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Soluble polymer-supported convergent parallel library synthesis†

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Soluble polymer-supported convergent synthesis has for the first time been successfully exploited for parallel library synthesis; sub-libraries of tripeptide iodoarenes and arylboronic acids reacted smoothly in a multipolymer Pd^{II}-catalyzed Suzuki coupling reaction to generate a library of bisaryl-linked hexapeptides.

Polymer-supported and polymer-assisted synthetic methodologies are now ubiquitous throughout the fields of combinatorial chemistry, high-throughput- and pure organic-synthesis.[1] Solid-phase chemistry, that utilizes cross-linked polymer supports, is by far the most widely accepted approach within these new synthetic spheres.[2] However, limitations imposed by the heterogeneous nature of the chemical synthesis, have meant that alternative soluble polymer supports are being increasingly exploited.[3,4]

An inherent property of all solid-phase-based synthetic methods is a stepwise, linear synthesis that by necessity leads to a divergent introduction of diversity. This limitation not only applies to the linear synthesis of oligomers, such as peptides or oligonucleotides, but also to the sequential derivatization of templates. Linear divergent synthesis can be performed both on the solid-phase and in solution, however, the more powerful convergent approach to synthesis of diversity can only be conducted in solution because with solid-phase techniques the combining components would be on mutually exclusive resin beads. Boger[5] and co-workers were the first to bring into sharp focus the clear benefits of convergent combinatorial synthesis and they applied it using solution-phase chemical methods for the generation of diaminodiacetic acid diamide and bisaryl libraries. However, to date, the question as to whether convergent synthesis could be performed effectively on a soluble polymer support had not been addressed (Fig. 1).

Herein, we have merged the power of convergent synthesis with the synthetic benefits of polymer-supported chemistry (*vide supra*). We have generated a parallel array of bisaryl-linked hexapeptides **L4** (81 members) using monomethoxy poly(ethylene glycol) MeO-PEG as a soluble polymer support (Scheme 1). The key convergent diversity-expanding step involves a high-yielding, homogeneous, soluble polymer–polymer Suzuki cross-coupling reaction between sub-libraries of PEG-supported tripeptide aryliodides **L1a–i** (9 members) and PEG-supported tripeptide arylboronic acids **L2a–i** to give a PEG-supported bisaryl-linked hexapeptide library **L3** (81 members).‡

The choice of MeO-(PEG)-OH of 5000 average molecular weight as the polymer support was guided by our extensive experience with this material that we have utilized previously both as a support for combinatorial[6] and organic synthesis,[7] and as a reagent[8] and catalyst support.[9]

A bisaryl-linked hexapeptide library was selected to illustrate this first polymer-supported convergent strategy because of the well-known benefits of a polymer-supported *versus* solution-phase synthesis of peptide oligomers. From the outset, we defined constraints on the reaction chosen for the key convergent step, specifically: a) it must be a high-yielding reaction: b) it must be amenable to modification for yield optimization: c) there should be few side-reactions. For these reasons the homogeneous Pd^{II}-catalyzed variant of the Suzuki cross-coupling reaction between iodoarenes and aryl boronic acids was selected.[10] We assumed that the tripeptide component of the library would have little effect on the cross-coupling reaction, therefore optimization of the multipolymer Suzuki reaction was investigated with the PEG-supported *para*-iodobenzoate **5a**, and PEG-supported boronic acid derivative **5b** (Scheme 2).

Formation of the bisaryl-linked PEG-dimer **6** was routinely determined by ^{1}H NMR using a comparison of the ratios of the integrated peak areas of biphenyl protons (δ 7.86) and the protons attached to the methylene carbon alpha to the terminal oxygen of the PEG-polymer support (δ4.59). The product yield was further confirmed by cleavage of the 1,4-bisaryl ester from the MeO-PEG support by transesterification (KCN–MeOH) to the bis-methyl ester **7**.

