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Regio-Reactive Resin: A Platform for Orthogonal Loading Using the Polymer Backbone and Cross-Linker

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Abstract—A new support for solid-phase combinatorial organic synthesis has been developed, which we term a regio-reactive resin (R^3-resin). The resin is based on a unique hydroxyl-functionalized cross-linker readily synthesized in two steps starting from 4-hydroxybenzaldehyde. The cross-linker's ease of synthesis and high purity enables the preparation of gel-type resins with regio-reactive orthogonal loading sites. The resin's swelling properties were investigated, and its potential utility was demonstrated via orthogonal reactivity of the pendant and cross-linker sites.

Solid-phase organic synthesis (SPOS) has been proven to be valuable for the preparation of a variety of compounds, especially in the field of combinatorial chemistry. While the development of both synthetic and analytical solid-phase techniques has occurred at an increasing rate, it is surprising that few new polymeric supports specifically designed for use in SPOS have been reported.[1] Typically, the solid supports used are lightly cross-linked divinyl benzene–polystyrene beads (e.g., Merrifield resin) or polyethylene glycol (PEG) grafted polystyrene beads (e.g., Tentagel),[2] both of which were developed for solid-phase peptide synthesis and subsequently adapted for SPOS. Recently, our laboratory reported the development of a new polystyrene resin designed specifically for use in SPOS which contains a tetrahydrofuran-derived cross-linker (Fig. 1).[3] Now commercially available under the trade name *J*anda*J*el™,[4] this resin has been shown to have superior swelling properties relative to traditional Merrifield resin due to the more 'organic solvent-like' interior and increased flexibility in the cross-linker. The uses of *J*anda*J*els™ have been demonstrated in both library synthesis[5] and for the preparation of solid-supported catalysts.[6]

Solid supports are composed of three major components: the polymer backbone (e.g., styrene), a cross-linker (e.g., divinylbenzene) and a functional monomer (e.g., 4-vinylbenzyl chloride for Merrifield resin). Thus far, this approach to polystyrene bead design has only allowed for attachment of molecules to the support at the pendant functional groups. To our knowledge, reports using a cross-linker with a reactive functional group have focused on site-isolation studies or incorporation of a catalyst containing polymerizable termini as the cross-linker.[7] We reasoned the cross-linker could be used as an additional site for attachment of molecules since the enhanced swelling properties of *J*anda*J*els™ would allow greatly increased interactions between the cross-linker and reagents. Herein, we report the synthesis of a novel regio-reactive resin, which we term 'R^3-resin', containing a secondary alcohol on the cross-linker and investigate its properties as a platform for orthogonal loading.

R^3-resin **1** was synthesized in three steps as shown in Scheme 1. Starting with 4-hydroxybenzaldehyde, reaction with epichlorohydrin in the presence of sodium hydroxide gave bis-aldehyde **2**,[8] which was then treated

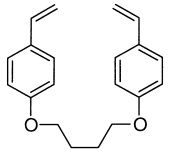

Figure 1. *J*anda*J*el™ Cross-linker.

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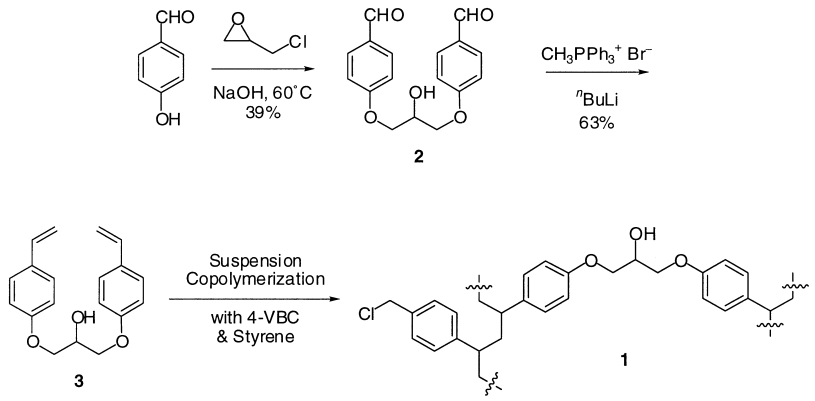


