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$EXPO_{3000}$—a new expandable polymer for synthesis and enzymatic assays

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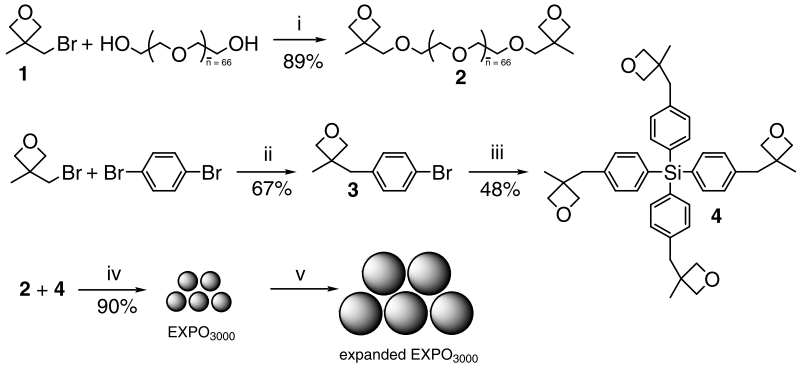
Abstract—A new polymer for synthesis and enzymatic assays is presented which combines moderate loading with the biocompatibility of poly(ethylene glycol)-based resins. The polymer displays low swelling in all solvents until selective cleavage of a silyl based crosslinker expands the polar resin to render it penetratable for enzymes (an example with a 27 kDa protease is given). An efficient alkylation procedure for derivatization of long PEG-chains is also presented. © 2002 Elsevier Science Ltd. All rights reserved.

New and specialized polymers for solid-phase synthesis continue to appear in the literature and they all present specific advantages, however, none of them meet all the requirements of compatibility for chemical synthesis, methods of analysis, enzymatic screening etc. The most commonly used polymer, polystyrene-divinylbenzene (PS-DVB), has good chemical resistance but is not compatible with aqueous solvents rendering it impossible to perform aqueous chemical and enzymatic reactions on polystyrene resins. PEG-grafted resins with a polystyrene core such as TentaGel are not fully compatible with enzymes because the core polymer consists of polystyrene with poly(ethylene glycol) tentacles.[1] However PEGA,[2] SPOCC[3] and HYDRA[4] resins are purely poly(ethylene glycol)-based resins with no aromatic core and have successfully been tested in solid-phase assays with many different proteases and receptors. Copolymerization of a bis-functionalized PEG_{3000} (**2**) and a highly functionalized crosslinker (**4**) gives $EXPO_{3000}$ (see Scheme 1). The crosslinker **4** was prepared by lithiation of 3-(4-bromo-benzyl)-3-methyloxetane (**3**) and subsequent reaction with tetrachlorosilane afforded **4** in 48% yield.

The physical properties of $EXPO_{3000}$ can be modified before, during or after synthesis to suit the properties of



Scheme 1. Preparation of $SPOCC_{3000}$ macro monomer (**2**), crosslinker (**4**) and $EXPO_{3000}$ resin. *Reagents and conditions*: (i) NaH, 40°C, DMF; (ii) *n*-BuLi, −78°C, THF; (iii) *n*-BuLi, $SiCl_4$, −78°C, THF; (iv) $BF_3{\cdot}OEt_2$, CH_2Cl_2; (v) TBAF/AcOH in THF or 10% CF_3SO_3H in CH_2Cl_2.

Keywords: expandable polymer; solid support; enzymatic assay; crosslinking; swelling.

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the polymer for either organic synthesis or enzymatic reactions. The new polymer displays several of the excellent properties of polystyrene resins such as low swelling and inertness towards many chemicals and has after expansion the polar and enzyme-compatible nature of PEGA and SPOCC resins. A cored dendrimer with a cleavable unit in the center has previously been described[5] but the effect on swelling was not mentioned.

