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Efficiencies of Reductive Amination Reactions on Different Solid Supports

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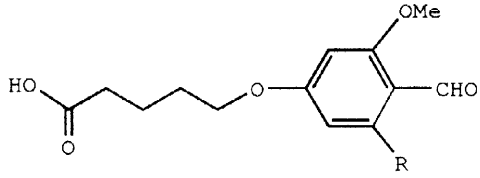
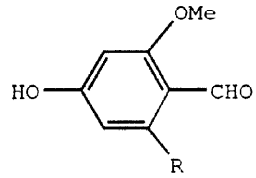
Abstract: *Rreductive amination on resins derivatized with 5-(4-formyl-3,5-dimethoxy-phenoxy)valeric acid linker (Barany linker, **1**)[1] has been reported to be less effective than on resin derivatized with its monomethoxy analog (**2**)[2] due to steric hindrance of the extra methoxy functional group within the molecule.[3] A study of these linkers indicate that the origins of such data is also related to the spacer and solid support used in the study. Depending on the linker, spacer and solid phase, yields from 10% to 75% were obtained under exactly the same reaction conditions.* © 1998 Elsevier Science Ltd. All rights reserved.

The choice of solid support and linker are important factors for success in solid phase synthesis.[4] Unlike solution phase synthesis, where an optimisation study will result in reproducible reactions, time consuming validation steps usually required in solid phase synthesis may only be specific for a particular solid support.[5] Here, we present a comparative study of reaction rates, yields, and purity to assess the effect of the solid phase and a six bond spacer arm in a model reductive amination. We compared aminomethylated and chloromethylated Synphase crowns[6] used with the Multipin method of solid phase synthesis,[7] with aminomethylated (1.0 mmol/g, 500-595 μm, Polymer Laboratories) and chloromethylated (0.99 mmol/g, 400-500 μm, Polymer Laboratories) resin beads. For comparative purposes, we chose resins with relatively large bead sizes as they can be considered individual "reactors" that allow syntheses of many different compounds in common reaction flasks but still allow separation and cleavage to give individual compounds. The resins and SynPhase crowns were derivatised with four linkers. Aminomethylated supports were derivatised with 5-(4-formyl-3,5-dimethoxy-phenoxy) valeric acid (**1**) and 5-(4-formyl-3-methoxyphenoxy) valeric acid (**2**) while the chloromethylated supports were derivatised with their corresponding analogues **3** and **4** under previously published conditions.[1,2] In principle, supports derivatised with **3** and **4** are functionally identical to **1** and **2** and any differences in outcome can be attributed to the six bond spacer arm.



1 R = OMe
2 R = H



3 R = OMe
4 R = H

Derivatized crowns and resins were subjected to a reductive amination reaction with benzylamine to give the amine bound linkers **6** (**a** or **b**) under identical reaction conditions.[8] The resulting products were acylated with Fmoc-(ß)-alanine using previously published conditions to afford 7 (**a** or **b**).[9] Under standard cleavage conditions (50% TFA/DCM), all 4 linkers liberated the target amide **8**.[10] The assigned structure and % purity of **8** were directly obtained by ES-MS[11] and HPLC analysis.[12]

