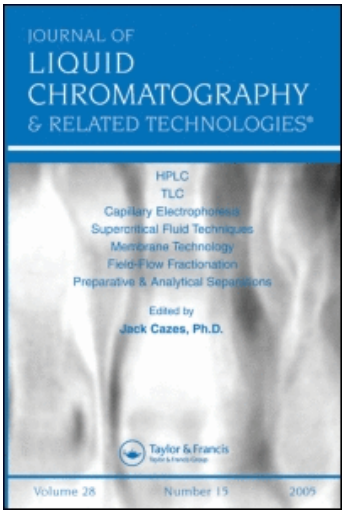

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PREPARATIVE LC AND NON-POROUS SILICA CHROMATOGRAPHY AS USEFUL TOOLS IN LARGE SCALE DENDRIMER SYNTHESIS

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ABSTRACT

The convergent synthesis of poly(benzyl ether) dendrimers was published by Frechet et al.[1] However, the coupling of the lower generation bromide with the 3,5-dihydroxy benzyl alcohol (monomer) involved the use of triphenylphosphine. The purification of higher generation dendrimers was difficult because triphenylphosphine was not easily separated from the desired product. A different approach was studied in our laboratory. A mesylate intermediate was developed, followed by the coupling of the monomer. Dendrimers generated from this process were separated using preparative LC chromatography. Non-porous silica (NPS) chromatography methods were developed to

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monitor the mesylation.[2] and coupling reactions and to confirm the purity of the preparative chromatography fractions.

Key Words: Dendrimer synthesis; Non-porous silica; Preparative LC

INTRODUCTION

This study reports on a simple strategy to isolate the higher generation dendrimers from reaction by-products, dendrimers with defects, using preparative chromatography. First, the separation was developed on an analytical column. Then the method was directly transferred to a preparative column without sacrificing resolution and separation efficiency. This approach involved three steps: (1) optimization of the analytical separation; (2) optimization of sample load using the analytical column; (3) scaling the separation to a preparative column. A convenient parameter of preparative chromatography is the loading factor. The loading factor can be expressed as a function of the retention factor at the peak maximum, k_{max} and the end of the peak k_0, at low sample mass:

$$L_f = \{1 - (k_{max}/k_0)^{1/2}\}^2 \tag{1}$$

If a given mass injected onto a column results in a large loading factor, the column is more overloaded than a column that gives a small loading factor. A large loading factor at a given load means a low loadability, while a small loading factor means a high loadability. High sample loadability generates better peak shape and higher resolution than low loadability. Therefore, one can judge the capacity of a packing for the analyte unambiguously from a single injection under overload conditions. The scaling to a preparative column was straightforward, provided the preparative column with the same high resolution packing as used in the scaling column was available. The scaling of all parameters of the separation should be done in proportion to the column volume.

The column load or injection volume and the flow rate are all scaled from the analytical column to the preparative column in proportion to the column volume. This results in identical chromatographic performance. Equations (2) and (3) calculate the flow rate and sample load increase proportional to column volume.[3]

$$\frac{\text{Flow(prep)}}{\text{Flow(scaling)}} = \frac{\text{Volume(prep)}}{\text{Volume(scaling)}} = \frac{\text{Length(prep)}}{\text{Length(scaling)}} \times \frac{r^2\text{(prep)}}{r^2\text{(scaling)}} \tag{2}$$

$$\frac{\text{Mass (prep)}}{\text{Mass (scaling)}} = \frac{\text{Length (prep)}}{\text{Length (scaling)}} \times \frac{r^2\text{ (prep)}}{r^2\text{ (scaling)}} \tag{3}$$

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The convergent synthesis of poly(benzyl ether) dendrimers was first reported by Fréchet et al.[1] However, the coupling of the lower generation bromide with the 3,5-dihydroxy benzyl alcohol (monomer) involved the use of triphenylphosphine. The purification of higher generation dendrimers was difficult because triphenylphosphine was not easily separated from the desired product.

