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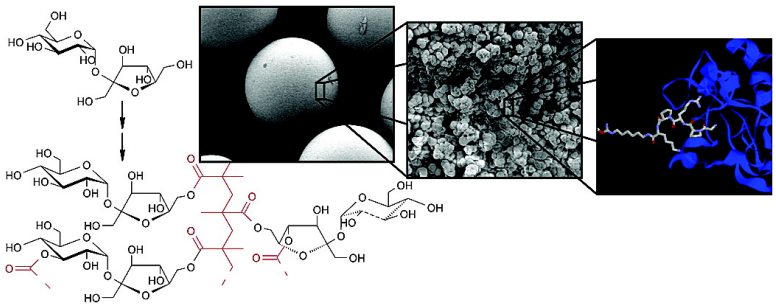

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SUBPOL: A Novel Sucrose-Based Polymer Support for Solid-Phase Peptide Synthesis and Affinity Chromatography Applications

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Abstract: A novel **SU**crose-**B**ased **Pol**ymer support (SUBPOL) with tailored morphology suitable for the use in solid-phase peptide synthesis (SPPS) is described, and its application as a hydrophilic affinity matrix for the specific removal of fibrinogen from human plasma is demonstrated. After suspension polymerization of partly methacrylated 2,1′:4,6-di-*O*-isopropylidene sucrose and subsequent removal of the protecting groups, hydrophilic spherical polymer beads were obtained. The morphology of the resulting resin was controlled by variation of the porogen as well as the average degree of substitution with respect to the methacryloyl groups of the monomer mixture. After introduction of amino groups for a permanent attachment of immobilized peptide ligands, prevention of unintended esterification during SPPS was achieved by silylation of remaining hydroxy groups. Alternatively, a Rink amide linker was introduced prior to SPPS to allow cleavage and subsequent analysis of the peptide assembled on the SUBPOL resins. Two hexapeptides of sequence kwiivw and hfffflw, consisting of D-amino acids, as well as a 19-mer peptide corresponding to the sequence GSGVRGDFGSLAPRVARQL of the VP1 protein from the foot-and-mouse disease virus (FMDV) were successfully prepared both manually or in a semi-automated process on SUBPOL resins according to the Fmoc/*t*Bu strategy. Yields and purities were comparable to peptides prepared on commercially available polystyrene resins. A specific affinity adsorbent containing the fibrinogen-binding pentapeptide GPRPK was prepared by SPPS on SUBPOL resins of different morphology, and the strong impact of the affinity matrix on adsorption performance was demonstrated.

Introduction

Since Cuatrecasas, Wilchek, and Anfinsen reported for the first time the purification of staphylococcal nuclease,[1] affinity chromatography has evolved as one of the most powerful and effective fractionation techniques for the purification of proteins. The unique interaction between the target molecule and a complementary ligand covalently attached to an insoluble matrix provides the specificity required for the isolation of biomolecules from complex mixtures, such as cell extracts or human blood.[2] The use of short synthetic peptides as affinity ligands provides distinct advantages for large-scale affinity purification compared to macromolecular proteins. Peptide ligands are accessible in high quantities and excellent purities by solid-phase peptide synthesis at much lower production costs and show satisfying chemical stability under conditions commonly employed for sterilization and regeneration of the adsorbent. Even short peptide sequences identified from phage-displayed or synthetic peptide libraries have shown high specificity and affinity toward several different target proteins, including fibrinogen,[3,4] IgG,[5] TNF-α,[6−9] anti-MUC1,[10,11] S-protein,[12] thrombin,[13] α1-

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proteinase inhibitor A1PI,[14] α-lactalbumin,[15] or human growth factor FVIII.[16]

Affinity chromatography separations are performed almost exclusively in aqueous solutions. Thus, peptides which are typically prepared on hydrophobic cross-linked polystyrene resins have to be cleaved from the support and coupled to a hydrophilic matrix.[17] The application of hydrophilic polymer supports that serve both purposes, i.e., being a suitable support for SPPS as well as an ideal matrix for affinity purification, seems to be a promising approach for a one-step production of specific adsorption materials.

In recent years, the increasing interest in resins that show good swelling properties in protic and aprotic polar solvents has led to the development of a broad range of novel hydrophilic supports for SPPS including poly(ethylene glycol)-polystyrene-grafted resins (PEG-PS and TentaGel),[18−20] poly(ethylene glycol)-polyacrylamide (PEGA),[21] cross-linked poly(ethylene glycol) (POEPOP,[22] SPOCC[23]), poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) (GMA-ST-EDMA),[24] cross-linked ethoxylate acrylate resin (CLEAR),[25] tetraethylene glycol diacrylate-*cross*-linked polystyrene[26] or cross-linked oligoethylene glycol monomethacrylates.[27] However, the majority of these resins does not meet the requirements for an ideal affinity matrix. The main drawbacks are their low mechanical strength as well as unspecific hydrophobic binding sites, as in the case of polystyrene-based supports. Additionally, various polysaccharide-based materials including cotton,[28] beaded cellulose (Perloza),[29] as well as cross-linked agarose (Sephadex L20,[30] HiTrap Sepharose[31]) have been tested as supports for SPPS. Although these polysaccharide gels are presently among the most preferred support materials for affinity chromatography,[32,33] their use in SPPS shows distinct limitations due to the presence of free hydroxy groups.[30] However, following a strategy where hydroxy groups are kept protected during SPPS to avoid side reactions, peptide ligands could directly be prepared on polysaccharide-based supports. Removal of the protecting groups after synthesis would result in a hydrophilic adsorbent.

Most resins currently employed for affinity chromatography consist of low cross-linked, highly swellable matrixes, such as agarose-based materials, the commercially most successful species to date. The degree of cross-linking is commonly between 4 and 6%, allowing maximum back pressures around 0.3 MPa. The situation is similar for composite resins, where the incorporation of flexible poly(ethylene glycol) chains is mostly responsible for the hydrophilic character of the matrix. These resins result in insufficient mechanical rigidity and, therefore, in an undesired back pressure at high flow rates if used in packed systems.[34,35] Other applications, although not packed, may exert high shear forces on the resins due to pumping components[36] and thus fraction the beads. It is therefore desirable to develop a polymer resin that can not only provide an efficient matrix for affinity chromatography but also to have the benefit of tailoring its morphology, depending on the application.

In this paper, we describe the development of a novel hydrophilic polymer resin consisting exclusively of sucrose moieties, whereas a synthetic strategy via protecting groups enables the fabrication of highly cross-linked, permanently porous matrixes with a broad bandwidth of pore sizes as well as gel-type species. Its successful application in SPPS, as well as its qualification for the use in affinity purification of proteins, is demonstrated.

Results and Discussion

Starting from sucrose as renewable, low-cost material, a novel hydrophilic polymer support (acronym: SUBPOL) has been designed. The morphology of the polymer support, such as pore size, pore size distribution, and porosity, plays a key role in the performance of affinity purifications. The accessibility of the immobilized ligands crucially depends on the pore size of the matrix. While a larger average pore radius increases the diffusion rate of proteins into the matrix, the correspondingly diminished surface area reduces the binding capacity of the adsorbents. To achieve this balance between pore size and surface area, porosity of the support has to be optimized for each specific protein. In this context, suspension polymerization represents an excellent method for producing spherical beads

