Sizing of amino acid based dendrimers in Langmuir monolayers

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The size and monolayer morphology of synthesised amphiphilic amino acid based dendrimers is studied in a Langmuir trough using Brewster Angle microscopy.

The synthesis of dendrimers remains an exciting challenge in view of their size, number of end-groups, properties and possible applications. Among the presently available large diversity of dendrimers,[1] we are especially interested in the synthesis, properties and applications of dendrimers which are obtained by repeated formation of amide bonds.[2] Recently we have contributed to this field by describing a novel class of amino acid based dendrimers and by expanding this to the synthesis of a diversity of dendrimers.[3]

Concomitant with increasing possibilities for the synthesis of dendrimers, there is an increasing desire to be able to study some of their fundamental properties. Examples of these properties include size, orientation of end-groups and presence of cavities.

As a promising approach towards realising part of these aims we turned to spreading of dendrimers at the water–air interface using a Langmuir trough.‡ We thought that this convenient technique would offer one of the few possibilities to directly determine their size and might also offer some perspective for the ordering of dendrimer end-groups.[4] Furthermore, by employing Brewster Angle microscopy (BAM) in combination with the Langmuir technique the monolayer morphology can be studied by direct imaging.[5]

Our prototype dendrimers were used for formation of Langmuir monolayers.[3a] The dendrimer itself is hydrophobic and insoluble in an aqueous system. The carboxylic acid moiety, which is obtained after saponification of the methyl ester, can then insert into the water surface. However, in order to provide sufficient space for a possible undistorted alignment of the dendrimers and their end-groups a fatty acid chain was connected first to the carboxylic acid moiety, which possibly could also lift the dendrimers from the water surface. For comparison purposes we have included the fourth generation dendrimer without a fatty acid chain.

The dendrimers were synthesised by the convergent method using 3,5-bis(2-*tert*-butoxycarbonylaminoethoxy)benzoic acid methyl ester **1**.[6] The synthesis of the dendrimers up to the fifth generation is depicted in Scheme 1.[3a] Briefly, amino acid ester **1** was used to prepare the 'surface' monomer **2** as well as the 'branching' monomer **3**. After successive BOP§ coupling and deprotection (by saponification) steps, subsequent generations **4**–**7** were obtained in a convenient manner. To each generation a fatty acid moiety was attached affording dendrimers **9**–**13** (Scheme 2). In addition, the fourth generation dendrimer **6** was saponified and **14** without a fatty acid chain was obtained for spreading experiments (Scheme 1).¶

Dendrimers **9**–**13** were spread on pure water in a Langmuir trough. In Fig. 1 the surface pressure–area isotherms of **9**–**13** at 20 °C are depicted. The isotherms correspond to a gaseous, liquid-expanded (LE) and liquid-condensed (LC) state.[7] In the isotherms of dendrimers **9** (1st generation), **10** (2nd generation) and **11** (3rd generation) a plateau was observed.

The lift-off area A_1, obtained by extrapolation of the first slope of the isotherm to the end of the two-dimensional gas-phase situation, afforded the molecular area occupied by one dendrimer molecule. Naturally, this area increased with the dendrimer generation and is the direct experimentally observed size of the dendrimer molecule on the water surface when the molecules start to interact with each other (Table 1).

From the relatively steep part of the isotherm, the condensed monolayer, the second intercept A_2 was obtained by extrapolation which gave the area of one dendrimer molecule in the condensed monolayer. The latter area was roughly one third of the area taken up by the dendrimer at the phase transition from the 'gas' to the liquid-expanded state (A_1) for the first to the third generation dendrimer, going up to about half for the fifth generation dendrimer. This might indicate that less change of structural organisation takes place in dendrimers of higher generations going from the first to the second phase transition.

The area taken up per Boc end-group varied between 13 and 18 Å^2 in the monolayer and is significantly larger in the two-dimensional 'gas' phase where it varied between 38 and 60 Å^2. An estimation of the 'theoretical' area of the Boc group by looking mainly at the *tert* butyl end amounts to 25 Å^2, at least indicating that the Boc end-groups were not arranged in some kind of densely packed orderly aligned fashion but were partly intertwined with the dendrimer skeleton.

