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Preparation and characterisation of a chromophore-bearing dendrimer

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Abstract

A dendrimer dye was prepared from a generation 2 polyamidoamine (PAMAM) dendrimer, which possesses 16 terminal amine groups. Reaction with 4-acetamidobenzene sulphonyl chloride yielded a fully substituted sulphonamide, as demonstrated by mass spectrometry and NMR. This was hydrolysed to the sulphanilamide hydrochloride, diazotised and coupled with 2-naphthol-3,6-disulphonic acid disodium salt, to yield an orange dye. The degree of substitution of chromophore units was ca. 85%, as determined by NMR and reductive titration. A pH 11 buffer was found suitable for gel permeation chromatography (GPC) analysis both of the initial dendrimer and of the dye product. GPC coupled with light scattering allowed molar masses to be determined.

Keywords: Dendrimer; Dye; Gel permeation chromatography

1. Introduction

Dendrimers have attracted much interest for their symmetry, high degree of branching and high density of terminal functional groups. The aim of this work was to prepare a dendrimer dye, for use as a characterisation standard and in physicochemical studies. The starting material was a generation 2 Starburst™ polyamidoamine (PAMAM) dendrimer [1], which has 16 terminal amine groups, on to which chromophore units were built. The objective was a product with the idealised structure shown in Fig. 1, referred to as Gen2-Orange. In Fig. 1 the structure is depicted in the azo form, but in reality the chromophores will exist as an equilibrium mixture of azo and hydrazone tautomers. The product incorporates sulphonic acid groups on the chromophores, as well as secondary and tertiary amine groups within the core, and so is an amphoteric polyelectrolyte, which poses particular difficulties in characterisation [2].

The synthetic approach, illustrated in Fig. 2, is a modification of the procedure used by Dawson et al. [3–5] to prepare polymeric dyes with a polyvinylamine backbone. The first step was a Schotten Bauman reaction of the dendrimer (i) with 4-acetamidobenzene sulphonyl chloride (ii) to yield the sulphonamide (iii). Acid catalysed hydrolysis of (iii) gave a solution of the sulphanilamide hydrochoride (iv). For characterisation purposes, samples were withdrawn and neutralised, to obtain a light brown precipitate of the sulphanilamide (v). Diazotisation was carried out on the acidic solution of (iv) to give the dendrimer diazonium salt (vi), which coupled rapidly with a cold aqueous alkaline solution of 2-naphthol-3,6-disulphonic acid disodium salt (vii), producing a dark red solution of Gen2-Orange (viii). Initial attempts at this synthesis gave rise to complex mixtures, and it proved necessary to purify and characterise intermediates. In this paper, the characterisation of the initial dendrimer (i), the sulphonamide (iii), the sulphanilamide (v) and the product (viii), is described.

2. Experimental

2.1. Preparation of dendrimer sulphonamide

Polyamidoamine (PAMAM) dendrimers were obtained as methanolic solutions (20 wt%) from Dendritech (Michigan, USA). Some samples were also purchased from Aldrich (Dorset, England), but were found to be less pure than those from Dendritech, as discussed in Section 3.1. The methanol was removed prior to use by rotary evaporation. A mixture of generation 2 dendrimer (10 g, 3 mmol), tetrahydrofuran (THF) (150 cm^3) and distilled water