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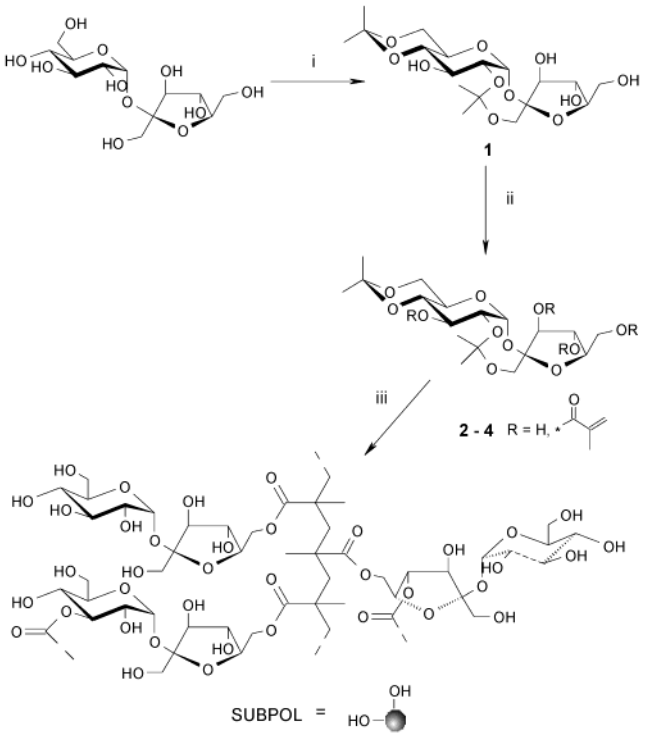
of tunable morphology in a size range between 10 and 1000 μm. Especially aqueous suspension polymerization offers a wide variety of suitable inert organic solvents (porogen) that influence the course of phase separation during the polymerization process, enabling control of the morphology of the resulting resins.[37] Polymerization of sugar-containing monomers such as glucose or sucrose methacrylates may also be performed by inverse suspension due to their hydrophilic nature, but the limited choice of suitable solvents to achieve the desired phase separation makes this method unfavorable. As an example, inverse suspension polymerization of sucrose methacrylates has been reported to result in rather weakly cross-linked polymer gels.[38] Using such monomers in aqueous suspension requires reduction of their solubility in water by introduction of protecting groups. In this context, polymerization and tailoring of the morphology of polymer supports based on 3-*O*-methacryloyl-1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose have successfully been carried out in aqueous suspension.[39] Glucose-based polymer supports may serve as alternative matrixes for SPPS and affinity chromatography. However, to avoid side reactions during solid-phase synthesis as well as unspecific adsorption during affinity purification, the use of nonreducing carbohydrates such as sucrose is preferred. Until now, the preparation of porous polymer supports from partly protected sucrose methacrylates by classical suspension polymerization has not been described.

Synthesis of Monomers. The selective introduction of *O*-isopropylidene-protecting groups by acetonation of sucrose under kinetically controlled conditions is well established and decreases the hydrophilicity by leaving only four out of eight hydroxy groups for subsequent esterification.[40] As an additional benefit, the amount of theoretically possible ester derivatives is reduced from 256 to a manageable number of 15. This facilitates the reproducibility of monomer synthesis and consequently polymer synthesis. 2,1′:4,6-di-*O*-isopropylidene sucrose (**1**) was prepared by protection of sucrose with a 4.5-fold excess of 2-methoxypropene in the presence of a catalytic amount of *p*-toluenesulfonic acid in dry DMF (Scheme 1). The crude reaction mixture typically consists of 58% of **1**, 10% of 4,6-*O*-isopropylidene sucrose, 24% of 2,1′:4,6-di-*O*-isopropylidene-6′-*O*-(1-methyl-1-methoxyethyl)sucrose, and 8% of an unknown derivative. Treatment of this mixture with 0.2% acetic acid for 5 min at room temperature leads to conversion of 2,1′:4,6-di-*O*-isopropylidene-6′-*O*-(1-methyl-1-methoxyethyl)sucrose to **1** by hydrolysis at the 6′-*O*-position, while 2,1′:4,6-di-*O*-isopropylidene groups remain stable under these mild conditions. Thin-layer chromatography showed that the unknown compound had exactly the same R_f value as **1** with different eluant systems and could therefore not be removed by conventional column chromatography. Reverse-phase HPLC-MS analysis revealed a molecular weight identical to **1**, indicating an isomeric form of different configuration.[41] Pure **1** was isolated after acetylation of the remaining hydroxy groups by using acetic anhydride in pyridine, recrystallizing the resulting tetra acetate, and subsequently removing the acetyl groups by treatment with sodium methanolate.[42] We observed that the separation of the isomeric species simplified subsequent analysis of the monomer mixtures but did not affect the properties of the resulting polymer.

Scheme 1. Synthesis of SUBPOL Resins[a]



[a] (i) 2-Methoxypropene, *p*-TosOH, DMF, 70 °C; 0.2% AcOH, rt; Ac_2O, pyridine, rt; NaOMe, MeOH, rt. (ii) Methacrylic anhydride, pyridine, 65 °C. (iii) AIBN, porogen, 80 °C; 60% AcOH, 60 °C.

Esterification of **1** with methacrylic anhydride was carried out in pyridine. Methacrylic acid formed during the reaction was neutralized as pyridinium salt, preventing the removal of the isopropylidene-protecting groups by acid hydrolysis. Hydrophobic monomer mixtures (compounds **2**−**4**) with various average degrees of substitutions (DS) were obtained and could be polymerized without an additional cross-linker (Scheme 1). To retain a maximum of free hydroxy groups for good hydrophilicity of the final polymer matrix, monomer mixtures with a lower DS are favorable. Therefore, reaction parameters, such as the amount of methacrylic anhydride and reaction time, were optimized, and different monomer mixtures of varying DS between 1.6 and 3.7 were prepared (Table 1). The mixtures were analyzed by gas chromatography (GC) after silylation of remaining hydroxy groups. Because of the diminished amount of possible derivatives after selective introduction of isopropylidene-protecting groups, well-resolved chromatograms were obtained (see Supporting Information). Because of the group-wise appearance of peaks, a certain degree of substitution could be appointed to the different fractions, enabling the calculation of the DS as well as the distribution of mono-, di-, tri-, and tetra-substituted products. The DS calculated from GC was

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Table 1. Influence of the Amount of Methacrylic Anhydride (MA) and of the Reaction Time on the Composition of the Resulting Monomer Mixtures

expt	monomer	equiv of MA	time (h)	tetra (%)	tri (%)	di (%)	mono (%)	**1** (%)	DS
A	2	2	3.5	2.9	8.2	53.2	20.2	15.5	1.6
B	3	4	3.5	10.6	23.2	53.1	8.8	4.3	2.3
C	[a]	4	24	18.5	29.2	50.0	2.3	0	2.6
D	[a]	8	3.5	21.7	37.3	38.3	2.7	0	2.8
E	4	8	48	65.0	35.0	0	0	0	3.7
F	[a]	16	3.5	38.6	39.4	22.0	0	0	3.2
G	[a]	16	24	51.7	37.4	10.9	0	0	3.4

[a] GC samples were taken directly from the reaction mixture, without any further workup.

confirmed by ^{1}H NMR spectroscopy, comparing the integration of the signals of methacryloyl CH_3 at 1.97−2.10 ppm to isopropylidene CH_3 at 1.23−1.60 ppm. Monomer mixture **2** with a low DS of about 1.6 showed already sufficient solubility in organic solvents such as toluene or butyl acetate, affording a yield of about 84%. We reached the optimum conditions by performing the esterification with 4 equiv of methacrylic anhydride over a period of 3.5 h to yield 96% of sucrose methacrylates **3** with a DS between 2.2 and 2.5. Because of the sterically hindered *O*-3 position,[43] a large excess of reagent (16 equiv), together with a reaction time of 24 h, was necessary for the preparation of highly substituted products (mixture **4**, DS > 3.0) (experiments F and G, Table 1). Alternatively, the amount of anhydride could be reduced to 8 equiv by further prolongation of the reaction time beyond 40 h, to give monomer **4** (experiment E, Table 1). With regard to the high tendency of highly methacrylated products to thermally induced polymerization, an increase of the reaction temperature was not considered. To prevent undesired, spontaneous polymerization, monomers were stored as 70−80% solutions in toluene until further use. The amount of toluene was quantified by ^{1}H NMR, comparing the integration of the signals of toluene CH_3 at 2.36 ppm to isopropylidene CH_3 at 1.23−1.60 ppm.

