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Highly-loaded amphiphilic polyimino resin: quench reagent and solid support for peptide synthesis

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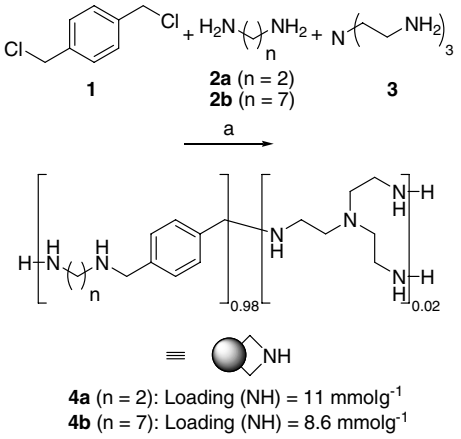
Abstract—We demonstrate herein that polyimino resin **4a** prepared by condensation of α,α′-dichloro-*p*-xylene, ethylenediamine and tris-(2-aminoethyl)-amine can be successfully exploited as a quench reagent for acids and electrophiles both in aqueous and organic solutions. Scope and limitations of such a resin as a solid support for peptide synthesis were also investigated.

Forty years ago, the pioneering work of Merrifield in the synthesis of peptides on divinylbenzene crosslinked polystyrene set the milestone of solid phase synthesis.[1] Later on, outstanding contributions from Janda, Sheppard, Meldal and others have placed a range of insoluble supports at the disposal of the scientific community.[2–9] These new solid supports may possess improved swelling capabilities in water and organic solvents, occasionally bear higher loading, and may also display increased stability under harsh reaction conditions used in organic synthesis, as compared to somewhat 'chimie douce' conditions of peptide synthesis.

Initially, we sought a new highly loaded, reticulated amino resin that might exhibit good swelling properties in hydrophobic and hydrophilic media, and we intended to use that resin as a scavenger reagent for acids and electrophiles. Indeed, there is currently a high level of interest in the development and use of functionalized polymers as scavenger reagents in synthesis.[10–14] Such quench reagents have been conceived to meet the requisite of the development of new synthesis technologies and to lessen the need for chromatographic purifications. However, methods for construction of such polymers, whilst effective, often require multistep polymerization and grafting processes and/or well defined polymerization conditions. Our goal was to make resin preparation as straightforward as possible.

Condensation of α,α′-dichloro-*para*-xylene (**1**) with diamines (**2**) and 2 mol % of tris-(2-aminoethyl)-amine (**3**, reticulating agent) in DMF/NEt_3 generated the desired polyimines (PI) in 64–85% yield as pale yellow to brown powders (Scheme 1).[15]

In both cases, IR shows a very broad absorption band at 3300–3400 cm^{-1} attributable to NH stretching. Resins loading based on theoretical nitrogen content should be 12.3 and 8.6 mmol g^{-1} with $n = 2$ and $n = 7$, respectively. Actual functional group loadings were 11 and 8.6 mmol g^{-1}, respectively, as measured by picric acid titration.[16]



Scheme 1. Reagents and conditions: (a) NEt_3, DMF, 85 °C, 2 days ($n = 2$: 64–80%, $n = 7$: 85%).

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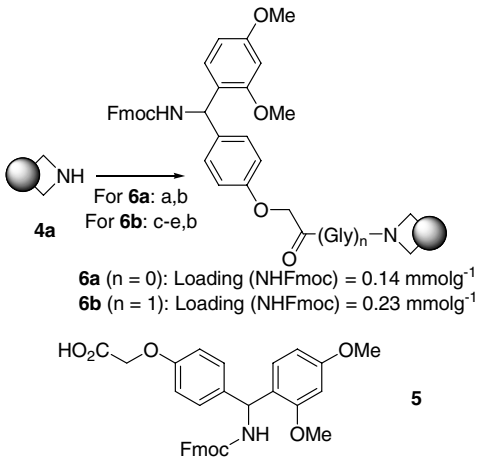
The success of organic reactions performed on a gel-phase resin is highly dependent on the accessibility of solvents, catalysts, and reagents to the interior of the resin. Therefore, the swelling properties of resins **4** were measured in polar and nonpolar solvents and were compared to those measured for commercially available polystyrene/divinylbenzene resin (PS). PI **4b** exhibits roughly the same swelling behavior as PS (Table 1).[17] It should therefore be regarded as a hydrophobic highly-loaded PI resin and may be utilized in the same way as amino functionalized PS resins. On the other hand, PI **4a** clearly behaves as an amphiphilic resin. Thus, **4a** was naturally preferred for scavenging purposes as a result of its higher loading and its superior resin-swelling ability in polar solvents.