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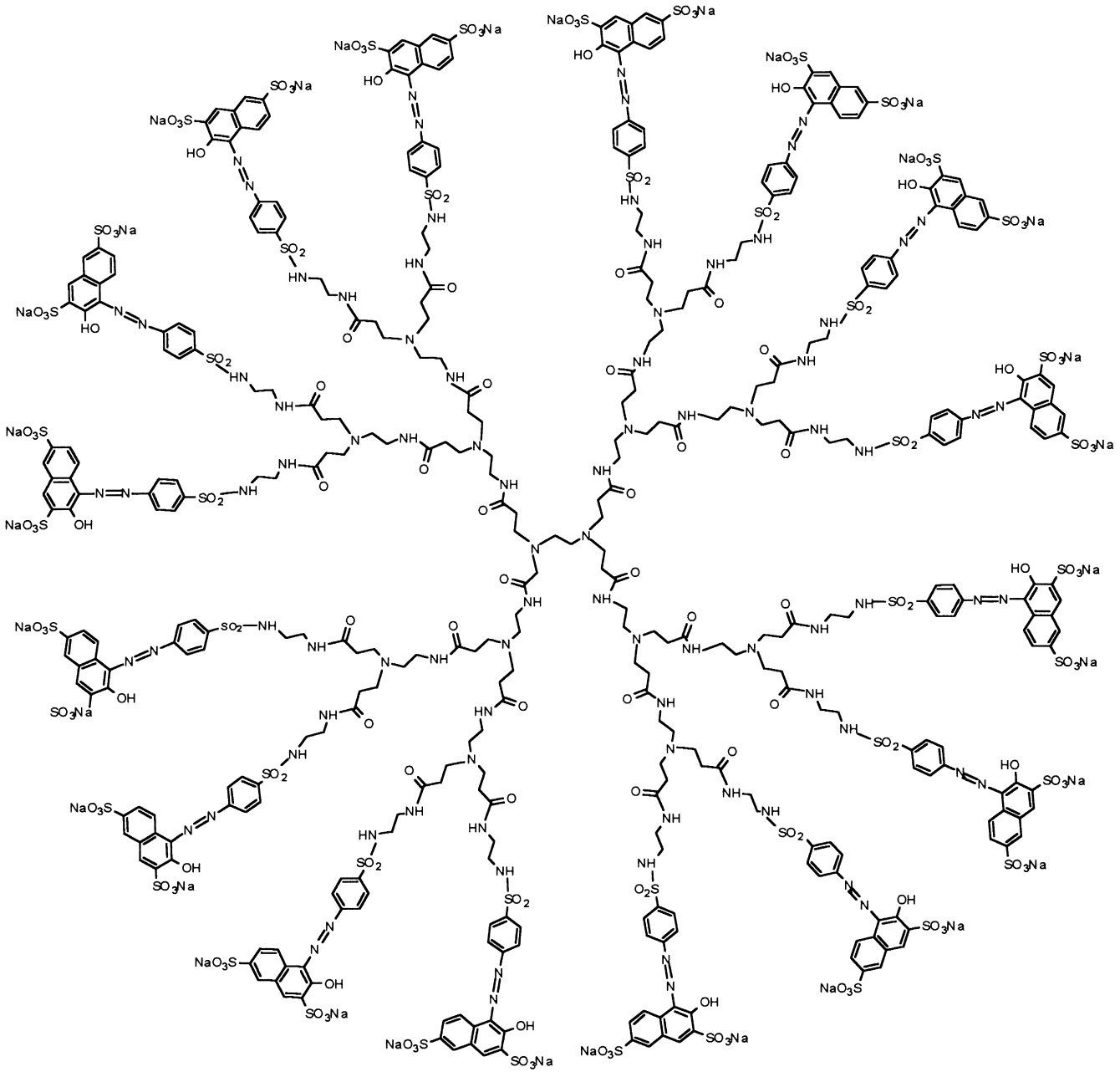

Fig. 1. Idealised structure of Gen2-Orange.

(200 cm^3) was stirred at room temperature until a colourless homogenous solution was obtained (20 min). Aqueous NaOH (5 M, 10 cm^3) was added dropwise to raise the pH to 11.5. 4-Acetamidobenzene sulphonyl chloride (ABSC) (12.64 g, 54 mmol, Lancaster synthesis) was divided into three equal portions. The first portion was added to the rapidly stirred solution and the decrease in pH monitored. After 20 min the pH steadied at 7.2 and further THF (50 cm^3) and aqueous NaOH (10 cm^3) was added, raising the pH to ca. 11. The procedure was repeated with the second portion of ABSC. After addition of the third portion of ABSC, the pH dropped gradually. Aqueous NaOH was added in portions (6×2 cm^3) until the pH remained constant with continued stirring. To isolate the product, THF was removed by rotary evaporation, causing the sulphonamide to separate out of solution as a white gum. The product was purified by repeated washing with distilled water in a sintered funnel under vacuum, then dried to constant weight in a vacuum oven. The sulphonamide was obtained in nearly 100% yield.

