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Solid-phase synthesis of aryl ethers on high loading dendrimer resin

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Abstract

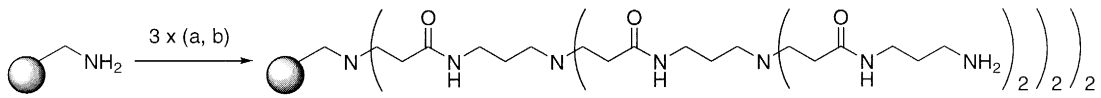
Resin-bound dendrimers have the potential to increase bead loading by at least one order of magnitude. In this letter we report the solid-phase synthesis of a library of aryl ethers using high-loading dendrimer beads, and compare the synthetic efficiency with that of TentaGel (Polystyrene-PEG) resin. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: dendrimers; solid-phase synthesis; high-loading beads; single-bead screening.

Ever since the concept of split and mix combinatorial synthesis was introduced in 1988,[1] and in particular the concept of one bead one compound,[2] this approach has been associated with the possibility of generating thousands or millions of compounds in only a relatively small number of synthetic steps. Unfortunately the full potential of this combinatorial chemistry technique has not been completely exploited for two main reasons: the small amount of substance linked to a single bead and the need of a tag or of a time-consuming deconvoluting analysis to identify the active compounds.

Indeed libraries that are usually reported in the literature are relatively small in number and usually obtained by parallel synthesis, although modern biological screening techniques are able to screen thousands of compounds in a very short time.

Many of the problems associated with single-bead screening could be solved if the loading on each single bead was increased. Currently the loading of a typical 100 μm resin bead with a substitution of 1 mmol/g is about 0.2 nmoles, increasing it 10–20 times would be enough for repeated biological screening.



Scheme 1. Synthesis of resin-bound gen [3.0] PAMAM dendrimers; conditions: (a) methyl acrylate, methanol; (b) 1,3-propanediamine, methanol

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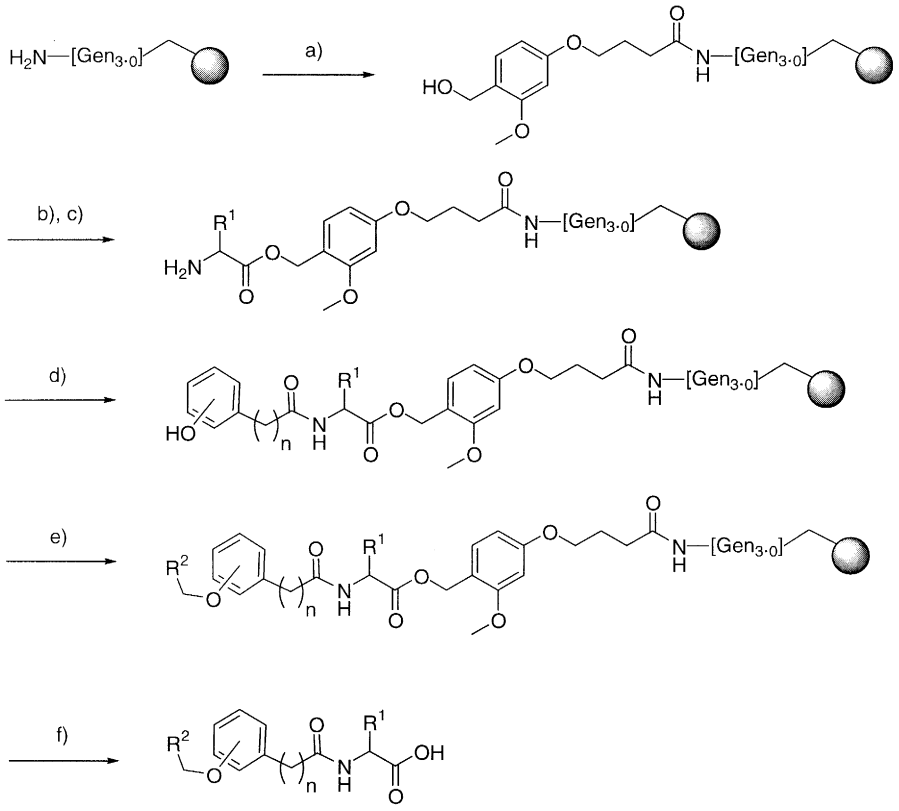


Scheme 2. Synthesis of a library of aryl ethers; conditions: (a) HMPB linker, DIC, HOBt, DCM, DMF; (b) Fmoc-amino acid, DIC, DMAP, DCM, DMF; (c) piperidine, DMF; (d) hydroxyacid, DIC, HOBt, DCM, DMF; (e) alcohol, DIAD, PPh_3, THF; (f) TFA, H_2O

