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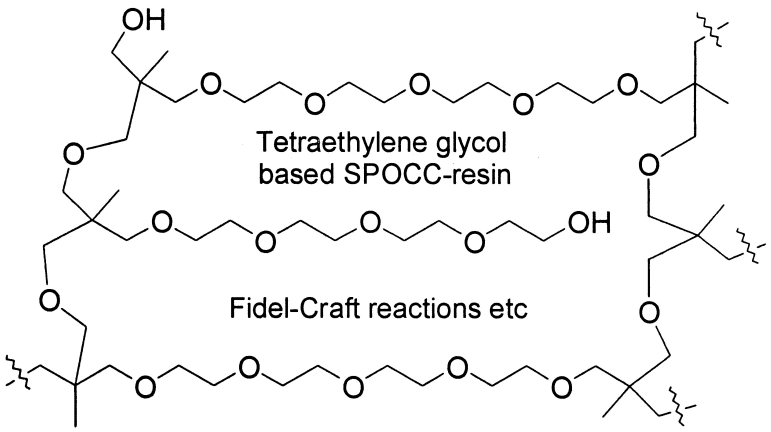

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SPOCC-194, a New High Functional Group Density PEG-Based Resin for Solid-Phase Organic Synthesis

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A novel polymer matrix for solid-phase synthesis, $SPOCC_{194}$ resin (**1**), was designed featuring a backbone of homogeneous tetraethylene glycol (TEG_{194}) macromonomer linked by quaternary carbon junctions and terminating in primary alcohol functionality. Beaded $SPOCC_{194}$ resin was effectively prepared by suspension polymerization of oxetanylated TEG macromonomer **5** in stirred silicon oil. Mechanically stable and inert to a diverse range of reaction conditions, $SPOCC_{194}$ possessed a high hydroxyl group loading (0.9−1.2 mmol/g) for substrate attachment and swelled effectively (∼2−4 mL/g) in a variety of organic and aqueous solvents. Developed for solid-phase synthesis at high reactant concentrations for driving organic and aqueous reactions to completion, $SPOCC_{194}$ exhibited high functional group density (mmol/mL) similar to that of low-loaded aminomethylated polystyrene−divinylbenzene copolymer (PS−1%DVB) yet significantly higher than that of $PEGA_{1900}$, $SPOCC_{1500}$, and TentaGel S. High-resolution MAS NMR spectra of Fmoc-derivatized $SPOCC_{194}$ indicate that monitoring of functional group transformation is possible. Moreover, by employment of a nonaromatic resin−linker combination, electrophilic chemistry, such as Lewis acid catalyzed glycosylation and Friedel−Crafts acylation, was selectively performed on substrate bound to $SPOCC_{194}$ resin. Such properties make $SPOCC_{194}$ resin a promising new polymer matrix for the support-bound construction of small organic molecules by parallel and combinatorial synthesis and the scavenging of solution-phase reactants or byproducts.

Introduction

The success of solid-phase chemistry is critically dependent on the chemical composition and physical properties of the polymer matrix.[1] Although the development of a universal support that possesses features ideally suited for all applications is unlikely, many polymers have proven to be effective for particular uses.[2] For example, polystyrene−divinylbenzene (PS−DVB)[3] has been widely used for solid-phase peptide synthesis (SPPS) and has more recently demonstrated utility for the polymer-supported preparation of particular organic molecules.[4] When prepared properly,[5] PS−DVB supports display excellent properties for chemical synthesis such as high loading, reasonable swelling in organic solvents, and physical stability. Drawbacks restricting the use of PS−DVB supports include poor compatiblity with aqueous solutions and polar solvents, a polymer matrix that is reactive under electrophilic chemical conditions, and relatively poor qualities for on-bead magic angle spinning (MAS) NMR analysis.[6] The aromatic nature of PS−DVB particularly limits its employment in the modification of support-bound substrate under common solution-phase chemistry, such as the Friedel−Crafts acylation[7] and related electrophilic reactions.[8]

A long-standing goal has thus been the development of a resin that alleviates the shortcomings of PS−DVB. With the objective of tailoring resins for the solid-phase synthesis of low molecular weight druglike molecules and peptide mimics, we and other groups have steadily arrived at several innovations that have promoted the application of the poly(ethylene glycol) (PEG) based resins for such purposes.[9] The PEG-grafted resin, TentaGel S,[10] and the PEG-cross-linked resins, such as PEGA,[11] POE−POP,[12] Janda-Jel,[13a] CLEAR,[13b] and SPOCC,[14] are aqueous compatible, and TentaGel, PEGA, POE−POP, and SPOCC resins are also generally suitable for MAS NMR analysis.[6] Furthermore, PEGA supports have proven to be useful for enzymology studies, such as the screening of libraries of peptide and peptide-based substrates and inhibitors.[16] Comparing these resins, we have found that superpermeable organic combinatorial chemistry (SPOCC)[14] resin is the most robust under various reaction conditions because it does not contain amide bonds, ester bonds, or polystyrene, which are important constituents in PEGA, CLEAR, TentaGel S, and JandaJel. Composed of primary ether bonds, quaternary carbon junction points, and primary

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alcohol functionality, SPOCC resins are the most chemically and physically stable PEG-cross-linked resins reported to date.