A different approach was studied at our laboratory to eliminate the use of triphenylphosphine. A mesylate intermediate was developed, followed by the

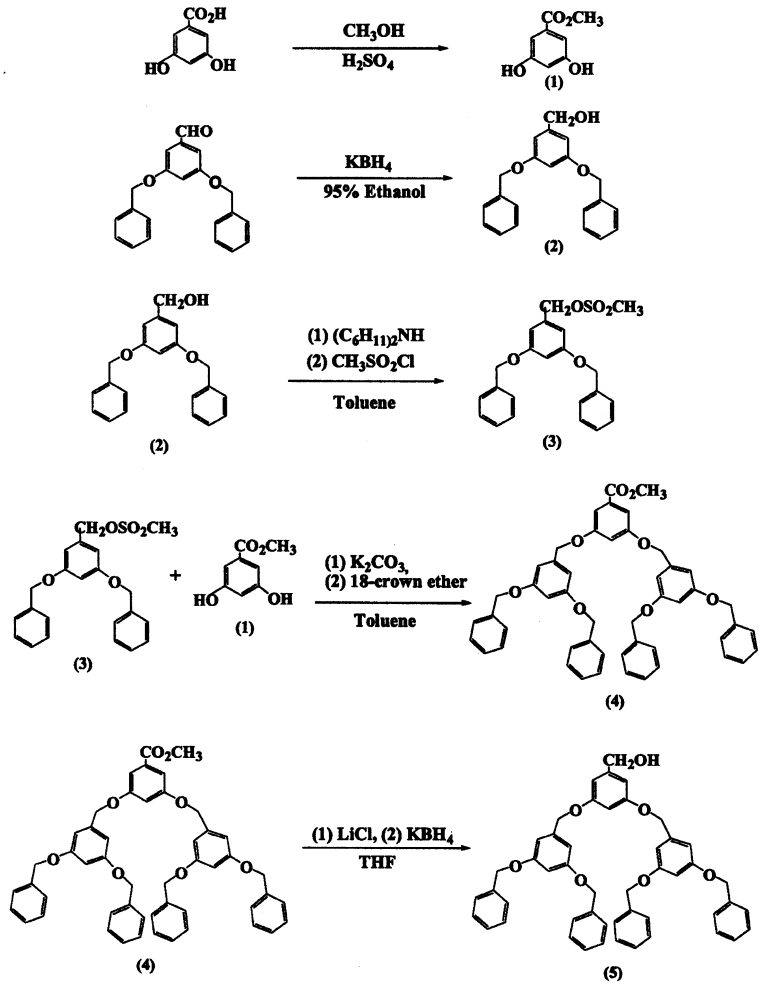

Figure 1. Synthetic scheme illustrating the mesylation route for poly(benzyl ether) dendrimers.

coupling of the monomer. The general synthetic pathway incorporating a mesylation reaction is shown in Figure 1. The alcohol form of the generation (n) branch, (2) to (3), can be quantitatively transformed to a mesylate intermediate followed by coupling of the monomer, (3) to (4), to give the next generation dendrimer. Dendrimers with defects and impurities were observed in this coupling step. LC/MS was used to identify the desired product. A RPLC method was used to optimize the reaction conditions and to monitor the completion of the reaction. Dendrimers generated from this process were separated using preparative LC chromatography. Non-Porous Silica (NPS) chromatography methods were developed to monitor the mesylation (2) and coupling reactions,[2] and to confirm the purity of the preparative chromatography fractions.

EXPERIMENTAL

HPLC

LC Instrumentation

The HPLC instrumentation consisted of a Hewlett Packard Model 1050 quaternary gradient pump with solvent degasser, autosampler, mode 1050 diode array detector, and IBM personal computer PL 300 with Hewlett Packard ChemStation version 6.04 [Revision A] software.

Columns

Analytical columns: a Luna C_{18} (2), 250×4.6 mm, 5 μm particle size and 100Å pore size from Phenomenex (Torrance, CA). A non-porous silica Micra (NPS) RP18 33×4.6 mm, with C_{18} 1.5 μm particle size, from Eichrom Industries, Inc. (Darien, IL,USA). Preparative column: a Luna 5 μ C18(2), 250×10 mm, packed with 5 μm particle size and 100 Å pore size from Phenomenex (Torrance, CA).

