SPOCC: A Resin for Solid-Phase Organic Chemistry and Enzymatic Reactions on Solid Phase†

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Abstract: SPOCC resin **1**, a novel, highly permeable, polar support for chemical and enzymatic solid-phase methods, is presented. The synthesis of SPOCC resin is based on the cross-linking of long-chain poly(ethylene glycol) (PEG) terminally substituted with oxetane by cationic ring-opening polymerization, affording a polymer containing only primary ether and alcohol C−O bonds. The polymer was prepared using $Et_2O{\cdot}BF_3$ as initiator either via bulk polymerization in solution or via suspension polymerization in silicon oil, the latter yielding a beaded resin. The polymerization reaction was investigated with respect to the effects of PEG chain length, the fraction of bisoxetanylated PEG, initiator amount, and temperature in order to vary the swelling, loading, and mechanical stability of the resin. Furthermore, the resin was derivatized with various functional groups and subsequently applied to peptide synthesis and organic reactions in both organic solvents and water. An N-terminal peptide aldehyde was generated on the solid phase and employed to synthesize peptide isosteres by nucleophilic addition of various ylides. Solid-phase glycosylation of peptides and enzymatic reactions were successfully performed with SPOCC resin. Enzymatic proteolytic cleavage of a resin-bound decapeptide on treatment with the 27 kDa protease subtilisin BNP′ demonstrated the accessibility of the interior of the SPOCC resin for enzymes.

Introduction

Solid-phase organic chemistry has evolved rapidly during the past few years mainly due to the enormous potential of peptide[1,2] and non-peptide libraries[3] in medicinal chemistry and chemical biology. The success of solid-phase organic chemistry depends crucially on the properties of the solid support.[4,5] Resins are preferred which are chemically inert to a broad range of reaction conditions, mechanically stable, and applicable in many solvents of different polarity.[5] In particular, resins swelling in water are attractive for use in enzymatic reactions and on-bead enzymatic assays.[6] The resins presently used for solid-phase organic chemistry are constructed predominantly from cross-linked polystyrene as polymer.[7] Although grafting of the polystyrene core with polar linear polymers such as poly(ethylene glycol) (PEG) may improve the swelling in polar solvents,[8−10] the PEG-grafted resins have shown limitations regarding their use in aqueous solvents and for enzymatic chemistry.[11] In contrast, a resin constructed with PEG as macromonomer can be fully compatible with water as first demonstrated by the synthesis of PEGA resin using radical polymerization of acrylamide-substituted PEG.[12,13] Characterized by high swelling volumes in both nonpolar solvents and water, PEGA has been successfully applied in the synthesis of difficult peptide sequences[12] as well as solid-phase enzyme reactions.[14] The favorable swelling properties of PEGA and other resins constructed by the cross-linking of PEG chains can be attributed to the stretched helical superstructures adopted by PEG in aqueous solution.[15] However, many organic reactions are not compatible with PEGA resin because of its rich abundance of amide functionality. For example, solid-phase glycosylation was hampered by the presence of the amide groups in the solid support[16,17] which interacted with both the oxocarbenium ion intermediate from the carbohydrate donor and the Lewis acids employed for activation. Similarly, strong bases cannot be used with this type of resin because they may readily deprotonate the amide nitrogen. Consequently, a PEG resin was developed based on polyoxyethylene/polyoxypropylene copolymer (POEPOP), which contained only ether bonds,[18] and this has been successfully applied in the solid-phase organic synthesis of peptide isosteres.[19] POEPOP could be prepared effectively by anionic

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† SPOCC resin = superpermeable organic combinatorial chemistry resin.

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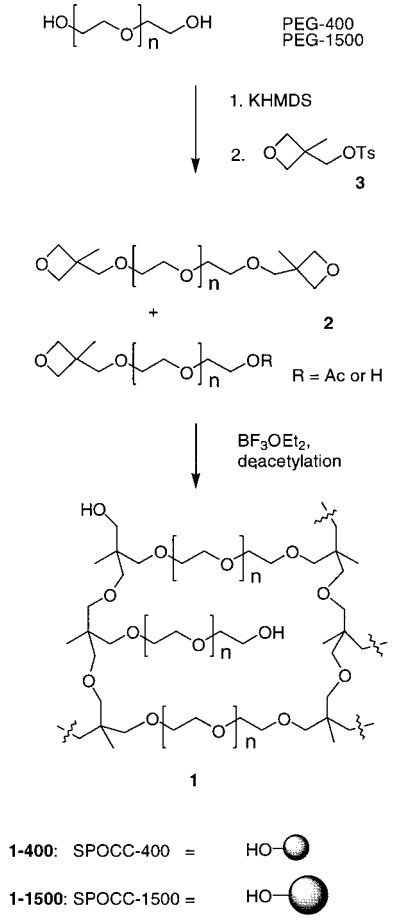
Scheme 1. Synthesis of SPOCC-400 and -1500 Resins **1**



polymerization of oxirane-derivatized PEG; however, this process produced a resin having functional groups comprised of an equal mixture of secondary and primary alcohols. A more homogeneous resin was preferred to ensure equal reactivity at all sites. Furthermore, POEPOP was chemically labile under extremely strong acidic or basic conditions due to the presence of secondary ether bonds formed during the polymerization process.

Results and Discussion

A novel cross-linked polymer was designed that contained exclusively primary ethers and alcohols: the SPOCC resin **1** (Scheme 1). The first resin synthesized by cationic ring-opening polymerization[20,21] for solid-phase organic chemistry, SPOCC resin **1** was prepared by Lewis acid-catalyzed polymerization of PEG chains of various length functionalized with four-membered oxetane rings. Oxetanes possess high reactivity for polymerization under acid catalysis combined with high stability toward basic and nucleophilic conditions.[22] Thus, basic conditions could be used for the alkylation reaction followed by acidic reaction conditions in the following polymerization step. High ring strain makes the oxetanes more reactive under cationic ring-opening polymerization than the corresponding five-membered tetrahydrofurans. On the other hand, oxetanes are less susceptible to nucleophilic ring-opening than the corresponding three-membered oxiranes.