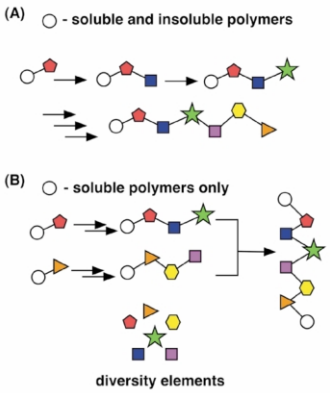
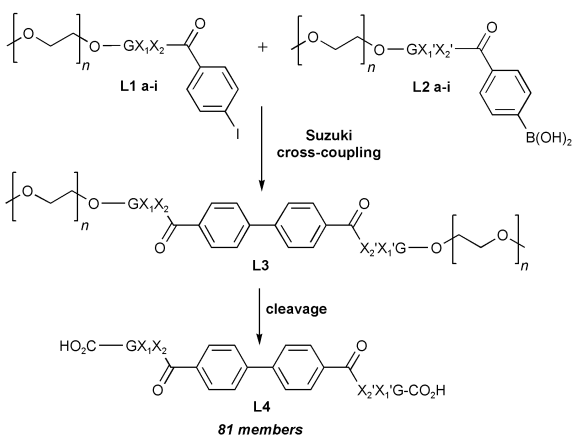


Fig. 1 Generalized scheme of polymer-supported linear *versus* convergent library synthesis. (**A**) Linear synthesis (**B**) convergent synthesis.



Scheme 1 Polymer-supported convergent approach to the bisaryl-linked hexapeptide library **L4** (81 member). G = glycine; X_1, X_2, X_1' and X_2' = Phe, Ala or Leu.

† Electronic supplementary information (ESI) available: detailed synthetic procedures for synthesis of **5b–7**, PEG-supported synthesis of **L1a--i** and **L2a–i**, purification of **L4**, ^{1}H-NMR spectra and LC/MS and HR-MALDIMS data of selected hexapeptide from **L4**. See http://www.rsc.org/suppdata/cc/b2/b210696e/

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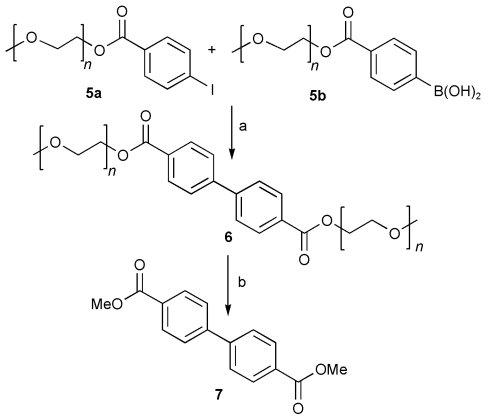

Scheme 2 Multipolymer approach to biaryl **7**. a) Conditions optimized Pd catalyst, base, solvent and temperature; b) KCN–MeOH.

Solvent effects did not appear to play a significant role in the polymer-polymer reaction as DMF, toluene, 1,4-dioxane and THF all gave similar yields (Table 1, entry 1–4). However, substantial improvements in the yields of **6** were observed when inorganic bases were used instead of Et_3N. Several inorganic bases, including K_2CO_3, Cs_2CO_3, KF, and K_3PO_4 were screened, and with the exception of KF (Table 1, entry 5), provided yields of **6** between 80 and 90% with few by-products (Table 1, entry 6–10). In addition, the reactions were faster with inorganic bases, being complete within 24 h using K_2CO_3, whereas up to 72 h was required with Et_3N at the same temperature (100 °C).

Three catalytic palladium sources were investigated, $PdCl_2$(dppf), $Pd(PPh_3)_4$, and $Pd(OAc)_2$. With K_2CO_3 as the base, no significant differences in yields were observed with the three palladium catalysts (Table 1, entry 8–10), therefore $PdCl_2$(dppf) was chosen due to its low air-sensitivity. The optimized reaction conditions ultimately comprised of MeO-PEG_{5000}-bound iodide **5a** and boronic acid **5b** (10 mM) with $PdCl_2$(dppf) (10 mol%) and K_2CO_3 (3 equiv.) in DMF at 100 °C for 24 h. These reaction conditions typically gave 95% yield of the PEG-supported biphenyl-containing product **6**, with negligible formation of byproducts.

