poly(propylene imine) dendrimers by high-performance liquid chromatography combined with electrospray mass spectrometry

Sj. van der Wal[a,*], Y. Mengerink[a], J.C. Brackman[a], E.M.M. de Brabander[a], C.M. Jeronimus-Stratingh[b], A.P. Bruins[b]

[a]*DSM Research, P.O. Box 18, 6160 MD Geleen, The Netherlands*

[b]*University Centre for Pharmacy, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands*

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Abstract

Separation methods for nitrile terminated poly(propylene imine) dendrimers were developed to monitor and optimize their large scale production. Detailed analyses of defects within a dendrimer generation were performed by HPLC at alkaline pH (sodium hydroxide) on a polymer-based column or at neutral pH (ammonium acetate) on a silica-based column combined with electrospray mass spectrometry. Feasibility of operation of HPLC–electrospray MS with a mobile phase containing non-volatile components is demonstrated. © 1998 Published by Elsevier Science B.V. All rights reserved.

Keywords: Dendrimers; Polymers; Mobile phase composition; Poly(propylene imine)

1. Introduction

Dendrimers are macromolecules with a tree-like architecture [1]. Their size and chemical formulation can be precisely controlled, so that highly spherical and uniform molecules with a dense outer shell of identical end groups are obtained. These properties make dendrimers attractive e.g., as a core for star-shaped polymers or as a host for the transportation of other molecules [2].

Poly(propylene imine) (POPAM) dendrimers of general structure 1,4-diaminobutane-dendrimer-R_z [R=NH_2(Am), CN and z=4–128] can be produced on a large scale from inexpensive starting materials [3] as depicted in Fig. 1. They are synthesized by Michael addition reactions between 1,4-diaminobutane (DAB) and four acrylonitrile molecules and subsequent hydrogenation of the four cyano groups to primary amino groups. This cycle of Michael addition and reduction is repeated until the desired amino-terminated molecular generation or nitrile-terminated half-generation is obtained.

Lower generations of dendrimers are nearly pure products as determined by a combination of IR and nuclear magnetic resonance (NMR) spectrometry. NMR appears very useful for the determination of the selectivity and conversion of the last reaction, while functional groups are detected by IR spectrometry.

Detection of defects by NMR or IR spectrometry is increasingly difficult in higher generations because

*Corresponding author. Tel.: +31 46 767697, Fax: +31 46 767244.

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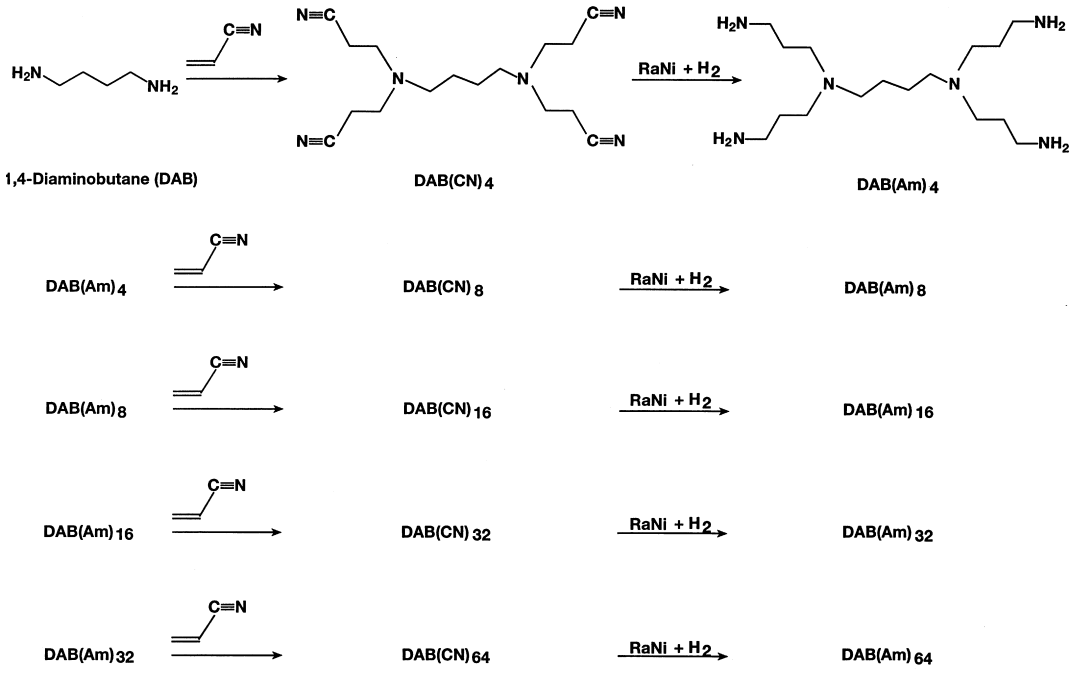

Fig. 1. Reaction scheme for the synthesis of poly(propylene imine) dendrimers.

the signals corresponding with a single defect are relatively weak.

Mass spectrometry (MS) is more suited for trace analysis, as was clearly demonstrated by Kallos et al. [4] who used electrospray (ESP) MS to analyze the fourth generation of a polyamidoamine dendrimer. Dvornic and Tomalia have followed the formation of successive generations of polyamidoamine dendrimers by means of ESP-MS and have identified the propagation of deficiencies through generations of dendrimers [5]. Schwartz et al. recorded ESP-MS spectra of polyamidoamine dendrimer generations 1–10 by means of a laboratory-built low-resolution, extended mass range quadrupole mass spectrometer [6]. Multiply charged ion spectra recorded on this extended mass range instrument demonstrated the complexity of the mixture of ideal structure and imperfect byproducts in generations 5–10. The molecular mass of ideal structure (IS) and imperfect byproducts in generations 1–4 were determined by the use of a commercial triple quadrupole instrument and associated software for deconvolution of multiply charged ion spectra [6]. Dendritic purity of amino- and cyano-terminated POPAM dendrimers has been assessed by this same method [7].