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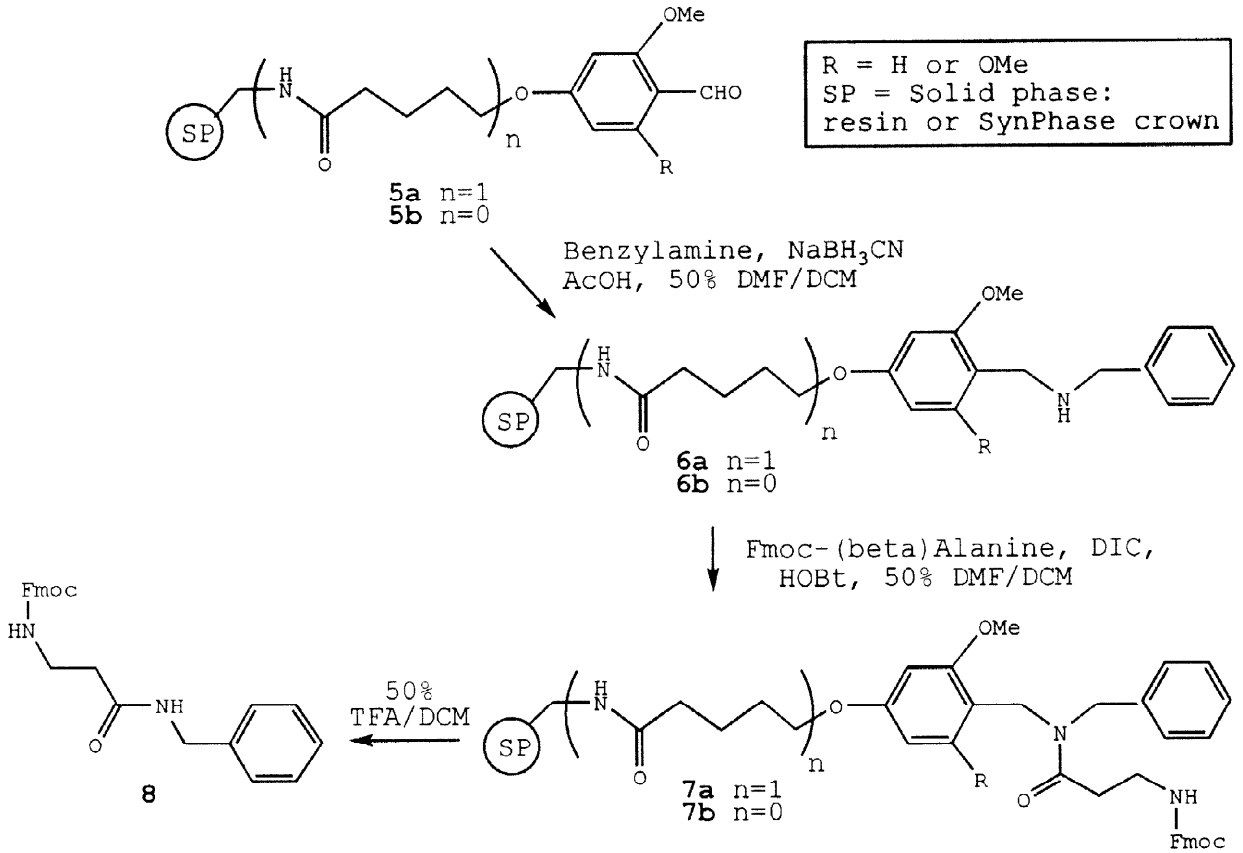

Scheme 1. Synthesis of the Model Molecule **8**

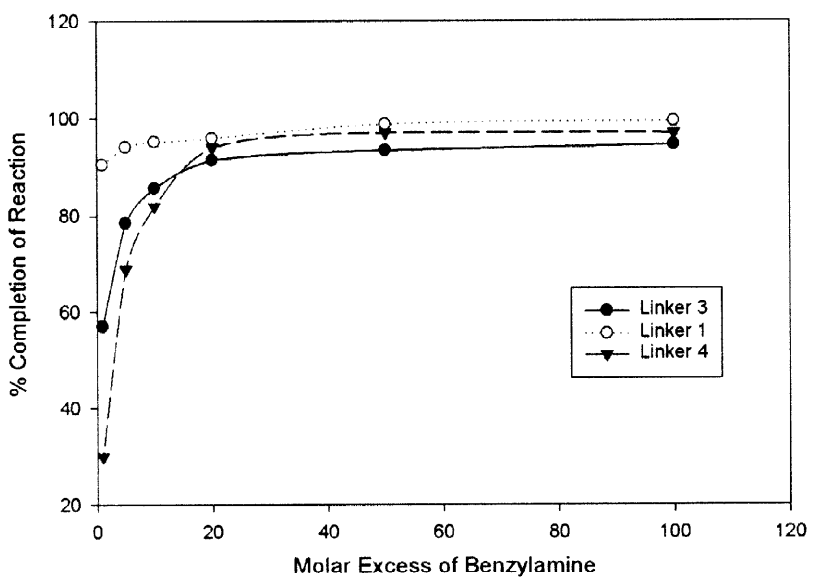

Figure 1. % Completion of Reaction vs. Molar Excess of Benzylamine on Resins Derivatized with Linker **1**, **3** or **4** (% completion = [HPLC[8] peak area of **8** /peak areas of **8** and by-product, Fmoc-ß-alanine]x100)

The % yield of this 4-step reaction was quantitated using HPLC analysis. In brief, the solution phase synthesis of **8** was carried out separately to establish a calibration curve of the HPLC peak area versus amount of amide **8** in the range of 0 to 10 nmoles (the HPLC response was linear in this range). The % yield of **8** obtained by solid phase synthesis was derived directly from the straight line equation y = 0.911x where y was amount of **8** and x was HPLC peak area at 214 nm. Assessment of the completion of the reductive amination reaction was based on the comparison of HPLC peak area of **8** and of the reaction by-product, Fmoc-ß-alanine. Incomplete reductive amination led to the formation of a hydroxyl group on the linker which was subsequently coupled with Fmoc-ß-alanine. An example of optimisation by multiple parallel synthesis showing dependence of % completion of reaction with varying molar excess of benzylamine is shown for resins in Figure 1.[13] Table 1 summarizes the yield and purity obtained from the reaction conditions for each linker and solid support combination which gave optimum purity.