Synthesis of PEG−Oxetane Macromonomers. Starting macromonomers for the polymerization were prepared by alkylation of PEG with the oxetane moiety. 3-Methyl-3-(alkoxymethyl)oxetanes have been reported to be favorable starting materials for cationic ring-opening polymerization because the dialkyl-substituted 3-position adjacent to the ring oxygen stabilizes the cationic intermediates formed during polymerization.[20,23] Furthermore, the presence of only secondary and quaternary carbon atoms in the polymerization product was expected to provide increased stability of the polymer. Therefore, 3-methyl-3-[[(4-tolylsulfonyl)oxy]methyl]oxetane (**3**)[24] was selected as the alkylating agent. This sterically demanding isopentyl building block cannot eliminate toluenesulfonic acid under the harsh, basic conditions required to alkylate PEG chains due to the absence of β-hydrogens. Macromonomers **2a** were synthesized from PEG chains of different lengths (PEG-400 and PEG-1500) to obtain resins with a wide range of swelling and loading properties. Macromonomers **2a** were metalated employing potassium bis(trimethylsilyl) amide (KHMDS). The hexamethyldisilazane (HMDS) formed in the metalation step was removed by coevaporation with DMF at elevated temperature. Alkylation was subsequently conducted with tosylate **3** at 75 °C. In the alkylation step potassium was a more favorable counterion than sodium, which gave a low yield of alkylated PEG monomer. Component **2a** was obtained with ≥95% conversion to bisoxetanylated PEG. The amount of free hydroxy groups after alkylation was assessed by acetylation using acetic anhydride/pyridine, yielding acetylated monomer **2b**, and subsequent comparison in the NMR spectra of the acetylated samples using the integration of the signals at 2.03, 4.31, and 4.47 ppm of acetyl CH_3 and ring CH_2 protons, respectively.

The Polymerization Reaction. Macromonomers (**2a,b**) were first polymerized in CH_2Cl_2 with boron trifluoride diethyl etherate ($Et_2O{\cdot}BF_3$) as a catalyst. Initially general conditions for cationic ring-opening polymerization of **2** were investigated. Previously, oxetanes had been polymerized at low temperature, using a minimum of Lewis acid catalyst in a nonpolar solvent.[20] Thus, reaction parameters such as temperature and the amount of Lewis acid were investigated to define the mildest conditions for effecting polymerization of macromonomers **2**. Acetylated macromonomer **2b**-1500 was dissolved in an equal volume of CH_2Cl_2 and treated with increasing amounts of $Et_2O{\cdot}BF_3$ at augmenting temperatures to determine the onset of polymerization. A temperature of at least +4 °C and 0.15 equiv of $Et_2O{\cdot}BF_3$ were found to be required for effecting polymerization. Subsequently, conditions were established to minimize the time needed for polymer formation as indicated by the inhibition of magnetic stirring. Shortest sticky times of less than 1 min were realized when 0.4−0.6 equiv of $Et_2O{\cdot}BF_3$ was used in diglyme (diethylene glycol dimethyl ether) at room temperature or at slightly elevated temperatures.

After optimal conditions were defined for the cross-linking polymerization of **2**, resins were synthesized for examination in solid-phase chemical and enzymatic reactions. The properties of the polymerization product could be varied by controlling the following parameters (Table 1): the PEG chain length, the amount and the type of Lewis acid catalyst added, the fraction of bisoxetanylated PEG, the presence of *O*-acetyl or hydroxy groups in **2**, and the temperature. After extensive curing, the bulk polymer was granulated, washed, and dried to yield resins **1**, which were characterized with respect to loading, swelling,

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Table 1. Influence of PEG Chain Length, Derivatization of Macromonomer, and Reaction Protocol on the Swelling of SPOCC Polymers

PEG length, Da	oxetane, %	chain protection −OR	reaction protocol	loading, mmol/g	swelling, mL/g		
					H_2O	DMF	CH_2Cl_2
400	>95	−H	A	0.6	2.2	3.9	5.2
400	>95	−Ac/−H	B	0.6	2.0	3.1	4.0
400	70	−Ac/−H	B	1.1	2.8	4.3	6.3
1500	>95	−H	A	0.4	8.4	8.5	12.4
1500	>95	−Ac/−H	C	0.4	5.1	5.4	7.9

and physical stability. As previously observed with POEPOP resin, the PEG chain length had a strong influence on the swelling of the SPOCC resin polymer. The PEG-1500-based resins attained much larger swelling volumes than PEG-400 resins. The amount of Lewis acid initiator exhibited a major influence on the resin properties. Polymers obtained with use of the minimum amount of $Et_2O{\cdot}BF_3$ (0.15 equiv) did not form physically stable resins; instead swelling and granulation gave a clear, translucent gel from which removal of solvent by filtration was problematic. When more than 0.7 equiv of Lewis acid was employed, initiation of polymerization was accompanied by an increase in viscosity; however, even after a prolonged reaction time, the resin never stabilized and remained in a highly viscous liquid state. Resins useful for synthesis were obtained by using Lewis acid in amounts ranging from 0.3 to 0.5 equiv. In the preferred method to yield a mechanically stable resin, Lewis acid was added at 0 °C and polymerization was initiated by warming to 25 °C and continued by a 48 h curing period at 50−70 °C. Furthermore, the loading of SPOCC resin **1** could be significantly enhanced by incomplete oxetanylation and subsequent acetylation of the starting monomers followed by polymerization and deacetylation (entry 5 in Table 1). At present, the maximum loading obtained by this method has been 1.2 mmol/g for SPOCC-400. In summary, SPOCC resin can be synthesized by variation of the reaction parameters to yield transparent resins with well-defined properties designed for different chemical and biochemical purposes.

Synthesis of Beaded SPOCC Resin. Suspension and dispersion polymerization methods for producing beads from acrylic monomers are well established.[25−27] These approaches offer a very attractive alternative to bulk polymerizations because they provide a means to produce beads of controlled and uniform size. Although water and highly polar organic solvents are usually used as the continuous phase for the polymerizations of hydrophobic monomers such as styrene, such solvents are incompatible with cationic ring-opening polymerization. An alternative approach was thus developed that avoided the use of dispersing agents which interfered with the cationic polymerization. This suspension polymerization technique employed emulsions of the monomers formed in silicon oil, which was immiscible with the PEG derivatives. The polymerization was carried out in acetonitrile to maintain the droplets floating in the silicon oil. Other solvents such as chloroform, dichloromethane, and tetrahydofuran did not yield droplets with optimal gravitational properties. The Lewis acid was added to the reaction mixture at −20 °C, and then the mixture was suspended in the silicon oil at room temperature. Attempts to add the Lewis acid directly to the emulsion resulted in bead aggregation. The size of the beads could be controlled by varying the rate of stirring during emulsion formation. Stirring at 200 rpm for 1 min appeared to be very efficient and afforded beads with good uniformity and reproducibility in the size range between 300 and 500 μm (Figure 1). Stirring at 2000 rpm yielded beads in the size range between 5 and 50 μm. Temperature influenced the outcome of the polymerization. Emulsion formation at 45 °C resulted in large amounts of aggregation, while at 0 °C hardly any polymerization occurred. When the silicon oil was maintained at 25 °C, the polymerization proceeded in a controlled manner to give the optimally beaded polymer.

