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Synthesis of a dendron and dendrimer consisting of MALDI matrix like branching units

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Abstract—The synthesis of a novel dendron and dendrimer with potential application in matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) is described. The dendrimer branching unit was designed to have structural similarities to cinnamic acid based matrices used in MALDI MS. Branching was incorporated into the structure by introducing a second acrylic acid side chain. Some intermediates of the monomer synthesis were shown to function as MALDI matrices. A second-generation dendron was assembled on a solid support employing an N-to-C directed convergent synthetic strategy. Coupling of the dendrons with a trivalent core molecule resulted in a second generation dendrimer. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Since the introduction of matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) in the late 1980s,[1] this techniques has become a valuable tool for the analysis of biological molecules. In MALDI analysis, the analyte is co-crystallized in a suitable matrix of highly UV absorbing small organic molecules. The excitation of the matrix by a laser pulse causes desorption and ionization of the matrix and the analyte molecules. Despite enormous research efforts, the mechanisms of this phenomenon are still not completely understood. In an attempt to enhance the desorption and ionization process, matrix molecules such as α-cyano-hydroxycinnamic acid[2] or sulfhydryl containing benzoic acid derivatives[3] have been covalently attached to the surface of a MALDI target. In contrast to conventional MALDI, the sample is added to the matrix coated target surface prior to MS analysis. This simplifies the sample preparation and may be of importance to automated high throughput MALDI analysis. However, the synthesis of dendrons and dendrimers consisting of matrix-like molecules as aids for MALDI mass spectrometry has not been investigated. A potentially useful avenue of investigation is the covalent attachment of a 'matrix dendrimer' to a MALDI target surface in order to form a film that can be used as an energy absorbing surface or as a photolabile tether between a covalently immobilized biomolecule and MALDI target.

In recent years, dendrimers have emerged from synthetic interest into tailor-made structures for specific applications.[4,5] The construction of dendrimers is carried out in a stepwise manner; whereby two fundamentally different synthetic routes can be employed. In a divergent strategy,[6] monomer units are successively attached to a core molecule which results in dendrimer growth. In a convergent approach,[7] the synthesis starts on the periphery and goes towards what will be the core of the dendrimeric wedge followed by a subsequent reaction with a core molecule. Traditionally, dendrimers were synthesized in solution by both of these strategies. In parallel with the development of dendrimer chemistry, there have been vast advances in the field of solid-phase and combinatorial chemistry which resulted in considerable progress in the solid-phase synthesis of dendrimers.[8–10] The major advantages are that unreacted soluble reagents can be removed by simple filtration and washing without sample loss. Furthermore, excess soluble reagents can be used to drive reactions to completion. Herein, we describe the design and synthesis of a dendrimer branching unit possessing MALDI matrix like properties and the convergent solid-phase synthesis to give a generation two dendron. We also demonstrate the assembly of dendrons with a core molecule in order to synthesize a second-generation dendrimer.

Keywords: MALDI matrix; dendrimer; solid phase synthesis; reverse synthesis.

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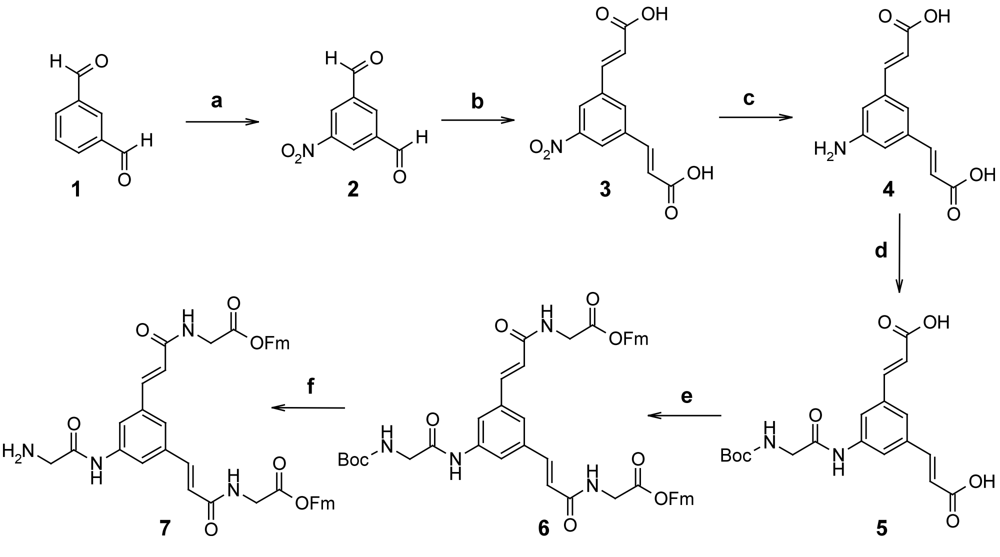
2. Design features