Scheme 1. Synthesis of R^3-resin **1**.

with methyltriphenylphosphonium bromide and *n*-butyl lithium to provide the distyryl cross-linking molecule **3** in 25% yield over two steps.[9] Although the overall yield is modest, the simplicity, ease of purification and low cost of the starting materials allow multi-gram quantities of the cross-linker to be synthesized rapidly. Suspension copolymerization[1b] of the cross-linker with styrene and 4-vinylbenzyl chloride (4-VBC) yielded R^3-resin **1**. This resin contains two orthogonal functional groups, a pendant benzyl chloride functionality on the polymer backbone (ca. 1.0 mmol/g) and a secondary alcohol on the cross-linker (ca. 0.17 mmol/g).

It has been shown that increased resin swelling leads to greater site accessibility.[10] The swelling of R^3-resin **1** is shown in Table 1 and is compared to commercially available Merrifield resin (2% cross-linked) and *J*anda*J*el^{TM}–Cl (2% cross-linked). This data agrees with observations previously made in this laboratory that more flexible cross-linkers impart a more 'organic solvent-like' interior to the resin and display a significant increase in swelling properties over conventional divinylbenzene cross-linked resins.

To show that the R^3-resin **1** sites could be reacted chemoselectively, a protected amino acid was loaded at the hydroxyl sites, followed by loading of an orthogonally protected amino acid onto the pendant benzyl chlorides. The hydroxyl loading and the chloride loading could then be independently determined from deprotection of the orthogonal protecting groups. The first attempt to selectively load an Fmoc-protecting amino acid onto the cross-linked site via a preformed symmetrical anhydride (from Fmoc-glycine and EDC) was not successful. Addition to both the cross-link as well as the pendant benzyl chloride was evidenced in the IR spectrum of the Fmoc-loaded beads by the presence of two new carbonyl peaks (1751 and 1724 cm^{-1}). However, the cross-link could be selectively functionalized with the pentafluorophenyl ester (OPfp) of Fmoc-glycine (Fig. 2) to give Fmoc-protected resin **4**.[11] In the IR spectrum of resin **4**, only one new carbonyl peak was observed (1724 cm^{-1}) and there was no apparent decrease in the intensity of the benzyl chloride stretch relative to the starting resin.

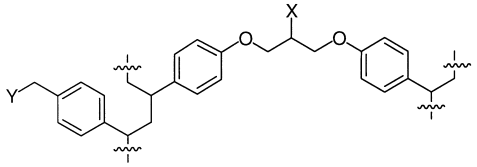
The cross-linked hydroxyl group loading was calculated to be 0.10 mmol/g (theoretical loading: 0.17 mmol/g) by removal of the Fmoc protecting group (20% piperidine/DMF) and quantitation of the UV absorbance of the dibenzofulvene–piperidine adduct at 290 nm. Fmoc-protected resin **4** was then treated with the cesium salt of Boc-glycine in order to acylate the pendant benzyl chloride sites and generate resin **5**.[12] The Boc protecting group was then removed (50% TFA/CH_2Cl_2) and a quantitative ninhydrin test[13] was performed to obtain the number of free amino groups, and therefore, number of chloride sites (1.0 mmol/g) on the resin.

To discount the possibility of *trans*-esterification during the addition of Boc-glycine, two different experiments were performed. First, the hydroxyl loading of Boc-deprotected resin **6** was determined by Fmoc deprotection as described previously (0.10 mmol/g). The loading

Table 1. Volumes of swollen resins (mL/g)[a]

Solvent	Merrifield Resin[3]	*J*anda*J*el^{TM}[3]	**1**
Dioxane	6.0	14.8	11.4
THF	6.4	14.0	9.8
DMF	4.8	10.4	7.4
Benzene	6.6	14.6	8.8
CH_2Cl_2	6.0	15.0	10.6

[a]Volumes were measured in syringes equipped with a sintered frit after equilibrating for 1 h.[10] Water, acetonitrile, and ethanol were also tested, but no significant swelling was observed. All resins studied were 2% cross-linked.