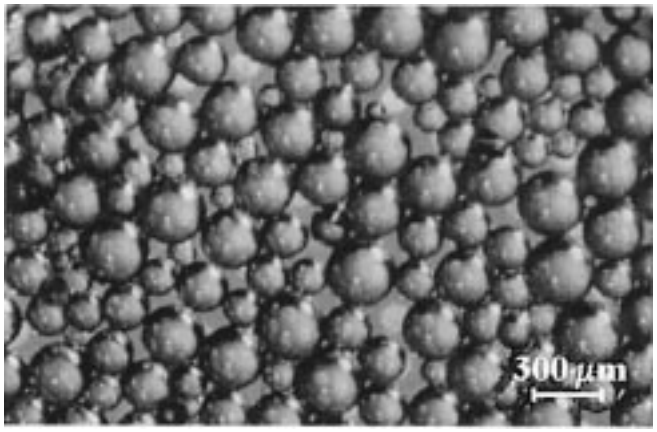

Figure 1. Beads of SPOCC-400 obtained by suspension polymerization.

Chemical Stability of the Resulting Polymer. Chemical stability was examined by exposing the resins to different reaction conditions and reaction times, and the results with SPOCC resin were compared with those of other resins used for solid-phase organic chemistry. No change of the SPOCC resin was observed under the reaction conditions 37% aqueous HCl (4 weeks), neat anhydrous hydrogen fluoride (45 min and 24 h), and BuLi (10 equiv in comparison to the determined resin loading), nor did heating the SPOCC resin with 20 equiv of thionyl chloride ($SOCl_2$) in toluene at reflux temperature for 72 h affect the resin stability. Under the same conditions the POEPOP resin was previously shown to dissolve after 10 min. Finally, SPOCC resin samples were exposed to 35% HBr in glacial acetic acid and to a mixture of 5% trimethylsilyl trifluoromethanesulfonate (TMSOTf) in 1:1 acetic anhydride/CH_2Cl_2, and the time for complete dissolution was measured and compared with values previously determined for the POEPS- and POEPOP resins. After 10 min POEPS was completely dissolved under both conditions. Similarly, POEPOP dissolved after 30 min in the HBr solution and after 3 min in the TMSOTf/Ac_2O solution. In contrast, SPOCC resin was much more stable and was dissolved by the HBr/HOAc and TMSOTf/Ac_2O solutions after 36 and 12 h, respectively. These results indicate that cleavage of the C−O ether bonds of the PEG chains in SPOCC resin occurs during extended treatment with these reagents.

Functionalization of the SPOCC Resin. After synthesis and workup the SPOCC resin was obtained in the form of a hydroxy-functionalized polymer. The conversion of the hydroxyl groups into amine and thiol functionalities was conducted to provide resins to be used for different types of chemistry. Initially, halogenated resin **4** was prepared by bromination of SPOCC resin **1** by a triphenylphoshine/imidazole/bromine system (Scheme 2. Three methods were then evaluated for introducing amino groups to yield **5**. Tritylamine and Hünigs base were reacted with the brominated resin **4** at 60 °C for 18 h to afford a resin with a loading of only 0.12 mmol/g as determined by analysis

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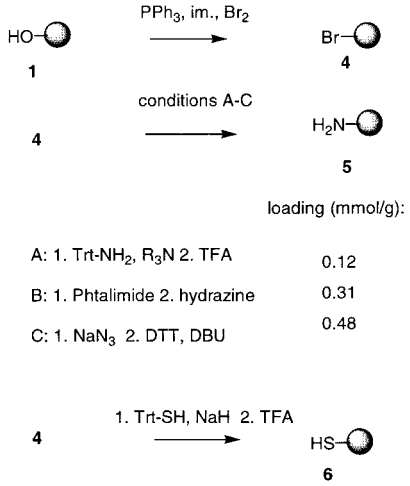
Scheme 2. Functionalization of SPOCC Resins **1**

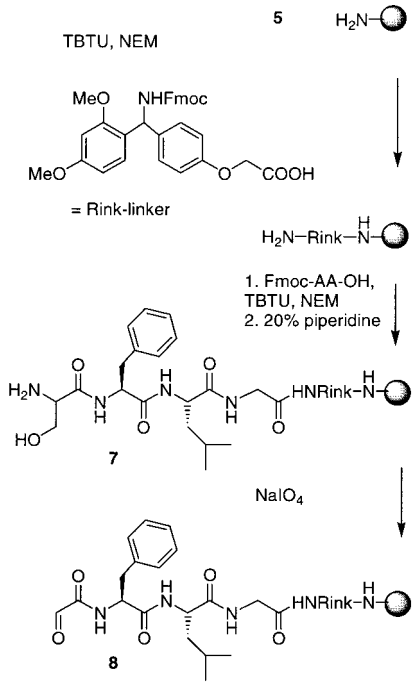


after acid-mediated trityl cleavage. Potassium phthalimide reacted with **4** at 60 °C for 18 h to yield an amine loading of 0.38 mmol/g after deprotection with hydrazine. The highest loading (0.45 mmol/g) was obtained by displacement bromine **4** with azide followed by reduction with dithiothreitol and DBU.[28]

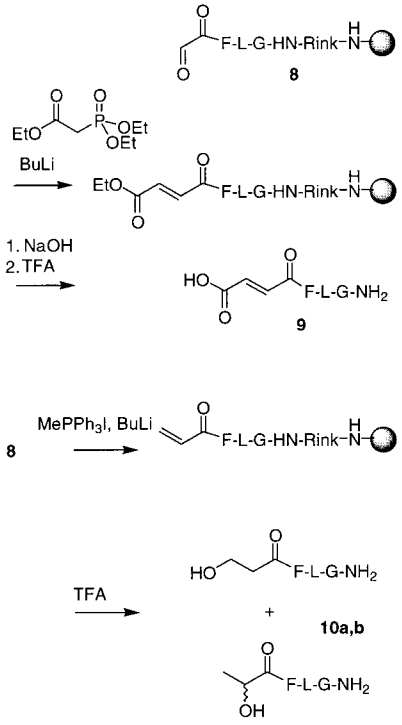
Thiol resin **6** was also prepared by the substitution of bromide **4** with trityl thiol in the presence of sodium hydride, followed by acidic trityl cleavage to yield a resin with a thiol loading of 0.53 mmol/g.

Chemical Synthesis on the SPOCC Resin. Organic synthesis and peptide chemistry were performed on resin **5** by using various linker strategies and reaction conditions to demonstrate the versatility of the SPOCC resin. Reactions of peptide aldehydes to introduce C−C bonds into the backbone as amide isosteres are of particular interest for generating potential protease inhibitors. This concept for the synthesis of peptide isosteres has been demonstrated recently on the POEPOP-400 resin.[19] However, the base-labile HMBA linker employed limited the use of strongly basic nucleophiles. To extend the scope, the acid-labile Rink linker was attached to SPOCC-400 resin **5** with a loading of 0.45 mmol/g by reaction with an Fmoc-protected Rink linker using *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU)[29] and *N*-ethylmorpholine (NEM) (Scheme 3). After deprotection of the linker, tetrapeptide **7** was assembled on the resin using Fmoc-amino acids and the same acylation conditions. The N-terminal serine residue was transformed into resin-bound peptide aldehyde **8** using periodate cleavage in water buffered with 0.01 M sodium phosphate at pH 7 to avoid cleavage of the acid-labile linker.[30] Aldehyde **8** was successfully reacted with ylides in Wittig-type and Horner−Wadsworth−Emmons-type reactions (Scheme 4). For example, triethyl phosphonoacetate deprotonated with less than 1 equiv of *n*-butyllithium added to the aldehyde resin to produce exclusively the trans isomer as determined by HPLC, MS, and NMR spectroscopy after treatment of the resin with 95% TFA/water to furnish compound **9**. The Wittig reaction between aldehyde **8** and the nonactivated methyltriphenylphosphonium iodide was performed as an example involving strongly basic conditions. The ylide was generated with less than 1 equiv of *n*-butyllithium at −78 °C to provide the characteristically

Scheme 3. Generation of N-Terminal Peptide Aldehyde **8** on the SPOCC Resin



Scheme 4. Nucleophilic Reactions on the SPOCC Resin



colored solution that was added to the resin at −40 °C. Cleavage with TFA/water yielded a 1:1 mixture of the 2- and 3-hydroxy products **10a** and **10b** resulting from hydration of the product acrylamido functionality.