Spreading of the fourth generation dendrimer **14**, *i.e.* the dendrimer without the fatty acid chain, gave a lift-off area of 613 Å^2, which is virtually identical to that found for dendrimer **12** having the fatty acid chain. The condensed monolayer area (A_2: 300 Å^2) of **14** is somewhat larger than that of **12** (253 Å^2), possibly indicating that the former underwent less further organisation than the latter upon increasing compression. Indeed, for dendrimer **14** close to the water surface, there are probably less alternative arrangements possible than for dendrimer **12**, containing the long, flexible, fatty acid chain.

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‡ A thermostatted (0.1 °C), computer controlled Lauda Langmuir balance FW2 installed on a vibration-free marble table.

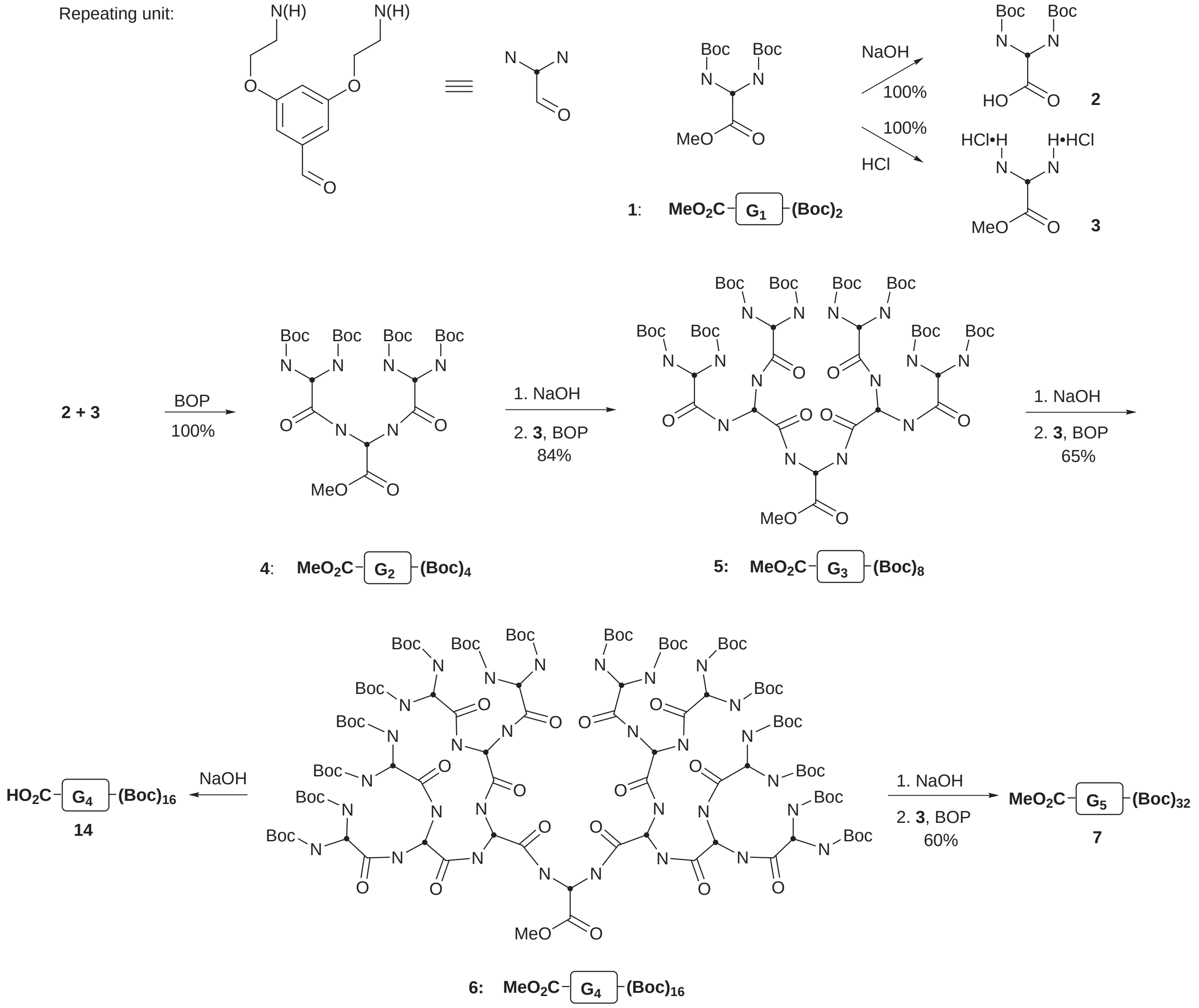
§ BOP reagent = benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate.