We first put our polymeric scavenger reagent to the test of acid and electrophile quenching from aqueous and organic solutions (Table 2). Aqueous solutions of sulfuric acid and hydrochloric acid could be efficiently neutralized with **4a**. Organic acid TsOH and electrophilic reagents Boc_2O and AcCl were also trapped quantitatively.

After having demonstrated that PI **4a** swells and reacts efficiently in a wide range of solvents, we embarked upon solid phase peptide synthesis (SPPS) using **4a**. First, we reacted the PI with excess Boc_2O and a catalytic amount of DMAP in order to evaluate the efficient loading for trapping electrophiles.[16] It was found that **4a** is capable of quenching 10.9 mmol of Boc_2O/g resin. Therefore, we concluded that most NH groups are indeed reactive toward electrophilic reagents.

Next, our choice was to attach commercially available Fmoc Rink amide linker **5** to imino functional groups (Scheme 2). Grafting was carried out by the conventional symmetrical anhydride method.[18] Unreacted imino functional groups were capped with Ac_2O, and functional loading in **6a** was measured by Fmoc release.[16] It was found to be rather low as compared to that of PI **4a**. Since we had demonstrated earlier that



Scheme 2. Reagents and conditions: (a) **5**, DIC, 0 °C, CH_2Cl_2 then **4a**, DMAP, DMF; (b) Ac_2O (capping); (c) FmocGlyOH, DIC, 0 °C, CH_2Cl_2 then **4a**, DMAP, DMF; (d) piperidine, DMF and (e) **5**, HBTU, HOBt, DIEA, DMF.

trapping of a smaller electrophilic reagent (Boc_2O) is quite efficient, the moderate grafting yield in this case was attributed to the large size of the intermediate anhydride. Furthermore, grafting technique using symmetrical anhydride consumes a lot of linker. We therefore embarked upon grafting cheaper and possibly less hindered glycine handle in-between the resin and linker. $(FmocGly)_2O$ trapping proved more efficient, yielding intermediate Fmoc–Gly–PI resin with 0.80 mmol NHFmoc groups per gram resin after acetic anhydride capping. Fmoc–Gly–PI resin was further functionalized with linker **5** yielding resin **6b**, which was used in model peptides syntheses.

Dipeptidamides **7** and **8**, as well as platelet aggregation inhibitor **9**,[19] were prepared on the solid phase using classical Fmoc SPPS method (Scheme 3).[18] HPLC purities were comparable to those obtained with commercially available Rink amide AM-PS resin, demonstrating the viability of Rink amide PI resin **6b**. Unlike first generation Rink amide PS resins, the link between the handle and the PI is stable under acidic conditions as TFA/TIS/water cleavage yielded pure peptides. Satisfactory yields in dipeptides were obtained. On the other hand, yield in tetrapeptidamide **9** was much lower than expected, presumably because the resin physical behavior slowly deteriorated as the peptide grew. Indeed, filtration of the crude reaction mixture became more and more difficult. Clearly, resin behavior should be improved if it is meant for peptide synthesis.

Table 1. Compared resin-swelling capabilities (mL g^{-1})

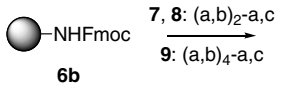
	Hexane	CH_2Cl_2	THF	DMF	MeOH	Water
Wang resin[a]	1.6[b]	5.4	6.0	5.2	1.6	1.6
PI **4a**	2.4	4.8	4.2	4.4	5.6	5.0
PI **4b**	2.5	4.9	4.3	4.3	2.0	1.8

[a] Hydroxymethyl PS.[17]
[b] Swelling ability in heptane.