Comparing the EXPO polymer with other resins, less solvent is needed to swell $EXPO_{3000}$ because the crosslinked polymer swells only a little, thus providing higher reagent concentrations and faster reactions. On the contrary, enzymatic assays require high swelling in water so enzymes can penetrate the polymeric network. The swelling of $EXPO_{3000}$ was measured in water, methanol, tetrahydrofuran, dichloromethane, *N*,*N*-dimethylformamide and toluene by the syringe method.[6] $EXPO_{3000}$ has low swelling (3–7 mL/g, see Fig. 1) in all solvents and can be expanded by selectively cleaving the crosslinking unit (**4**) within the polymer to increase swelling ~threefold.

The resin is expanded by strong acidic conditions or fluoride ions. This gives a higher swelling (10–20 mL/g) which render the resin permeable to enzymes thus enabling on-bead screening for substrates or inhibitors of proteases.

A substrate for Subtilisin Carlsberg was synthesized on the photolabile linker attached to $EXPO_{3000}$ (**5**). The activity of the enzyme towards the resin bound substrate was investigated before and after resin expansion (Scheme 2[†]). The resin bound peptides were cleaved off the resin and analyzed by HPLC. Enzyme incubation of the fully crosslinked resin led to no substrate cleavage due to the low swelling of the resin (Fig. 2, part B). Expansion of the resin with tetrabutylammonium fluoride (TBAF) allowed the protease to penetrate the resin and cleave the substrate (Fig. 2, part C) leading to a smaller peptide fragment (H-NPLAV-NH2, identified by co-injection on HPLC with a pure sample). This proves that Subtilisin Carlsberg (a 27 kDa protease) cannot enter the fully crosslinked resin but can easily cleave a substrate on the resin upon silyl–carbon bond cleavage.

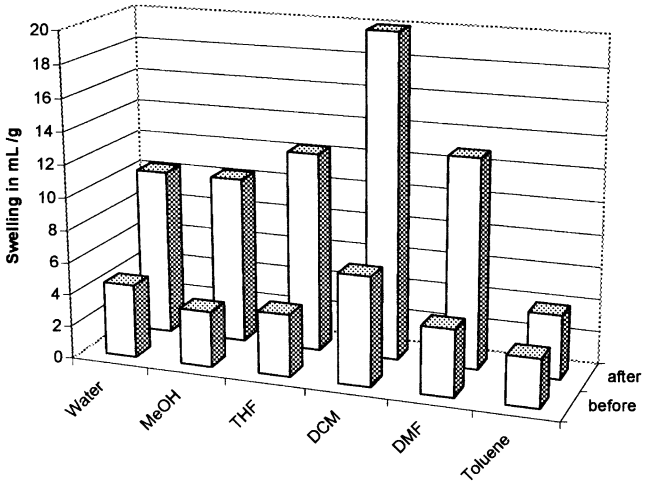
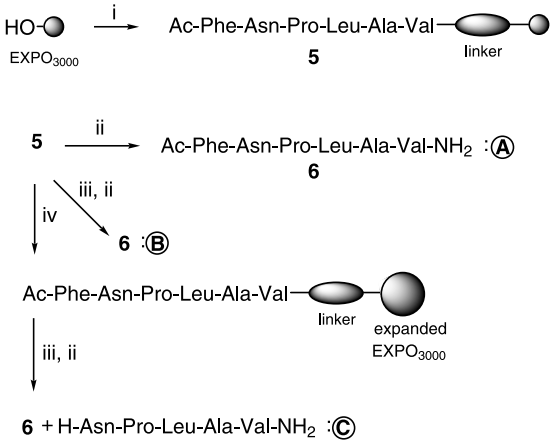


Figure 1. Swelling of $EXPO_{3000}$ before and after expansion.



Scheme 2. Synthesis of Subtilisin substrate and enzymatic assays. A, B and C refer to the HPLC traces in Fig. 2. *Reagents and conditions*: (i) SPPS; (ii) h*v*; (iii) Subtilisin Carlsberg (50 nM) for 30 min; (iv) TBAF/AcOH in THF.