In addition to the olefination chemistry to yield peptide isosteres, electrophilic reactions promoted by Lewis acids were investigated on the SPOCC resin. For example, glycopeptide **13** was prepared via solid-phase glycosylation[17] of the pentapeptide Fmoc-ASFLG-SPOCC resin (**11**) that was synthesized directly on the resin using the Fmoc-amino acids and TBTU/NEM conditions (Scheme 5). Serine was incorporated without side-chain protection. Tetra-*O*-acetylgalactopyranosyl trichlo-

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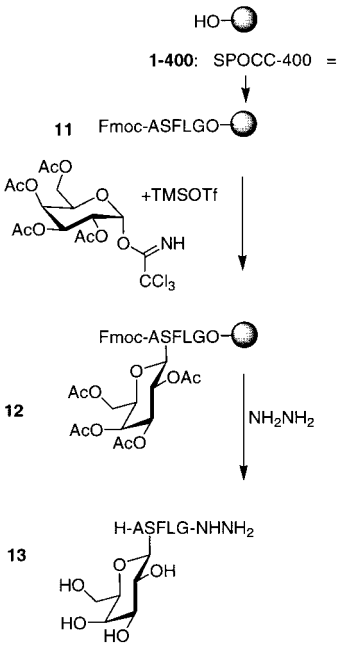
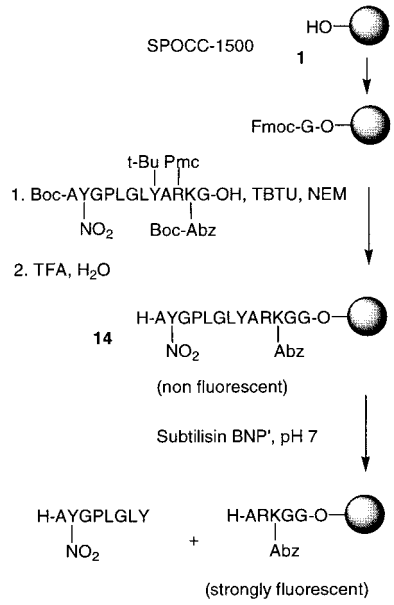
Scheme 5. Synthesis of Glycopeptides by Solid-Phase Glycosylation



roacetimidate[31] was added to the Fmoc-protected peptide resin and activated with TMSOTf to effect glycosylation. No linker was used, and the first amino acid, Fmoc-glycine, was directly attached to the resin by an ester bond. Therefore, after two cycles of glycosylation (each with 5 equiv) the glycopeptide was cleaved from resin **12** with hydrazine to afford galactopyranosyl peptide **13** as the C-terminal peptide hydrazide in good yield.

Enzyme Reactions with SPOCC-1500 Resin. Enzymatically catalyzed reactions on solid supports deserve particular interest because of their potential use in enzyme-assisted synthesis and on-bead screening of substrate and inhibitor libraries. The feasibility of enzyme reactions with SPOCC-1500 resin was examined with a decapeptide protease substrate that was synthesized on the solid support. Hydroxyl-functionalized SPOCC resin **1** was acylated with Fmoc-Gly-OH using 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT) and *N*-methylimidazole (MeIm) in CH_2Cl_2 for activation.[32] After deprotection of the amino group, a fully protected nonapeptide fragment (AY(3-NO_2)GPLGLYARK(Abz)G) was coupled to yield resin **14**. The 3-nitrotyrosine and N^{ϵ}-(2-aminobenzoyl)-lysine residues were incorporated to serve as a resonance energy transfer pair of chromophores for visual detection of substrate cleavage (Scheme 6).[33] The substrate containing resin **14** was treated with a solution of the 27 kDa protease subtilisin (0.1 μM in a 0.01 M pH 7 phosphate buffer). After 10 min, bright fluorescence was observed using a fluorescence microscope. Furthermore, the emission of light was uniform from the entire volume of the resin particles, indicating the reaction to be complete after a reaction time of 30 min. To elucidate the nature of the enzyme reaction, Edman degradation, peptide cleavage, and HPLC analysis were conducted and proved the digestion of the starting substrate to be complete with no detection of residual decapeptide. Residual fragments were cleaved off the resin and purified by HPLC. They were identified by Edman

Scheme 6. Enzymatic Reactions on SPOCC-1500 Resin: Cleavage of Decapeptide Substrate **14** by Proteases



degradation and MALDI-TOF-MS. The resin contained only the pentapeptide H-ARK(Abz)GG-OH.

On the other hand, the reaction of resin **14** bearing a substrate for the matrix metalloprotease MMP-9 (a considerably larger protease of 72 kDa) was not cleaved as shown by the absence of fluorescence and a complete recovery of the decapeptide after cleavage and analysis by HPLC−MS. The enzyme was highly active with the substrate in solution. Presumably, the enzymatic reaction on the solid phase was impeded by the resin excluding the larger protease, indicating the importance of the permeability of the polymer in solid-phase enzyme reactions. Furthermore, the size limit for enzyme reactions on the new SPOCC resins may be further expanded by the use of longer PEG chains as starting materials as recently demonstrated by the complete cleavage of the identical substrate with MMP-9 employing a PEGA-6000 resin (unpublished results).

Conclusions

A polar polymeric resin (SPOCC) equally suited for general organic chemistry and for enzyme reactions has been presented. Cationic ring-opening polymerization was demonstrated as the preferred method for SPOCC resin preparation. It enabled various parameters such as swelling, loading, and mechanical stability to be modified in the resulting polymer. Oxetanes served as the reactive moieties, allowing both macromonomer alkylation without formation of elimination byproducts and Lewis acid-catalyzed polymerization. Acetylation of residual hydroxy groups facilitated the polymerization. SPOCC resins possess only primary ether and alcohol functionalities. The resin is therefore inert to most reaction conditions required in organic chemistry, even to strongly acidic or basic conditions. SPOCC polymers proved to be susceptible only to PEG backbone decomposition under conditions effecting cleavage of primary ether bonds and after reaction times substantially longer than those destroying other PEG-based resins. Prepared as beaded polymers with hydroxyl, amino, and thiol functionalities, the novel resins were used for solid-phase peptide synthesis and for organic chemistry. The syntheses of glycopeptide derivatives and peptide isosteres were demonstrated. Furthermore, complete enzymatic digestion of a peptide substrate bound to the solid support was achieved. As indicated by these experiments, the

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novel resin should be suitable for the generation of peptide isostere libraries and for identification of new protease inhibitors on the solid support.