Originally intended for use in both organic synthesis and solid-phase bioassaying, SPOCC polymers were initially designed to have a balance of chemical and physical properties for both applications. Although SPOCC could be effectively used in peptide synthesis as well as for some organic chemistry such as Wittig and Horner−Wadsworth−Emmons-type reactions,[14] prior to the on-bead assaying of resin-bound substrate, its high swelling capacity and moderate loading restricted the use of SPOCC in concentration-sensitive chemistry. A comparison between resin loading and swelling in DCM illustrates the low concentrations of active sites available when contemporary PEG-based resins are used in their ideal swelling volumes: TentaGel S (0.03 mmol/mL), CLEAR (0.04 mmol/mL), JandaJel (0.09 mmol/mL), $PEGA_{1900}$ (0.015 mmol/mL), and $SPOCC_{1500}$ (0.025 mmol/mL). Considerable reactant dilution is necessary to enable resin solvation with these PEG-based resins. This poses a limitation when expensive reagents and valuable intermediates must be employed in high excess as solution-phase reactants. Because high reactant concentrations are desirable for driving solid-phase reactions to completion, higher-loaded/lower-swelling resins such as PS−DVB are still generally preferred for solid-phase organic synthesis when reactions can be carried out in compatible organic solvents.

Ideally, a PEG-based polymer for organic synthesis would possess a high-loading yet would swell well in small volumes of organic and aqueous solvents. Such a polymer would bead effectively to provide resin of homogeneous size and shape such that chemistry may be performed uniformly on support-bound substrate. Also, at the molecular level, the resin should also be chemically pure and ideally homogeneous in terms of macromonomer chain length.

In an attempt to meet these criteria, $SPOCC_{194}$ was developed for general use in solid-phase organic chemistry. This resin is derived from a well-defined tetraethylene glycol (TEG_{194}) macromonomer, which has previously been used as a spacer on PS−DVB,[15] and maintains the chemical inertness and physical stability of SPOCC resins prepared with longer-chain macromonomer mixtures; however, $SPOCC_{194}$ possesses an order-of-magnitude higher loading capacity to swelling volume ratio (0.3 mmol/mL in DCM). Furthermore, the preparation of beaded $SPOCC_{194}$ resin proceeds more quickly by controlled suspension polymerization in stirred silicon oil. The merits of $SPOCC_{194}$ have been validated by performing peptide and selective electrophilic chemistry, such as glycosylation and Friedel−Crafts acylation, on support-bound substrate.

Results and Discussion

Preparation and Properties of $SPOCC_{194}$. A cross-linked tetraethylene glycol (TEG_{194}) polymer, $SPOCC_{194}$ (**1**), was prepared according to an improved procedure derived from the reported method for SPOCC resin synthesis with longer PEG macromonomers[14] (Figure 1). Instead of a Boltzman-like distribution of PEG chain lengths, as used for the preparation of $SPOCC_{400}$ and $SPOCC_{1500}$ resins, homogeneous TEG (**4**) was employed to synthesize macromonomers of defined composition, as characterized by NMR spectroscopy. Oxetane moieties were attached to the TEG chains and were cross-linked to furnish a resin possessing only primary hydroxyl groups. Because toluenesulfonate and methanesulfonate oxetane derivatives left residues that complicated resin purification,[14] alkylation of the macromonomer was performed with oxetane bromide **3** after deprotonation of TEG with sodium hydride in THF. Bromide **3** was synthesized from treatment of 3-hydroxymethyl-3-methyloxetane **2** with triphenylphosphine and bromine in DCM and was purified by vacuum distillation.[17] Typically, mono- and bisoxetanylated TEG were produced as a mixture with 75% incorporation of the oxetane moiety. The remaining hydroxyl groups were then capped with acetic anhydride and pyridine in DCM. After aqueous workup, the TEG oxetane macromonomers were purified and decolorized by continuous extraction into hexane.

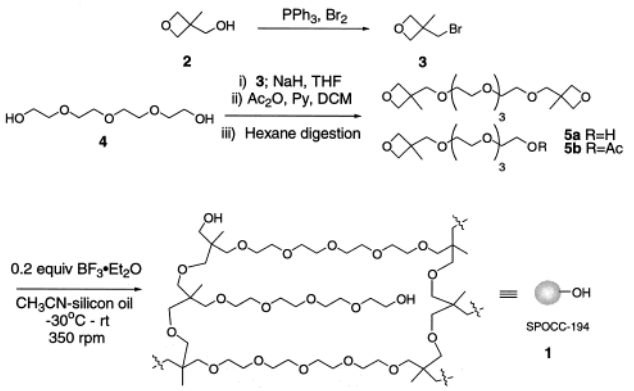

Figure 1. Synthesis of $SPOCC_{194}$ resin.