Solid Support	Size	Loading (/ g resin or / crown)	Linker	Yield (/100 mg resin or /crown)	% yield[a]	% Purity
Resin PS-CH_2-Cl	400-500 µm	0.99 mmol	**3**	10.2 µmol	10	91
Resin PS-CH_2-NH_2	500-595 µm	1.00 mmol	**1**	32.4 µmol	32	98
Resin PS-CH_2-Cl	400-500 µm	0.99 mmol	**4**	28.6 µmol	30	93
Crown PS-CH_2-Cl	I- series	16.4 µmol	**3**	6.4 µmol	39	98
Crown PS-CH_2-NH_2	I- series	30 µmol	**1**	21.8 µmol	73	99
Crown PS-CH_2-NH_2	I- series	30 µmol	**2**	22.5 µmol	75	98

[a] Overall yields (in 4-step reaction from **1**, **2**, **3** & **4** were determined in triplicate and based on the initial loading of crown or resin.

Table 1. Synthesis of Compound **8** on Resin and Crown Solid Supports

From this study, the following results were obtained. (i) Within one type of solid support and linker, those with the 6 bond (valeric acid) spacer arm gave higher yields than those without this spacer. Kinetic studies (see Figure1) also indicated that those with the valeric acid spacer reacted faster and gave better results than those without this spacer. At least 20 molar excess of reagent was required for **3** and **4** to approach completion of the reductive amination reaction within 4 h. In comparision, only 5 molar excess was required for **1** to go to completion. Even one equivalent of benzylamine gave >90% reaction completion indicating the importance of this spacer. (ii) It has been commented that linkers **1** and **3** are more hindered than **2** and **4** due to steric effects of the extra methoxy group and, consequently, give lower yield and purity on conventional PS resins.[3] Such experimental results have been confirmed on these polystyrene beads where replacement of linker **3** by **4** significantly improved product yield. On crowns, however, there are no observable differences in yield and purity obtained from linkers **1** and **2**. (iii) Decrease in overall purities with linkers **3** and **4** on resin beads were the result of a HOBt peak in the HPLC and not incomplete reductive amination. In these cases, the lower purities are indications of poorer washing characteristics of these resins. (iv) In contrast, the yields obtained on the two solid supports were very different. SynPhase crowns consistently gave better yields in all cases. As optimised reaction conditions were applied in this comparative study and repeated a number of times, the variation in yields cannot be the result of incomplete reactions. The cleavage conditions were extensive, using TFA/DCM for 2 hrs with repeated washing in DCM to remove the cleaved product. The data clearly indicates that efficiency in solid phase synthesis is dependent on the differences in diffusion rates, mobility, etc, that come with different solid supports, spacer and linker combinations.[4, 15] The model study also indicates that steric

effects attributed to differences in the linker may originate at a more fundamental level and can be minimised by appropriate selection of spacer and solid support combinations.

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References and Notes:

Abbreviations: DIC: diisopropylcarbodiimide, DMAP: dimethylaminopyridine, DMF: dimethylformamide, ES-MS: electrospray mass spectrometry, Fmoc: 9-fluorenyl-methoxycarbonyl, HOBt: 1-hydroxybenzotriazole, PIP: piperidine, TFA: trifluoroacetic acid, TNBSA: 2,4,6-trinitrobenzene sulfonic acid.

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10. Characterization of compound **8**: Rt = 8.8 min.[12] Melting point = 178°C
Ion spray MS[11] m/z 401.2 $[M + H]^+$, 418.2 $[M + NH_4]^+$.
11. Mass spectrometer analysis (MS): Ion-spray MS was conducted with Perkin-Elmer Sciex API III using 0.1% acetic acid in 60% acetonitrile.
12. Reverse phase high performance liquid chromatography (RP-HPLC) was conducted with Rainin, Microsorb-MV Cat.# 86-200-F3, 50x4.6 mm column using gradient mobile-phase 0-100% B over 11.5 mins. Flow rate: 1.5 ml/min.(Solvent A: 0.1% ortho-phosphoric acid in water; Solvent B: 0.1% ortho-phosphoric acid in 90% acetonitrile). Detection: 214 nm.
13. The derivatised resin **5a** or **5b** (50 mg) was swollen in 3 ml of DCM:DMF (50:50) for 20 mins then treated with a series of reductive amination solutions at 60°C for 4h. The resins were cooled to room temperature and then filtered, washed with DCM:DMF (2x), DMF (x) and DCM (2x) and used immediately for the next step. The reductive amination solution was prepared as described in Ref 8 with six different benzylamine concentrations (100x, 50x, 20x, 10x, 5x and 1x molar excess) while maintaining constant $NaBH_3CN$.
14. At diluted reductive amination solution, two compounds were isolated in the reaction mixture: Product 8 (Rt = 8.8 min) and the by-product (Fmoc-ß-Alanine, Rt = 7.6 min)
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