Experimental Section

General Procedures. All solvents were stored over molecular sieves. DMF was distilled under reduced pressure prior to use. Solid-phase peptide chemistry and solid-phase organic chemistry were performed in plastic syringes. Flat-bottom PE syringes were equipped with sintered Teflon filters (50 μm pores), Teflon tubing, and valves for applying suction to the syringes from below.[34] For moisture-sensitive and low-temperature reactions such as the Wittig olefination, commercially available reaction vials with septa and screw caps were employed (Reacti-Vial, Pierce, Rockford, IL). Resin loadings were determined by Fmoc cleavage and optical density measurement at 290 nm and were calculated employing a calibration curve. In the case of the starting hydroxyl resin the loading was measured after esterification with Fmoc-Gly using MSNT as condensing agent and MeIm as base.[32] Starting loadings were calculated from the measured loadings and the molar mass increase (ΔM) employing the formula $\text{loading}_{\text{start}} = \text{loading}_{\text{measd}}/(1 - \text{loading}_{\text{measd}}\Delta M/1000)$ and expressed in millimoles per gram (the division by 1000 is required since the molar mass increase is used as grams per mole).

Analysis of all solid-phase reactions was performed after product cleavage from a resin sample. When the Rink linker was used, a small portion of the resin (1−2 mg) was weighed in an Eppendorf tube and treated with TFA/water (95%, 50 μL, 1 h). The resin was dried in a centrifuge in vacuo for 15 min and resuspended in acetonitrile/water (1:1, 50 μL). A sample of this solution (10−20 μL) was examined on analytical HPLC (8 × 200 mm C-18 column, Millipore Delta PAK 15 μm) with detection at 215 and 280 nm using a photodiode array detector (Waters M 991). Eluents A (1% TFA in water) and B (10% of A with 1% TFA in acetonitrile) were used in a linear gradient (0% B → 100% B in 50 min). Preparative HPLC was performed using a 25 × 200 mm semipreparative RP-18 column (Millipore Delta Pak 15 μ), employing a linear gradient starting with 85% A and 15% B, a slope of 0.5% per min, and a flow rate of 10 mL/min. Collected fractions were analyzed by ES/MS (positive mode on a Fisons VG Quattro Instrument). Alternatively a MALDI-TOF mass spectrum (Finnigan MAT 2000) was recorded employing α-cyano-4-hydroxycinnamic acid or 1,4-dihydroxybenzoic acid as matrix. The calculated molecular weights of all products are the averaged molecular masses. Functionalized resins were investigated with MAS solid-phase NMR on a 3−5 mg sample placed using a nanoprobe tube using a Varian unity INOVA 500 MHz instrument and different deuterated solvents. Solution-phase NMR was performed on a Bruker DRX 250 MHz instrument or a Varian 500 MHz instrument. Chemical shifts were calibrated relative to the signal of tetramethylsilane (0 ppm) or internal solvent signals (3.30 and 36.067 ppm for d_4-MeOD, 2.49 ppm for d_6-DMSO).

Preparation of SPOCC Resins. (3-Methyloxetan-3-yl)methyl 4-Toluenesulfonate (3). 4-Toluenesulfonyl chloride (20 g, 105 mmol) was dissolved in CH_2Cl_2 (50 mL) and pyridine (50 mL). With cooling in an ice bath, 3-(hydroxymethyl)-3-methyloxetane (100 mmol, 9.9 mL) was added dropwise. The temperature was increased to 20 °C overnight, the solution was diluted with CH_2Cl_2 (100 mL), and the solvent was extracted with water. The organic phase was dried with magnesium sulfate and filtered, and the volatile solvents were removed by evaporation. The remaining material was concentrated several times with toluene to remove the residual pyridine and with chloroform to remove the toluene. The crude product was sufficiently pure for further use. Yield: 22 g of a white crystalline solid (92%). TLC: R_f (petroleum ether/ethyl acetate, 1:1) 0.56. The spectroscopic data were in accordance with those in the literature.[24]

Bis[(3-methyloxetan-3-yl)methyl]PEG (2a). Poly(ethylene glycol) (10 mmol; -400 or -1500) was dried carefully by removal of water through coevaporation with toluene and then dissolved in a 1:1 toluene/DMF solution (30 mL). With stirring, potassium hexamethyldisilazane (KHMDS; 22 mmol) was added at room temperature, and after 15 min, the volatiles including the HMDS were removed in a 50 °C water bath on a rotary evaporator. The residue was dissolved in DMF (15 mL), treated with portions of tosylated oxetane **3** (24 mmol) at room temperature, and heated for 12 h at 75 °C. After cooling to ambient temperature, water (2 mL) was added, and the mixture was stirred for 15 min to hydrolyze unreacted alkylating agent. The solvents were removed at 40 °C under reduced pressure. The remaining slurry was resuspended in CH_2Cl_2 (10 mL), filtered through a 2 cm layer of kieselguhr (Celite, prewetted with CH_2Cl_2 and compressed) on a fritted glass filter, and washed with CH_2Cl_2 (50 mL). Evaporation gave a yield of 90% of **2a**. NMR spectroscopy on the acetylated product indicated the alkylation of >95% of the PEG hydroxyl groups with oxetane rings; NMR spectroscopy on the product indicated less than 5% of remaining tosylate salts. The product obtained by the described filtration method was of higher purity than that obtained by a precipitation procedure. When less alkylating reagent **3** was employed (15 and 18 mmol), the percentage of oxetanyl groups decreased (66%, 80%).

Data for 2a-400. ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.28$ (s, 3 H, 3-Me), 3.52 (s, 2 H, 3-CH_2O), 3.62 (br s, 16 H, PEG-CH_2), 4.31 (d, $^2J = 5.7$ Hz, 2 H, 2-CH_2), 4.47 (d, $^2J = 5.7$ Hz, 2 H, 2-CH_2). ^{13}C NMR (MHz, $CDCl_3$): $\delta = 21.33$ (3-methyl), 70.5−70.95 (PEG-CH_2), 76.56 (3-CH_2O−), 80.12 (2-CH_2).

Data for 2a-1500. ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.28$ (s, 3 H, 3-Me), 3.52 (s, 2 H, 3-CH_2O), 3.62 (br s, 34 H, PEG-CH_2), 4.31 (d, $^2J = 5.7$ Hz, 2 H, 2-CH_2), 4.47 (d, $^2J = 5.7$ Hz, 2 H, 2-CH_2).