Chromatographic Procedure

An isocratic RPLC method was developed on an analytical column to monitor and optimize the reaction conditions. Then, the method was directly transferred to a preparative column, with the adjusted flow rate and sample injection size based on the theoretical value calculated from Equations 2 and 3. The mobile phase solvents were water (mobile phase A) and acetonitrile (mobile phase B) each containing 0.05% trifluoroacetic acid. The signal was scanned from

200 to 350 nm in order to record UV spectra and the UV detection was at 210 nm. The LC condition used for each method was:

Analytical method #1, column, Luna C_{18} (2), 250×4.6 mm; mobile phase composition, A : B (50 : 50); flow rate, 1.0 mL/min; injection size, 10 μL.

Analytical method #2, column, NPS RP18, 33×4.6 mm; mobile phase composition, A : B (70 : 30); flow rate, 0.5 mL/min; injection size, 0.2 μL.

Preparative LC method, column, Luna 5 μ C18(2), 250×10 mm, mobile phase composition, A : B (10 : 90); flow rate, 5 mL/min; injection size, 500 μL.

MS Instrumentation

Electrospray ionization was performed in the positive mode with nitrogen gas flow of 440 L/hour. The response was optimum with a 3.50 kV capillary voltage, a source voltage of 19 V, and source temperature of 180°C. The instrument was operated at unit resolution in the scan mode, scanning from mass 100 to 1000. The Navigator LC-MS was interfaced with a computer workstation running ThermoQuest's Excaliber 1.1 software.

Chemicals

Potassium carbonate, 18-crown ether, methyl 3, 5-dihydroxybenzoic acid, anhydrous toluene, and sulfuric acid were purchased from Aldrich (Milwaukee, WI, USA). The chemicals, whose purity is listed by Aldrich, were used as is without further purification. HPLC grade acetonitrile and methanol were purchased from Allied Signal (Burdick and Jackson). Trifluoroacetic acid, anhydrous, protein sequencing grade, was purchased from Pierce (Rockford, IL, USA). Water was purified with the Milli-Q Water System (Millipore, Bedford, MA, USA).

Sample Preparation

For monitoring purposes, a 100 μL aliquot was withdrawn from the reaction mixture using an 18 gauge, 6 inch hypodermic needle attached to a 1 mL Becton, Dickinson & Co. (Franklin Lakes, NJ) glass syringe. The solution was diluted to 2 mL with acetonitrile. The sample solution was filtered through an Acrodisc 13CR PFTE 13 mm, 0.2 μm pore size syringe filter purchased from Pall Gelman Laboratory (Ann Arbor, MI), and transferred into a HPLC vial. For preparative LC, the crude products were re-dissolved in ethyl acetate (5 mg/mL) and 500 μL of the solution was directly injected to the LC without filtration. The desired fraction was collected. The combined solution was then evaporated to dryness

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using a rotaevapor. The white solid residue was then placed in a vacuum oven and dried at 40°C at 2 mm Hg overnight.

Synthesis

Procedure for Coupling of (Gn) Mesylate with Methyl 3,5-dihydroxybenzoate

To a mixture of 3, 5-dibenzyloxy benzyl methyl sulphonate, (3), (2.0 equivalents), K_2CO_3 (2.5 equivalents) and 18-crown ether (0.4 equivalents) in a minimum amount of anhydrous toluene required to dissolve the above reagents was added methyl 3, 5-dihydroxybenzoate, (1), (1.0 equivalents). The reaction mixture was stirred under N_2 and gently refluxed for 55 hours. Methylene chloride (100 mL) and 0.5 N HCl (100 mL) were added to the mixture to remove the excess K_2CO_3. The aqueous layer was extracted twice with an equal volume of methylene chloride. The combined organic layers were washed once with a saturated NaCl, then dried over anhydrous $MgSO_4$. The solution was filtered, and removal of the solvent in vacuo gave white solid residues. The crude product was purified by preparative liquid chromatography (Prep-LC) to give 4. The yield was 68% Mp 133–134°C. In the FTIR spectrum (KBr pellet) absorptions at 3031 cm^{-1} (Ar C−H, st), 2912 cm^{-1} (C−H aliphatic, as st), 2873 cm^{-1} (C−H aliphatic, sy st),1713 cm^{-1} (C=O, st), 1241 cm^{-1} (C−O−C, as st), 1164 cm^{-1} (C−O−C, sy st). The combination bands of 835 cm^{-1} and 725 cm^{-1} are characteristic of 1, 3, 5-trisubstituted benzene (C−H out of plane bending vibration). The ^{1}H-NMR spectrum ($CDCl_3$, TMS) shows Δ 7.399(m, Ar_2−H, Ar_1−H, Ar_0−H, 22H), 6.765 (t, Ar_1−H, Ar_0−H, 7H), 5.034 (s, Ar_2−CH_2−O, 8H), 5.004(s, Ar_1−CH_2−O−, 4H), 3.907 (s, COO−CH_3, 3H). The ESI mass spectrum confirms the G_2 ester mass with the $[M+H]^+$, 773 and the m/z 303, the 3, 5 dibenzyloxybenzylic fragment ion. The elemental analysis calculated for $C_{50}H_{44}O_8$; theory: C, 77.70; H, 5.74, found: C, 77.23; H, 5.69.