Polymer Synthesis and Characterization. Spherical porous polymer beads were prepared by aqueous suspension polymerization (Scheme 1). For tailoring the morphology, an inert solvent (porogen) was added to the monomer mixture. The porogen should act as a good solvent for the monomer but as a rather poor solvent for the polymer network to influence the onset of phase separation during the polymerization process and consequently the pore size of the polymer support.[37] Since the formation of pores depends on both the polarity of the porogen and the polarity of the monomers, both aspects were investigated. The morphology of the polymer resins was analyzed by nitrogen sorption and Hg intrusion. Using nitrogen sorption may result in pore sizes of up to approximately 100 nm (micro- and mesopores) to be covered. Mercury intrusion on organic polymers is only reliable above approximately 10 nm because of high compression forces, and it covers meso- and macropores. For a comprehensive characterization of the morphology, both methods should be applied, and the results should be regarded as complementary.[44] Carbohydrate-methacrylate-based supports are known to show drastically reduced BET surface areas after deprotection of the isopropylidene groups.[39] The most probable reason might be the blocking of micro- and small mesopores due to hydrogen bonding and/or rearrangement of the sugar moieties during hydrolysis, while large meso- and macropores remain unaffected. In polar solvents these hydrogen bonds are likely to be broken, and therefore, the surface area would correspond more to the one from protected polymers. Nitrogen sorption and Hg intrusion were performed on isopropylidene-protected supports because of the ease of their characterization in the protected state, while swelling studies were carried out after deprotection because it is an essential property during application. Results of porosity measurements are summarized in Table 2. The data indicate that for monomer mixture **3**, the onset of phase separation is delayed with increasing polarity of the porogen (toluene:*n*-octane = 1:1 → toluene). This results in smaller pores (1330 → 14 nm) and consequently a larger BET surface area (11 → 343 m^2/g) (SUBPOL resins IV−VIII). The same effect is apparent for the different monomer mixtures polymerized with the same porogens. Using toluene as solvent, the monomer mixture **2** (highest polarity) leads to large pores of 159 nm (SUBPOL I), monomer mixture **3** (medium polarity) yields small pores of 14 nm (SUBPOL VIII), whereas monomer mixture **4** (lowest polarity) does not develop pores and results in a solvent-expanded gel-type polymer (SUBPOL XI). Similar results were obtained with butyl acetate instead of toluene. The rather polar porogen toluene:*n*-octanol = 3:7 causes the reverse effect, i.e., the polar monomer mixtures **2** and **3** were polymerized to gel-type polymers (SUBPOL III and X), whereas the polymer from mixture **4** developed permanent pores (SUBPOL XIII). Scanning electron microscopy (SEM) images illustrating the morphology of polymer resins SUBPOL I, IV, and VIII are shown in Figure 1. An example of a SEM image of whole beads (SUBPOL VIII) is shown in Figure 2.

The isopropylidene-protecting groups were removed by shaking the beads in aqueous acetic acid.

In addition to morphology, the swelling properties of the support contribute significantly to the accessibility of immobilized ligands. The expansion of the polymer network provides additional space to large biomolecules. To study the swelling behavior, SUBPOL resins were swollen in water and DMF for 12 h. In both cases, the amount of absorbed solvent calculated from the gain in weight of the polymer beads was between 1.4 and 2.6 mL/g (Table 2).

The amount of accessible hydroxy groups was determined by treating 100, 150, and 200 mg of resin with trimethylsilylimidazole in pyridine. The formation of imidazole was quantified by GC with fluorene as internal standard. Using this method on polymer beads prepared from monomers **2** and **3**, we determined values of 10.1 and 7.4 mmol OH/g resin, respectively, corresponding to approximately 70% of the theoretical amount of hydroxy groups. However, this method was not applicable for gel-type polymers due to adsorption of imidazole. Thus, in case of polymer beads derived from monomer **4**, only 42% of the theoretical amount of hydroxy groups could be determined.

The chemical stability of the unprotected resins toward reaction conditions applied in routine Fmoc solid-phase peptide chemistry was confirmed by exposing the beads to neat trifluoroacetic acid (TFA) and to a solution of piperidine in DMF for 3 h, respectively. In both cases, neither a loss of weight

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Table 2. Influence of the Porogen and of the Average Degree of Substitution (DS) of the Monomer Mixture on the Porosity and Swelling Properties of the Resin

SUBPOL	monomer (DS)	porogen	BET surface area, m^2/g	pore diameter[a] Hg (N_2), nm	pore volume[a] Hg (N_2), cm^3/g	swelling, mL/g H_2O	swelling, mL/g DMF
I		toluene	61	159 (9.4)	0.85 (0.11)	2.2	2.4
II	2 (1.6)	butyl acetate	80	14 (5.8)	0.18 (0.07)	1.6	2.1
III		toluene: *n*-octanol (3:7)	[b]	[b]	[b]	2.5	2.6
IV		toluene: *n*-octane (1:1)	11	1330 (8.2)	0.10 (0.01)	1.7	2.0
V		toluene: *n*-octane (6:4)	43	1097 (8.7)	0.81 (0.05)	1.8	2.0
VI		toluene: *n*-octane (7:3)	263	252 (6.3)	0.94 (0.02)	2.0	2.1
VII	3 (2.3)	toluene: *n*-octane (8:2)	318	42 (6.9)	0.70 (0.33)	1.6	1.7
VIII		toluene	343	14 (8.2)	0.14 (0.50)	1.4	1.7
IX		butyl acetate	319	26 (8.3)	0.52 (0.43)	1.9	2.3
X		toluene: *n*-octanol (3:7)	[b]	[b]	[b]	2.2	2.2
XI		toluene	[b]	[b]	[b]	0.9	1.0
XII	4 (3.7)	butyl acetate	[b]	[b]	[b]	1.3	1.8
XIII		toluene: *n*-octanol (3:7)	469	19 (4.3)	0.28 (0.29)	2.2	2.6

[a] Hg intrusion analysis gives the medium diameter of pores exceeding 10 nm and being, therefore, mainly responsible for the diffusion rate of proteins. In contrast, the average pore diameter assessed by N_2-sorption (shown in parentheses) covers small meso- and micropores that hardly contribute to protein adsorption. [b] Solvent-expanded gel-type polymers.

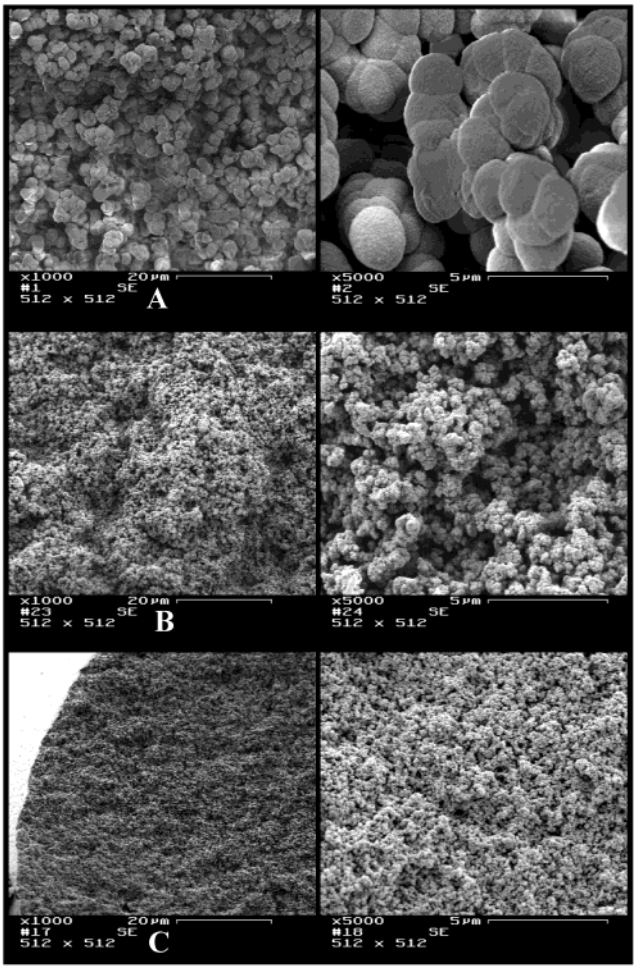


Figure 1. Scanning electron micrographs showing the inner porous structure of broken SUBPOL particles at different magnifications (×1000, left; ×5000, right). (A) SUBPOL IV (pore size: 1330 nm). (B) SUBPOL I (159 nm). (C) SUBPOL VIII (14 nm).

nor a significant difference in the FT-IR spectra in comparison to the untreated resins was observed (see Supporting Information).