2.2. Preparation of dendrimer sulphanilamide

A mixture of generation 2 dendrimer sulphonamide (10 g,

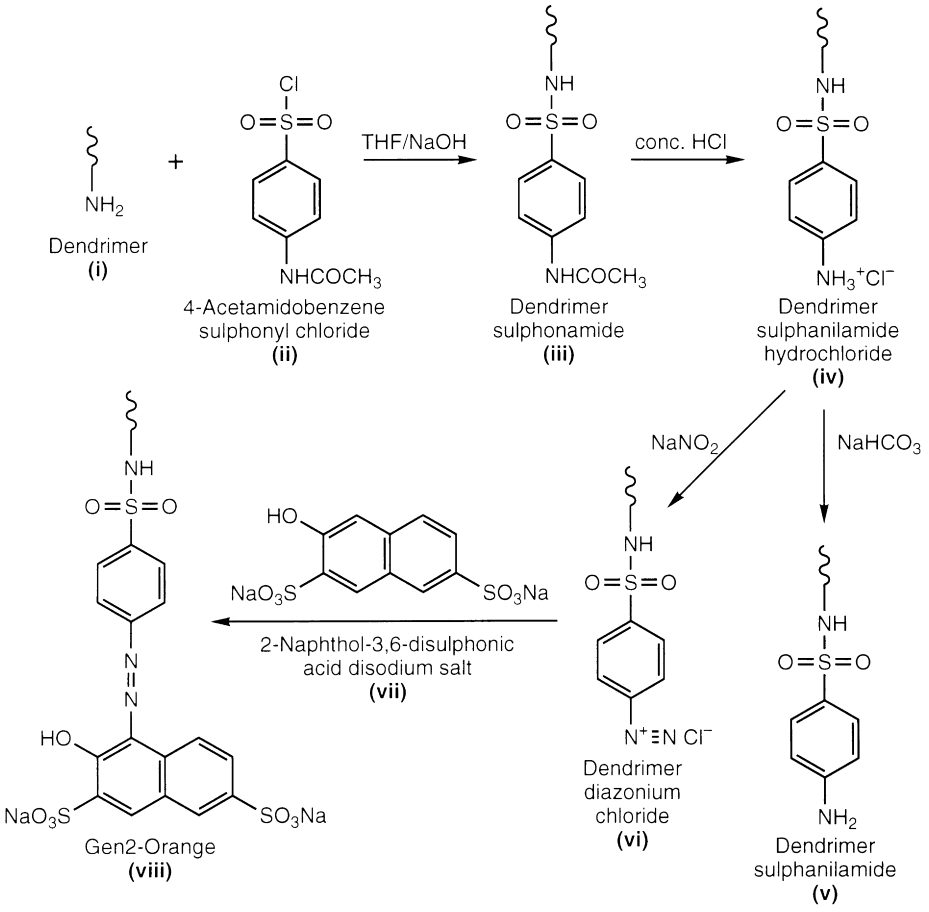

Fig. 2. Synthetic scheme for the preparation of Gen2-Orange.

1.6 mmol), distilled water (350 cm^3) and aqueous HCl (12 M, 4 cm^3) was stirred and heated under reflux for 2 h, then cooled to room temperature. A sample of the solution (54 cm^3) was withdrawn for isolation of the sulphanilamide and the remainder of the solution used for diazotisation.

To isolate the sulphanilamide from the hydrochloride solution, aqueous NaOH (2 M) was added slowly whilst monitoring the pH. The solution became cloudy at pH 7.8, and eventually a brown/yellow gum separated from the solution (pH 9.9, 40 cm^3 NaOH added in total), which was allowed to settle without stirring. The product was filtered through a sinter under vacuum, purified by repeated washing with distilled water (5×20 cm^3) and dried in a vacuum oven.

2.3. Preparation of dendrimer diazonium salt

Generation 2 dendrimer sulphanilamide hydrochloride solution (300 cm^3) was cooled to <5°C and stirred for 15 min. Aqueous $NaNO_2$ (2 M, 14.5 cm^3, 29.1 mmol, pre-cooled to <5°C) was added as rapidly as possible, with vigorous stirring. The solution was stirred for a further 5 min, maintaining the temperature at <5°C, then tested for the presence of excess nitrous acid using potassium iodide/starch indicator paper. Aqueous sulphamic acid (20 wt%) was added (5 cm^3 at a time, 25 cm^3 in total) until excess nitrous acid was neutralised.

2.4. Preparation of dendrimer dye

2-Naphthol-3,6-disulphonic acid disodium salt (10.12 g, 29.05 mmol) was dissolved in aqueous NaOH (1 M, 200 cm^3) and cooled to <5°C. The cold solution of generation 2 dendrimer diazonium salt was added dropwise, with rapid stirring, over 20 min. Throughout the addition the pH was maintained at 13–13.5 by the addition of 5 M aqueous NaOH and the temperature was kept <5°C. After the addition, the dark red solution was stirred for a further 45 min, then filtered under vacuum. The solution was purified by exhaustive dialysis against distilled water, using a dialysis membrane with a 1000 molecular weight cut-off (Spectra Por/7, Fisher Scientific). The resulting cloudy orange solution was freeze dried to give an orange dendrimer dye (7.35 g).

2.5. Gel permeation chromatography

Aqueous gel permeation chromatography (GPC) was carried out with two TSK gel columns in series

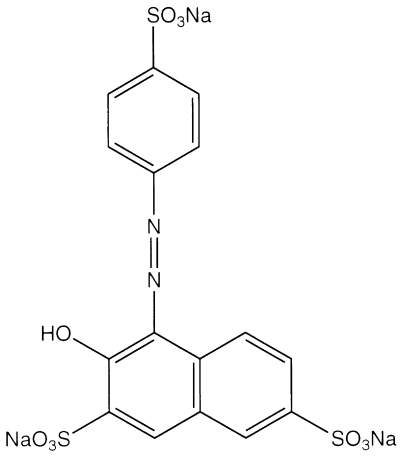

Fig. 3. Structure of 2,7-naphthalenedisulphonic acid, 3-hydroxy-4-[(4-sulphonyl)azo]-, trisodium salt, used for reductive titration calibration.