The research reported here is focused on bead-loading enhancement via a dendrimerisation process using our published solid-phase synthesis of polyamidoamino (PAMAM) dendrimers (DendroGel).[3,4]

Synthesis started directly on custom synthesised 160 μm diameter (dry beads, 220 μm swollen in DCM) TentaGel beads which had an initial loading of 0.85 nmol/bead. Generation [3.0] PAMAM dendrimers were obtained as outlined in Scheme 1 by repeated treatment with methylacrylate in methanol (24–48 hours) and 1,3-propanediamine in methanol (24–48 hours) to give up to 20 g of derivatised resin (5 million beads). Dendrimer synthesis efficiency was monitored throughout the synthesis by ninhydrin test,[5] and Fmoc analysis[6] and gave a final loading of 4.0 nmol/bead, reasonably close to the theoretical maximum (6.8 nmol/bead) based on manufacturer's loading. These beads were the starting point for all subsequent chemistry and in this paper we report their use for the synthesis of a small library of aryl ethers via the Mitsunobu reaction.[7]

The linker 4-(4′-hydroxymethyl-3′-methoxy)phenoxybutyric (HMPB) was first attached to the resin using DIC/HOBt followed by an Fmoc-protected amino acid which was coupled onto the linker with DIC/DMAP. After removal of the protecting group, a hydroxyphenyl carboxylic acid was attached using DIC/HOBt. These steps were all straightforward, although the amounts of reagent and the reaction times were crucial to avoid phenoxy ester formation in the last reaction (reactant 1.0 equiv., HOBt 1.5 equiv., DIC 1.0 equiv., less than three hours) (Scheme 2).

A number of different reaction conditions were investigated for the Mitsunobu condensation of the resin-bound phenol with an alcohol. DIAD/PPh_3 was observed to be the most efficient, while

TMAD/PBu_3[8,9] or other combinations gave much poorer results. It was also observed that washing the resin with dry THF before reaction gave much higher conversions.

Results were encouraging: most of the reactions could be driven to completion by repeating the coupling. Some difference in reactivity was observed in relation to the alcohol used in the coupling, but not to the amino acid component nor to the phenolic acid used; only the reaction with *N*-Boc-ethanolamine showed starting material remaining in the HPLC trace.

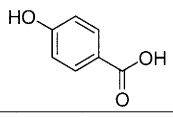
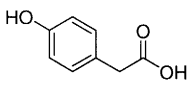
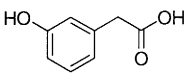
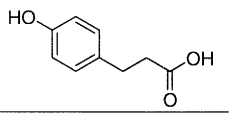
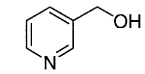
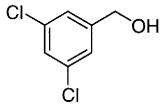
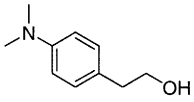
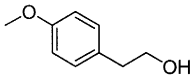
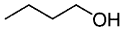
Before synthesising a small library, a Mitsunobu condensation was performed in parallel with normal TentaGel resin and our dendrimer resin using four different alcohol building blocks. The scaffold on the resin was kept constant, with glycine and 4-hydroxyphenylacetic acid used as the building blocks. Four different alcohols were used: benzyl alcohol, pyridyl-2-carbinol, 3,5-dichlorobenzylalcohol and *N*-Boc-ethanolamine. The results are given in Table 1. It is clear that much better yields were obtained with the dendrimer resin than TentaGel although the purity was high in all cases. It was initially believed that the dendrimerised resin was more efficient because of the basicity of the dendrimer aiding the deprotonation of the resin bound phenol (it is known in literature that amines can enhance the Mitsunobu reaction).[10] However, when *N*-methyl morpholine (10 equiv., 30 equiv. or as solvent) or a commercially available Gen[1.0] PAMAM dendrimer was added to the reaction mixture similar or worse results were obtained. One possible explanation could be the amount of water trapped within the resin, however both dendrimerised and normal TentaGel resin were carefully dried before the condensation (the resin was kept over P_2O_5 overnight); while it might be expected that the dendrimerised resin would have a higher tendency to trap water.

Table 1
Comparison of the reactivity of TentaGel and dendrimerised resin towards Mitsunobu conditions

	Conversion (%)	
Alcohol	Normal TentaGel	Dendrimerised
Benzyl alcohol	91	91
3,5-Dichlorobenzyl alcohol	45	93
N-Boc ethanolamine	0	67
Pyridyl-2-carbinol (1^{st} coupling)	0	100
Pyridyl-2-carbinol (2^{nd} coupling)	57	100