¶ The convergent method allows the preparation of pure single molecular weight monodisperse dendrimers, which were fully characterised by ^{1}H and ^{13}C NMR, FAB and electron-spray mass spectra, HPLC and GPC.

Table 1 Experimental and theoretical areas (Å^2) of **9**–**14** in the monolayer at $T = 20$ °C

Dendrimer, generation	Number of end-groups	Theoretical area[a]	'gas' phase, lift-off area: $A_1^{b,c}$	A_1(Boc)	Condensed monolayer area $A_2^{b,c}$	A_2(Boc)	A_1/A_2
9, 1	2	50	104 ± 1	52	29 ± 0	15	3.6
10, 2	4	100	240 ± 2	60	74 ± 0	18	3.2
11, 3	8	200	366 ± 6	46	102 ± 6	13	3.6
12, 4	16	400	620 ± 15	39	253 ± 3	16	2.5
13, 5	32	800	1210 ± 0	38	585 ± 5	18	2.1
14, 4	16	400	613 ± 3	38	300 ± 0	9	2.0

[a] The theoretical area per end-group, *i.e.* Boc group, is *ca.* 25 Å^2. [b] For comparison: A_1 and A_2 for stearic acid are 24 and 20 Å^2, respectively. [c] Two independent experiments.



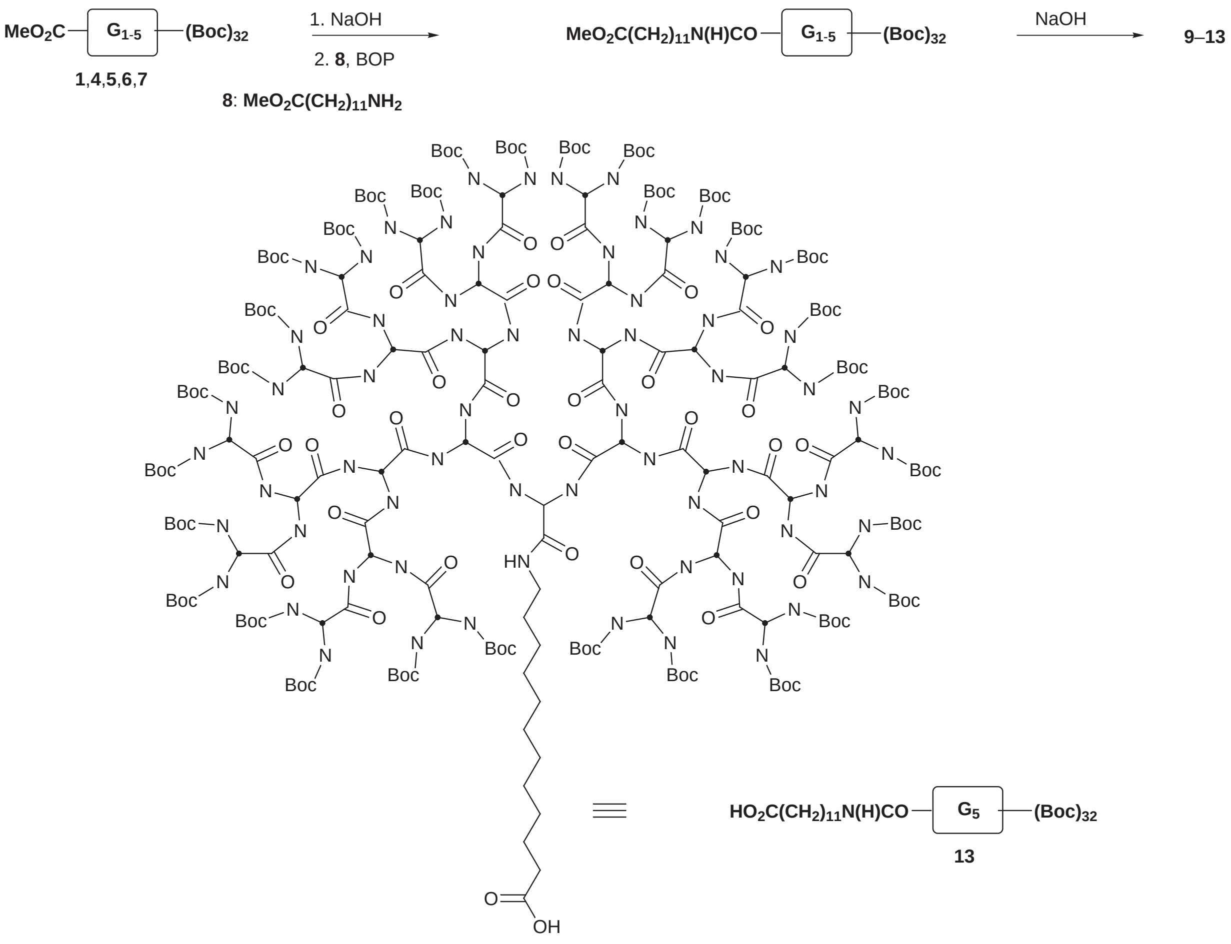
Scheme 1 Convergent synthesis of the first to fifth generation amino acid based dendrimer