For medical applications, biocompatibility of the polymer support is mandatory. Cytotoxicity assays were performed with extracts from the various SUBPOL matrixes to assess the possible release of cytotoxic compounds from the polymers. None of the matrixes tested showed cytotoxic effects (data not shown).

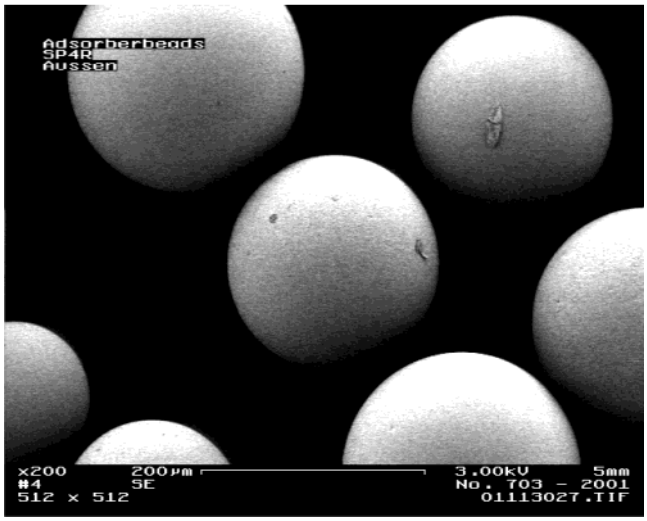
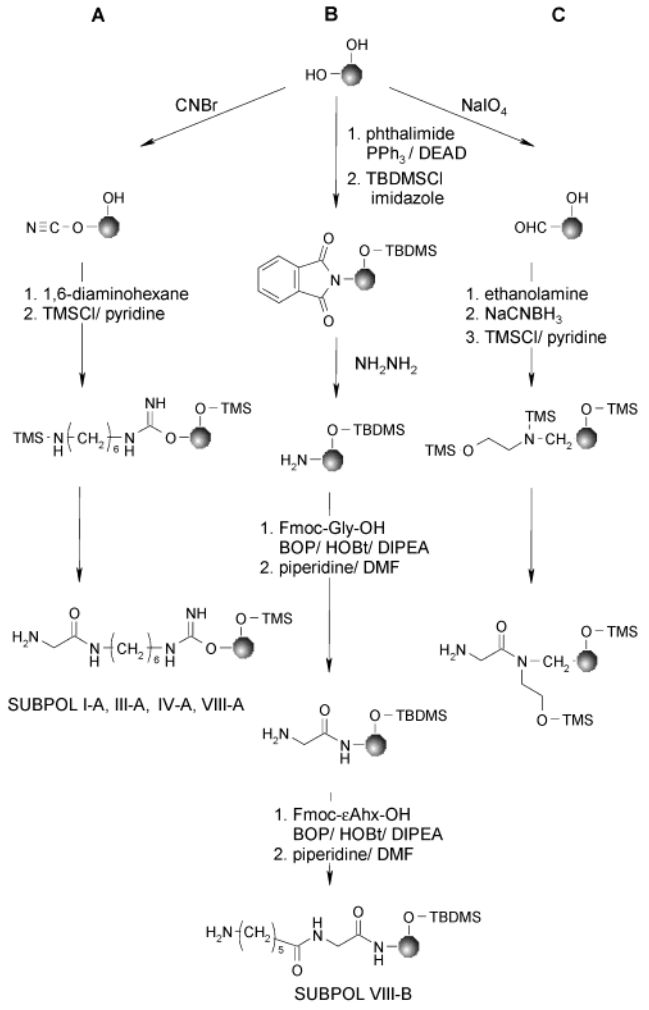


Figure 2. Scanning electron micrograph showing spherically beaded SUBPOL particles obtained after suspension polymerization. Magnification: 200-fold.

Functionalization of SUBPOL Resins. For affinity chromatography, a chemically stable attachment of the ligands to the affinity matrix is required. As shown previously, ester functionalities of the tight polymer network are resistant toward acidic conditions as a result of a certain sterical hindrance.[25] However, ester bonds exposed on the surface of the polymer may be more sensitive to hydrolysis by strong acids, thus resulting in deleterious loss of the peptide during the cleavage of peptide side-chain protecting groups by treatment with TFA. To obtain permanently immobilized peptides, amino groups were introduced to form acid-stable amide bonds between the C-terminus of the peptide and the polymer support.

Three strategies for introduction of amino groups were compared with regard to achievable loading capacities and reproducibility: First, activation of hydroxy groups and subsequent coupling of diamines; second, direct conversion of hydroxy groups to amino groups; and third, periodate oxidation of sugar diols followed by reductive amination of aldehyde

Scheme 2. Introduction of Amino Groups and Spacer Arms (A) by Cyanogen Bromide Activation and Diamine Coupling, (B) by Conversion of Hydroxy Groups to Amino Groups via the Mitsunobu Reaction, and (C) by Reductive Amination after Periodate Oxidation of Sugar Diols.



groups (Scheme 2, parts A−C). Cyanogen bromide activation of hydroxy groups is among the most widely used methods in polysaccharide chemistry.[45] The resulting highly reactive cyanate ester groups enable the coupling of peptides or enzymes via their amino functionalities to form isourea bonds. According to path A of Scheme 2, the activation was carried out by adding a solution of cyanogen bromide in dry acetonitrile to the polymer beads (SUBPOL I, III, IV, and VIII), previously swollen in 1 M aqueous sodium carbonate, shaking for 2 min, and thoroughly washing with water.[46] The amount of cyanate ester groups was determined spectrophotometrically according to a slightly modified procedure described by Kohn et al.[47] A substitution between 420 and 500 μmol cyanate ester groups/g support could be achieved. A prolongation of the reaction time over 12 min led to a significant decrease of active groups below 220 μmol/g. This can be explained by partial hydrolysis of cyanate ester groups to inert carbamates under the basic activating conditions that are necessary to neutralize liberated HBr. To retain best reactivity, 1,6-diaminohexane was coupled immediately after activation by shaking the activated beads with a diamine solution in bicarbonate buffer for 12 h.

Hydroxy groups of the SUBPOL VIII resin were partly converted to amino groups according to Mitsunobu et al.[48] by treatment with a mixture of phthalimide, triphenylphosphine, and diethylazodicarboxylate in dry THF, followed by hydrazinolysis (Scheme 2B).

After oxidation of sugar diol groups using sodium periodate solution in the dark, amination of resulting aldehyde groups was achieved by treating the oxidized polymer with 1 M aqueous ethanolamine solution and subsequently with sodium cyanoborohydride for reduction of the imine (Scheme 2C).

To prevent undesired esterification during SPPS, the amino-functionalized resins, except those obtained after Mitsunobu reaction, were treated with chlorotrimethylsilane (TMSCl) in pyridine to protect remaining hydroxy groups and to simultaneously activate amino groups for subsequent acylation (Scheme 2, parts A and C). The TMS group is stable under the basic conditions required for Fmoc chemistry and could easily be removed together with the peptide side-chain-protecting groups at the end of the synthesis. Thereby, the polymer support regained its hydrophilic character. The amount of reactive amino groups was quantified spectrophotometrically via the Fmoc-piperidine color complex at 300 nm in dichloromethane after coupling with Fmoc-Gly-OH and subsequent cleavage of the Fmoc group.[17c,49] Comparison of the three methods for amino group introduction shows that CNBr activation results in a capacity of 450−510 μmol of amino functionalities per gram of polymer, confirming the quantitative conversion of cyanate ester groups (Scheme 2A). The Mitsunobu reaction gave similar results in terms of loading (500 μmol NH_2/g resin) and reproducibility, but was technically more challenging as it required exclusion of moisture during the whole procedure. As a benefit, this strategy allows selective protection of remaining hydroxy groups by silylation, since amino groups are protected as phthalimides. Therefore, the *tert*-butyldimethylsilyl (TBDMS) group was introduced to provide higher stability under weakly acidic conditions as compared to the TMS-protecting group (Scheme 2B). Using the third strategy for amino group incorporation, namely reductive amination, we could achieve the highest substitution (570 μmol NH_2/g resin; Scheme 2C). The main disadvantage of this strategy is the modification of the polymer matrix by oxidation of sugar moieties, resulting in a barely predictable alteration of affinity performance. Hence, CNBr activation proved to be the most reliable method for the introduction of amino groups. Moreover, by varying the chain length of the diamine, this method allows a very simple introduction of additional spacers, which may be necessary to improve the flexibility of the immobilized peptides.