The building block for the dendrimer synthesis was designed to have structural similarities to a family of organic molecules used as matrices in MALDI MS. Members of that family are analogues of cinnamic acid such as 3,4-dihydroxycinnamic acid (caffeic acid), 3-methoxy-4-hydroxycinnamic acid (ferulic acid) and 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid). The common cinnamic acid structure was the basis of the design of a MALDI matrix like dendrimer branching unit. To obtain a branching multiplicity of two (type AB_2), a second arm was introduced into the cinnamic acid structure by placing an additional acrylic acid moiety in the *meta* position and the methoxy group was replaced with an amino group to afford 3-amino-5-(carboxyvinyl)-phenyl acrylic acid **4**. In the design, it was envisaged that the nucleophilicity of the aromatic amine and the reactivity of the acrylic acids might present problems during the synthesis of the dendron. Thus, in the design strategy a glycyl moiety was attached to both the amino and carboxylate termini to afford the amino acid which would be prepared as the 9-fluorenylmethyl ester for utilization in solid phase dendron synthesis. The structure of the designed building block dictates the synthetic route for the solid phase dendron assembly, which would involve N to C synthesis and the use of hyperacid labile attachment (trityl based) to the support to produce the protected dendron for attachment to the core molecule. Our strategy involved the activation of the carboxylic acid, which is immobilized on the solid support followed by treatment with the free amine. This 'reverse' coupling is not very widely studied and would require optimization. As an adjunct, the extended conjugated system in the monomeric unit was required for enhanced absorption of the laser light in UV-MALDI.

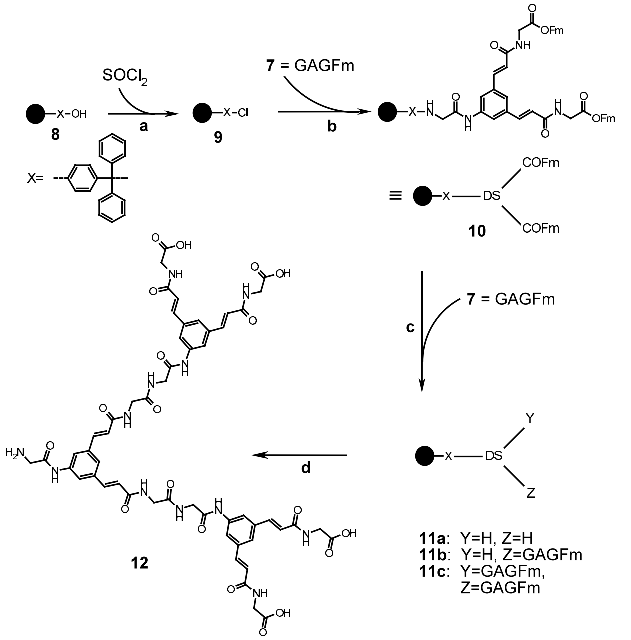
3. Results and discussion

The synthesis of the 9-fluorenylmethyl protected building block **7** (GAGFm) is shown in Scheme 1. Isophthalaldehyde **1** was nitrated using nitric acid and sulphuric acid to afford the 5-nitro-isophthalaldehyde **2**.[11] Condensation of 5-nitro-isophthalaldehyde with malonic acid under basic conditions gave 2-carboxyvinyl-5-nitrophenylacrylic acid **3**. The nitro group was subsequently reduced with ferrous sulphate to yield 3-amino-5-(carboxyvinyl)-phenyl acrylic acid **4**.[12] **4** was then acylated with *tert*-butoxycarbonyl glycine anhydride to afford the diacid **5**, which was subsequently treated with 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyl uronium tetra-fluoroborate (TBTU), diisopropylethylamine (DIPEA) and glycine fluorenylmethyl ester to yield the orthogonally protected building block **6**. Finally, the *tert*-butoxycarbonyl group was removed with trifluoroacetic acid to give the monomeric unit **7**.