Beaded SPOCC resin was prepared by $BF_3{\cdot}OEt_2$-catalyzed cationic ring-opening suspension polymerization of the precooled TEG-oxetanylated macromonomer in silicon oil at room temperature. In contrast to the beading of high molecular weight PEG macromonomers, which required the use of surfactants for suspension polymerization in silicon oil,[18] $SPOCC_{194}$ polymerization proceeded rapidly and gave beaded resin without any additives. After overnight curing, acetate saponification, and thorough washing to remove silicon oil, spherical $SPOCC_{194}$ beads were obtained that had uniform spherical shape and a white to slightly off-white color (Figure 2). The bead size was controlled by adjustment of the polymerization stirring rate, and the size distribution could be further narrowed by a sieving process.

The $SPOCC_{194}$ resin was inert to a range of extreme conditions, including 12 N HCl, neat TFA, *n*-butyllithium in THF, sodium in liquid ammonia, and heating in thionyl chloride at reflux. The hydroxyl (OH) group loading of $SPOCC_{194}$ was typically measured in the range 0.9−1.2 mmol/g. Easy to weigh out and transfer, the resin swelled in a range of solvents with a typical volume of about 2−4 mL/g as determined by the syringe method[19] (Figure 3A). In water, $SPOCC_{194}$ swelled to a slightly lesser extent than in polar organic solvents. The swelling of $SPOCC_{194}$ in DCM (∼4 mL/g) was comparable with low-loaded aminomethylpolystyrene (AMPS−1% DVB, 6 mL/g) yet was

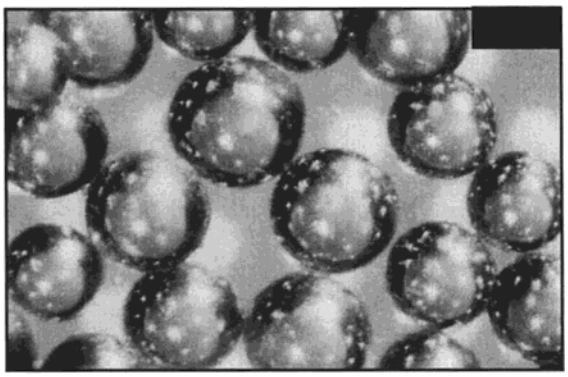

Figure 2. Microscope image of beads of $SPOCC_{194}$ obtained by suspension polymerization in silicon oil at room temperature after sieving.

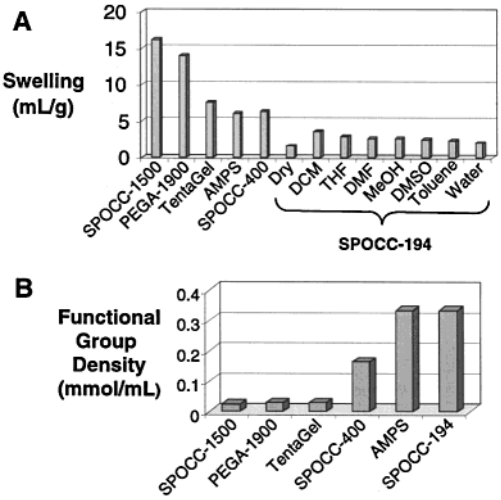

Figure 3. Comparison of AMPS, $SPOCC_{194}$, $SPOCC_{400}$, $SPOCC_{1500}$, $PEGA_{1900}$, and TentaGel S resins: (A) swelling estimates in DCM, unless otherwise indicated; (B) expression of functional group density in mmol/mL DCM. $SPOCC_{400}$ swelling data were generated from granulated resin prepared by bulk polymerization.

considerably lower than that of $SPOCC_{1500}$, and $PEGA_{1900}$ (16 mL/g and 14 mL/g, respectively).

The relationship between resin swelling and loading in terms of mmole per volume (mmol/mL) is useful for developing optimimal reaction parameters. From a comparison of the ratio of loading to swelling volume, $SPOCC_{194}$ exhibited a functional group density similar to that of AMPS in DCM (~0.3 mmol/mL) yet possessed approximately an order-of-magnitude more functional groups per resin swelling volume than TentaGel S (0.03 mmol/mL DCM), CLEAR (0.04 mmol/mL DCM), $PEGA_{1900}$ (0.015 mmol/mL DCM), and $SPOCC_{1500}$ (0.025 mmol/mL DCM). The functional group density of $SPOCC_{400}$ was approximately half that of $SPOCC_{194}$.