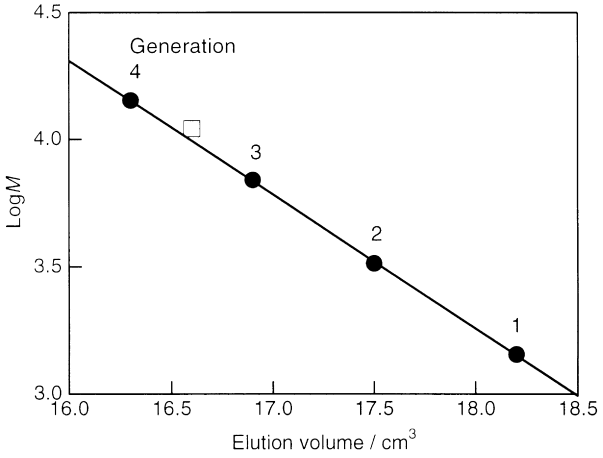

Fig. 5. GPC calibration plot (●) for dendrimers and (□) experimental result for Gen2-Orange product.

(G3000PW and G4000PW). The eluent was a pH 11 buffer (0.05 M $NaHCO_3$/0.1 M NaOH/distilled water, 50:23:27 vol.). Most experiments were carried out at Avecia using a Waters 150-CV Plus Gel Permeation Chromatograph with refractive index (RI) detection, in conjunction with a Wyatt Technologies' mini-DAWN Light Scattering (LS) Instrument. Some experiments were carried out at Manchester University with RI (Waters 410) and ultraviolet (UV, Waters LC Spectrophotometer) detectors. Refractive index increments were measured using a Wyatt Technologies' Optilab DSP Interferometric Refractometer.

2.6. Mass spectrometry

Electrospray ionisation (ESI) mass spectrometry was carried out using a Micromass Platform Quadrupole II spectrometer, with an acetonitrile/water (50:50 vol.) solvent mixture and a cone voltage of 80 kV. Matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) mass spectrometry was carried out using a Micromass Tof Spec 2E spectrometer, with 2,5-dihydroxy benzoic acid as the matrix and dimethyl sulphoxide as the solvent.

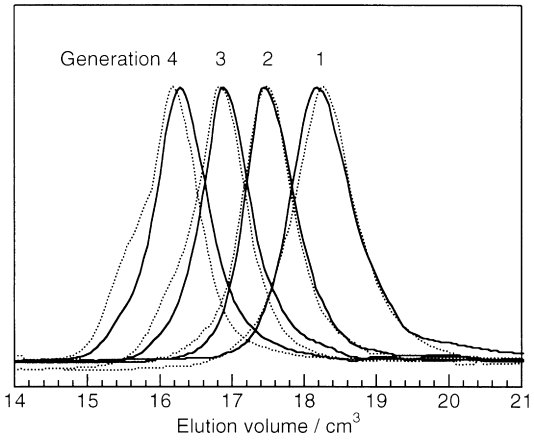

Fig. 4. GPC curves for dendrimer generations 1–4: (—) RI and (- - -) LS detection.

2.7. Nuclear magnetic resonance spectroscopy

^{13}C nuclear magnetic resonance (NMR) spectroscopy was carried out on a Varian Unity 500 Spectrometer operating at 125.8 MHz with a pulse interval of 5 s. Samples (300 mg) of dendrimer sulphonamide and sulphanilamide were dissolved in deuterated dimethyl sulphoxide (3 cm^3) and samples of generation 2 dendrimer and dendrimer dye were dissolved in D_2O (3 cm^3). In the case of the dendrimer dye, two drops of 40 wt% NaOD were added to suppress protonation of amines. So that accurate peak integral comparisons could be made, spectra of the sulphonamide, sulphanilamide and dye product were obtained with suppression of nuclear Overhauser enhancement (NOE). For the dye product, pulse intervals up to 20 s were investigated, but a 5 s interval was found to be sufficient.