RESULTS AND DISCUSSION

The coupling reaction was monitored using the analytical method #1 (Figure 2). The major product of the coupling reaction was found to be the generation 2 poly(benzyl ether) dendrimer, (4) with the protonated molecular ion, $[M+H]^+$, 773 (Figure 3). The desired product (4) was successfully separated from the dendrimer defects and impurities, using the preparative LC method described in this paper (Figure 4). The preparative LC chromatography was found to be a better technique than column chromatography or flash chromatography for purification purposes. The advantages are: (1) easily automated, (2) better

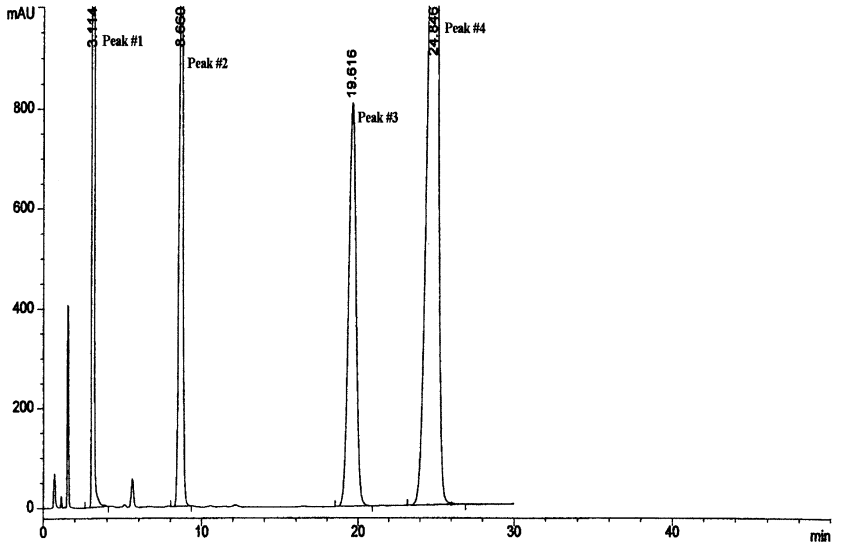

Figure 2. The coupling reaction monitored by analytical method #1: peak #1 toluene; peak #2 impurity; peak #3 dendrimer defect; peak #4 desired dendrimers.