The monolayer behaviour of dendrimers **9** to **13** was also studied by Brewster Angle microscopy (BAM). After spreading, none of the compounds showed formation of domains and the molecules do not aggregate after spreading of the chloroform solution of the dendrimer,|| or crystallise before compression. The first and second generation dendrimers **9** and **10**, respectively, showed the presence of a plateau in the isotherm that was not observed with the higher generation dendrimers **12** and **13** (Figs. 1 and 2). Although a plateau is present in the isotherm of **11**, it is less distinct.

At the onset of the plateau (18 mN m^{-1}) the first generation dendrimer **9** started to crystallise and small regular nuclei appeared, which were observed by BAM [Fig. 3(*a*)]. As the compression was continued, the surface pressure remained constant and the formation of nuclei progressed towards the formation of domains until a complete probably polycrystalline layer was obtained, which finally collapsed at a surface pressure of 26 mN m^{-1}.

In the BAM image of dendrimer **10** the formation of nuclei was also observed, however there is a noticeable difference.

|| The dendrimer solutions contained 1 mg ml^{-1} of dendrimer in chloroform.



Scheme 2 Synthesis of dendrimers **9**–**13**

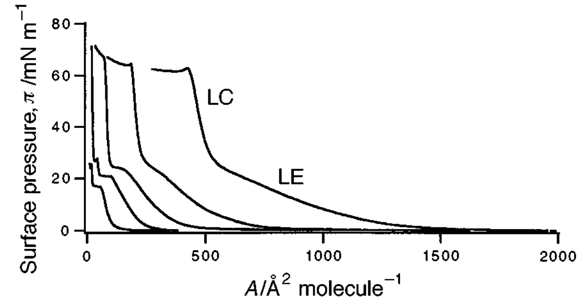


Fig. 1 Surface pressure–area isotherm at 20 °C of compounds **9**–**13** (from left to right)

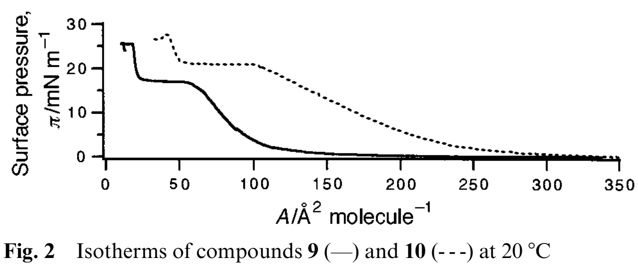


Fig. 2 Isotherms of compounds **9** (—) and **10** (- - -) at 20 °C

After the first incline of the isotherm the pressure remained constant at $\pi = 22$ mN m^{-1}. Simultaneously, nucleation cores became visible. During the plateau in the isotherm further nuclei formation was observed, but the pressure started to rise before a fully covered image was observed, *i.e.* in the BAM image only *domains* of crystallised structures appeared, which is in contrast to observations with dendrimer **9**. At $\pi = 28$ mN m^{-1} the monolayer of dendrimer **10** collapsed (Fig. 2). Apparently, the rate of compression is higher than the rate of crystallisation. During compression the higher generation dendrimers **11**, **12** or **13** gave only homogenous BAM images indicative of the absence of crystallisation. Clearly, the higher generation dendrimers **11**, **12** and **13** fail to crystallise.