Acylation Yields on $SPOCC_{194}$, $SPOCC_{1500}$, TentaGel S, and AMPS. Relative reaction rates on typical preparations of $SPOCC_{194}$, $SPOCC_{1500}$, TentaGel S, and AMPS resins were compared using the model peptide coupling reaction of Boc-Val-OSu with resin-bound Ile-Phe in a minimal amount of DMF to permit solvation (coverage) of the beads. Because the average bead diameter of each resin varied with their commercial specifications, this comparison did not reflect intrinsic differences in reagent diffusion and reactivity;

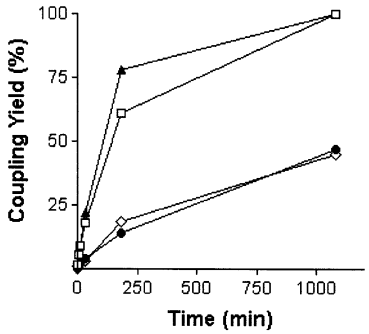

Figure 4. Comparison of reaction rates on AMPS (filled triangles), $SPOCC_{194}$ (open squares), $SPOCC_{1500}$ (open diamonds), and TentaGel S (filled circles). An amount of 1.5 equiv of Boc-Val-OSu was dissolved in a minimal amount of DMF to permit coverage of the beads. Shown are time courses of the reactions at 1, 5, 10, 30, 180, and 1080 min intervals, and the incorporation of valine was determined by amino acid analysis (AAA).

however, by measurement of reaction conversion (valine incorporation) at 1, 5, 10, 30, 180, and 1080 min using amino acid analysis of peptide−resin samples, the relative reactivity was found to relate to the resin functional group density (Figure 4). When large excesses and high reactant concentration (50-fold excess of Fmoc-Val-O-Pfp at 0.45 M in DMF) were employed, all of the resins yielded quantitatively the resin-bound tripeptide, Val-Ile-Phe, after 30 min. On the other hand, when 1.5 equiv of Boc-Val-OSu was employed, the reactant concentration was influenced significantly by resin swelling: $SPOCC_{194}$ (0.39 M), AMPS (0.41 M), $SPOCC_{1500}$ (0.048 M), and TentaGel S (0.043 M). The coupling reaction proceeded more quickly with AMPS and $SPOCC_{194}$, which exhibited respectively 99.9% and 99.7% completion after 1080 min. In contrast, the coupling reaction proceeded more slowly on $SPOCC_{1500}$ and TentaGel S, which both showed only 45% completion after 1080 min. These differences reflect the importance of high resin functional group density (mmol/mL) for rapid reaction conversion with a limiting reagent.

MAS NMR Properties of $SPOCC_{194}$. The analysis of solid-supported compounds with conventional NMR probe techniques generally leads to broad resonances due to sample heterogeneity. Although high-resolution magic angle spinning (HR-MAS) NMR spectroscopy can provide sharper resonances,[21] spectral quality is contingent on the polymer matrix. Resins that provide greater mobility of bound compound generally yield ^1H NMR signals with narrower line widths,[6,20a] keeping in mind that narrow NMR resonances can only be generated if both the resin-bound compound and the resin itself are well solvated. The HR-MAS NMR spectra measured at different spinning rates using Fmoc-derivatized $SPOCC_{194}$, TentaGel S, and AMPS were compared by evaluating the multiplet splitting of the Fmoc aromatic resonances. The Fmoc aromatic resonances are ideally split into two doublets and two triplets, as was observed in spectra of Tentagel−Fmoc, independent of spinning speed (4000−10000 Hz). For the $SPOCC_{194}$ resin, these resonances appear as relatively sharp singlets, whereas for the AMPS−Fmoc, these singlets are broader and overlapped with the aromatic styrene resonances. For the AMPS−Fmoc, broader resonances were observed and remained relatively unchanged

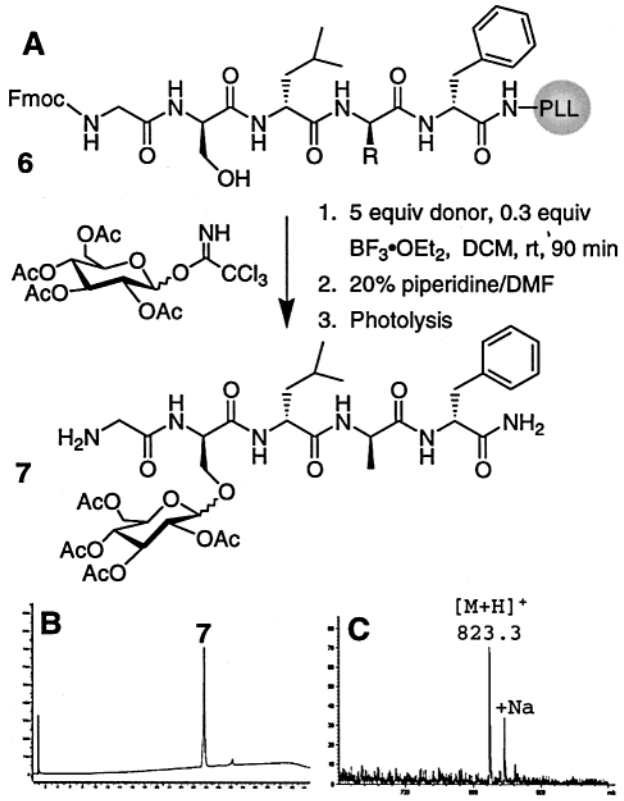

Figure 5. (A) Solid-phase glycosylation of a $SPOCC_{194}$-bound peptide. PLL indicates photolabile linker. (B) Reversed-phase HPLC analysis of the crude product. (C) Electrospray mass spectrum of the crude product.