MeO-PEG-bound tripeptides were prepared by standard N^{α}-Boc peptide chemistry. Each coupling reaction was monitored by the Kaiser ninhydrin test.[11] Each MeO-PEG-supported peptide coupling proceeded to >98% based on routine ^{1}H NMR analysis. The first amino acid coupled to MeO-PEG was fixed as Gly to minimize potential epimerization during the coupling reaction in the presence of DMAP. The second and third residues were randomized, either as Ala (A), Phe (F) or Leu (L). After tri-peptide synthesis, the PEG-supported tripeptides were split into two portions and the N^{α}-termini of the tripeptides were coupled with either 4-iodobenzoic acid or 4-carboxyphenylboronic acid, giving the PEG-supported iodide sub-library **L1a–i** (9 members) and PEG-supported boronic acid sub-library **L2a–i** (9 members).

The hexapeptide-library **L3** (81 members) was then prepared, in parallel, using the optimized polymer–polymer Suzuki cross-coupling reaction condition *vide supra*. ^{1}H NMR of selected members of **L3** confirmed that the reaction proceeded in high yield across the whole library. The **L3** hexapeptides were cleaved from the PEG support with aqueous LiOH. The resulting bis-acids **L4** were separated from the MeO-PEG by non-covalent attachment onto a weakly basic anion-exchange resin (IRA-67). The library of peptides **L4** was then released from the resin (1 M HCl in acetonitrile) and further purified by reversed-phase HPLC. The purities of all crude members of **L4** were determined by HPLC/MS and found to be 50–95%, with yields ranging from 72–95%. The structure of certain **L4** members was confirmed by ^{1}H-NMR spectroscopy and HR-MALDI MS.† The reduced purity and yield of certain library members of **L4**, constructed from **L1a–i** and **L2a–i** where $X_1X_2 \neq X_1'X_2'$, was a result of their containing a byproduct in small amounts (7–24%). This byproduct is the bisaryl-linked hexapeptide arising from a competing homocoupling of **L1a–i**, that gives a X_1X_2-X_2X_1–containing hexapeptide in addition to the required X_1X_2-$X_2'X_1'$-containing hexapeptide from the heterodimeric coupling. This byproduct was routinely separated by HPLC.§

In summary, we have demonstrated the first successful merging of polymer-supported chemistry with convergent parallel synthesis and illustrated it by producing an 81 member bisaryl-linked hexapeptide library **L4**. This methodology highlights the unique applicability of soluble polymers as supports in parallel library synthesis and should have universal applicability within the spheres of combinatorial chemistry and high-throughput synthesis.

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Table 1 Optimization of the polymer–polymer Suzuki cross-coupling reaction between **5a** and **5b**

Entry	Catalyst	Base	Solvent	Yield (%)
1	$PdCl_2$(dppf)	Et_3N	THF	60
2	$PdCl_2$(dppf)	Et_3N	Toluene	50
3	$PdCl_2$(dppf)	Et_3N	1,4-Dioxane	40
4	$PdCl_2$(dppf)	Et_3N	DMF	60
5	$PdCl_2$(dppf)	KF	DMF	55
6	$PdCl_2$(dppf)	Cs_2CO_3	DMF	85
7	$PdCl_2$(dppf)	K_3PO_4	DMF	80
8	$PdCl_2$(dppf)	K_2CO_3	DMF	90
9	$Pd(PPh_3)_4$	K_2CO_3	DMF	85
10	$Pd(OAc)_2$	K_2CO_3	DMF	90

Notes and references

‡ *Experimental*: MeO-PEG_{5000}-supported tripeptide iodide library **L1a–i** (20 mg, 4 μmol; 10 mM), MeO-PEG_{5000}-supported tripeptide boronic acid **L2a–i** (20 mg, 4 μmol; 10 mM), K_2CO_3 (1.6 mg, 12 μmol), $PdCl_2$(dppf) (0.33 mg, 10 mol%) and degassed DMF (0.4 mL) were added to thick-walled glass vials. The vials were sealed under Ar and the reaction mixture was stirred at 100 °C for 24 h. The reaction mixture was then cooled to room temperature and added into cold ether (10 mL). The precipitated PEG-bound hexapeptides **L3** were separated by centrifugation, washed with cold ether (10 mL) and dried under reduced pressure.

§ While the homodimer was separated by LC to increase the purity of each member of the **L4** array, it should be noted that the byproduct is also a member of **L4**. Therefore, given that we know its constitution and the amount present there is no need for it to be removed.

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