An efficient alkylation of PEG-chains with 3-bromomethyl-3-methyl-oxetane[7] (**1**) is presented since previously reported procedures[8] were less successful on PEG_{3000} because the water content was difficult to control with the longer polyethylene glycols. Instead the PEG was dried under high vacuum at 100°C to remove all traces of water,[‡] treated with NaH in DMF and then the alkylbromide (**1**). This Williamson ether synthesis was a very clean reaction affording 89% of the product with 98% incorporation of oxetane groups. Remaining tosylate from the previously reported procedure[8] was thereby avoided.

Fully crosslinked $EXPO_{3000}$ was acylated with 2-(*N*-*tert*-butoxycarbonyl-amino)benzoic acid and studied by magic angle spinning NMR. The resin performed extremely well giving well resolved signals with very narrow line widths of the Abz resonances (2-aminobenzoic acid, Fig. 3) similar to other PEG-based resins.[8] The long and flexible tethers of the resin provided good resolution and even *meta* couplings in the aromatic ring could be detected.

[†] Enzymatic assay: expanded and fully crosslinked $EXPO_{3000}$ derivatized with the substrate were swollen in H_2O, washed with H_2O (3×) and buffer (50 mM bicine, 2 mM $CaCl_2$) (3×). They where incubated for 30 min with Subtilisin Carlsberg (50 nM) and then washed with H_2O, 2% TFA, H_2O, 2% $NaHCO_3$ and H_2O (3× each). An aliquot of each resin was cleaved by photolysis and the product subjected to HPLC analysis as presented in Fig. 2.

[‡] The dried PEG_{3000} contained approx. 50 ppm water compared to 850 ppm when used directly.

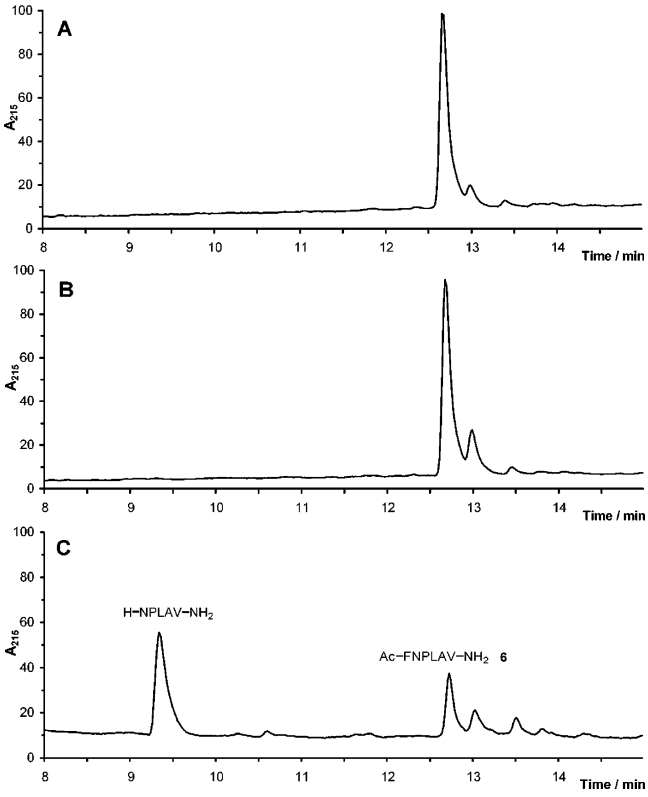


Figure 2. HPLC traces of cleavage products A, B and C from Scheme 2. **A**: substrate Ac-Phe-Asn-Pro-Leu-Ala-Val-NH_2; **B**: cleavage product from enzyme treatment of fully crosslinked resin (**5**); **C**: cleavage product from enzyme treatment of expanded resin.