A small library of ethers was then synthesised by varying the phenolic acid and the alcohol, although in principle a ternary library could have been generated by varying the amino acid. The library was synthesised in a semi-automated fashion on an Argonaut QUEST 210 using four different phenolic acids (with different electronic properties and position of the -OH group) and five different alcohols (with different reactivity and steric hindrance). The results obtained are summarised in Table 2. All reactions worked very well in terms of purity (determined by HPLC with diode array UV detection) and yield (determined by ^{1}H NMR via d_5-DMSO calibration).[11] Only in the case of 4-(dimethylamino) phenethyl alcohol was the cleavage yield below 70%. All the members of the library were characterised by LC-MS, ^{1}H NMR, ^{13}C NMR and single bead MS (all but two compounds ionised under the experimental conditions). A typical ^{1}H NMR of a crude compound is shown in Fig. 1, and shows the efficiency of synthesis on the dendrimer-based beads. The absolute amount of compound released by a single bead was determined via HPLC calibration of compound **1b**. Cleavage from 10 beads afforded enough material

Table 2
Synthesis of a 20-member library of aryl ethers; single-bead MS, yield (calculated by NMR via d_5-DMSO quantification) and HPLC purity (bold) are reported for each compound. [a]The expected MS peak was obtained only with higher concentration of compound

		1 	**2** 	**3** 	**4** 
a		287.2 86% **91%**	301.2 82% **97%**	301.3 84% **85%**	315.3 79% **95%**
b		354.1 84% **100%**	368.2 82% **95%**	368.2 84% **97%**	(380.2)[a] 76% **98%**
c		343.2 68% **100%**	357.3 67% **95%**	357.3 62% **67%**	371.3 66% **88%**
d		330.1 78% **100%**	344.1 83% **91%**	344.1 78% **83%**	358.2 82% **94%**
e		252.1 85% **97%**	266.2 88% **92%**	(266.2)[a] 75% **84%**	280.2 78% **91%**

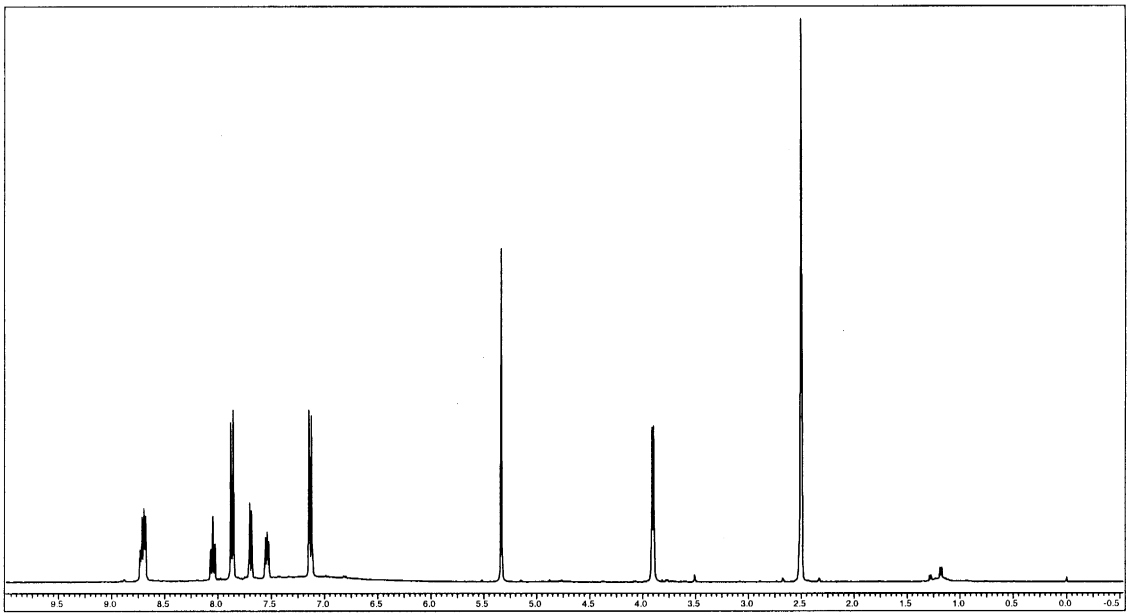


Fig. 1. Crude ^{1}H NMR of compound **1a** of the library of aryl ethers

for conventional HPLC analysis and gave a resin loading of 3.5 nmol/bead. Taking into account both synthesis and compound cleavage this value agrees very well with the initial loading of the dendrimer resin of 4 nmol/bead and indicates the efficiency of the chemistry.

The synthesis of the library proved the robustness of our resin: agitation in most automated systems is by inert gas bubbling while in the QUEST it is by magnetic agitation. Although this agitation method is more vigorous, the beads were robust enough to survive without breakage. These beads are now used in single-bead biological screening.

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11. This is an approximate method of quantification. The amount of residual protonated DMSO present in the NMR solvent (constant in each bottle) is quantified with a known quantity of *p*-nitrophenol via the respective ^{1}H NMR signal integrations. This is then used as an internal standard in the calculation of the quantity of compound released by a known amount of resin.