The direct infusion of a dendrimer solution into an ESP-MS system followed by deconvolution of multiply charged ion mass spectra is a quick and convenient method for the detection of imperfections in a synthetic product. A deconvoluted molecular mass profile has a limited clean range, since spurious signals outside this clean range are produced by the deconvolution algorithm [8]. Therefore, a complete branch cleaved off from the ideal structure cannot be detected in a deconvoluted spectrum since its molecular mass is approximately one fourth (or a lower fraction) of the molecular mass of the ideal structure. The same is true for the products formed by the condensation of two or more dendrimers, having a molecular mass well above the molecular mass of the IS.

Furthermore, MS cannot discriminate between isomeric dendrimer imperfections. Mixtures of generations of amino-terminated POPAM dendrimers

can be separated by size-exclusion chromatography [9]. Capillary electrophoresis (CE) and on-line CE–MS have been used by Stöckigt et al. [10] for the separation and identification of imperfections in the nitrile terminated second half-generation $DAB(CN)_8$. Investigation by CE–MS of impurities in higher generations was not reported [10].

Here we present the analysis of a series of nitrile terminated half-generations of POPAM dendrimers by reversed-phase high-performance liquid chromatography (HPLC)–ESP-MS, using both neutral and alkaline eluents.

2. Experimental

Experiments were done with samples before the production was optimized as described in Ref. [11] and were used for confirmation of the identity of the defects only. Solvents and buffer materials were of HPLC grade quality or equivalent. Water was taken from a Milli-Q purification system (Millipore, Milford, MA, USA). Inertsil columns 250 mm×6 mm I.D. (GL Sciences, Tokyo, Japan) were obtained from Applied Science Group (Emmen, The Netherlands); the PLRP-S column (125 mm×4 mm I.D.) was from Polymer Laboratories (Church Stretton, UK). Optimization of HPLC phase systems was performed on an HP 1090 liquid chromatograph equipped with an autosampler and a diode array UV absorbance detector (Hewlett-Packard, Boblingen, Germany).

HPLC–MS experiments carried out on a system consisting of two Kratos SF400 pumps with high-pressure gradient formation (Applied Biosystems, Foster City, CA, USA), a Rheodyne 7025 injector (Rheodyne, Cotati, CA, USA) with a 20-μl loop, an SPH99 column oven (Spark Holland, Emmen, The Netherlands), an IonSpray (pneumatically assisted electrospray) MS interface [12] and a Nermag R3010 triple quadrupole MS system with a custom-built atmospheric pressure ionization source equipped with a gas curtain [13]. A pneumatically controlled splitter transfers 5–10 μl/min of the HPLC eluent via a 60 cm×75 μm I.D. fused-silica capillary into the ionspray interface which is operated at 3.5 kV. Mass spectra were recorded from m/z 110 to 1900 at 2.9 s per scan under control of the Nermag Sidar data system. The sampling orifice (nozzle) was held at +70 V for most of the LC–MS experiments. To increase sensitivity for $DAB(CN)_{64}$ the voltage applied to the ion sampling orifice was increased from 70 to 170 V. Fragmentation of $[M+zNa]^{z+}$ ions was not observed at 170 V orifice voltage. The skimmer located behind the sampling orifice was at +25 V in all experiments.

The presentation of ions observed together with calculated molecular masses of various components in Tables 1–4 may seem inconsistent. The discrepancy is due to three different definitions for the mass of an ion and molecular mass, viz. nominal mass, accurate monoisotopic mass and average (chemical) molecular mass. Nominal mass is calculated using the rounded numbers $C=12$, $H=1$ and $N=14$. Accurate monoisotopic mass is calculated using the accurate mass of the isotopes ^{12}C, ^{1}H and ^{14}N. Average chemical molecular mass is calculated with the use of the average mass of each element, including its naturally occurring isotopes such as ^{2}H, ^{13}C and ^{15}N.

For singly charged ions below m/z 1000 nominal mass is adequate and convenient. With increasing molecular mass the accurate monoisotopic mass differs by more than 1 u from the nominal mass and the isotope distribution of a molecule becomes broader, while the top of the envelope of this distribution corresponds to the average chemical molecular mass. For example: for a neutral molecule $DAB(CN)_{16}$ $C_{88}H_{144}N_{30}$, nominal mass is 1620, monoisotopic mass is 1621.22, average molecular mass is 1622.32. For multiply charged ions the measurement of ion mass to the nearest 0.1 m/z value is to be preferred. However, due to limitations in hardware and software of the Nermag Sidar data system there is no provision for full scans at 0.1 m/z step size. Scans were taken with 1.0008 u steps (mass defect 0.08 per 100 u) to accommodate for the actual accurate m/z value of singly charged dendrimer ions. Rounded integer m/z values for singly and multiply charged ions have been compiled in the tables. All molecular mass values in the tables give the average chemical molecular mass rounded to integer numbers.

Table 1
Evaluation of mass spectra of $DAB(CN)_8$

Peak in Fig. 5	Neutral phase system				Alkaline phase system				Molecular mass	Proposed identity
	Retention time (min:s)	Ions observed			Retention time (min:s)	Ions observed				
		$z=1$ (m/z)	$z=2$ (m/z)	$z=3$ (m/z)		$z=1$ (m/z)	$z=2$ (m/z)	$z=3$ (m/z)		
1	6:35	565			26:47	588			565	Cyclic product
2	8:37	759	380		22:42	782	402		759	Monoamide, IS+18
3	9:10	688	345		24:07	711	367		688	IS−53
4	11:53	578			23:06	601			578	IS−53−110
5	11:56	741	371		27:02	764	393		741	Ideal structure (IS)
6	14:22	675			28:43	698	360		675	Cyclic product+110
7	16:30	851	426		28:30	874	448		851	IS+110
8	16:36	565			29:24	588	305		565	Cyclic product
9	16:39	631			26:42	654	338		631	IS−110
10	app. 17:00		654	436	31:25	1328	676	458	1306	Dimer
11	17:58		599		31:31		621	423	1196	Dimer−110
12	18:02	730	366		29:47	753	388		730	Monopropane, IS−11
13	app. 19:00		708	473	32:17		731	495	1416	Dimer+110
14	19:18		599		31:31		621	423	1196	Dimer−110
15					23:06	659			635	IS−53−53
16					29:40	711			688	IS−53
17					34:00		958	647	1871	Trimer

3. Results and discussion

3.1. Separation

HPLC phase systems with UV-transparent aqueous eluents with non-polar stationary phases are the most suitable for polar compounds that are hard to detect by UV absorbance except at low wavelengths, such as the POPAM dendrimer half-generations.