Batch solid-phase dendrimer synthesis was carried out in a manual sintered flow reactor (Scheme 2). A trityl alcohol PEG-PS (polyethylene glycol–polystyrene) resin (NovaSyn® TGT), was utilized for solid phase dendron assembly. The trityl alcohol resin **8** was treated with thionyl chloride at room temperature under a N_2 atmosphere to yield the chlorotrityl resin **9**.[13] As the chlorotrityl resin is susceptible to hydrolysis it was used immediately. The chlorotrityl resin **9** (0.26 mmol/g) was treated with half an equivalent of GAGFm **7** in the presence of DIPEA. This reaction was monitored by HPLC analysis of the **7**, which indicated that half of the sites on the resin were acylated. The remaining unreacted sites on the resin were capped with methanol. This procedure effectively reduced the resin loading to 0.013 mmol/g in order to prevent steric problems



Scheme 1. Synthetic pathway of MALDI matrix like dendrimer branching unit. *Reagents*: (a) $(NH_4)_2SO_4$, conc. H_2SO_4, fuming HNO_3; (b) (i) malonic acid, pyridine, piperidine; (ii) glacial acetic acid; (c) ammonium hydroxide, H_2O, $FeSO_4$; (d) $(Boc\text{-}Gly)_2O$, DMF; (e) H-Gly-OFm, TBTU, DIPEA, dioxane; (f) TFA, CH_2Cl_2, H_2O.



Scheme 2. Solid phase synthesis of the G-2 dendron. *Reagents*: (a) $SOCl_2$, CH_2Cl_2; (b) **7**, DIPEA, DMF; (c) (i) 20% piperidine; (ii) **7**, HOAt, DIPCDI, DMF; (d) (i) 20% piperidine; (ii) CH_2Cl_2, TFA, H_2O, TIPS.

during the solid-phase synthesis. To couple the next generation of the building block **7**, the fluorenylmethyl esters were removed employing 20% piperidine in DMF[14] and the piperidinium counter ion was removed by washing with diluted acetic acid (0.5%). The trityl linker was found to be completely stable to the treatment with 0.5% acetic acid. The parameters investigated to achieve optimized coupling of the second generation of **7** were the reagent concentration, the reaction temperature and time. It was found that 15 equiv. of the building block **7**, 20 equiv. of 1-hydroxyazabenzotriazole (HOAt) and 40 equiv. of diisopropylcarbodiimide (DIPCDI) gave acceptable acylation yields. Coupling reactions in solid-phase peptide synthesis are usually undertaken at room temperature in order to minimize side reactions and racemization. Since our acylation reactions failed to reach completion at room temperature we conducted the reactions at 37°C, which resulted in successful and efficient acylation as determined by reverse-phase HPLC (Fig. 1). The peak area percentage of the total chromatogram was used to plot the graph. The second largest HPLC peak was related to deprotected **11b**, i.e. only one branching unit being coupled to the generation 1. The coupling reaction was regarded as being complete after 4 h.

The standard procedure for final resin cleavage from the trityl solid support with acetic acid (20% v/v) or TFA (1% v/v) solution in trifluoroethanol (20% v/v) and CH_2Cl_2[15] was slow and therefore unsuitable. However, successful cleavage was achieved with TFA/CH_2Cl_2 (1:4). Final purification of **12** was performed by size exclusion chromatography using an ammonium bicarbonate buffer (purity 94.5%).

All intermediates of Scheme 1 were tested for their potential to function as MALDI matrices. In fact, **3**, **4** and **5** showed the ability to promote desorption and ionization of cytochrome C (12.2 kDa) when co-deposited on a MALDI target. MALDI spectra of cytochrome C were successfully recorded after co-crystallizing 0.8 μl of the intermediate **3**, **4** or **5** (10 mg/ml in 70% acetonitrile/water) with 2 pmol of the protein. However, in initial experiments the observed MALDI signal intensities for cytochrome C were weaker than those obtained from conventional sinapinic acid/cytochrome C co-crystals and higher laser power was required for desorption. Expectedly, compounds **2**, **6** and **7** did not exhibit any MALDI matrix like properties as they lack the ability to donate a proton for protein ionization. The dendron, **12**, was also co-deposited with cytochrome C on a MALDI sample