as spinning speed was varied. For the $SPOCC_{194}$, an effect on the peak height is seen between spectra acquired with a spinning rate of 4000 and 6000 Hz, with 6000 Hz giving similar peak height for all four resonances. The observed increase in peak height with an increase in spinning speed may be attributed to MAS, which is known to reduce dipolar interactions at higher spinning speeds. As expected, the data obtained show the spectra quality to be in the order TentaGel S > $SPOCC_{194}$ > AMPS. Nevertheless, the quality of the spectra obtained with $SPOCC_{194}$ indicated that in most cases functional groups may be analyzed using HR-MAS NMR spectroscopy.

Chemistry on $SPOCC_{194}$: Solid-Phase Glycosylation. Initially, $SPOCC_{194}$ resin was used to synthesize glycopeptide **7**, which involved the reaction of a peptide hydroxyl group with a glycosyloxycarbenium ion.[21] The Fmoc−OPfp-3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (Dhbt−OH) chemistry was used to assemble N^{α}-Fmoc-protected GS(OH)-LAF pentapeptide[22a] on $SPOCC_{194}$ derivatized with a photolabile linker (4-{4-[1-(9*H*-fluoren-9-ylmethoxycarbonylamino)ethyl]-2-methoxy-5-nitrophenoxy}butanoic).[22b] The unprotected serine hydroxyl group was readily glycosylated using 2,3,4,6-tetra-*O*-acetyl-a-D-glucopyranosyl trichloroacetimidate as a donor and $BF_3 \cdot Et_2O$ as a Lewis acid catalyst at room temperature for 90 min in anhydrous DCM (Figure 5A).[21] After glycosylation, the Fmoc group was removed with 50% piperidine in DMF and the glycopeptide was cleaved from the solid support by UV irradiation. Examination of crude cleaved material by reversed-phase HPLC showed a major product that demonstrated the expected mass

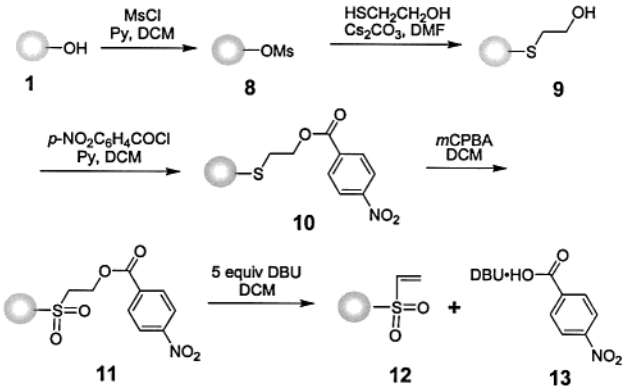

Figure 6. β-Eliminating sulfone safety-catch linker system on $SPOCC_{194}$.

spectral analyses for glucosylated peptide **7** by ES-MS and MALDI-TOF (parts B and C of Figure 5).

Friedel−Crafts Acylation of Resin-Bound Substrates. To further demonstrate the utility of $SPOCC_{194}$, we examined a chemical reaction that would be incompatible or impossible on contemporary polystyrene-based supports, such as AMPS and TentaGel S. The Friedel−Crafts acylation of a resin-bound aromatic substrate using aluminum trichloride was selected because the aromatic nature of polystyrene-based resins precludes the use of such chemistry for modification of bound substrate.[23] The sulfone-based safety-catch β-elimination system was examined as a nonaromatic linker,[24] which was attached by thiolysis of permethanesulfonated $SPOCC_{194}$ with β-mercaptoethanol in DMF (Figure 6). Acylation with *p*-nitrobenzoyl chloride and pyridine in DCM gave resin-bound ester **10**, which was shown to be removable after oxidation of the thioether to the sulfone with excess *m*-chloroperbenzoic acid in DCM at room temperature followed by β-elimination with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). This protocol provided the DBU salt of *p*-nitrobenzoic acid (**13**) in 65% isolated yield based on the initial loading estimate. Because the ester linkage was susceptible to premature cleavage with nucleophiles and strong acids, we examined next the β-elimination of phenolic ether derivatives from this linker after their acylation under the Friedel−Crafts conditions. On-bead FT-IR analysis indicated the formation of a keto moiety after Friedel−Crafts acylation of the phenolic ether; however, resin cleavage using the above strategy provided only trace amounts of the acylated phenol product as detected by ES-MS analysis of the released material. β-Elimination of a tertiary amine was next investigated for removing the product from the Friedel−Crafts acylation (Figure 7).[25] Permethanesulfonated $SPOCC_{194}$ safety-catch linker **14** was displaced with Boc-piperazine in DMF and oxidized to sulfoxide **15** using 30% peroxide. After Boc group removal with 95% TFA, the piperazine was alkylated with benzyl bromide. After sulfoxide oxidation to the sulfone with *m*CPBA in DCM, the amines were quaternized with methyl iodide[25] to give intermediate **16**. The resin was dried and then subjected to Friedel−Crafts acylation conditions with *p*-nitrobenzoyl chloride in the presence of $AlCl_3$ in nitrobenzene. Ketone **17** was obtained after elimination with DBU and validated the selective Friedel−Crafts chemistry.