Acetylation of Mixtures of Mono- and Bisoxetanylated PEG (2b). Residue **2a** (10 g) was dissolved in pyridine (20 mL), treated with acetic anhydride (10 mL), and stirred at room temperature for 24 h. Solvents were removed under reduced pressure to give the product in a quantitative yield. The ^{1}H NMR spectrum of the product was recorded, and the degree of acetylation was quantified by using the integration of the signals at 2.03, 4.31, and 4.47 ppm of acetyl CH_3 and ring CH_2 protons, respectively.

Data for 2b-400. ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.28$ (s, 3 H, 3-Me), 2.03 (s, Ac), 3.52 (s, 2 H, 3-CH_2O), 3.62 (br s, 16 H, PEG-CH_2), 4.31 (d, $^2J = 5.7$ Hz, 2 H, 2-CH_2), 4.47 (d, $^2J = 5.7$ Hz, 2 H, 2-CH_2).

SPOCC Resin 1. Procedure A. Under argon, oxetanylated PEG-1500 or -400 (**2a**; 1−20 mmol) or acetylated derivative **2b** was dissolved in CH_2Cl_2 (equal volume), and the solution was cooled to −20 °C, stirred with a magnetic stirbar, and treated with boron trifluoride diethyl etherate (0.15−0.3 equiv). The reaction mixture was warmed gradually to determine at which temperature polymerization occurred (−10 °C, 2 h; 0 °C, 2 h; 4 °C, 2 h). Finally, the viscosity of the solution increased and magnetic stirring stopped (sticky point) after the solution was kept at 4 °C for 30 min. The polymer was kept at this temperature for 2 days and an additional day at room temperature. For workup the polymer was cut into pieces, swollen (CH_2Cl_2, 2 h), and then granulated through a metal sieve (1 mm pore size) employing a pestle. The granulated resin was washed thoroughly (CH_2Cl_2, THF, DMF, water, 3 M HCl, water, 0.5 M NaOH, water, DMF, THF, CH_2Cl_2) and dried in vacuo. Resin loading was determined as described in the General Procedures, and the swelling volumes in different solvents were determined following a literature procedure[35] (see Table 1). In the case of partially acetylated macromonomers (**2b**) the resin was treated with 0.5 M NaOH overnight; nanoprobe MAS-NMR of the dried resin proved the disappearance of any acetyl signals in the ^{1}H spectrum.

Procedure B. A solution of oxetanylated PEG-1500 (**2a**) in CH_2Cl_2 (1 mL/g monomer) was cooled to 0 °C, treated with $Et_2O{\cdot}BF_3$ (0.4 equiv), warmed to room temperature, and stirred until the sticky point was reached after 10 min, as indicated by the increased viscosity halting the magnetic stirrer. After stirring stopped the reaction was maintained at an oil bath temperature of 60 °C for 2 d, cooled to room temperature, and worked-up as described in Procedure A.

Procedure C. A solution of oxetanylated PEG-400 (**2a,b**) in diglyme (1 mL/g monomer) was stirred at room temperature and treated slowly with $Et_2O{\cdot}BF_3$ (0.4 equiv). Stirring stopped after 1 min. The reaction was warmed to 70 °C for 2 d, cooled to room temperature, and worked-

(34) Christiansen-Brams, I.; Meldal, M.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1461−1471.

(35) Auzanneau, F.-I.; Meldal, M.; Bock, K. *J. Pept. Sci.* **1995**, *1*, 31−44.

up as described in Procedure A. The same procedure was employed for PEG-1500 monomers (**2a,b**) dissolved in 1,2-dichloroethane (1 mL/g monomer), reaching the sticky point after 5 min.

Suspension Polymerization. Under argon, a solution of macromonomer **2b-400 (**600 mg, 1.21 mmol) in dry acetonitrile (0.3 mL) was cooled to −20 °C, treated with $Et_2O{\cdot}BF_3$ (0.06 mL, 0.48 mmol), and then added to silicon oil (75 mL) at room temperature with emulsification by stirring at 200 rpm for 1 min. Stirring was slowed to a minimum, and the reaction was allowed to proceed overnight. The slurry of beads was filtered off and washed with 5 mL volumes of each of the following solutions: 1 M aqueous HCl, water, MeOH, DMF, and CH_2Cl_2. The beads were air-dried for 1 h and then dried under vacuum to yield 490 mg (83%) of material having a loading of 0.5 mmol/g, exhibiting swelling volumes of 3.3 mL/g (water), 3.2 mL/g (DMF), and 4.6 mL/g (CH_2Cl_2).

Functionalization of SPOCC Resins. Bromo-SPOCC Resin (4). Resin **1** (1 g, 0.6 mmol) was suspended in CH_2Cl_2 (10 mL) and treated with triphenylphosphine (787 mg, 5 equiv) and imidazole (204 mg, 5 equiv). After the reagents had dissolved, the suspension was cooled to 10 °C in a water bath and treated dropwise with bromine (155 μL, 5 equiv). The water bath was removed, and after being stirred overnight at room temperature, the resin was filtered and washed with DMF, water, 10% sodium thiosulfate solution, water, DMF, THF, and CH_2Cl_2 (3 × 20 mL each). The bromine content was 0.59 mmol/g as determined by elemental analysis.

Amino-SPOCC Resin (5). Resin **4** (1 g, 0.59 mmol) was suspended in a solution of sodium azide in DMSO (390 mmol, 10 equiv, 10 mL). The mixture was warmed to 60 °C for 18 h, cooled to room temperature, filtered, and washed thoroughly with DMF, water, and DMF. Reduction was effected employing a 0.5 M solution of 1,4-dithiothreitol (DTT) in DMF (10 mL), containing 0.1 M 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The resin was filtered and washed with DMF, THF, and CH_2Cl_2. The resin loading was determined by spectrophotometric measurement of Fmoc cleavage at 290 nm after functionalization of a resin sample with Fmoc-succinimide (10 equiv, 4 h). The measured loading was 0.44 mmol/g, corresponding to a loading of 0.48 mmol/g for the resin employed.

Thiol-SPOCC Resin (6). Triphenylmethylthiol (77 mg, 10 equiv) in THF (1 mL) was cooled to 4 °C, treated with sodium hydride (9 mg of 60% NaH, 8 equiv), stirred for 15 min, and added under argon to bromo-SPOCC resin **4** (44 mg, 0.028 mmol) in a reaction vessel (ReactiVial, 0.6 mL). After the solution was stirred for 16 h at room temperature, the resin was filtered, washed with THF, DMF, water, DMF, THF, and CH_2Cl_2, and dried in vacuo. The trityl protecting group was cleaved by washing with 10% TFA in CH_2Cl_2. TFA washings were collected until yellow color was no longer detectable (82 mL) and measured spectrophotometrically at 410 nm. The loading was calculated from the amount of cleaved trityl cation by employing a calibration curve. Finally the resin was washed with DMF, THF, and CH_2Cl_2, and dried to yield 42 mg of resin **6** with a loading of 0.54 mmol/g.