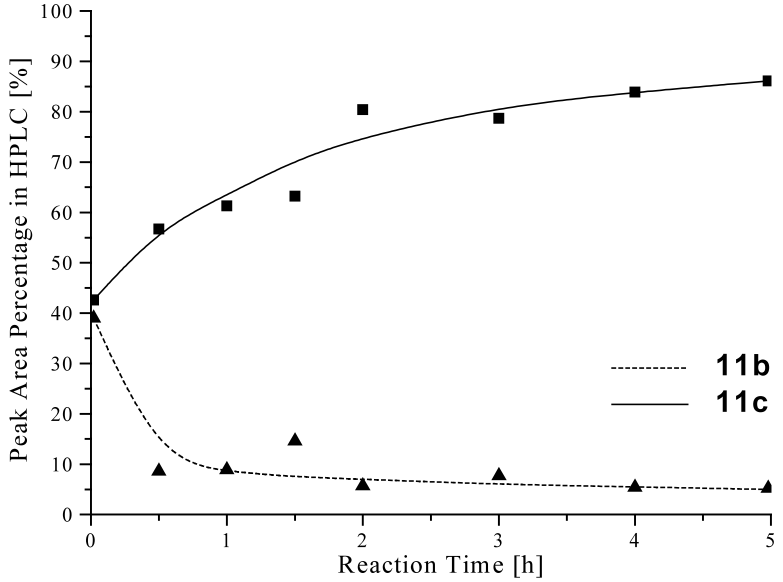


Figure 1. Reaction kinetic of acylation of resin bound branching unit GAGFm at 37°C, **11c** = complete G-2 dendron, **11b** = one branching unit missing.

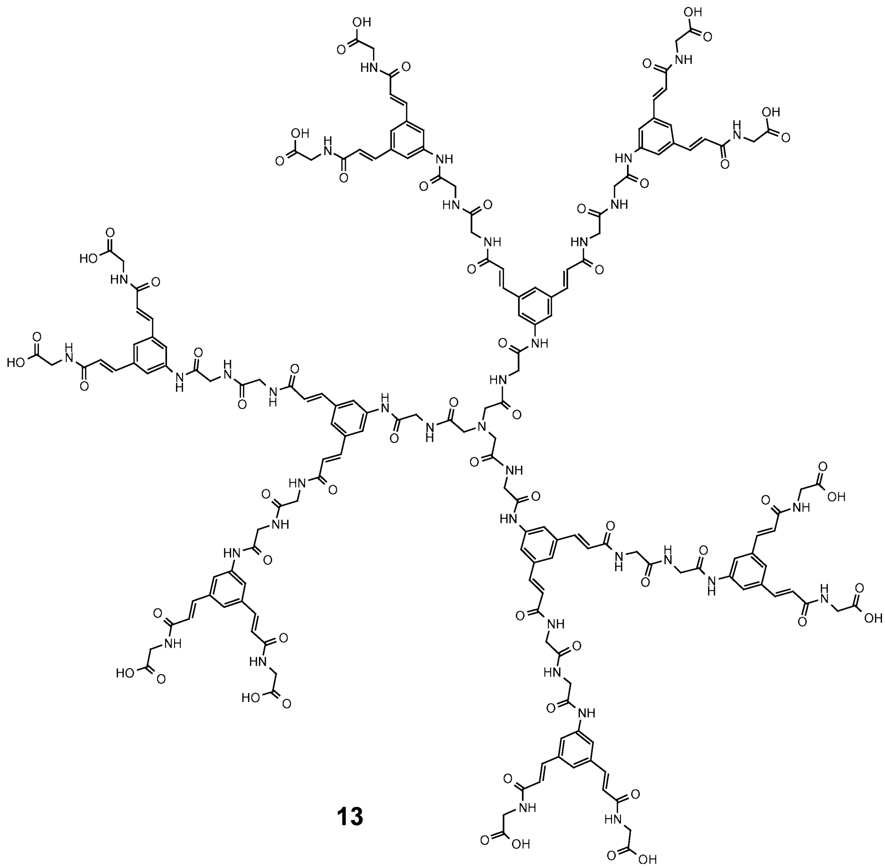


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plate. However, no signal for cytochrome C was observed which was related to the exclusion of the protein from crystals of **12** upon drying due to dissimilar physico-chemical characteristics.

The dendrons were covalently attached to a suitable core to give a complete dendrimer, a strategy known from conventional convergent dendrimer growth.[7] Treatment of the trivalent core tris-succinimidyl aminotriacetate with the dendron **14** failed to produce the final dendrimer **13**. However, on addition of HOAt, the dendrimer was successfully prepared. To drive the reaction to completion and to overcome steric problems during the assembly, the reaction was performed at 60°C and 9 molar equiv. of **14** and HOAt were utilized.