BAM studies were also performed at 40 °C. Again all compounds gave no domains after spreading, *i.e.* no aggregation or crystallisation before compression. During compression crystallisation was not only observed in the images of dendrimers **9** and **10**, but also in the third and fourth generation dendrimers **11** and **12**, respectively. In addition to crystallisation and/or the occurrence of domains, meandering structures were observed with dendrimers **10**–**12**, but not with **9**. The fifth generation dendrimer **13** did not show any crystallisation and/or aggregation. Therefore, it seemed that the increased mobility at a higher temperature, *i.e.* 40 °C, allowed for an easier orientation of the third and fourth generation dendrimer for crystallisation. Interestingly, two different monolayer areas (A_2) were observed at $T = 20$ °C and at $T = 40$ °C for the third to fifth generation dendrimers. The area at the higher temperature for the fourth and fifth generation dendrimer was about half that observed at $T = 20$ °C indicating that either a better or different packing was achieved at a higher temperature (illustrated by **13** in Fig. 4), since at the higher temperature the crystallisation can better accommodate the compression.

In conclusion, we have shown that investigation of Langmuir monolayers is a convenient technique for determining the size of an individual dendrimer molecule at the beginning of the liquid-expanded state, *i.e.* when a coherent monolayer starts to be present as well as at the liquid-condensed phase when the monolayer is highly ordered. The attachment of a fatty acid chain was not required *per se* for successful spreading since saponification of the methyl ester of one of our dendrimers, *i.e.* **14**, seemed to be sufficient for study of the Langmuir monolayers. Finally, the observed areas are smaller than the

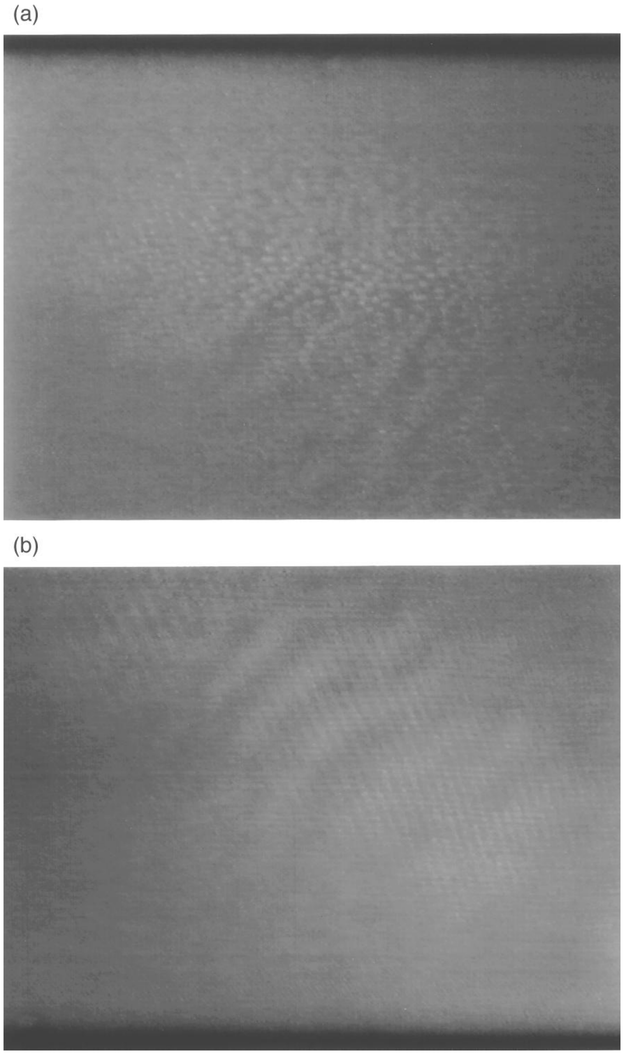


Fig. 3 BAM image of compound **9** (a) in the plateau (at $\pi = 18$ mN m^{-1}) and (b) in the monolayer area ($18 < \pi < 26$ mN m^{-1})

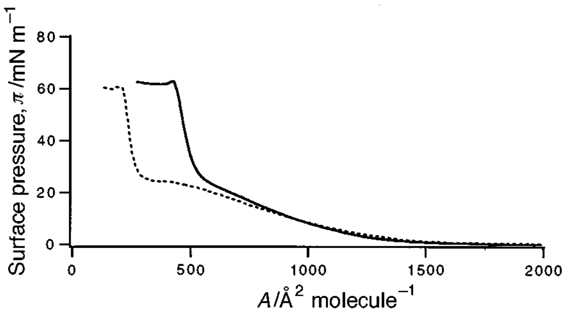


Fig. 4 Isotherms of **13** at $T = 20$ °C (—) and at $T = 40$ °C (- - -)

theoretical areas taken up by the Boc groups or the aromatic residues of the dendrimer skeleton (data not shown), which indicates that at least a simple parallel arrangement is absent.

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