To a large extent the ionic character of the POPAM dendrimers dominates their chromatographic behavior: capacity ratios increase upon raising the pH of the eluent in a reversed-phase system. More subtle differences in ionization (and thus exposed hydrophobic surface) of byproducts relative

Table 2
Evaluation of mass spectra of $DAB(CN)_{16}$

Neutral phase system				Alkaline phase system				Molecular mass	Proposed identity
Retention time (min:s)	Ions observed			Retention time (min:s)	Ions observed				
	$z=1$ (m/z)	$z=2$ (m/z)	$z=3$ (m/z)		$z=2$ (m/z)	$z=3$ (m/z)	$z=4$ (m/z)		
				26:08	788			1530	Monoamide−110
				26:34	843	569		1640	Monoamide, IS+18
				27:21	752			1459	IS−53−110
				27:35	828	559		1611	IS−11 (monopropropane)
20:21		785	524	27:37	808	546		1569	IS−53
20:25		701		28:27	723	490		1402	IS−220
22:41		756	505	29:06	779			1512	IS−110
20:25	1622	812	541	29:14	834	563		1622	Ideal structure (IS)
				32:17		936		2738	Product of 1st gen. dimer
				32:58		1046	791	3068	Dimer

Table 3
Evaluation of mass spectra of $DAB(CN)_{32}$

Retention time (min:s)	Ions observed $z=3$ (m/z)	Molecular mass	Proposed identity
34:00	1116	3279	IS − 2x53
34:08	1121	3293	Monoamide − 110
34:12	1157	3403	Monoamide
34:55	1097	3222	IS − 53 − 110
34:55	1134	3332	IS − 53
35:06	1004	2944	IS − 440
35:22	1078	3165	IS − 220
35:26	1079	3169	IS − 2x53 − 110
35:26	1041	3055	IS − 330
35:42	1151	3385	Ideal structure (IS)
35:46	1115	3275	IS − 110

to the main product (IS, ideal structure) are likely, but hard to predict, since they depend on the conformation of the molecules, which in turn is dependent on the organic modifier, its concentration, the buffer and ionic strength. Acid–base dissociation constants for the first two generations of nitrile terminated POPAM dendrimers were calculated previously [9]. The pK_a in water of tertiary amino groups adjacent to a nitrile terminated extension is approximately 2 to 4. The strongly basic tertiary amino groups in the core of the molecule are protonated below pH 8.

At pH<6 the large number of protonated amino groups cause tailing peaks on silica based reversed-phase packing materials.

Table 4
Evaluation of mass spectra of $DAB(CN)_{64}$

Peak in Fig. 7	Retention time (min:s)	Ions observed						Molecular mass	Proposed identity
		$z=1$ (m/z)	$z=2$ (m/z)	$z=3$ (m/z)	$z=4$ (m/z)	$z=5$ (m/z)	$z=6$ (m/z)		
1	2:30	366						343	
2	3:05	419						396	
3	3:05	807						784	
4	3:55	750						727	
5	3:55	860						837	
6	5:00		856	578				1665	
7	6:20		827					1608	
8	6:20		882	595				1718	
9	15:00								$DAB(CN)_{32}$
10	26:00				1324	1064	890	5203	IS − 15x110 − 53
11	27:20				1310			5147	IS − 16x110
12	27:20				1337	1075		5258	IS − 15x110
13	27:20				1364			5368	IS − 14x110
14	28:30				1419			5588	IS − 12x110
15	31:00				1544			5976	IS − 53 − 880
16	31:30				1502			5919	IS − 990
17	31:50				1530			6029	IS − 880
18	32:30				1557			6139	IS − 770
19	32:50				1585	1250		6249	IS − 660
20	32:50				1654			6250	IS − 53 − 330
21	broad at 33:20				1681	1350		6637	IS − 53 − 220
22	33:30				1709	1372		6747	IS − 53 − 110
23	33:30				1737			6857	IS − 53
24	34:00				1612	1294		6359	IS − 550
25	33:50 through 35:00				1640	1316		6469	IS − 440
26	35:00				1667	1339		6580	IS − 330
27	35:00				1695	1360		6690	IS − 220
28	36:00				1722	1383		6800	IS − 110
29	36:05				1750	1405	1175	6910	IS (ideal structure)

Two pH ranges remain of interest for separation: pH 6–8 and pH>8.

At neutral pH good selectivity and peak shape for $DAB(CN)_{16}$ samples was obtained only with the recently introduced reversed-phase materials made of very pure silica and with deactivated silica packing materials with a polar coating (e.g., BioSil TSK125 and Supelcosil ABZ). However, these materials show similar degradation of chromatographic behavior. Upon the installation of a new column there is no need for the addition of triethylamine to the eluent since peak tailing is not observed. After a few hours of repeated injections of dendrimer solutions retention increases and peak tailing becomes unacceptable so that triethylamine needs to be added to the eluent in order to reestablish acceptable chromatographic performance.

In clean samples of dendrimers a limited number of side-products is present, that can be separated from the main component under isocratic conditions. A low concentration of triethylamine in the eluent (e.g., 200 μl/l) causes a slight elevation of the baseline of the UV detector at low wavelengths (<230 nm) and constitutes no problem otherwise. At pH 6–8 the difference in ionization of the tertiary amines within the core of the dendrimer translates into a different selectivity for isomeric defects: when the pH of the eluent is increased from 6.0 to 6.7 selectivity changes and the capacity ratios increase (Fig. 2).