Peptide Synthesis. In a parallel approach to enable cleavage of the peptide from the polymer support for subsequent analysis, we introduced the Fmoc-Rink linker by direct coupling of *p*-[(*R*,*S*)-α-[1-(9H-fluoren-9-yl)-methoxyformamido]-2,4-dimethoxybenzyl]-phenoxyacetic acid[50] to the 6-aminohexyl-glycine spacer before starting SPPS (Scheme 3). All peptide

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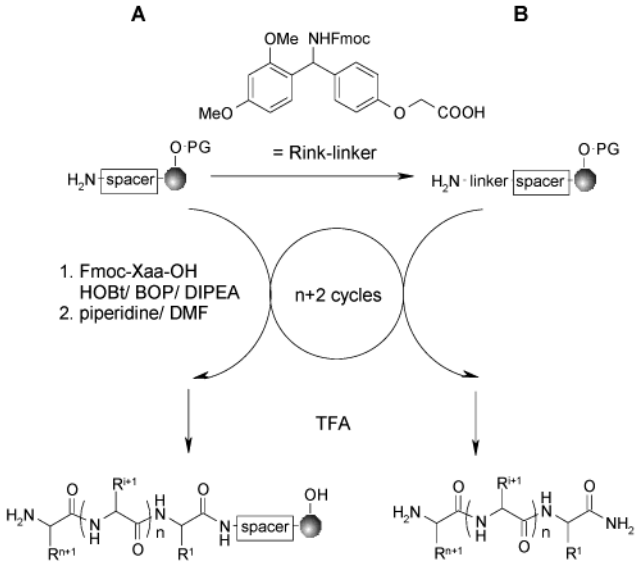
Scheme 3. General Procedure for SPPS of Peptide Ligands[a]



[a] (A) Preparation of the adsorbent. (B) Preparation of a cleavable peptide through Rink amide linker.

Table 3. Peptides and Resin-Bound Peptides

entry	sequences
5	kwiivw
6	hfffIw
7	GSGVRGDFGSLAPRVARQL
8	GPRPK
9	GPRPK_SUBPOL VIII-A
10	GPRPK_SUBPOL VIII-B
11	GPRPK_SUBPOL I-A
12	GPRPK_SUBPOL IV-A
13	GPRPK_SUBPOL III-A
14	GPRPK_POEPOP

syntheses on the different resins were performed using standard Fmoc/*t*Bu chemistry. The crude peptides were characterized by MALDI-TOF, and their purity was determined by reverse-phase HPLC. In case of permanently immobilized peptides, deprotection of the side-chain functionalities was accomplished in the same manner, but with the N-terminus of the peptide still under Fmoc protection. Finally, the amount of polymer bound peptide was quantified spectrophotometrically after Fmoc cleavage, and the results were compared to the initial amount of amino groups. In all cases, an acceptable overall decrease between 14 and 18% could be observed, indicating the sufficient stability of the peptide linkage to the polymer support.

Batchwise Synthesis of Hexapeptides. Two hexapeptides corresponding to the sequences kwiivw (**5**) and hfffIw (**6**), identified from a synthetic peptide library and consisting exclusively of D-amino acids, have been reported in the literature to inhibit binding of TNF-α to its receptor p55 (Table 3).[9] Both peptides were synthesized manually on SUBPOL VIII-A resin and on a polystyrene resin bearing the common Rink amide linker. Fmoc-Gly-OH and Fmoc-Ser(*t*Bu)-OH were coupled to serve as additional spacers prior to the attachment of the Fmoc-Rink linker. During synthesis of peptide **5** on SUBPOL VIII-A, each coupling step was monitored by subsequent quantitative spectrophotometric Fmoc analysis that confirmed the same efficiency as for the synthesis on the commercial resin. The TMS groups on the hydroxy groups of the polymer matrix were removed simultaneously with the side-chain-protecting groups

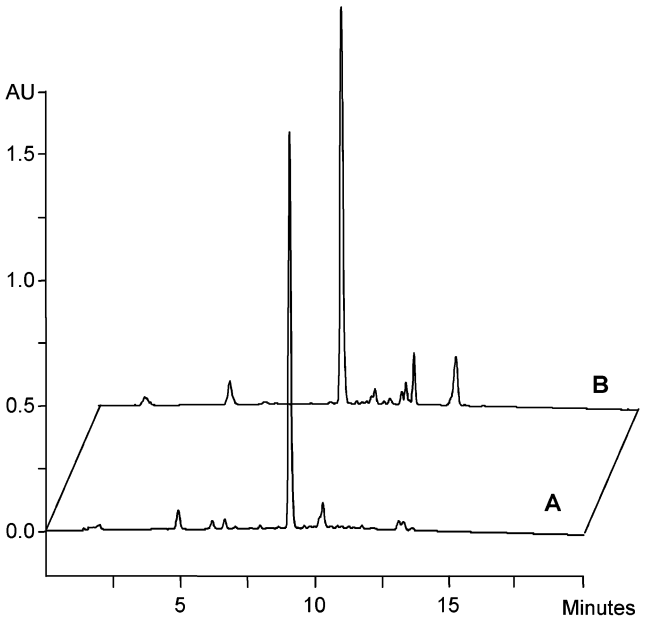


Figure 3. RP-HPLC chromatograms of crude peptide kwiivw (**5**) prepared by manual batchwise synthesis (A) on SUBPOL VIII-A resin and (B) on Rink amide resin.

of the peptide by acid hydrolysis using reagent K.[51] Peptides bound via the Rink linker were cleaved and analyzed by RP-HPLC and MALDI-TOF. Figure 3 shows the HPLC chromatogram of crude peptide **5** after the cleavage from the resin. For both peptides, the purities of crude products were higher than 80%.

Synthesis of a 19-mer Peptide. The synthesis of a 19-mer immunogenic epitope derived from the foot-and-mouth disease virus (FMDV) corresponding to the sequence GSGVRGDFGSLAPRVARQL (**7**) (Table 3) has been already reported on different resins, including Wang polystyrene[52] and POEPOP.[53] To demonstrate that SUBPOL resins are equally suitable for SPPS of longer peptides, the FMDV peptide was prepared on SUBPOL VIII-A using a multiple semi-automated peptide synthesizer.[54] The N-terminus of the peptide was acetylated. Figure 4 displays the HPLC chromatogram of crude peptide **7** obtained after cleavage from SUBPOL VIII-A with a purity of more than 75%. The amino acid composition was also confirmed by amino acid analysis.