2.8. Reductive titration

A dye sample (100 mg) was dissolved in distilled water (100 cm^3) with aqueous NaOH (2 M, 5 cm^3) in a modified 250 cm^3 flask bearing a side-arm test tube with a narrow tube leading into the solution. The test tube was filled with solid CO_2 and sealed at the top. The solution was heated to boiling and titrated against aqueous $TiCl_3$ (2 M), the end point being the discharge of the red/orange colour. The Ti^{3+} ($TiCl_3$) is oxidised to Ti^{4+}, producing hydrogen, which reduces the azo bond to the corresponding amines.

$$R_1\text{–}N{=}N\text{–}R_2 + 4H \rightarrow R_1\text{–}NH_2 + H_2N\text{–}R_2$$

A low molar mass analogue of the dendrimer dye, 2,7-naphthalenedisulphonic acid, 3-hydroxy-4-[(4-sulphonyl)-azo]-, trisodium salt (CA registry number 50880-65-4) (Fig. 3), was prepared following the same procedure as

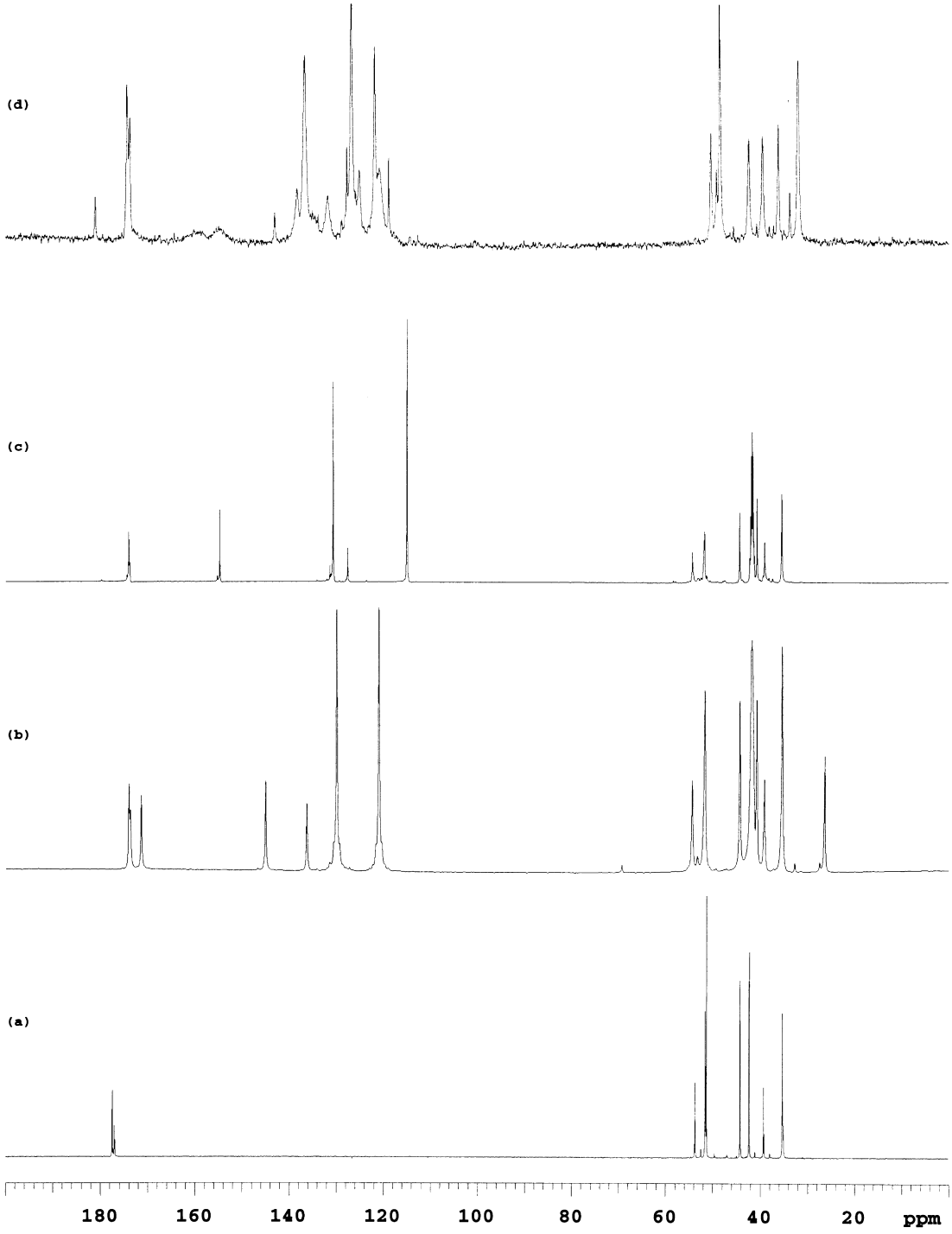

Fig. 6. ^{13}C NMR spectra for (a) generation 2 dendrimer, (b) sulphonamide, (c) sulphanilamide and (d) Gen2-Orange product.