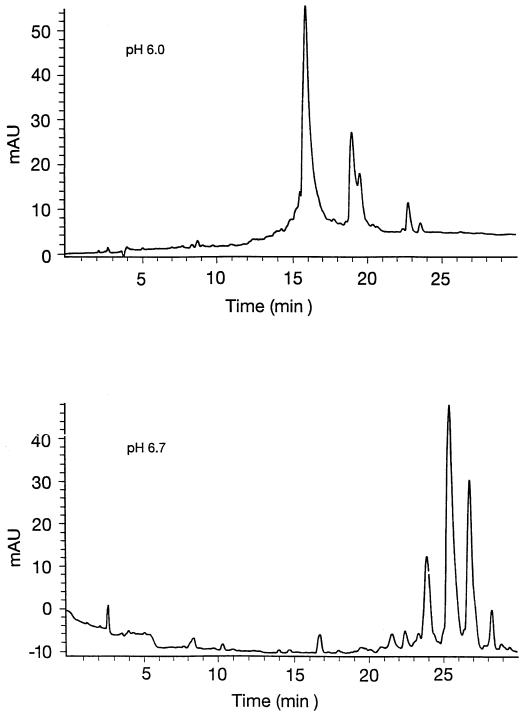

Fig. 2. Changes in selectivity with pH of the mobile phase at pH 6.0 and pH 6.7. Column: 250×6 mm Inertsil ODS-2; eluents: A=50 m*M* phosphate buffer; B=acetonitrile; gradient: 40 to 60% B in 30 min at 2 ml/min; temperature: 25°C; detection: 220 nm; sample: 5 μl of 20 mg/ml off-spec batch of $DAB(CN)_{16}$ in acetonitrile.

Similar selectivity at neutral pH is obtained with polystyrene–divinylbenzene columns, but lower resolution is observed due to lower efficiency of these polymer based columns.

The apparent instability of the neutral pH systems made us search for an alternative phase system. The wide range of expected side products in higher generations calls for a more suitable second phase system that may also be put to use in multidimensional separation. The change in selectivity with pH suggests the operation of this second phase system in the high pH range.

Silica packings dissolve at an alkaline pH, so polystyrene–divinylbenzene based reversed-phase columns were used. Separations obtained for the nitrile terminated dendrimers are shown in Fig. 3. Peak shape is greatly improved, when compared with separations on a silica based column presented in Fig. 2. Detection by UV absorbance at 220 nm combines simplicity with an acceptable signal-to-noise ratio and appears suitable for routine purity assessment. UV absorbance detection at this low wavelength is not very discriminating, however. The impurity profiles in the neutral and alkaline pH systems differ considerably and make the assignment of expected structures to peaks in liquid chromatograms highly speculative. Identification of impurities by combined LC–MS is necessary for guidance towards improved production of dendrimer generations.

3.2. On-line HPLC–MS

Mass spectrometrists have always preferred volatile buffer systems. Eluents containing 0.1 *M* ammonium acetate are the first choice for thermospray

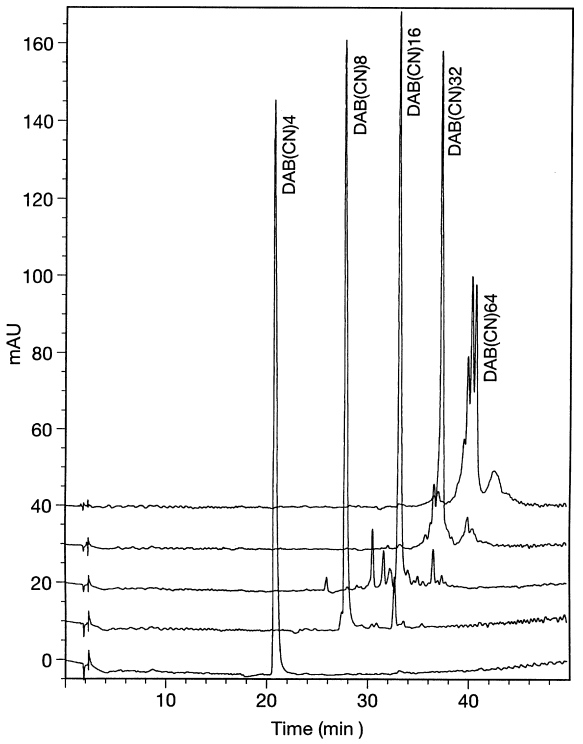

Fig. 3. Separation of poly(propylene imine) dendrimers at alkaline pH. Column: 125×4 mm PLRP-S; eluents: A=50 m*M* NaOH in water–acetonitrile (95:5); B=acetonitrile; gradient: 0 to 95% B in 55 min at 1 ml/min; temperature: 40°C; detection: 220 nm; samples: 5 μl of 4 mg/ml nitrile terminated dendrimer in acetonitrile.

ionization. In ESP ionization the efficiency of release of ions from charged droplets is reduced when the ammonium acetate concentration is increased from 1 m*M* to 100 m*M*. Unfortunately, we are forced to use a high ammonium acetate concentration in order to achieve adequate separation on a reversed-phase silica column.

Non-volatile components are usually avoided in on-line LC–MS. When thermospray or continuous flow fast atom bombardment (CF-FAB) are employed it is certain that non-volatile residues are left behind after evaporation of the LC eluent and will plug the thermospray vaporizer or the capillary channel of a CF-FAB probe tip. In the case of ESP ionization however, nebulization and solvent evaporation take place in free space inside the atmospheric pressure ionization source. The question now is whether ions can be introduced effectively into the vacuum system and mass analyzer, while neutral solid particles can be kept away from the entrance aperture (orifice or nozzle) into the vacuum. ESP sources equipped with a gas curtain (Fig. 4) can tolerate non-volatile components in the eluent since all neutrals including neutral buffer salt particles are swept away from the atmospheric lens and the ion sampling nozzle, while ions are allowed to pass through the gas curtain into the ion sampling orifice (nozzle) (Fig. 4). The chance of contaminating the ion sampling orifice is reduced further by diagonal positioning of the pneumatically assisted ESP interface and by aiming the aerosol beam at a position 1 cm from the nozzle orifice (Fig. 4). The prolonged use of a 50 m*M* NaOH solution for sample elution through the PLPR-S column has not presented a problem of nozzle orifice blockage or loss of sensitivity in our ion source. It should be noted that separation of ions from nonvolatile neutrals takes place in free space by the opposing action of curtain gas flow and electric field. Sodium hydroxide and sample ions are not forced to pass together through a narrow bore channel that gradually gets contaminated by the deposition of non-volatile materials.