Preparation and Examination of a Fibrinogen Adsorber. The pentapeptide GPRPK **8** has previously been reported to show a high specificity and affinity for fibrinogen.[3a] An affinity adsorber prepared by immobilization of this peptide on fractogel, a cross-linked polymethacrylate, was used for the purification of fibrinogen from human plasma.[3b] Moreover, first clinical results demonstrate that diminished plasma viscosity, as a consequence of fibrinogen removal, can lead to improved microcirculation of blood in patients suffering from atherosclerosis.[55] To examine the qualification of SUBPOL resins as

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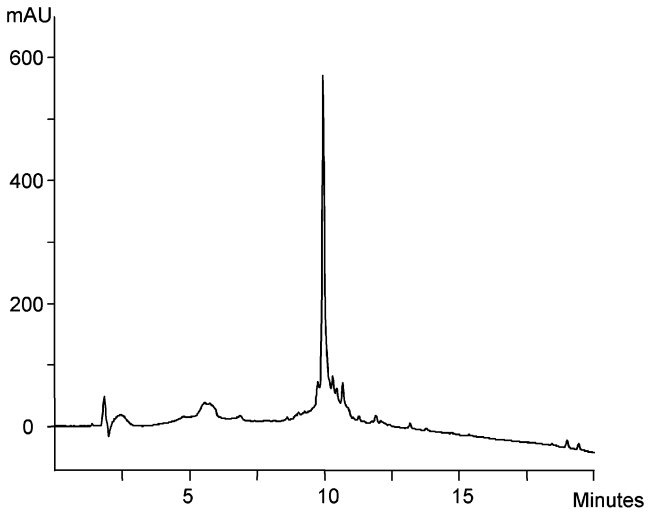

Figure 4. RP-HPLC chromatogram of crude FMDV peptide corresponding to the sequence GSGVRGDFGSLAPRVARQL (**7**) prepared by semi-automated synthesis on SUBPOL VIII-A resin.

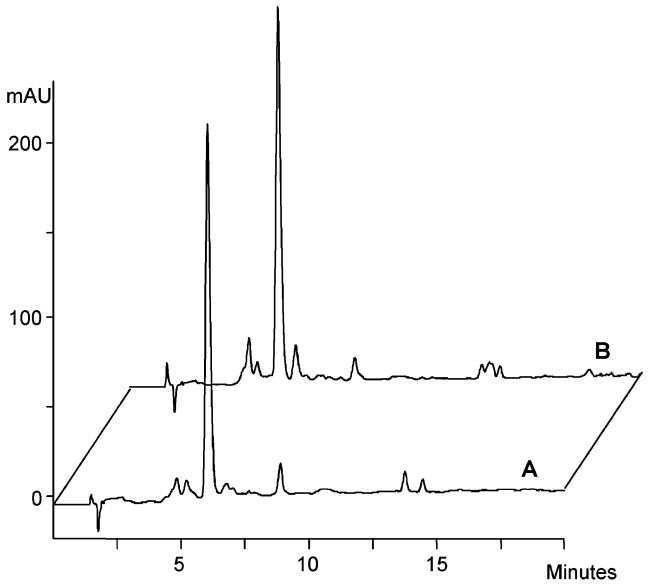

Figure 5. RP-HPLC chromatograms of crude fibrinogen-binding peptide GPRPK (**8**) prepared by manual batchwise syntheses on (A) SUBPOL VIII-A and (B) SUBPOL VIII-B resins.

hydrophilic affinity matrixes and to investigate the influence of the porosity of the support on the affinity purification performance, this pentapeptide was synthesized on amino-functionalized SUBPOL resins of various morphologies (I-A, III-A, IV-A, VIII-A, VIII-B). HPLC chromatograms of crude peptide **8** prepared on SUBPOL VIII-A and VIII-B resins are shown in Figure 5. The adsorbents were examined with respect to adsorption capacity and specificity for fibrinogen. To estimate the contribution of free hydroxy groups to unspecific fibrinogen adsorption, the results were compared to an adsorbent consisting of a highly swelling gel-type POEPOP resin carrying the same peptide sequence in a comparable amount. In all cases, specific adsorption of fibrinogen in comparison to the corresponding matrix was observed (Figure 6). The sugar-based matrixes devoid of the peptide showed a slightly higher nonspecific adsorption than POEPOP resin. This might be the result of additional interactions between free hydroxy groups of the polymer supports and fibrinogen side-chain functionalities. In

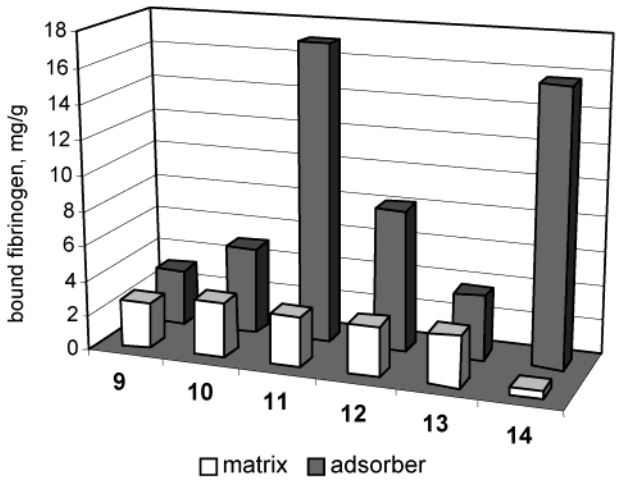

Figure 6. Fibrinogen adsorption by adsorbents based on pentapeptide GPRPK (**8**) covalently linked to SUBPOL resins of different morphology (**9**−**13**; Table 3) and to POEPOP resin (**14**) in comparison to the underivatized matrixes.

this study, the strong impact of the morphology of the polymer support on the affinity performance was clearly evidenced. In case of GPRPK_SUBPOL VIII-A resin **9** (Table 3), distinct size exclusion of fibrinogen (Stokes radius = 10.8 nm)[56] was observed because of its small average pore size of about 14 nm. Thus, despite a large surface area (343 m^2/g) (Table 2), only a rather low adsorption capacity could be achieved. Nevertheless, this porosity might be better suited for the adsorption of smaller proteins such as TNF-α (Stokes radius = 2.3 nm),[57] which has not yet been tested. Adsorbent **10**, based on SUBPOL VIII-B resin where NH_2 groups were introduced by Mitsunobu reaction, showed slightly higher specific adsorption capacity than its analogous matrix SUBPOL VIII-A, possibly due to additional expansion of the polymer network by chemical modification of the matrix during Mitsunobu reaction. This assumption was confirmed by comparing FT-IR spectra analysis of SUBPOL resins before and after exposition to hydrazine solution (Scheme 2B). The increased intensity of the OH absorption band at 3500 cm^{-1} relative to the carbonyl band at 1735 cm^{-1} indicated partial hydrolysis of ester groups during hydrazinolysis of phthalimide moiety (see Supporting Information). The best results were obtained with GPRPK_SUBPOL I-A resin **11**. Large pores (159 nm) allowing unrestricted diffusion of large proteins such as fibrinogen, together with a sufficiently big surface area (61 m^2/g), proved to be the ideal combination to achieve excellent adsorption capacity (Table 2). In the case of GPRPK_SUBPOL IV-A **12** resin which exhibits very large pores (1330 nm), adsorption capacity was reduced because of the correspondingly smaller surface area (Table 2). Within the series of SUBPOL resins, we also generated an adsorbent **13** based on a gel-type matrix (SUBPOL III-A) and compared its behavior to that of GPRPK_POEPOP **14**. These two polymers have a different degree of cross-linking. In contrast to the highly swelling POEPOP resin, the highly cross-linked and less-swelling gel-type SUBPOL III provides insufficient accessibility to immobilized peptide ligands for large proteins such as fibrinogen

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(Figure 6). Although POEPOP resin has a good adsorption capacity, its use in affinity chromatography is hampered by the lack of sufficient mechanical resistance, thus strengthening the importance of the new SUBPOL polymers.

Conclusions

The novel family of sucrose-based resins (SUBPOL) presented in this paper was found to meet the requirements of both SPPS and affinity chromatography. The selective introduction of isopropylidene-protecting groups facilitated reproducible synthesis of organic soluble sucrose methacrylates which could be polymerized in aqueous suspension in the presence of suitable porogens. This allowed the tailoring of the morphology of this new material in a very broad range to optimize the performance of affinity purification for each specific protein. Three different strategies were developed to introduce amino functionalities for a stable attachment of peptide ligands to the polymer support. To avoid side reactions during SPPS, silanes were used as selective and temporary protection of the residual hydroxy groups and were easily removed after peptide synthesis to regain the hydrophilic character of the matrix. Several peptides of different length, including a fibrinogen-binding peptide for affinity chromatography, were successfully prepared on SUBPOL resins in yields and purities comparable to those obtained from commercially available polystyrene resins. The influence of the new SUBPOL affinity matrix on the adsorption capacity was studied by comparing fibrinogen-binding adsorbents of various morphologies. We obtained the optimum performance with SUBPOL I resin. Finally, we believe that this novel family of resins provides an additional benefit to commonly employed affinity polymers because of its flexible design in morphology and represents an attractive alternative especially for applications that exhibit high pressure or shear forces. Furthermore, the use of these resins paves the way for a simplified "on-resin" affinity screening of peptide-based ligands.