used for the dendrimer dye and used for calibration. A calibration factor, K, (moles azo corresponding to unit volume $TiCl_3$ solution) was determined using

$$K = \frac{m_s}{M_s V_s},$$

where m_s is the mass of the standard dye, M_s is the molar mass of the standard dye (532 g mol^{-1}) and V_s is the titre for the standard dye solution. The molar mass per azo bond in the dendrimer dye, M_a, was calculated using

$$M_a = \frac{m_d}{KV_d},$$

where m_d is the mass of dendrimer dye and V_d is the titre for the dendrimer dye solution. Since the molar mass for the

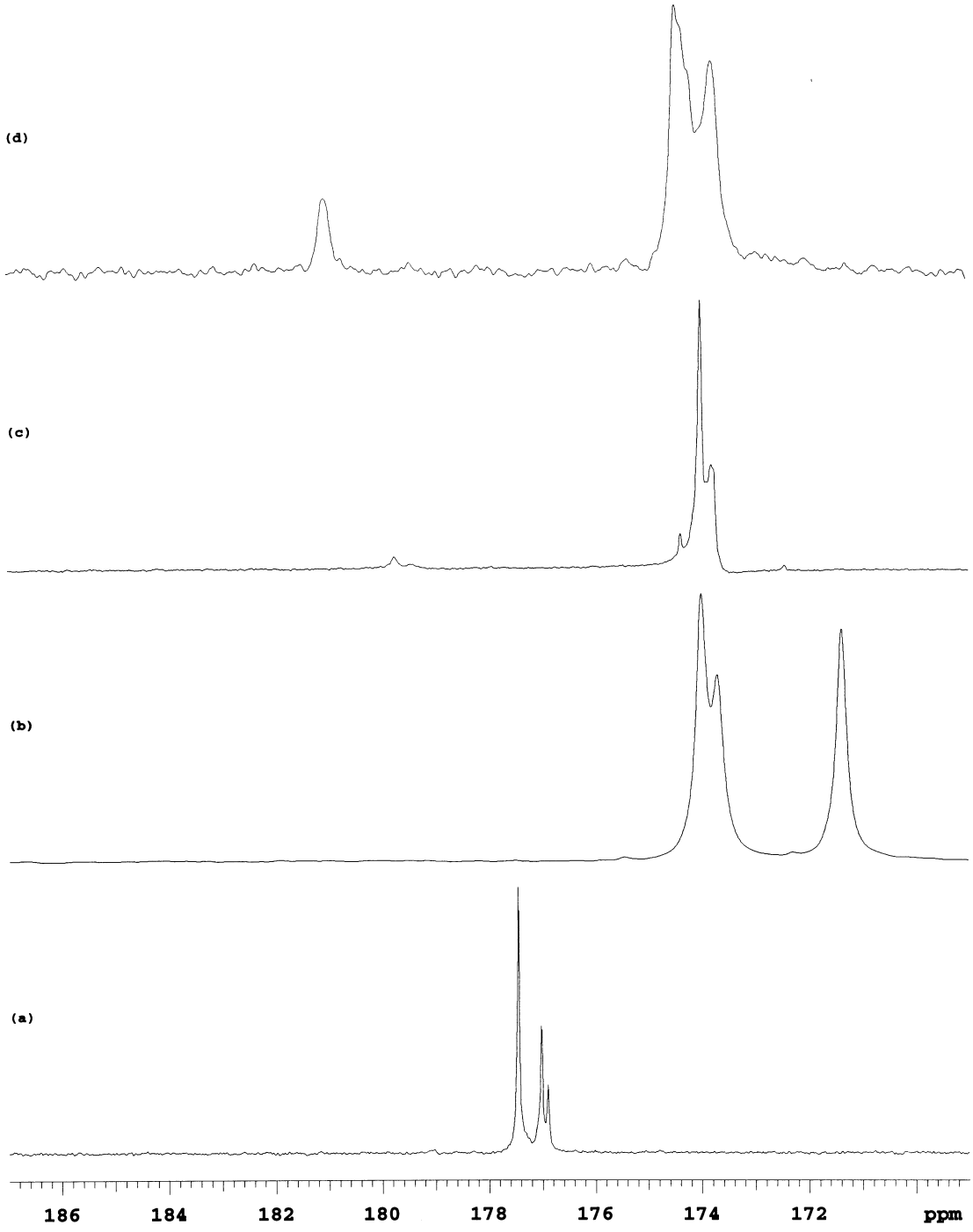

Fig. 7. Carbonyl region of ^{13}C NMR spectra for (a) generation 2 dendrimer, (b) sulphonamide, (c) sulphanilamide and (d) Gen2-Orange product.