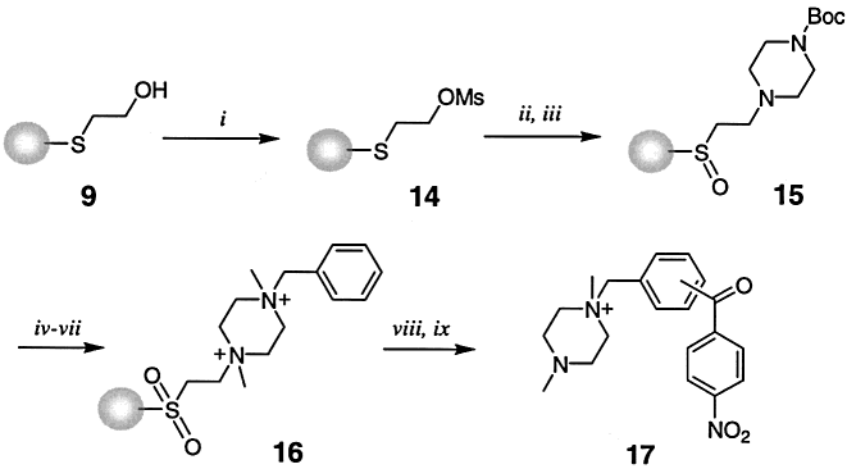

Figure 7. Friedel−Crafts acylation chemistry of $SPOCC_{194}$ using a sulfone-based safety-catch linker strategy for the release of tertiary amines. Conditions are the following: (i) 20 equiv of mesyl chloride, Py/DCM; (ii) Boc-piperazine, 10% TEA/DMF, 45 °C, overnight; (iii) 30% H_2O_2 and 5% acetic acid; (iv) TFA; (v) BzlBr, DBU, 45 °C, overnight; (vi) *m*CPBA/DCM (15 mg/mL), room temp, 2 h; (vii) CH_3I, DMA, 3 days; (viii) 5 equiv of *p*-NO_2−C_6H_4−COCl, 25 equiv of $AlCl_3$, anhydrous nitrobenzene, 8 h; (ix) 5 equiv of DBU/DMF, room temp, overnight.

Conclusion

$SPOCC_{194}$ resin (**1**), a beaded, chemically inert polymer matrix, has been prepared for application in solid-phase organic synthesis. By employment of oxetanylated TEG macromonomers to synthesize **1**, a stable cross-linked PEG-based polar resin was constructed that exhibited a high loading/swelling ratio. Because functional group pseudo-dilution was minimized, higher reaction yields were observed when employing a limited amount of reagent, relative to those observed with PEG-based resins that exhibited low loading/swelling ratios such as $SPOCC_{1500}$ and TentaGel S. The MAS NMR spectral quality of $SPOCC_{194}$ indicated that in most cases it should be possible to analyze functional groups directly on-bead. By employment of a nonaromatic β-elimination safety-catch linker, $AlCl_3$-catalyzed Friedel−Crafts acylation was selectively performed on substrate attached to $SPOCC_{194}$ resin. In comparisons with contemporary resins, $SPOCC_{194}$ resin exhibited superior qualities for solid-phase synthesis including facile beading, a high loading/swelling ratio, compatibility in organic and aqueous solvents, and inertness under electrophilic reaction conditions. In light of such properties, $SPOCC_{194}$ resin is a promising new polymer matrix for application in the support-bound construction of organic molecules by parallel synthesis and combinatorial techniques and for the scavenging of solution-phase reactants or byproducts.

Experimental Section

Preparation of $SPOCC_{194}$ Resin. 3-Methyl-3-(bromomethyl)oxetane (Oxetane Bromide, 2). This was prepared in one step from 3-(hydroxymethyl)-3-methyloxetane (**1**) using bromine and triphenylphsophine in DCM and purified by vacuum distillation (55 °C at 17 mbar) in 35% yield according to the previously reported procedure.[17] ^{1}H NMR (250 MHz, $CDCl_3$): δ 4.45 (q, J = 5.95 Hz, 4H), 3.65 (s, 2H), 1.44 (s, 3H).