Table 2. Acids and electrophiles scavenging with **4a**

Reagent	Amount of reagent	Solvent	Trapping conditions[a] (h)	Reagent remaining[b] (%)
H_2SO_4	0.2 mmol (0.5 N)	H_2O (pH = 0.3)	1	<1 (pH = 6)
HCl	0.4 mmol (0.5 N)	H_2O (pH = 0.3)	1	<1 (pH = 6)
TsOH	0.4 mmol	$CDCl_3$	2	<2
Boc_2O	0.4 mmol	$CDCl_3$	DMAP, 12	Not detected
AcCl	0.4 mmol	$CDCl_3$	4	<2 AcOH

[a] Trapping conditions: **4a** (150 mg, 4 equiv).
[b] The amount of acid left in aqueous solutions was determined by pH measurement. The proportion of acid or electrophile left after scavenging in organic solution was evaluated using pentamethylbenzene as an NMR internal standard.



H-Phe-Phe-NH_2.TFA	**7** (94%)
H-Leu-Phe-NH_2.TFA	**8** (91%)
H-Arg-Gly-Asp-Tyr(Me)-NH_2.2TFA	**9** (35%)

Scheme 3. Reagents and conditions: (a) Fmoc cleavage: piperidine/DMF; (b) Fmoc-amino acid grafting: Fmoc–AA–OH, HBTU, HOBt, DIEA, DMF and (c) peptide release: TFA/TIS/water.

In conclusion, we have depicted a simple one-step access to highly loaded, amphiphilic, reticulated polyimines. Such resins show very good potential as a scavenger reagent for acids and electrophiles. Further improvement of resin physical stability may lead to the development of PI as support for SPPS. Indeed, despite low linker grafting yield, preparation of Rink functionalized PI is quite straightforward and we demonstrated that such polyimines display the required chemical stability for peptide synthesis.

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References and notes

1. Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149–2154.
2. Delgado, M.; Janda, K. D. *Curr. Org. Chem.* **2002**, *6*, 1031–1043.
3. Bergbreiter, D. E. *Curr. Opin. Drug Discovery Dev.* **2001**, *4*, 736–744.
4. Vaino, A. R.; Janda, K. D. *J. Comb. Chem.* **2000**, *2*, 579–596.
5. Toy, P. H.; Reger, T. S.; Janda, K. D. *Aldrichim. Acta* **2000**, *33*, 87–93.
6. Bergbreiter, D. E. *Med. Res. Rev.* **1999**, *19*, 439–450.
7. See also: Badyal, J. P.; Cameron, A. M.; Cameron, N. R.; Coe, D. M.; Cox, R.; Davis, B. G.; Oates, L. J.; Oye, G.; Spanos, C.; Steel, P. G. *Chem. Commun.* **2004**, 1402–1403.
8. See also: Sasikumar, P. G.; Kumar, K. S.; Rajasekharan Pillai, V. N. *J. Pept. Res.* **2003**, *62*, 1–10.
9. See also: Spanka, C.; Clapham, B.; Janda, K. D. *J. Org. Chem.* **2002**, *67*, 3045–3050.
10. Eames, J.; Watkinson, M. *Eur. J. Org. Chem.* **2001**, 1213–1224.
11. Ley, S. V.; Baxendale, L. R.; Bream, R. N.; Jackson, P. S.; Leach, A. G.; Longbottom, D. A.; Nesi, M.; Scott, J. S.; Storer, R. L.; Taylor, S. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3815–4195.
12. Hodges, J. C. *Synlett* **1999**, 152–158.
13. See also: Ghanem, N.; Martinez, J.; Stien, D. *Eur. J. Org. Chem.* **2004**, 84–89.
14. See also: Ghanem, N.; Martinez, J.; Stien, D. *Tetrahedron Lett.* **2002**, *43*, 1693–1695.
15. Stien, D. WO2004103516, 2004, Fr. Patent 03 06102, 2003.
16. Rahali, H.; Ghanem, N.; Griffe, L.; Rahali, R.; Stien, D. *New J. Chem.* **2004**, *28*, 1344–1346.
17. Santini, R.; Griffith, M. C.; Qi, M. *Tetrahedron Lett.* **1998**, *39*, 8951–8954.
18. Chan, W. C.; White, P. D. In *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*; Chan, W. C., White, P. D., Eds.; Oxford University Press: Oxford, 2000; pp 41–76.
19. Adams, S. P.; Feigen, L. P.; Miyano, M. U.S. Patent 4,857,508, 1989; Ling, R.; Yoshida, M.; Mariano, P. S. *J. Org. Chem.* **1996**, *61*, 4439–4449.