Experimental Section

General. All reagents and solvents were obtained from commercial suppliers and used without further purification. Tetrahydrofuran (THF) and dichloromethane (DCM) were carefully distilled prior to use. Fmoc-protected amino acids and Rink amide resins were purchased from Applied Biosystems (Foster City, CA) and NovaBiochem (Läufelfingen, Switzerland), respectively. POEPOP resin (substitution, 0.82 mmol/g) was prepared as described by Renil and Meldal,[22a] using poly(ethylene glycol) 1000 (PEG_{1000}) obtained from Fluka.[50] RP-HPLC analysis was performed on a C_{18} column (5 μm, 150 mm × 4.6 mm) using a linear gradient of (A) 0.1% TFA in water and (B) 0.08% TFA in acetonitrile, 5−65% B in 20 min at 1.2 mL/min flow rate. Chromatograms were recorded at 210 nm. MALDI-TOF mass analysis was performed on a linear MALDI-TOF Bruker instrument using α-cyano-4-hydroxycinnamic acid as matrix. Amino acid analysis was performed on an Applied Biosystems 130A separation system coupled to an Applied Biosystems 420A derivatizer. Manual batchwise peptide syntheses were carried out in 2 mL syringes equipped at the bottom with porous frits purchased from Roland Vetter Laborbedarf (Ammerbuch, Germany). Mercury porosimetry was performed on a Micromeritics Autopore II 9220 apparatus. Nitrogen adsorption was carried out on a Micromeritics ASAP 2010 apparatus. Visualization of carbohydrates on TLC was achieved by using a mixture of AcOH, H_2SO_4, and 4-methoxybenzaldehyde (100:2:1 v/v) and heating. GC analysis was done after silylation of free hydroxy groups using a mixture of trimethylsilylimidazole:pyridine 8:2 on a Perkin-Elmer 8500 gas chromatograph using FID. Mixtures obtained after introduction of isopropylidene groups were analyzed using a DMS column (25 m, ∅ = 0.32 μm, liquid phase 0.3 μm, Perkin-Elmer). For methacrylated derivatives, a permaphase SE-54 column (25 m, ∅ = 0.32 μm, liquid phase 1 μm, Perkin-Elmer) was applied. Fourier transform infrared (FT-IR) spectra of resins (KBr pellets) were recorded using a Biorad FTS-135 IR apparatus. Images of SUBPOL resins were taken by scanning electron microscopy (SEM) using a Gemini DSM 982 apparatus.

Abbreviations. Symbols and abbreviations for amino acids and peptides are in accord with the recommendations of the IUPAC-IUB Commission on Nomenclature (*J. Biol. Chem.* **1972**, *247*, 977). Other abbreviations are: AIBN, 2,2′-azobis(2-methylpropionitrile); Boc, *tert*-butyloxycarbonyl; BOP, benzotriazole-1-yl-oxy-tris-(dimethylmino)-phosphonium hexafluorophosphate; *t*Bu, *tert*-butyl; DEAD, diethyl azodicarboxylate; DIPEA, diisopropylethylamine; DTT, dithiothreitol; Fmoc, fluorenylmethyloxycarbonyl; HOBt, *N*-hydroxybenzotriazole; MTT, tetrazolium salt 3,[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; TIPS, triisopropylsilane, TMS, trimethylsilyl; TBDMS, *tert*-butyldimethyl silyl; TFA, trifluoracetic acid.

2,1′:4,6-Di-*O*-isopropylidene Sucrose (1). A solution of sucrose (40 g, 117 mmol) in dry DMF (440 mL) was stirred with powdered (Kahn) Drierite (20 g) at 70 °C under nitrogen for 15 min. 2-Methoxypropene (38 g, 527 mmol) and *p*-toluenesulfonic acid (50 mg) were added, the mixture was stirred at 70 °C for 40 min, and then the reaction was stopped by addition of triethylamine (4 mL). The reaction mixture was filtered over Hyflo to remove the Drierite and evaporated to dryness. The yellow slurry was resuspended in water (400 mL), acetic acid (1 mL) was added, and the solution was stirred for 5 min at room temperature. After addition of sodium carbonate (10 g), the solution was evaporated to dryness, and the residue was dissolved in EtOAc (250 mL). The solution was dried over sodium sulfate and filtered, and the solvent was removed by evaporation. GC analysis of the obtained crude product mixture **1a** (36.5 g, 74%) gave 75% of **1**, 8% of 4,6-*O*-isopropylidene sucrose, and 17% of a currently unidentified byproduct. Column chromatography on silica gel using EtOAc as eluant afforded the product mixture **1b** (82% **1**, 18% unknown derivative) as a colorless solid (33.4 g, 68%).

3,3′,4′,6′-Tetra-*O*-acetyl-2,1′:4,6-di-*O*-isopropylidene Sucrose (1c). Acetic anhydride (142 mL, 1.5 mol) was added to a solution of mixture **1b** (80 g, 82% of **1**, 155 mmol) in dry pyridine (400 mL), and the mixture was stirred for 24 h at room temperature. Pyridine and acetic anhydride were removed by repeated coevaporation with toluene (3 × 100 mL). The yellow oily residue was recrystallized from diethyl ether to give **1c** as colorless crystals (57.2 g, 97 mmol, 62%). TLC: R_f ($CHCl_3$/MeOH, 98:2) 0.73. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.23, 1.36, 1.41, 1.43 (4s, 12H, $(CH_3)_2C$); 2.01, 2.02, 2.06, 2.20 (4s, 12H, CH_3C═O); 3.47 (d, $^2J_{HH}$ = 12.49 Hz, 1H, H-1a′); 3.61 (dd, $^3J_{HH}$ = 9.64 Hz, 1H, H-4); 3.65 (dd, $^3J_{HH}$ = 10.43 Hz, 1H, H-6a); 3.77 (dd, $^3J_{HH}$ = 3.40 Hz, $^3J_{HH}$ = 10.69 Hz, 1H, H-2); 3.79−3.86 (m, 1H, H-5); 3.92 (dd, $^3J_{HH}$ = 5.90 Hz, $^2J_{HH}$ = 10.43 Hz, 1H, H-6b); 4.00 (d, $^2J_{HH}$ = 12.49 Hz, 1H, H-1b′); 4.17−4.26 (m, 2H, H-5′, H-6a′); 4.36 (dd, $^3J_{HH}$ = 9.60 Hz, $^2J_{HH}$ = 15.70 Hz, 1H, H-6b′); 5.12 (d, $^3J_{HH}$ = 6.08 Hz, 1H, H-3′); 5.18 (dd, $^3J_{HH}$ = 9.64 Hz, 1H, H-3); 5.27 (dd, $^3J_{HH}$ = 4.64 Hz, 1H, H-4′); 6.05 (d, $^3J_{HH}$ = 3.40 Hz, 1H, H-1). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 19.3, 21.1, 21.2, 21.2 (4q, $(CH_3)_2C$); 21.3, 24.2, 25.8, 29.6 (4q, CH_3C═O); 62.4 (t, C-6); 64.6 (d, C-5); 65.5 (t, C-6′); 66.3 (t, C-1′); 70.8 (d, C-3); 71.7 (d, C-4); 72.1 (d, C-2); 77.2 (d, C-3′); 77.4 (d, C-4′); 80.0 (d, C-5′); 91.8 (d, C-1); 100.0−101.8 (2s, $(CH_3)_2C$); 104.9 (s, C-2′); 170.1, 170.9 (4s, C═O).