dendrimer dye, M_d, is

$$M_d = xM_a = M_b + xM_c,$$

where M_b is the molar mass of the core and M_c is the molar mass of a chromophore unit, the average number of chromophore units per dendrimer molecule, x, can be calculated using

$$x = \frac{M_b}{M_a - M_c}.$$

2.9. Elemental analysis

Analysis for C, H, N, S and Na was carried out by the

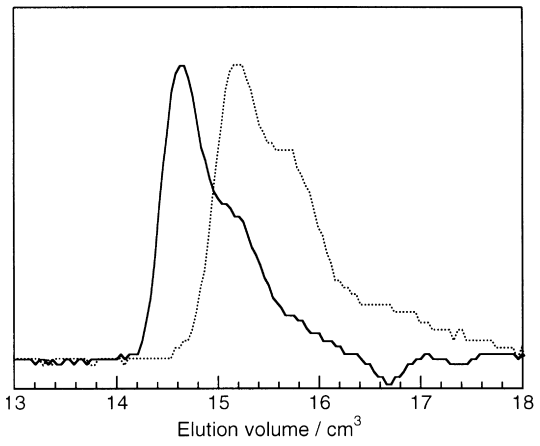

Fig. 8. GPC curves (RI detection, Manchester University instrument) for impure batches of (- - -) generation 1 dendrimer and (—) generation 2 dendrimer.

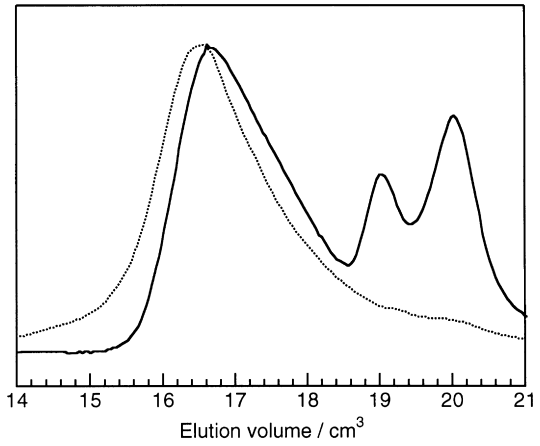

Fig. 9. GPC curves for Gen2-Orange product: (—) RI and (- - -) LS detection.

Microanalytical Service, Department of Chemistry, University of Manchester.

3. Results and discussion

3.1. Dendrimers

GPC curves for dendrimer generations 1 to 4 from Dendritech are shown in Fig. 4. Reasonably symmetrical peaks were obtained with a pH 11 buffer as eluent. A high pH buffer was essential for aqueous GPC, as protonation of amine groups at lower pH gave rise to strong adsorption effects. For the dendrimer samples, the logarithm of the theoretical molar mass was found to depend linearly on GPC peak elution volume, as can be seen in Fig. 5. Table 1 lists refractive index increments, dn/dc, and gives values of molar mass, M, determined by GPC-LS. It can be seen from Table 1 that the experimental M agree reasonably with values calculated for the theoretical structures.

The ^{13}C NMR spectrum of the generation 2 dendrimer (Fig. 6a) showed C=O resonances at δ 177.5, 177.0, and 176.9, and CH_2 resonances at δ 53.8, 51.6, 51.3, 44.2, 42.3, 39.2, 35.3, 35.2 and 35.1. The carbonyl region is expanded in Fig. 7a. The intensities of the three C=O peaks were, in order of increasing frequency, in the ratio 1:2:4, as expected for generation 2 dendrimer. ESI mass spectrometry of the generation 2 dendrimer (theoretical $M = 3256$ g mol^{-1}) gave peaks at 1086, 815, 652, 543, and 466, attributable to the 3 + , 4 + , 5 + , 6 + and 7 + molecular ions, respectively. Elemental analysis gave (theoretical values in parentheses): C 51.8% (52.4); H 9.0% (8.9); N 24.7% (24.9).

The above results indicate that the sample of generation 2 dendrimer used in the present work (Lot No. 0598-11-E2.0-LD) was reasonably pure. However, significant batch-to-batch variation was found for dendrimer samples. Fig. 8 shows GPC curves for different samples of generation 2 (Lot No. 24725JQ) and generation 1 dendrimer, obtained from Aldrich. The curves exhibit shoulders at elution volumes corresponding to lower generations. This was confirmed for the Aldrich generation 2 dendrimer by ESI mass spectrometry, which, as well as having peaks expected for the generation 2 dendrimer, gave peaks at 715, 477 and 358, which may be attributed to the 2 + , 3 + and 4 + ions, respectively, of the generation 1 dendrimer (theoretical $M =$ 1430 g mol^{-1}). One might also expect some incompletely substituted dendrimer to be present, although definitive evidence for this was not obtained.

