



Site–site interactions within high-loading PAMAM dendrimer resin beads

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Abstract—Combinatorial chemistry and solid-phase synthesis have revolutionised the process of drug discovery in the last decade. Ever since the concept of split and mix synthesis was introduced in 1988, and in particular the concept of *one bead one compound*, this approach has been associated with the possibility of generating thousands or millions of compounds in only a relatively small number of synthetic steps. The demand for high loading resins has therefore increased over the last few years. Our research has focussed on bead-loading enhancement via a dendrimerisation process. Dendrimer resin beads have shown compatibility for many chemical conditions, however high functional group density could produce undesirable site–site interactions. © 2003 Elsevier Science Ltd. All rights reserved.

The ability to ‘insulate’ molecules from one another by attaching them to an inert, rigid matrix provides a valuable alternative to the high-dilution principle for the suppression of undesirable bimolecular reactions when highly reactive species or bifunctional compounds are employed, or when large rings are synthesised. The ‘pseudodilution’ effect attributed to cross-linked polystyrene resins has been successfully exploited in the past for the syntheses of cyclic peptides¹ and threaded macrocycles,² for the Dieckmann cyclisation of mixed esters,³ the monoacylation and monoalkylation of esters,⁴ the monoreaction of symmetrical bifunctional compounds⁵ and the preparation of supported *o*-benzyne⁶ and for the attachment of titanocene species with high tendency to polymerisation.⁷ However, it has been demonstrated that site–site reactions occur to some extent, depending not only on cross-linking levels but also on resin loading⁸ and kinetic relationships between the desired and competitive reactions.⁹

We have recently reported the synthesis of polyamidoamine (PAMAM) dendrimer resin via an iterative two-step process involving exhaustive Michael addition of methyl acrylate to a solid-supported primary amine and subsequent displacement of the methyl ester moieties with a symmetrical diamine such as 1,3-

diaminopropane.¹⁰ Increasing mobility of the growing polyamidoamine branches can lead to truncated dendrimers where both amino groups of the symmetrical diamine react with two different resin-bound methyl esters. This side reaction was observed starting from generation 4.0 dendrimer resin, due to its higher flexibility and increased functional group density compared to the lower generations. Generation 3.0 dendrimer resin, with an eightfold increase in loading of TentaGel (PS-PEG) resin, was therefore considered to offer the best compromise between bead loading and reactivity/site–site interactions. Generation 3.0 PAMAM dendrimer resin was subsequently used under many different chemical conditions, and compounds such as amidines,¹¹ ethers,¹² benzodiazepines, tetrazoles and biaryls¹³ were successfully prepared via solid-phase synthesis. The experimental conditions applied confirmed chemical inertness, mechanical robustness, good swelling properties and suitability for single bead screening of this novel solid support.

In this communication site–site interactions of active sites on generation 3.0 PAMAM dendrimer resin are investigated. Two different classes of reactions were carried out, the first being intramolecular dipeptide cyclisation/release and the second being nucleophilic displacement of a resin-bound electrophile by a long chain symmetrical diamine. This choice was made in order to compare dendrimer resins with commercial polystyrene and TentaGel resins.

Keywords: solid-phase synthesis; dendrimer resin; site–site interactions.

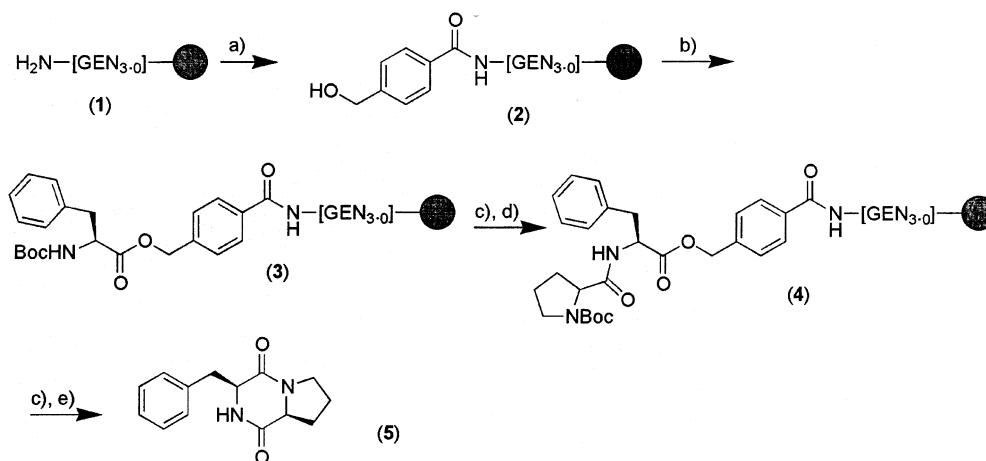
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In the first experiment (Scheme 1) generation 3.0 dendrimer resin (**1**) (1.0 mmol/g, 4.0 nmol/bead) was coupled with 4-hydroxymethylbenzoic acid. The resulting hydroxymethyl-functionalised resin (**2**) was reacted with N-Boc-phenylalanine and, after Boc deprotection, coupled with N-Boc-proline. Trifluoroacetic acid cleavage of the Boc protecting group of resin (**4**) and suspension in neat triethylamine at 70°C overnight gave the desired diketopiperazine (**5**)¹⁴ as the only product, as determined by HPLC analysis, in 53% yield after semi-preparative HPLC purification. Treatment of the resulting resin with 1 M NaOH/dioxane (1:1) did not release any additional product deriving from cross-linking reactions. An analogous experiment (Scheme 2) was carried out coupling N-Boc-proline and then N-Boc-phenylalanine to resin (**2**). After Boc deprotection of resin (**6**), diketopiperazine formation occurred at room temperature in DCM/triethylamine 1:1. Again, compound **5** was the only material released from the resin (98% HPLC purity) and was isolated in 60% yield after semi-preparative HPLC. Treatment of the resulting resin with 1 M NaOH/dioxane 1:1 confirmed no inter-molecular reactions took place during the cyclisation/release step.