Bis[(3-methyloxetan-3-yl)methyl]tetraethylene Glycol (5). Tetraethylene glycol (**3**, TEG, 5.0 g, 25.7 mmol) was dried by azeotropic removal of water from concentration of 50 mL volumes of acetonitrile and toluene on a rotary evaporator and then stored for 3 days in vacuo over fresh P_2O_5. The colorless oil was diluted with 20 mL of anhydrous 1:1 DMF/THF, treated slowly with NaH (103 mmol, 400 mol %, a 60% suspension in mineral oil that was removed by washing with dry hexane), stirred vigorously at 37 °C for 3 h under nitrogen, treated over 3 min with oxetane bromide **2** (400 mol %, 103 mmol), and stirred at 37 °C overnight. After cooling to ambient temperature, the reaction mixture was treated carefully with 3 mL of water, stirred for 15 min, and concentrated in vacuo. The residue was digested into 50 mL of DCM, stirred, filtered, and concentrated in vacuo. The oil was diluted with DCM (200 mL) and washed with 5% citric acid and brine (2 × 50 mL). The aqueous phases were back-extracted with DCM (4 × 50 mL). The combined organic phases were dried with Na_2SO_4 and concentrated to an oil, which was digested with hexane (8 × 100 mL). The collected hexane digestions were concentrated in vacuo to furnish 7.3 g (78%) of a pale-yellow oil. ^{1}H NMR (250 MHz, $CDCl_3$): δ 4.50 (d, J = 5.65 Hz, 4H), 4.33 (d, J = 5.65 Hz, 4H), 3.64 (br m, 16H), 3.53 (br d, 4H), 1.30 (t, J = 2.51 Hz, 6H). ^{13}C NMR: δ 80.05, 76.52, 70.93, 70.65, 70.61, 70.48, 39.87, 21.30.

Acetylation of Oxetanylated Tetraethylene Glycol (5). A solution of bis[(3-methyloxetan-3-yl)methyl]tetraethylene glycol in 40 mL of 1:1 DCM/pyridine was treated with acetic anhydride (5 mL), stirred overnight at room temperature, and evaporated in vacuo to give the acetate. The degree of acetylation was quantified by comparing the integrations of the acetylmethyl singlet (2.06 ppm) and the oxetane methylene doublets (4.33 and 4.50 ppm) in the ^{1}H NMR spectrum. On measurement of the acetate constituent, the yield of oxetane incorporation was inferred to be in the range 75−80%.

Suspension Polymerization. A solution of acetylated macromonomer **5** (1 g, 2.76 mmol) in dry acetonitrile (1 mL) was cooled to −42 °C, treated with BF_3·Et_2O (97 mL, 0.773 mmol, 0.2 equiv), and then quickly added dropwise to a silicon oil bath (75 mL) at room temperature stirred at

450 rpm. Emulsification was allowed to occur, and polymerization continued with stirring at room temperature for 20 h. The slurry of $SPOCC_{194}$ beads was filtered onto a sintered glass filter and washed with 50 mL volumes of each of the following solutions: DCM, MeOH, 1:1 MeOH/DMF, DMF, THF, MeCN, and MeOH. Unreacted oxetane groups were ring-opened on heating the beads in 4 M HCl at reflux for 3 h. Acetate groups were cleaved on stirring the beads with 4 M NaOH at room temperature for 18 h. Oxetane ring opening and acetate hydrolysis were monitored by observing the disappearance of resonances at 4.3−5 and 2.1 ppm, respectively, in the ^{1}H MAS NMR spectrum of the resin. Beads were sieved between 106 and 212 mm to provide 0.76 g (76%) of $SPOCC_{194}$ resin as uniformly shaped and sized beads. This procedure was scaled up for the preparation of 5 g batches of $SPOCC_{194}$.

Solid-Phase Glycosylation. The $SPOCC_{194}$-bound pentapeptide **6**, N^a-Fmoc-Gly-Ser(OH)-Leu-Ala-Phe, was synthesized as described above. Following the last coupling reaction, the resin was thoroughly washed with DMF, THF, and DCM (8 × 5 mL/g resin), dried in vacuo over P_2O_5 overnight, and then stored at −20 °C. Glycosylation was performed in dry DCM under an argon atmosphere in a syringe fitted with a Teflon filter that allowed the addition of solvents and catalyst under an inert atmosphere. The pentapeptide resin **6** (19 mg, 21 mmol) and the 2,3,4,6-tetra-*O*-acetyl-a-D-glucopyranosyl trichloroacetimidate donor (105 mmol, 5 equiv) were both dried overnight under high vacuum in the reaction vessel. Dry DCM (200 mL) was injected into the syringe to swell the resin. After 30 min, $BF_3{\cdot}Et_2O$ (3.9 mL, 31.5 mmol; 0.3 equiv to 2,3,4,6-tetra-*O*-acetyl-a-D-glucopyranosyl trichloroacetimidate donor) was injected and the slurry was briefly mixed. After 90 min, suction was applied and the resin was washed with DCM (10 volumes). The above procedure was repeated. The Fmoc group was removed with 50% piperidine/DMF (2 × 15 min), and after washing with DMF (5 × 5 mL/g resin) and DCM (7 × 5 mL/g resin), the glycopeptide was cleaved by irradiation with a Hg UV lamp in 3% AcOH/MeOH for 2 h at room temperature. Glycopeptide **7** was analyzed by RP-HPLC (R_t = 28.3 min; purity estimate, 92%). Mass spectral analysis of the major peak showed the following. ESI-MS: 823.3 $[M_r + H]^+$, 845.3 + Na. MALDI-TOF MS: 844.68 (M_r + Na); calcd for $C_{37}H_{54}N_6O_{15}$, 822.36 Da (monoisotopic).