Mass spectra obtained with the Inertsil column and neutral eluent system are very different from the spectra obtained with the PLRP-S column and alkaline eluent. In ESP combined with the neutral-phase system the dendrimers are ionized by the attachment of one or more protons donated by

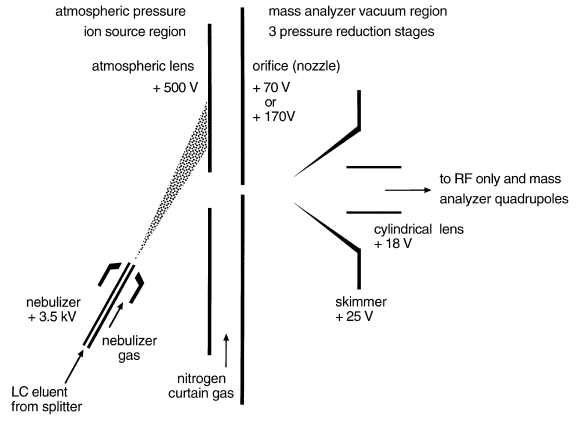

Fig. 4. Schematic diagram of the atmospheric pressure ionization source with pneumatically assisted electrospray interface.

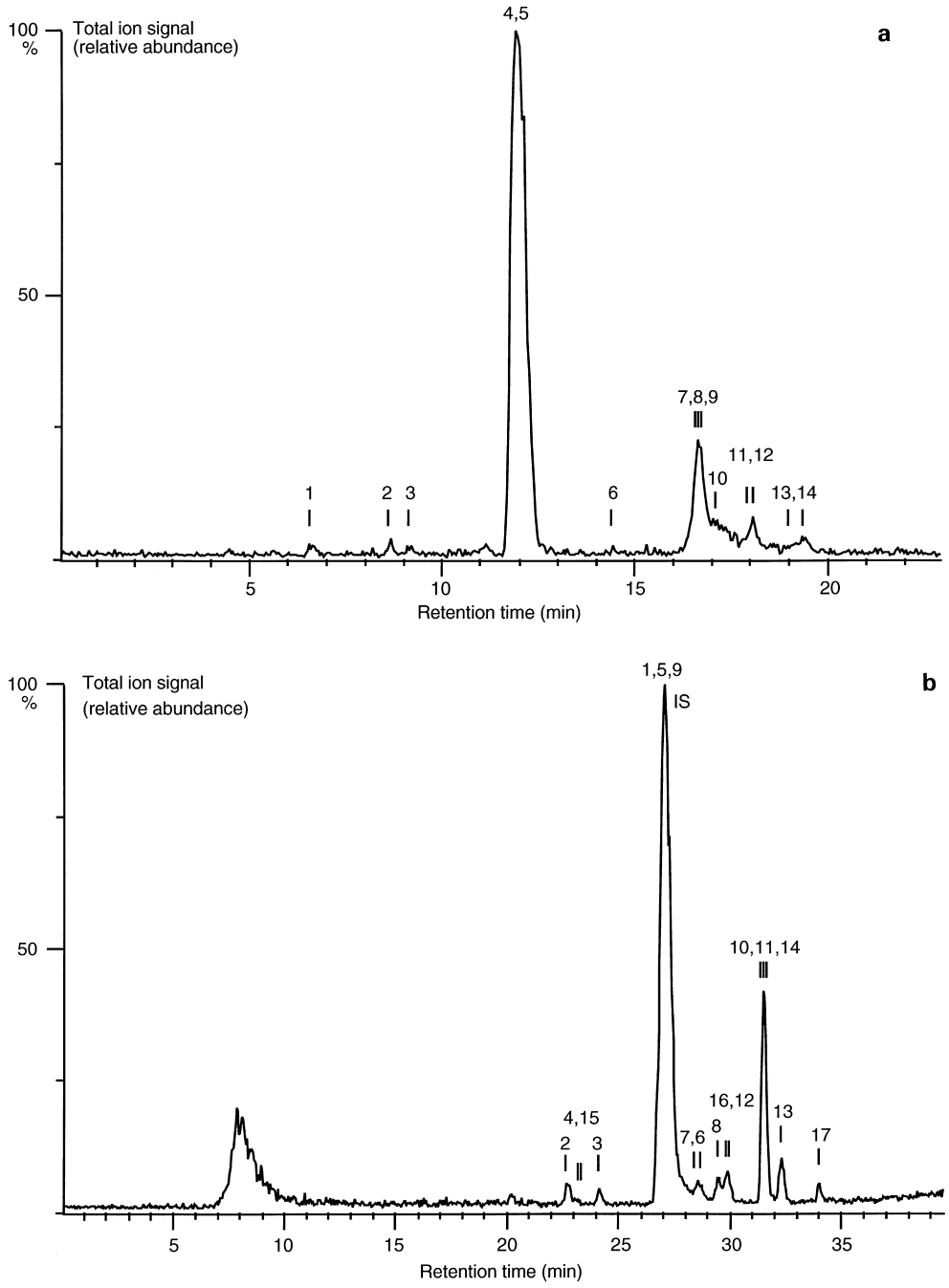

Fig. 5. Chromatograms with mass spectrometric detection (reconstructed TIC trace of the m/z range 305–1900) of $DAB(CN)_8$ with (a) neutral and (b) alkaline phase systems. The numbers in this figure correspond to those in Table 1. Conditions: (a) sample: 3 mg/ml in acetonitrile; column: 250×6 mm Inertsil ODS-2; eluents: A = 100 m*M* ammonium acetate, pH 6.7; B = acetonitrile; gradient: 20 to 50% B in 30 min at 2 ml/min; temperature 50°C; (b) sample: 5 mg/ml in acetonitrile; column: 125×4 mm PLRP-S, chromatographic conditions as in Fig. 3.