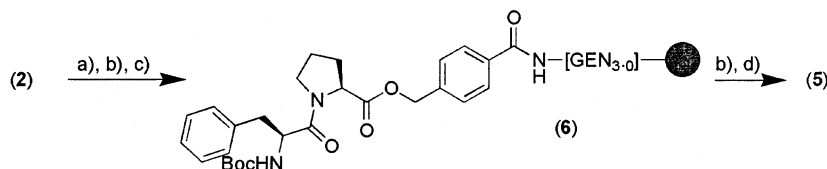
A second investigation (Scheme 3) was carried out in parallel with generation 3.0 dendrimer resin (**1**) and TentaGel resin. The Rink linker was attached using standard protocols and the resulting resins (**7a,b**) were

coupled with 4-chloromethylbenzoic acid. Both resins (**8a,b**) were then suspended in DMF and reacted with variable amounts of 1,9-diaminononane at different temperatures. The resins were then treated with trifluoroacetic acid and the released compounds analysed by HPLC, MS and NMR. The results are summarised in Table 1. In all cases, together with the desired product (**9**),¹⁵ formation of the cross-linked product (**10**)¹⁶ was observed; in some cases also the double cross-linked product (**11**)¹⁷ was isolated. Dendrimer resin showed a higher extent of cross-linked products when critical conditions were chosen (i.e. lower number of equivalents of diamine and higher temperatures, entries 3, 4, 5 and 6). However, under standard conditions, no appreciable differences were observed between TentaGel and dendrimer resin (entries 1 and 2).

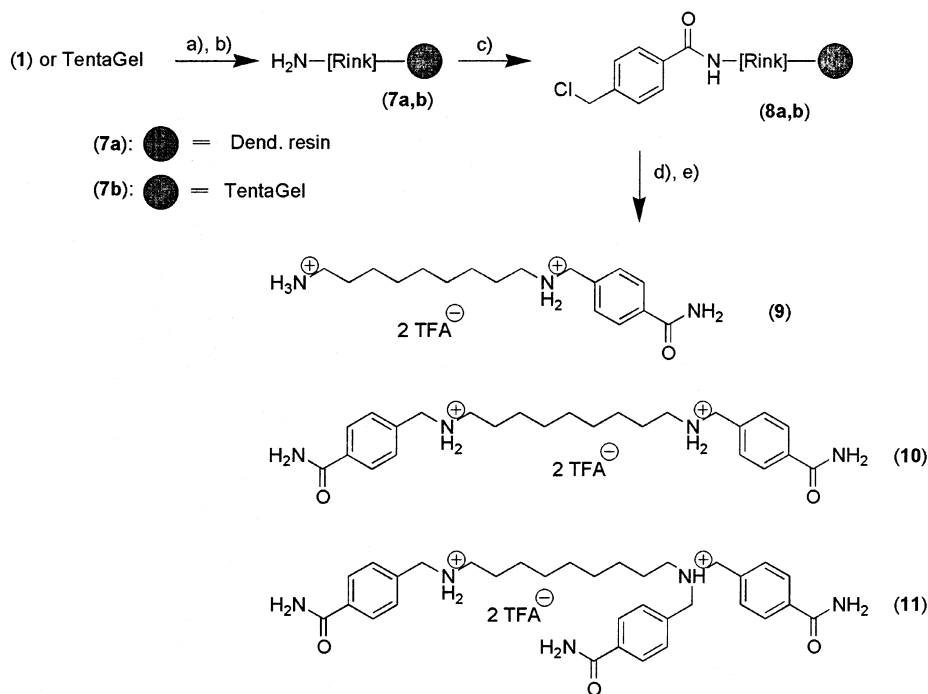
In conclusion, it has been proven that, beside inertness, robustness and swelling properties, site-site interactions are not a critical issue for PAMAM dendrimer resins, with behaviour similar to that of normal TentaGel resin, if routine conditions are applied. Once more, it has been demonstrated that dendrimer resin can be successfully used in solid-phase organic synthesis, combining reactivities comparable to commercial resins with high loadings suitable for single bead screenings and without appreciable undesired reactions.