4: X = FmocHN–CH_2–CO_2–; Y = Cl–
5: X = FmocHN–CH_2–CO_2–; Y = BocHN–CH_2–CO_2–
6: X = FmocHN–CH_2–CO_2–; Y = H_2N–CH_2–CO_2–

Figure 2. Orthogonally protected R^3-resins.

experiments were then performed in the opposite sequence, that is, loading of Boc-glycine onto the benzyl chloride sites followed by loading of Fmoc-glycine onto the hydroxyl sites, and the assays repeated. Identical loadings were found for both the chloride (1.0 mmol/g) and hydroxyl (0.10 mmol/g) sites. Since *trans*-esterification is only possible in the first set of loading experiments, and the determined loadings from both experiments agree, it is apparent that this side reaction is not occurring and the loadings are accurate.

In summary, we have synthesized a unique cross-linker containing a secondary alcohol and this was used to prepare a novel regio-reactive resin (R^3-resin) possessing two orthogonal loading sites. We envision that this resin will be useful in SPOS applications, including encoding and tagging strategies, novel sensors, or the preparation of high-loading solid supports.

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8. Synthesis of **2**: 4-Hydroxybenzaldehyde (20 g, 163.8 mmol) was added to a solution of sodium hydroxide (6.55 g, 163.8 mmol) in water (300 mL). The solution was warmed to 60 °C and epichlorohydrin (5.27 mL, 81.9 mmol) was added slowly over 2 h. The reaction was then allowed to stir an additional 3 h at 60 °C, at which time the precipitate was filtered, washed with water, and air dried. The crude product was recrystallized from methanol/water (1:1) to yield 9.16 g (39%) of the desired product as yellow needles. Mp 142.5–143 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.91 (s, 2H), 7.85 (d, J = 9 Hz, 4H), 7.05 (d, J = 9 Hz, 4H), 4.47 (t, J = 4.8 Hz, 1H), 4.27 (m, 4H), 2.56 (broad s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 190.73, 163.23, 131.99, 130.41, 114.81, 68.87, 68.38. HRMS (MALDI-FTMS) m/z calcd for $[M+Na]^+$ 323.089, found 323.0888.

9. Synthesis of **3**: To a solution of methyltriphenylphosphonium bromide (7.17 g, 20.1 mmol) in THF (60 mL) at −78 °C was added *n*-butyl lithium (1.6 M in hexane, 12.6 mL) and the resulting ylide allowed to warm to room temperature. Bisaldehyde **2** (1.9 g, 6.69 mmol) in THF (50 mL) was added slowly to the ylide and the reaction was stirred for 12 h at room temperature. The mixture was poured into 30 mL of water and the organic layer separated. The aqueous layer was extracted with diethyl ether (2×30 mL) and organic layers combined. The combined layers were then washed with brine (4×25 mL), dried over $MgSO_4$, and concentrated. Purification by flash chromatography on silica (94:6 methylene chloride/diethyl ether) gave the desired compound **3** (1.25 g, 63%) as a white solid. Mp 118–118.5 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (d, J = 6.7 Hz, 4H), 6.90 (d, J = 6.7 Hz, 4H), 6.66 (dd, J = 10.6 Hz, 17.5 Hz, 2H), 5.61 (d, J = 17.53 Hz, 2H), 5.14 (d, J = 10.6 Hz, 2H), 4.14–4.17 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.16, 136.07, 131.07, 127.45, 114.58, 111.97, 68.78. HRMS (MALDI-FTMS) m/z calcd for $[M+H]^+$ 297.1485, found 297.1480.

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