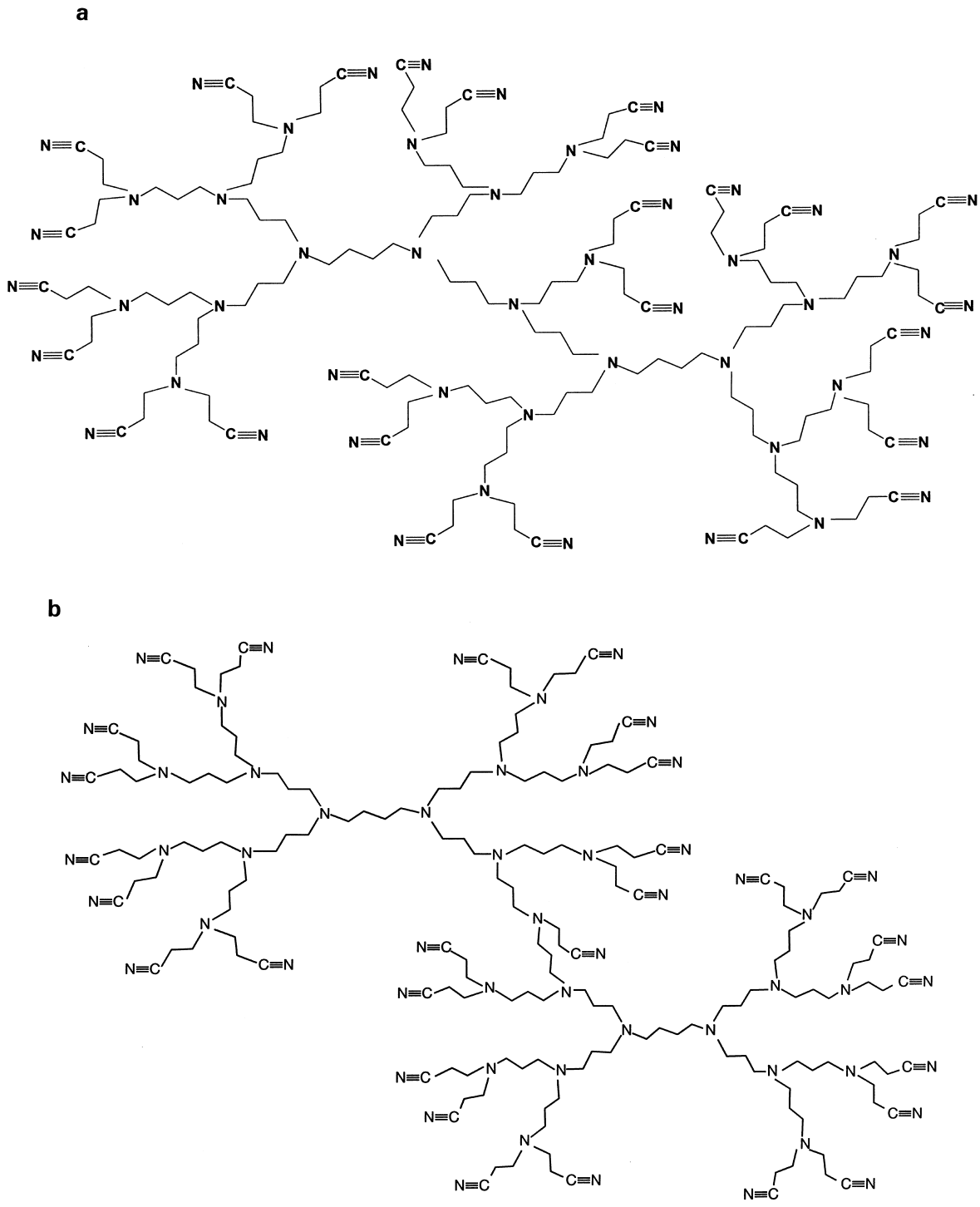

Fig. 6. Proposed structures of the products of the Michael addition reaction of acrylonitrile (a) with ''dimeric'' DAB(Am)$_4$ (M_r = 2738) and (b) with dimeric DAB(Am)$_8$ (M_r = 3068).

ammonium ions in solution. In the alkaline phase system protonation does not take place and the sample molecules are ionized by single or multiple attachment of sodium ions. $[M+zNa]^{z+}$ ions are formed in high abundance, together with a small number of $[M+zNa]^{z+}\cdot(NaOH)_n$ cluster ions with $n=1$ and $n=2$.

Four major side reactions in dendrimer synthesis have been observed earlier [3,5]: (a) single Michael addition of only one acrylonitrile, resulting in compounds lacking 53 u; (b) retro-Michael addition during hydrogenation giving a 110 u deficit; (c) cyclic structures formed by intramolecular elimination of ammonia during hydrogenation; and (d) formation of ''dimers'' and ''trimers'' by intermolecular elimination of ammonia during hydrogenation.

LC–MS chromatograms of a $DAB(CN)_8$ sample with neutral and alkaline phase systems are shown in Fig. 5. The molecular masses and proposed identities of the compounds eluting in the successive peaks are given in Table 1.

The most abundant imperfection in this sample is the IS−110 compound (IS=ideal structure). It elutes together with the main product in the alkaline phase system and would go undetected if only UV-absorbance had been used. Furthermore, the compound that is formed when the double Michael addition is not completed, having a deficiency of 53 u, is present at low level and is detected by the use of the alkaline eluent system but is not observed as clearly when the neutral eluent is used (Table 1, Fig. 5). Two isomeric compounds (M_r 565) consistent with cyclisation during hydrogenation of the first generation are found at 6:35 min and 16:36 min during elution with the neutral eluent system. For the analysis of oligomeric side products (M_r=1306 and 1871) only the alkaline phase system shows efficient chromatography. At 22:24 min in Fig. 5b a component with IS+18, the monoamide, is seen. Some unexpected side products are those with one methyl end group instead of a nitrile end group and products with an excess of 110 u. This last group of error structures occurs for the ideal structure (M_r=741+110), as