Organic Synthesis with SPOCC Resins. *p*-[α-(L-Seryl-L-phenylalanyl-L-leucylglycylamido)-2,4-dimethoxybenzyl]phenoxyacetylamido-SPOCC Resin (7). Fmoc-protected Rink linker (208 mg, 0.4 mmol), TBTU (122 mg, 0.38 mmol), and 4-ethylmorpholine (NEM) (83 μL, 0.5 mmol) were dissolved in DMF (3 mL). After an activation period of 10 min, this solution was added to resin **5** (210 mg, 0.1 mmol) and reacted for 3 h. After the reaction solution was washed with DMF (5 × 10 mL), the Fmoc group was cleaved with 20% piperidine in DMF (2 and 16 min treatments), and the resin was washed again. The deprotected amine was coupled, deprotected, and coupled with 3 equiv of the Fmoc-amino acids Gly, Leu, Phe, and Ser, using TBTU (93 mg, 0.29 mmol) and NEM (66 μL, 0.4 mmol) for activation as described for the linker above. After final Fmoc deprotection the product was cleaved of a resin sample (2 mg) using 95% TFA in water for 2 h, and analyzed by HPLC (retention time 24.0 min) and MALDI-MS [calcd ($M = C_{20}H_{31}N_5O_5$), found (MH^+, MNa^+, MK^+) *m*/*z* 422, 444, 460]. The final peptide loading was 0.36 mmol/g.

***p*-[α-[(*N*-Glyoxalyl-L-phenylalanyl)-L-leucylglycylamido]-2,4-dimethoxybenzyl]phenoxyacetylamido-SPOCC Resin (8)**. Resin **7** (200 mg, 0.072 mmol) was treated for 1 h with an aqueous solution of $NaIO_4$ (46 mg, 3 equiv) in sodium phoshate buffer (2.5 mL of 0.01 M, pH 7). The resin was filtered, washed with water, DMF, THF, and CH_2Cl_2, dried, and analyzed after resin cleavage as described above. HPLC: retention time 25.6 min. MALDI-MS: calcd ($M = C_{19}H_{26}N_4O_5$) 390.44, found (MNa^+, MH_2ONa^+) *m*/*z* 413.3, 431.4.

***p*-[α-[[*N*-(Ethylfumaroyl)-L-phenylalanyl]-L-leucylglycylamido]-2,4-dimethoxybenzyl]phenoxyacetylamido-SPOCC Resin (9)**. Lyophilized resin **8** (90 mg, 0.032 mmol) was treated with toluene (1 mL) and triethyl orthoformate (0.5 mL) for 2 h and washed with dry toluene (6 × 2 mL). In a separate round-bottom flask triethyl phosphonoacetate (32 μL, 5 equiv) was dissolved in toluene (1 mL), the solution was cooled to 0 °C, and *n*-butyllithium (4.5 equiv, 1.6 M solution in hexane) was added. After 10 min the solution was added to the resin and allowed to react at ambient temperature for 90 min. After being washed (DMF, THF, CH_2Cl_2) and dried, the resin was treated with NaOH (0.1 M) for a period of 2 h, washed, and dried again. Cleavage of an analytical sample and HPLC analysis were conducted as described in the General Procedures. Preparative cleavage of 30 mg of resin yielded 3.3 mg (64%). HPLC: retention time 27.0 min. MALDI-MS: calcd ($M = C_{21}H_{28}N_4O_6$) 432.48, found (MH^+) *m*/*z* 433.4. ^{1}H NMR (250 MHz, d_4-MeOD): δ = 0.88−0.98 (2 d, 6 H, Leu-Me), 1.6−1.75 (m, 3 H, Leu), 3.0, 3.25 (2 dd, 2 H, Phe-CH_2), 3.8−4.0 (2 d, 2 H, ^2J = 17.8 Hz, Gly-α), 4.4−4.5 (dd, 1 H, Leu-α), 4.85 (dd, 1 H, Phe-α), 6.68, 7.06 (2 d, 2 H, $^3J_{trans}$ = 15.5 Hz, olefinic protons), 7.15−7.4 (m, 5 H, aromatic protons).

α-[*N*-(2-hydroxypropionyl)-L-phenylalanyl]-L-leucylglycylamide (10a). A suspension of methyltriphenylphosphonium iodide (69 mg, 0.171 mmol) in THF (2 mL) was cooled to −50 °C and treated with a 1.6 M solution of *n*-butyllithium (0.154 mmol) to furnish a yellow-orange solution. After 20 min of stirring under argon, the solution was warmed to −10 °C, treated with resin **6** (96 mg, 0.034 mmol), and allowed to react for 2 h. Washing and drying the resin followed by cleavage of the product (95% TFA, 2 h) afforded a mixture of 2- and 3-hydroxypropionyl products **10a** and **10b** (5.3 mg, 40%). HPLC: retention time 25.6 min. ESI-MS: calcd ($M = C_{20}H_{30}N_4O_5$) 406.22 Da, found (MH^+, MNa^+) *m*/*z* 407.4, 429.3.

Data for 10a (2-Hydroxy Product). ^{1}H NMR (250 MHz, d_4-MeOD): δ = 0.88−0.98 (m, 6 H, Leu-Me), 1.28 (m, 3 H, Me-CHOH), 1.6−1.75 (m, 3 H, Leu), 3.0−3.2 (2dd, 2 H, Phe-β), 3.82 (dd, 2 H, ^2J = 17.9 Hz, Gly-α), 3.90 (m, 1 H, C*H*OH), 4.4−4.5 (dd, 1 H, Leu-α), 4.6−4.75 (dd, 1 H, Phe-α).

Data for 10b (3-Hydroxy Product). ^{1}H NMR (250 MHz, d_4-MeOD): δ = 0.88−0.98 (m, 6 H, Leu-Me), 1.6−1.75 (m, 3 H, Leu), 2.0−2.2 (m, 2 H, C*H*$_2$−CH_2OH), 3.0−3.2 (2 dd, 2 H, Phe-β), 3.82 (dd, 2 H, ^2J = 17.9 Hz, Gly-α), 3.30 (m, 1 H, C*H*$_2$OH), 4.4−4.5 (dd, 1 H, Leu-α), 4.6−4.75 (dd, 1 H, Phe-α).