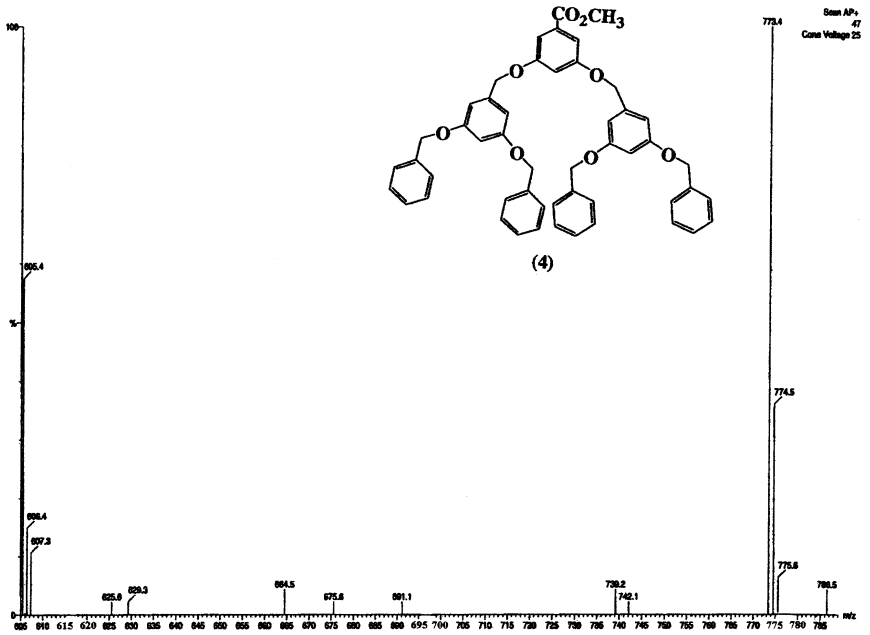

Figure 3. LC/MS data identified peak #4 as the desired product.

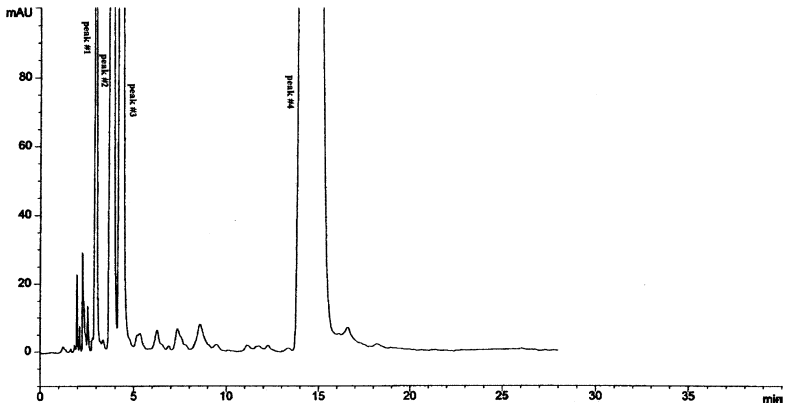

Figure 4. Shows the separation efficiency using a preparative LC column: peaks #1 toluene; peak #2 impurity; peak #3 dendrimer defect; peak #4 desired dendrimers.

separation due to more theoretical plates, (3) less solvent volume consumed, (4) safer to operate, and (5) it can be scaled for the process environment.

To confirm the purity of the preparative chromatography fractions, an NPS column was used to rapidly screen each fraction collected. The method employed a short NPS column, 33 mm in length, which enabled the analysis to be completed in less than three minutes with minimum solvent consumption. Figure 5 shows a pure product collected from the preparative LC. Structure identification of the dendrimer defect and impurities from the reaction mixture has presented quite a challenge. The

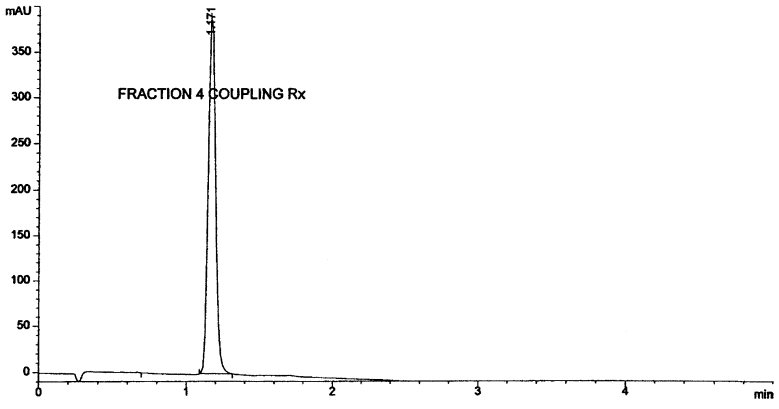

Figure 5. Shows the purity of the desired product monitored by NPS column using the analytical method #2.