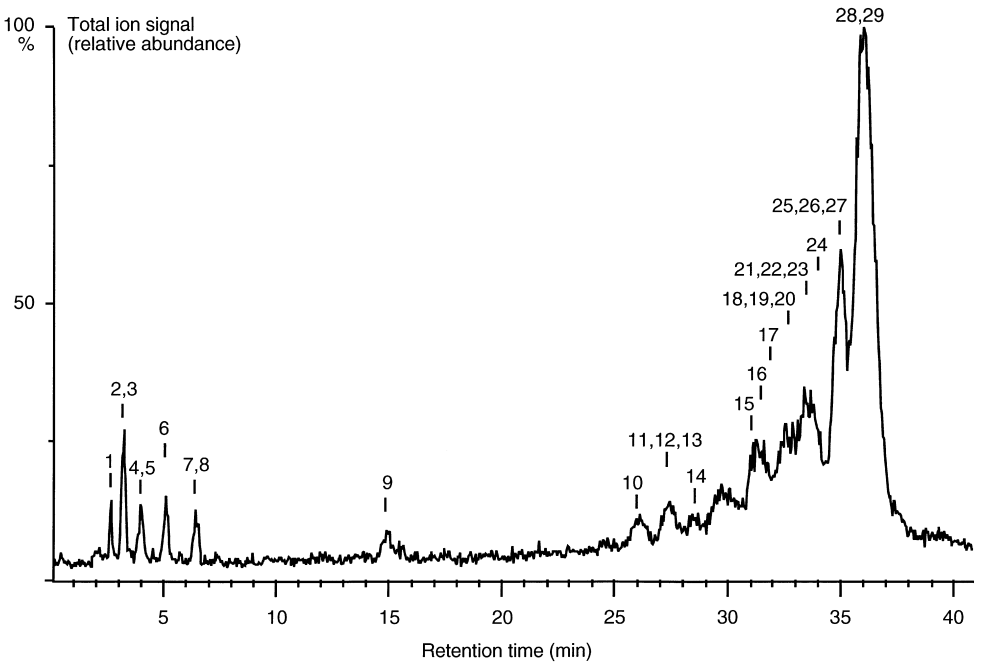

Fig. 7. Reconstructed TIC trace of the m/z range 305–1900 of $DAB(CN)_{64}$ obtained by LC–MS at alkaline pH; the numbers in this figure correspond to those in Table 4. Sample: 5 mg/ml in acetonitrile; column: 125×4 mm PLRP-S, chromatographic conditions as in Fig. 3, except for the gradient: 55 to 65% acetonitrile in 40 min.

well as for the cyclic (M_r=565+110) and the dimeric (M_r=1306+110) side products and its formation is at present not well understood.

Table 2 shows the elution times, MS data and proposed identities of the components of a $DAB(CN)_{16}$ sample. Due to different conditions during synthesis the number and levels of side products in this sample are considerably lower than in the $DAB(CN)_8$ sample discussed above. The major side products, however, are IS−53 and IS−110. Minor amounts of byproducts identified as molecules built up from first and second generation dimers were detected (Fig. 6).

In a $DAB(CN)_{32}$ sample the same series IS−110*n* was present (Table 3). A relatively large concentration of the IS−53 defect (earlier eluting than the main product by the presence of a secondary amine) and even defects lacking two propionitrile groups (IS−106) were found. Dimers did not appear to be present in this particular sample.

Chromatograms of $DAB(CN)_{64}$ samples with MS (Fig. 7) or UV absorbance detection (Fig. 3) indicate that higher generations of dendrimers are increasingly complex mixtures. Since synthesis does not include a separate purification procedure the percentage of perfect product in a production run is expected to decrease progressively with each (half-)generation as the number of functional groups increases by:

$$c(\mathrm{p})/c(\mathrm{s}) = y^n$$

where c(p)=concentration of perfect product; c(s)=concentration of perfect product from previous reaction step; n=number of reactions used to produce product; and y=yield of the reaction.

Conversion of $DAB(CN)_{32}$ into $DAB(CN)_{64}$ takes place via reduction of 32 nitrile groups and 64 Michael addition reactions, a total of 96 reactions. Even if each reaction proceeds with 99% yield no more than 40% perfect product is expected in the synthesis of $DAB(CN)_{64}$ starting from pure $DAB(CN)_{32}$.

The probability that compounds coelute from an HPLC column increases with the generation of dendrimer, as the number of potential isomers increases and selectivity of separation decreases. In this respect HPLC–UV is inadequate for the analysis of $DAB(CN)_8$ and later generations. The use of MS

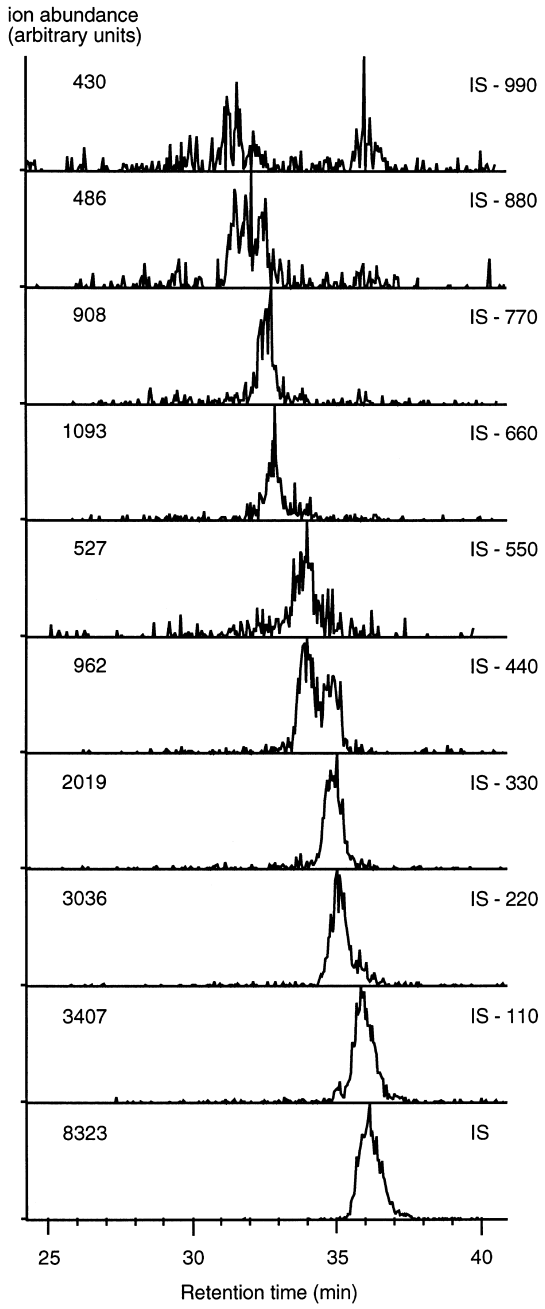

Fig. 8. Extracted ion current profiles of quadruply charged ions of the series of imperfections IS−110*n* in $DAB(CN)_{64}$. For reconstructed TIC trace and chromatographic conditions see Fig. 7.