General Sulfone Safety-Catch Linker Preparation. After neutralization in 30% DIPEA in DMF, $SPOCC_{194}$ resin (0.1 mmol) was permethanesulfonated using 100 mol % of methanesulfonyl chloride and pyridine (0.5 mL) in dry DCM (1 mL) at room temperature (2 × 1 h). Resin **8** was washed with DCM (3 × 15 mL/g) and DMF (3 × 15 mL/g), then swollen in DMF (1 mL), treated with β-mercaptoethanol (0.5 mmol) and Cs_2CO_3 (0.5 mmol), and left at room temperature overnight. The resin was washed with 15 mL/g of the following solvents: DMF, H_2O, DMF, THF, and DCM. The resin was dried in vacuo to give $SPOCC_{194}-S-CH_2CH_2-OH$ resin **9**, which was stored at −20 °C.

Friedel−Crafts Acylation on $SPOCC_{194}$: Synthesis of 1,4-Dimethyl-1-[4-(4-nitrobenzoyl)benzyl]piperazin-1-ium, 17. $SPOCC_{194}-S-CH_2CH_2-OH$ resin (0.06 mmol) was prepared as described above. The resin was washed with DMF and DCM and treated twice with 20 equiv of mesyl chloride (1.2 mmol) in pyridine/DCM (1:1) at room temperature for 3 h. Following a DMF wash, the resin was treated with Boc-piperazine (1.2 mmol) in a 10% triethylamine/DMF solution at 45 °C. After 6 h, 5 equiv of DBU (0.3 mmol) was added and left overnight. The resin was washed extensively with DMF and MeOH. The linker thioether was oxidized to the sulfoxide by treatment with 30% H_2O_2 and 5% acetic acid.[29] The piperazine Boc group was then removed with neat TFA for 10 min. The presence of free secondary amines on the resin was clearly evident from the chloranil test. Alkylation of the piperazine was performed using a 10% solution of benzyl bromide in DMF with 20 equiv of DBU (0.3 mmol) added, and the mixture was left overnight at 45 °C. After a washing with DMF, the chloranil test for free secondary amine was negative. FT-IR: NO_2, ν 1529.1 cm^{-1}. The resin was then treated with methyl iodide (125 mL, 2 mmol) in dimethylacetamide (DMA) at 50 °C for 3 days.[30] Oxidation to the linker sulfoxide to the sulfone was performed by suspending the resin twice in *m*CPBA in DCM (15 mg/mL) at room temperature for 2 h. FT-IR: SO_2, ν 1267.9 cm^{-1}. The resin was washed thoroughly with DCM, DMF, MeOH, THF, MeOH, and DCM and was dried in vacuo over P_2O_5. For Friedel−Crafts acylation, aluminum trichloride ($AlCl_3$, 1.5 mmol) and recrystallized *p*-nitrobenzoyl chloride (0.3 mmol) in anhydrous nitrobenzene were cooled in an acetone dry-ice bath for 10 min under argon. The nitrobenzene solution was then added to the resin under argon and left to warm gradually to room temperature over 3 h. The resin was then heated to 30 °C for 5 h and then washed with CCl_4, DMF, MeOH, 2-propanol, water, MeOH, DCM, and chloroform. The product was cleaved from the resin with 3 equiv of DBU in DMF at room temperature overnight and was extracted from the beads with 50% acetonitrile/water and $(CD_3)_2SO$. Overall isolated yield after 11 steps and HPLC purification was 12%. Higher yields were not expected because of difficulties with on-resin piperazinium formation.[30] ^{1}H NMR (250 MHz, $CD_3CN-CD_3OD-(CD_3)_2SO$): δ 8.43 (d, J = 8.48 Hz, $2H_{ar}$), 8.03 (d, J = 8.79 Hz, $1H_{ar}$), 7.92 (d, J = 8.48 Hz, $2H_{ar}$), 7.80 (d, J = 8.79 Hz, $2H_{ar}$), 4.79 (s, 2H, CH_2), 3.98 (m, 4H, CH_2), 3.43−3.48 (m, 4H, CH_2), 3.08 (s, 3H, CH_3), 2.55 (s, 3H, CH_3). ^{13}C NMR: δ 194.557 (C=O), 153.73 (C_{ar}), 142.12 (C_{ar}), 137.73 (C_{ar}), 133.84 (C_{ar}), 132.23 (C_{ar}), 131.15 (C_{ar}), 130.37 (C_{ar}), 129.04 (C_{ar}), 124.03 (C_{ar}), 66.54 (CH_2), 58.83 (CH_2), 45.11 (CH_2), 48.2 (CH_3), 37.82 (CH_3). ESI-MS, M_r: 354.4; calcd for $C_{20}H_{24}N_3O_3$, 354.18 (monoisotopic).

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Supporting Information Available. Experimental details and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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