Scheme 1. Reagents and conditions: (a) 4-hydroxymethylbenzoic acid, DIC, HOBT, DMF; (b) Boc-Phe-OH, DIC, DMAP, DMF; (c) TFA, DCM, then 20% DIPEA/DCM; (d) Boc-Pro-OH, DIC, HOBT, DMF; (e) Et₃N, reflux.



Scheme 2. Reagents and conditions: (a) Boc-Pro-OH, DIC, DMAP, DMF; (b) TFA, DCM, then 20% DIPEA/DCM; (c) Boc-Phe-OH, HATU, HOAt, DMF; (d) Et₃N, DCM.



Scheme 3. *Reagents and conditions:* (a) Fmoc-Rink linker, DIC, HOBT, DMF; (b) 20% piperidine, DMF; (c) 4-chloromethylbenzoic acid, DIC, HOBT, DMF; (d) 1,9-diaminononane, DMF; (e) TFA, DCM.

Table 1. Results of the reaction between chloromethylated resins (**8a**) and (**8b**) and 1,9-diaminononane (**12**) under different conditions

Entry	Resin	Conditions	(9) ^a (%)	(10) ^a (%)	(11) ^a (%)
1	TentaGel	(12) (6.0 equiv.), 20°C, 48 h	63	37	—
2	Dend. Resin	(12) (6.0 equiv.), 20°C, 48 h	62	38	—
3	TentaGel	(12) (4.0 equiv.), 50°C, 48 h	29	55	16
4	Dend. Resin	(12) (4.0 equiv.), 50°C, 48 h	14	50	36
5	TentaGel	(12) (2.0 equiv.), 50°C, 48 h	21	56	23
6	Dend. Resin	(12) (2.0 equiv.), 50°C, 48 h	8	39	52

^a Area % determined by HPLC at 220 nm. This is not an absolute measurement of the relative amount of compounds **(9)**, **(10)** and **(11)**, since their molar absorbance increases with the number of aromatic rings and amide bonds.

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References

- Acknowledgements**
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 14. Analytical data for compound **5**: ^1H NMR (CDCl_3 , 400 MHz) 7.36 (2H, t, $J=7$), 7.32 (1H, t, $J=7$), 7.23 (2H, d, $J=7$), 5.68 (1H, s), 4.28 (1H, dd, $J=10, 3$), 4.09 (1H, t, $J=7$), 3.70–3.52 (3H, m), 2.79 (1H, dd, $J=14, 10$),

- 2.40–1.84 (4H, m); ^{13}C NMR (CDCl_3 , 100 MHz) 165.2, 165.1, 135.8, 129.4, 129.3, 127.7, 59.3, 56.4, 45.6, 36.9, 28.5, 22.7; HR-MS (EI+) 244.1213 (–0.6 ppm).
15. Analytical data for compound **9**: ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) 9.05 (2H, s), 8.02 (1H, s), 7.92 (2H, d, $J=9$), 7.79 (3H, s), 7.55 (2H, d, $J=9$), 7.42 (1H, s), 4.19 (2H, t, $J=6$), 2.90 (2H, s), 2.76 (2H, m), 1.60 (2H, m), 1.50 (2H, m), 1.26 (10H, s); ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz) 167.0, 158.0 (quart, $J=33$), 134.8, 134.4, 129.4, 127.5, 116.3 (quart, $J=295$), 49.2, 48.3, 46.3, 28.3, 28.1, 26.7, 25.6, 25.5, 25.0; HR-MS (FAB+) 292.2387 (0.5 ppm).
16. Analytical data for compound **10**: ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) 8.96 (4H, s), 8.01 (1H, s), 7.92 (4H, d, $J=9$), 7.56 (4H, d, $J=9$), 7.44 (1H, s), 4.18 (4H, m), 2.89 (4H, s), 1.60 (4H, m), 1.25 (10H, s); ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz) 167.2, 158.0 (quart, $J=33$), 135.0, 134.7, 129.6, 127.7, 116.3 (quart, $J=295$), 49.5, 46.6, 28.5, 28.4, 25.9, 25.3; HR-MS (FAB+) 425.2912 (1.2 ppm).
17. Analytical data for compound **11**: ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) 10.2 (1H, s), 8.90 (2H, s), 8.05–8.01 (3H, m), 7.93 (6H, m), 7.60 (4H, s), 7.56 (2H, d, $J=9$), 7.47–7.42 (3H, m), 4.40 (4H, s), 4.19 (2H, t, $J=6$), 2.89 (4H, s), 1.67–1.57 (4H, m), 1.25–1.16 (10H, m); ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz) 167.7, 157.8 (quart, $J=33$), 134.5, 132.9, 132.7, 128.1, 127.7, 127.4, 127.3, 116.3 (quart, $J=295$), 52.8, 52.3, 48.4, 28.9, 28.8, 28.7, 26.7, 26.3, 26.2; HR-MS (FAB+) 558.3429 (2.6 ppm).