The objective was to produce a low-swelling, expandable polar resin by copolymerization of a conventional bisfunctionalized long chain PEG with a short cleavable crosslinker containing silyl-carbon bonds. In summary, $EXPO_{3000}$ has excellent swelling properties for organic synthesis[§] and it combines low swelling with the good hydrophilic properties of PEGA and SPOCC resins. The polymer has a moderate loading of 0.4–0.6 mmol/g and after expansion it is suitable for enzymatic assays of chemical libraries.

[§] The aromatic residues of $EXPO_{3000}$ constitute only 4% measured by weight whereas TentaGel contains 30–50% polystyrene. Preparation of $EXPO_{3000}$: **2** (675 mg, 0.21 mmol) was dissolved in dry CH_3CN and concentrated in vacuo three times to remove water azeotropically. Then it was dissolved in CH_2Cl_2 and concentrated in vacuo again. To this was added the crosslinker **4** (29 mg, 0.043 mmol) and diluted with CH_2Cl_2 under argon to a final concentration of ~0.3 M of **2**. $BF_3{\cdot}Et_2O$ (16 μL, 0.13 mmol) was added by syringe and the reaction was stirred for 1 h at 25°C and then heated to 70°C. The sticky point for the polymer was reached at 40°C. It was cured 16 h at 70°C under argon. The clear resin was swollen in CH_2Cl_2 and grinded through a metal sieve (1 mm^2), washed with CH_2Cl_2, THF, DMF, H_2O, DMF, THF, CH_2Cl_2 (3× each) and lyophilized to give $EXPO_{3000}$ (633 mg, 90% yield). Loading was measured to 0.51 mmol/g resin.

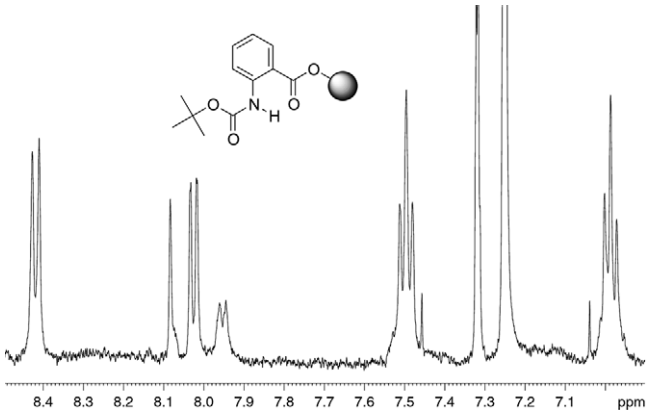


Figure 3. Magic angle spinning ^1H NMR spectrum (500 MHz) of aromatic region of Boc-Abz derivatized $EXPO_{3000}$ resin (fully crosslinked) in $CDCl_3$.

Acknowledgements

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References

1. Smith, H. K.; Bradley, M. *J. Comb. Chem.* **1999**, *1*, 326–332.
2. Meldal, M. *Tetrahedron Lett.* **1992**, *33*, 3077–3080.
3. Rademann, J.; Grøtli, M.; Meldal, M.; Bock, K. *J. Am. Chem. Soc.* **1999**, *121*, 5459–5466.
4. Groth, T.; Grøtli, M.; Lubell, W. D.; Miranda, L. P.; Meldal, M. *J. Chem. Soc., Perkin Trans. 1* **2000**, 4258–4264.
5. Schultz, L. G.; Zhao, Y.; Zimmerman, S. C. *Angew. Chem., Int. Ed. Engl.* **2001**, *40*, 1962–1966.
6. Auzanneau, F.-I.; Meldal, M.; Bock, K. *J. Pep. Sci.* **1995**, *1*, 31–44.
7. Blaskovich, M. A.; Evindar, G.; Rose, N. G. W.; Wilkinson, S.; Luo, Y.; Lajoie, G. A. *J. Org. Chem.* **1998**, *63*, 3631–3646.
8. Grøtli, M.; Gotfredsen, C. H.; Rademann, J.; Buchardt, J.; Clark, A. J.; Duus, J.Ø.; Meldal, M. *J. Comb. Chem.* **2000**, *2*, 108–119.