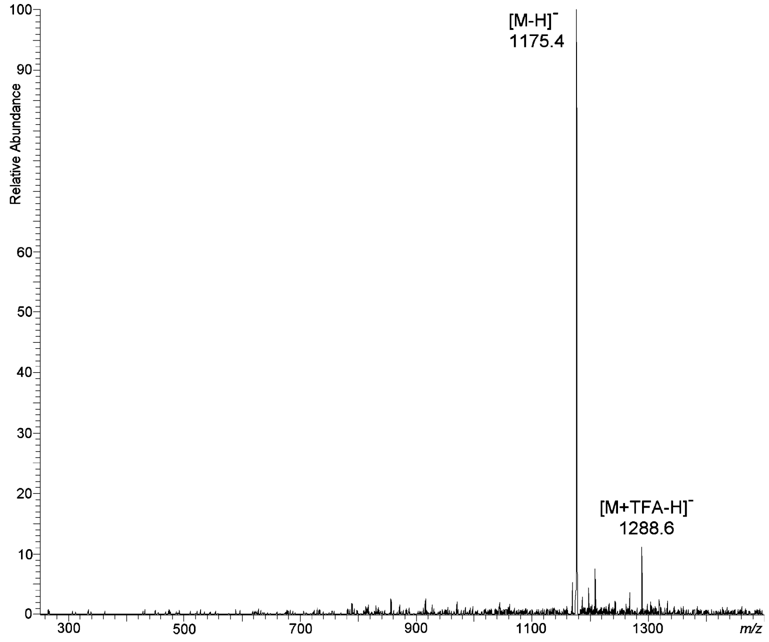

Figure 2. Negative ion mode ESI spectrum of G-2 dendron (M, **12**).

The products of all synthetic steps were analyzed by direct infusion electrospray ionization iontrap and MALDI-TOF mass spectrometry. Exemplarily, Fig. 2 shows the negative ion mode ESI mass spectrum of the generation 2 dendron, **12**.

In conclusion, we have designed a novel dendrimer and dendron, which is derived from MALDI matrices to possess matrix properties for MALDI MS. A second generation dendron has been successfully synthesized on a solid support at elevated temperature and in the N-to-C (reverse) direction. All products were characterized by HPLC and by MALDI and ESI mass spectrometry. The dendron has been attached to a core molecule to produce a dendrimer. These novel derivatives have been shown to possess matrix characteristics and further studies are currently being carried out.

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References

1. Karas, M.; Hillenkamp, F. *Anal. Chem.* **1988**, *60*, 2299–2301.
2. Hutchens, T. W.; Yip, T. T. *Rapid Commun. Mass Spectrom.* **1993**, *7*, 576–580.
3. Voivodov, K. I.; Ching, J.; Hutchens, T. W. *Tetrahedron Lett.* **1996**, *37*, 5669–5672.
4. Matthews, O. A.; Shipway, A. N.; Stoddart, J. F. *Prog. Polym. Sci.* **1998**, *23*, 1–56.
5. Fischer, M.; Vögtle, F. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 884–905.
6. Buhleier, E.; Wehner, W.; Vögtle, F. *Synthesis* **1978**, 155–158.
7. Hawker, C. J.; Frechet, J. M. J. *J. Am. Chem. Soc.* **1990**, *112*, 7638–7647.
8. Lebreton, S.; Monaghan, S.; Bradley, M. *Aldrichim. Acta* **2001**, *34*, 75–83.
9. Swali, V.; Wells, N. J.; Langley, G. J.; Bradley, M. *J. Org. Chem.* **1997**, *62*, 4902–4903.
10. Mahajan, A.; Chhabra, S. R.; Chan, W. C. *Tetrahedron Lett.* **1999**, *40*, 4909–4912.
11. Jennings, K. F. *J. Chem. Soc.* **1957**, 1172–1175.
12. Ackerman, J. H. US Patent 3,856,853: Iodinated 5-substituted-1,3-benzenediacrylic and dipropionic acids; Sterling Drug Inc.: USA, 1974.
13. Harre, M.; Nickisch, K.; Tilstam, U. *React. Funct. Polym.* **1999**, *41*, 111–114.
14. Henkel, B.; Zhang, L. S.; Bayer, E. *Liebigs Ann.-Recl.* **1997**, 2161–2168.
15. Barlos, K.; Chatzi, O.; Gatos, D.; Stavropoulos, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 513–520.