[*N*-(9-Fluorenylmethoxycarbonyl)-L-alanyl]-L-seryl-L-phenylalanyl-L-leucylglycyl-SPOCC Resin (11). SPOCC-400 resin **1** (326 mg, 0.58 mmol/g loading, 0.19 mmol) was reacted twice with a solution of Fmoc-Gly-OH (339 mg, 3 equiv), MSNT (338 mg, 3 equiv), and *N*-methylimidazole (MeIm; 68 μL, 2.25 equiv) in CH_2Cl_2 (4 mL) each time for 45 min. After Fmoc deprotection with treatments using 20% piperidine in DMF for 2 and 16 min, the glycinyl residue was elongated by couplings, deprotections, and coupling 3 equiv of the Fmoc-amino acids Leu, Phe, Ser, and Ala, which were preactivated for 15 min with TBTU (2.9 equiv, 177 mg) and NEM (4 equiv, 127 μL) in DMF (4 mL) and reacted with the resin for 3 h. A small resin sample (2 mg) was cleaved with 0.1 M NaOH solution (50 μL, 2 h) and analyzed by HPLC and MALDI-MS. HPLC: retention time 28 min. MALDI-MS: calcd ($M = C_{23}H_{35}N_5O_7$) 493.6 Da, found (MNa^+) *m*/*z* 517.7.

[*N*-(9-Fluorenylmethoxycarbonyl)-L-alanyl][*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-L-seryl]-L-phenylalanyl-L-leucylglycyl-SPOCC Resin (12). Resin **11** (100 mg, 0.04 mmol) was lyophilized from dry toluene (3 mL) in a speed vac overnight and treated with a solution of tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate[31] (100 mg, 5 equiv) in CH_2Cl_2 (1.5 mL). Under argon, trimethylsilyl trifluoromethanesulfonate (TMSOTf; 120 μL of a 1 M solution in CH_2Cl_2) was added, and the mixture was allowed to react for 1 h. The resin was filtered, washed with CH_2Cl_2, THF, DMF, THF, and CH_2Cl_2, and dried in vacuo. The glycosylation procedure was repeated, and analysis was conducted with HPLC and MALDI-MS after cleavage

with NaOMe in MeOH (0.02 M, 2 h). HPLC: retention time 20.1 min. MALDI-MS: calcd (M = $C_{29}H_{45}N_5O_{12}$) 655.7 Da, found (MNa^+) *m*/*z* 656.

L-Alanyl[*O*-(*β*-D-galactopyranosyl)-L-seryl]-L-phenylalanyl-L-leucylglycine Hydrazide (13). Resin **12** (35 mg, 4 *μ*mol) was treated for 3 h with a 10% hydrazine solution in water. Yield: 4.8 mg of compound **13** (55%). HPLC: retention time 22.0 min. MALDI-MS: calcd (M = $C_{29}H_{47}N_7O_{11}$) 669.7 Da, found (MNa^+) *m*/*z* 694. ^{1}H NMR, 250 MHz, d_4-MeOD): δ = 0.88−0.98 (2 d, 6 H, Leu-Me), 1.45 (d, ^3J = 7 Hz, 3 H, Ala-Me), 1.6−1.75 (m, 3 H, Leu), 3.0, 3.25 (2 dd, 2 H, Phe-CH_2), 3.4−3.6 (dd, m, 2 H, Gal-2-H, Gal-5-H), 3.7−4.1 (m, 8 H, Ala-α, 2 Ser-*β*, 2 Gly-α, Gal-3-H, Gal-4-H, 2 Gal-6-H), 4.29 (d, ^3J = 7.2 Hz, 1H, *β*-Gal-H-1), 4.3−4.4 (dd, 1 H, Leu-α), 4.7 (2 dd, 2 H, Phe-α, Ser-α), 7.15−7.4 (m, 5 H, aromatic protons).

Enzymatic Reactions on the SPOCC Resin. L-Alanyl-(3-nitro-L-tyrosinyl)-L-glycyl-L-prolinyl-L-leucylglycyl-L-leucyl-L-tyrosinyl-L-alanyl-L-arginyl-[N^ϵ-(2-aminobenzoyl)-L-lysinyl]glycylglycyl-SPOCC Resin (14). SPOCC-1500 **1** (prepared by procedure C, 65 mg, 0.027 mmol) was treated twice with a solution of Fmoc-Gly-OH (41 mg, 5 equiv), MSNT (40 mg, 5 equiv), and MeIm (8 *μ*L, 3.75 equiv) in CH_2Cl_2 (4 mL) for 45 min, filtered, and washed with CH_2Cl_2 and DMF. The Fmoc group was cleaved (20% piperidine in DMF for 2 and 16 min), and the resin was washed with DMF. Protected nonapeptide Fmoc-AY(3-NO_2)GPLGLY(^{t}Bu)AR(Pmc)K(*N*-Boc-Abz)-G-OH (43 mg, 3 equiv) in DMF (4 mL) was preactivated with TBTU (6.8 mg, 2.9 equiv) and NEM (3.7 *μ*L, 4 equiv) for 15 min, added to the resin, and reacted for 3 h. The resin was washed with DMF, THF, and CH_2Cl_2, dried, and treated with 95% TFA for 10 min and for 2.5 h to remove side chain protecting groups. The resin was washed with 95% acetic acid (four times, 5 min), 5% triethylamine in DMF (three times, 2 min), DMF (two times, 2 min), THF, and CH_2Cl_2 and dried in vacuo. Cleavage of a resin sample (2 mg, 0.1 M NaOH, 2 h) afforded material for analysis. HPLC: retention time 32.0 min. MALDI-MS: calcd (M = $C_{68}H_{99}N_{19}O_{19}$) 1486.7 Da, found (MH^+, $MNa^+ - H_2O$) *m*/*z* 1487, 1493.

I. Substrate Cleavage with Subtilisin. Resin **14** (2 mg) was treated with a solution of subtilisin BNP′ (Novo Nordisk, Bagsvaerd, Denmark; 10^{-7} M = 100 nM of the 27 kDa protein) in pH 7 phosphate buffer (50 mmol of NaH_2PO_4 in H_2O). After 15 min strong fluorescence was observed under UV irradiation. After 3 h the resin was washed with water, DMF, THF, and CH_2Cl_2 and dried. One portion of the enzyme-treated resin (1 mg) was cleaved with NaOH (50 *μ*L of a 0.1 M solution, 2 h) and analyzed by HPLC and MALDI-MS. The other portion of the resin (1 mg) was subjected to Edman degradation. The HPLC chromatogram indicated complete cleavage of the starting peptide substrate and yielded one peptide product. HPLC: retention time 21.0 min. ES-MS: calcd (M = $C_{26}H_{42}N_{10}O_7$) 606.7 Da, found *m*/*z* 607.6. Edman degradation (three cycles): A, Abz; R; K.

II. Substrate Cleavage with Matrix Metalloprotease-9 (MMP-9). Resin **14** (2 mg) was treated with a solution of MMP-9 (recombinant, 72 kDa enzyme, obtained from the Center for Clinical and Basic Research (CCBR), Ballerup, Denmark) (100 and 275 nM protease) in pH 7.72 buffer (buffer 17, obtained from CCBR, Ballerup, Denmark) for 24 h. In neither case was any significant fluorescence observed. Cleavage and HPLC analysis as described in (I) yielded exclusively the starting peptide substrate.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra (250 MHz) of macromonomers **2a** and **2b**, ^{1}H MAS NMR spectra (500 MHz) of resins **1**, and HPLC chromatograms of the enzymatic cleavage of compound **14** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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