alone appears to be inappropriate for reliable quantification: there is a considerable discrepancy between quantification by peak height measurements from mass spectra obtained by infusion of a sample solution into the mass spectrometer on the one hand and quantification by HPLC–MS and HPLC–UV on the other hand. Dimeric side products and cyclic structures were not found by MS alone, while the dimers were found in size-exclusion chromatography of both cyano-terminated and amino-terminated dendrimer generations [9]. Undesired suppression effects and/or preferential ionization of certain components apparently take place during ESP ionisation of a complex mixture infused into the mass spectrometer. Evaluation of the mass spectra at the apex of each peak in Fig. 7 revealed the complexity of the mixture of structural defects. All spectra clearly showed the presence of coeluting materials during LC–MS analysis of $DAB(CN)_{64}$. Extracted ion current profiles of the quadruply charged ions corresponding to the ideal structure (IS) and defective structures IS−110*n* in Fig. 8 demonstrate that a number of components appear to coelute in a pair-wise manner. It is interesting to note that defective structures IS−110, IS−220 and IS−330 elute as single peaks, while IS−440 is clearly split into two closely eluting peaks. It is not clear of course, if chromatographic resolution is high enough to permit the conclusion that a single peak in a profile in Fig. 8 represents no more than one isomer.

Dendrimer imperfections reported in earlier publications and in this paper are composed of the core molecule (diaminobutane in this paper) and side-arms in which one or more branches are absent. The intramolecular elimination of NH_3 from an amino-terminated generation gives rise to cyclic products while intermolecular condensation products of two or three dendrimer molecules leads to ''dimers'' and ''trimers''. The first few components in the chromatogram presented in Fig. 7 correspond to a new series of imperfections (peaks 1–8 in Fig. 7, Table 4) in $DAB(CN)_{64}$. The molecular masses of these components reveal that these byproducts do not contain the core molecule. These byproducts can be formed if a complete side-arm or part of a side-arm is split off from amino-terminated $DAB(Am)_{32}$. After exhaustive Michael addition of all amino groups to acrylonitrile the molecular mass series 396, 837, 1718 is formed. The molecular mass series 343, 784, 1665 can be explained by the assumption that Michael addition to acrylonitrile by the hindered secondary amino group has not taken place. The

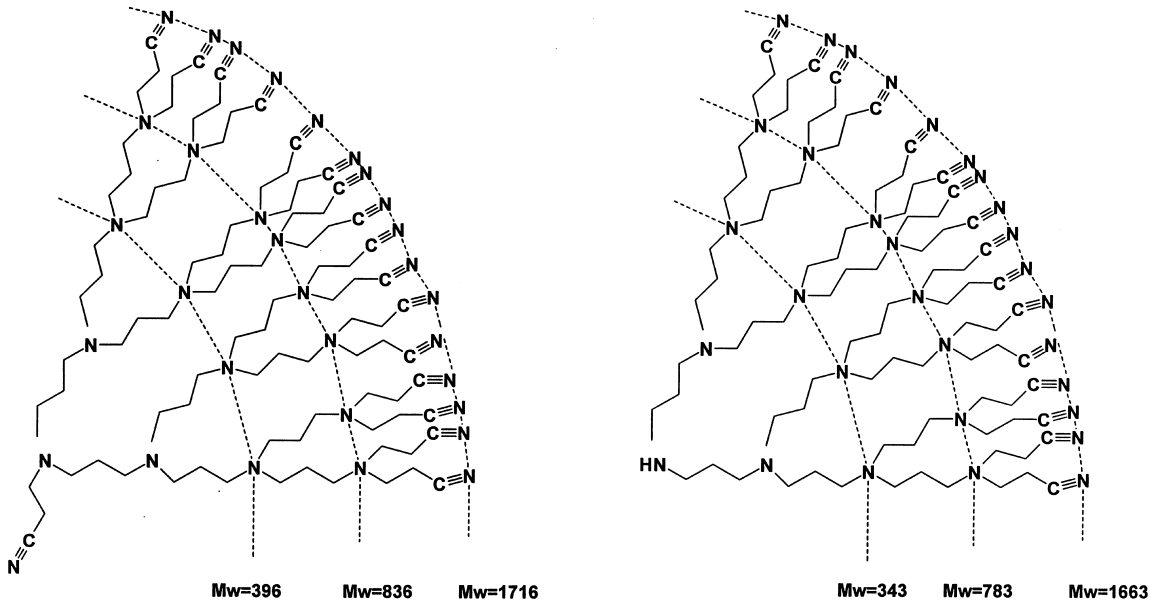

Fig. 9. Proposed structures for the peaks numbered 1–8 in Fig. 7 Table 4. Mw = Nominal molecular mass.

formation of these low-molecular-mass side products in a high-molecular-mass dendrimer is depicted in Fig. 9.

Finally, dendrimers appear to act as very effective adsorbents to components of the LC system: the peak at 15 min in Fig. 7 corresponds to $DAB(CN)_{32}$, presumably carried over from the previous run via the injector.

4. Conclusions

The separation and identification of imperfections in higher generations of dendrimers is performed by LC–MS. The presence of 50 m*M* sodium hydroxide in the HPLC eluent does not present a problem to the ion source of the mass spectrometer since the ion sampling orifice is effectively protected by a nitrogen gas curtain. The reconstruction of extracted ion current profiles from data files created by the recording of full-scan mass spectra reveals the presence of a large number of imperfections when the experiments are performed on samples from pre-optimization runs. Some imperfections are present as two or more isomers, but the present study cannot reveal the position of an imperfection in each isomer. HPLC alone is insufficient for assessment of the purity of the generations of $DAB(CN)_n$ since LC–MS demonstrates that most chromatographic peaks consist of two or more coeluting components. MS alone can give a quick analysis of imperfections having a molecular mass fairly close to that of the ideal structure. For a complete analysis of imperfections HPLC has to be combined with on-line ESP-MS, and extensive post-run data processing is required for selective detection and identification of each imperfection.

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