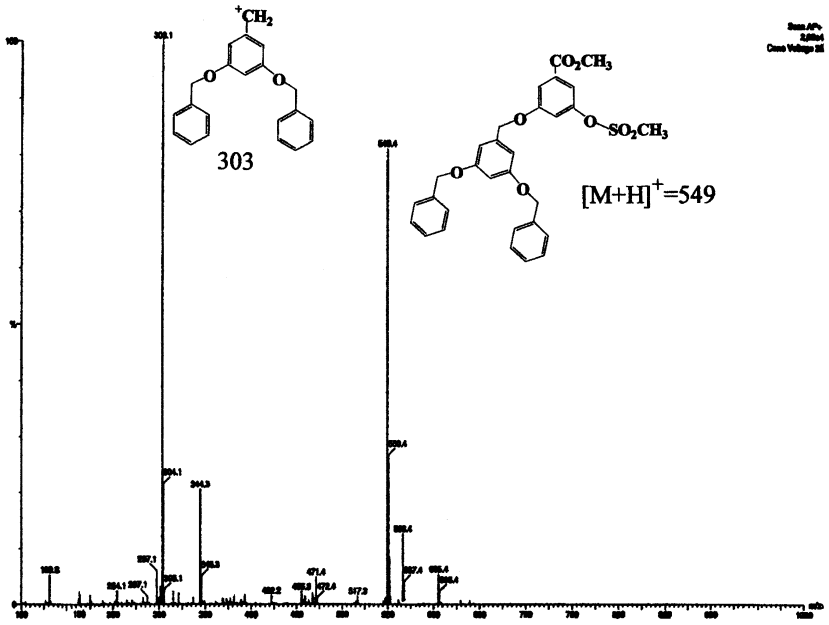

Figure 6. LC/MS data for peak #2.

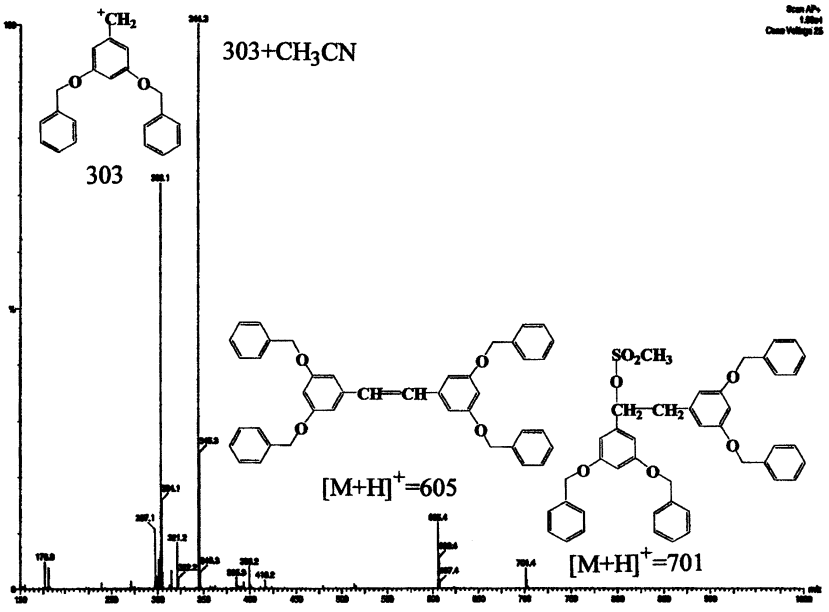

Figure 7. LC/MS data for peak #3.

postulated molecular structures are shown in Figures 6 and 7. The syntheses of the starting materials, 3, 5-dibenzyloxy benzyl methyl sulphonate (3) and methyl 3, 5-dihydroxybenzoate (1), were described in a previous publication.[2]

CONCLUSION

A preparative LC chromatography method was developed to support a large scale dendrimer synthesis. The poly(benzyl ether) dendrimer was successfully purified from the undesired byproducts using this preparative LC method. The method is simple to operate and can be fully automated. The method can be easily scaled-up for the process environment using the theory described in section 1 (introduction). Molecular weight identification, confirmed by LC/MS, supports the application of the preparative LC method as a better purification technique than flash chromatography and/or column chromatography.

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