3.2. Generation 2 dendrimer sulphonamide

ESI mass spectrometry of the generation 2 dendrimer

Table 1
Refractive index increments, theoretical molar masses and experimental molar masses for dendrimer generations 1–4 and for generation 2 dendrimer dye product. Estimated uncertainties: (dn/dc), ±5%; M, ±15%

Dendrimer	dn/dc	M (g mol^{-1}) theoretical	M (g mol^{-1}) from GPC-LS
Generation 1	0.143	1430	1630
Generation 2	0.149	3256	3640
Generation 3	0.159	6909	7170
Generation 4	0.166	14,215	15,340
Gen2-Orange dye	0.203	11,486 (100% substitution) 10,800 (88% substitution)	10,990

sulphonamide (theoretical $M = 6411$ g mol^{-1}) gave peaks at 2137, 1603, 1282, 1069, and 917, attributable to the 3 +, 4 +, 5 +, 6 + and 7 + ions, respectively. This provided strong evidence of quantitative reaction to the sulphonamide. Further evidence came from MALDI-TOF mass spectrometry, which gave peaks at 6408 and 3205. The ^{13}C NMR spectrum (Figs. 6b and 7b) showed peaks at δ 174 and 173.7 (dendrimer C=O); δ 54.4, 51.7, 44.4, 39.2 and 35.4 (dendrimer CH_2); δ 171.4 (NH*C*=OCH_3); δ 145.1 (aromatic C–S); δ 136.3 (aromatic C–N); δ 129.9 and 121.0 (aromatic C–H); δ 26.4 (NHC=O*C*H$_3$). Comparison of the dendrimer and acetyl C=O integrals confirmed a quantitative substitution. Elemental analysis (theoretical values in parentheses): C 49.3% (50.6); H 6.4% (6.2); N 15.9% (16.2); S 8.6% (8.0).

3.3. Generation 2 dendrimer sulphanilamide

The ^{13}C NMR spectrum of the generation 2 dendrimer sulphanilamide (Figs. 6c and 7c) showed peaks at δ 174.0, 173.7, 153.0, 129.5, 125.0, 113.0, 54.4, 51.7, 44.4, 39.2 and 35.4. The absence of peaks in the region of δ 171 and 26 indicated the loss of the C=OCH_3 group. Elemental analysis (theoretical values in parentheses): C 49.3% (49.8); H 6.7% (6.4); N 17.8% (18.1); S 8.7% (8.9).

3.4. Generation 2 dendrimer orange dye

The product was an orange colour in the solid state and exhibited good solubility in water at alkaline pH, giving deep red solutions. A bathochromic shift on increasing pH arises from anion formation (p. 101 of [6]). Precipitation occurred at pH less than 8, which may be attributed to interactions between protonated amine groups in the dendrimer core and sulphonic acid groups on the substituent chromophores.

GPC results (RI and LS detection) for the Gen2-Orange product are illustrated in Fig. 9. Further GPC using a UV detector confirmed that the high molar mass material was strongly UV absorbing. On the basis of the idealised structure (Fig. 1) the product is expected have a hydrodynamic volume intermediate between generation 3 and generation 4 dendrimers. Consequently, the peak elution volume lies between those for generation 3 and generation 4 dendrimers, as can be seen in Fig. 5. The molar mass determined by GPC-LS (1.1×10^4 g mol^{-1}) is close to, arguably a little lower than, that expected for the fully substituted dendrimer dye (Table 1). The degree of substitution was determined by two different methods, NMR and reductive titration, as discussed below.

The ^{13}C NMR spectrum (Figs. 6d and 7d) showed peaks at δ 181.0 (aromatic C=O from substituent); δ 174.4 and 173.7 (dendrimer C=O); δ 50.5, 49.4, 48.6, 42.5, 39.6, 36.3, 33.9 and 32.1 (dendrimer CH_2); δ 143.0, 138.3, 135.0, 133.8, 131.8, 128.8, 127.8, 126.8, 125.2, 121.9, 120.9 and 118.9 (aromatic CHs from substituents). The degree of substitution determined from the ratio of aromatic (substituent) to aliphatic (dendrimer core) carbons was 82%. For comparison, reductive titration with $TiCl_3$ gave an average number of chromophore units per dendrimer molecule, $x = 14$, corresponding to a degree of substitution of 88%. Both techniques confirm a high degree of substitution (ca. 85%).

Attempts to obtain a mass spectrum of the product were unsuccesful. Elemental analysis (theoretical values for 100% substitution in parentheses): C 40.8% (41.6); H 5.9% (3.6); N 10.4% (11.0); S 9.9% (13.4); Na 2.3% (6.4). The overall yield was low (41%), possibly because of loss of material through the dialysis membrane.

The results demonstrate that a highly substituted dendrimer dye can be prepared with 2-naphthol-3,6-disulphonic acid disodium salt as a coupling reagent. Coupling reactions were also attempted with 4-amino-5-hydroxy-2,7-naphthalene-disulphonic acid, monosodium salt, and with *N*-phenyliminodiacetic acid, but insoluble products were obtained.

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