Hydrolysis of the Acetyl Groups. To a solution of **1c** (60 g, 102 mmol) in dry methanol (400 mL) sodium (150 mg) was added under nitrogen, and the reaction mixture was stirred at room temperature for 12 h. The reaction was quenched by purging the solution with carbon dioxide for 10 min. The solvent was evaporated and the residue suspended in DCM. The solution was filtered over Hyflo, dried over sodium sulfate, and evaporated to dryness to give **1** as colorless crystals (41.1 g, 97 mmol, 95%). TLC: R_f ($CHCl_3$/MeOH, 9:1) 0.53. ^{1}H NMR

(400 MHz, $CDCl_3$) δ = 1.32, 1.41, 1.47, 1.48 (4s, 12H, $(CH_3)_2C$); 3.46 (d, $^2J_{HH}$ = 12.45 Hz, 1H, H-1a′); 3.54 (dd, $^3J_{HH}$ = 9.57 Hz, 1H, H-4); 3.58−3.62 (m, 1H, H-6a′); 3.64 (dd, $^2J_{HH}$ = 10.65 Hz, 1H, H-6a); 3.73 (dd, $^3J_{HH}$ = 3.22 Hz, $^3J_{HH}$ = 9.08 Hz, 1H, H-2); 3.82 (dd, $^3J_{HH}$ = 5.12 Hz, 1H, H-6b′); 3.84−3.91 (m, 2H, H-5, H-6b); 3.93 (dd, $^3J_{HH}$ = 7.59 Hz, 1H, H-3′); 4.00 (m, 1H, H-5′); 4.10 (dd, $^3J_{HH}$ = 9.08 Hz, H-3); 4.33 (d, $^2J_{HH}$ = 12.45 Hz, 1H, H-1b′); 4.56 (dd, $^2J_{HH}$ = 8.05 Hz, 1H, H-4′); 6.23 (d, $^3J_{HH}$ = 3.22 Hz, 1H, H-1).^{13}C NMR (400 MHz, $CDCl_3$) δ = 19.5, 24.7, 25.6, 29.4 (4q, $(CH_3)_2C$); 61.8 (t, C-6′); 62.5 (t, C-6); 64.4 (d, C-5); 67.0 (t, C-1′); 69.1 (d, C-3); 73.3 (d, C-4′); 73.7 (d, C-4); 74.1 (d, C-2); 79.1 (d, C-5′); 82.6 (d, C-3′); 91.6 (d, C-1); 100.5 (s, C-2′); 102.9, 103.4 (2s, $(CH_3)_2C$).

Optimization of Reaction Parameters for the Preparation of Partly Methacrylated 2,1′:4,6-Di-*O*-isopropylidene Sucrose. Methacrylic anhydride (experiment A: 0.37 g, 2.4 mmol; B, C: 0.74 g, 4.8 mmol; D, E: 1.48 g, 9.6 mmol; F, G: 2.96 g, 19.2 mmol) was added to a solution of **1** (0.5 g, 1.2 mmol) in dry pyridine (experiment A: 5 mL; B, C: 10 mL; D, E: 15 mL; F, G: 20 mL) containing 4-methoxyphenol (1 mg), and the solution was stirred at 65 °C. Reaction times were 3.5 h (experiments A, B, D, F), 24 h (C, G), and 48 h (E). An analytical sample (0.5 mL) was silylated by addition of trimethylsilylimidazole (0.3 mL) at 50 °C for 5 min and analyzed by gas chromatography (Table 1).

Partly Methacrylated 2,1′:4,6-Di-*O*-isopropylidene Sucrose (2). Methacrylic anhydride (7.3 g, 47 mmol) was added to a solution of **1** (10 g, 24 mmol) in dry pyridine (200 mL) containing 4-methoxyphenol (20 mg), and the mixture was stirred at 65 °C for 3.5 h. Excess methacrylic anhydride was hydrolyzed by stirring the reaction mixture for 12 h at room temperature after addition of water (300 mL). The solution was extracted with EtOAc (4 × 200 mL), and the combined organic phases were washed with saturated bicarbonate solution (100 mL) and ice cold 10% NaOH (100 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated several times with toluene to remove residual pyridine. To prevent undesired, spontaneous polymerization, the obtained monomer mixture **2** (10.3 g, 81%, DS = 1.6), a yellow slurry, was stored as 70−80% solution in toluene until further use.

Partly Methacrylated 2,1′:4,6-Di-*O*-isopropylidene Sucrose (3, 4). The reaction and workup was conducted under the same conditions as for monomer mixture **2** using methacrylic anhydride (14.6 g, 95 mmol and 29.2 g, 190 mmol) and pyridine (200 and 40 mL) to give monomer mixtures **3** (13.3 g, 96%, DS = 2.3) and **4** (14.5, 92%, DS = 3.7), respectively.

3,3′,4′,6′-Tetra-*O*-methacryoyl-2,1′:4,6-Di-*O*-isopropylidene Sucrose. This derivative is one of the components of the novel monomer precursors of SUBPOL resins, which was isolated from mixture **4** by column chromatography on silica gel using $CHCl_3$ as eluant and characterized in detail by 2D NMR spectroscopy (See Supporting Information). TLC: R_f ($CHCl_3$) 0.21. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.22, 1.33, 1.40, 1.40 (4s, 12H, $(CH_3)_2C$); 1.88, 1.89, 1.89, 2.04 (4s, 12H, CH_3−C=); 3.51 (d, $^2J_{HH}$ = 12.31 Hz, 1H, H-1a′); 3.58−3.70 (m, 2H, H-4, H-6a); 3.70−3.78 (m, 1H, H-5); 3.83 (dd, $^3J_{HH}$ = 3.62 Hz, $^3J_{HH}$ = 9.10 Hz, 1H, H-2); 3.94 (dd, $^3J_{HH}$ = 4.55 Hz, $^2J_{HH}$ = 9.91 Hz, 1H, H-6b); 4.12 (d, $^2J_{HH}$ = 12.31 Hz, 1H, H-1b′); 4.30−4.45 (m, 2H, H-5′, H-6a′); 5.10−5.23 (m, 2H, H-3, H-3′); 5.41 (dd, $^3J_{HH}$ = 2.81 Hz, $^3J_{HH}$ = 4.15 Hz, 1H, H-4′); 5.50, 5.52, 5.61, 5.74 (4s, 4H, =CH_2); 6.02, 6.09, 6.11, 6.30 (4s, 4H, =CH_2); 6.05 (d, $^3J_{HH}$ = 3.62 Hz, 1H, H-1). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 18.2, 18.3, 18.3, 18.4 (4q, CH_3−C=); 18.9, 23.8, 25.4, 29.1 (4q, $(CH_3)_2C$); 62.0 (t, C-6); 64.1 (d, C-5); 64.5 (t, C-6′); 66.1 (t, C-1′); 71.0 (d, C-3); 71.4 (d, C-4); 71.8 (d, C-2); 77.2 (d, C-3′); 78.1 (d, C-4′); 80.2 (d, C-5′); 91.6 (d, C-1); 99.4−101.3 (2s, $(CH_3)_2C$); 104.9 (s, C-2′); 124.9, 125.9, 127.0, 127.9 (4t, CH_2=); 135.1, 135.3, 135.8, 136.4 (4d, CH_2=C); 166.1, 166.3, 166.4, 166.7 (4s, C=O). Anal. Calcd for $C_{34}H_{46}O_{15}$: C, 58.78; H, 6.62. Found: C, 58.30; H, 6.56.

General Procedure of Suspension Polymerization. The aqueous phase consisting of water (144 g), hydroxyethyl cellulose (MW: 300 000, DP: 1250, MS: 2.0, 0.23 g, 0.16 w %), and NaCl (14.4 g, 10 w %) was placed into a 250-mL parallel-sided flanged gastight glass vessel fitted with a metal stirrer carrying two impellers and purged with nitrogen for 10 min. The organic phase consisting of the monomer mixture (9 g, 50 w %), the porogen (9 g, 50 w %), and AIBN (0.1 g), purged with nitrogen, was added, and the agitation speed was adjusted to 350 rpm. The polymerization was carried out at 80 °C for 6 h. The spherical beads generated were filtered and washed with water (1 L) and methanol (300 mL). To remove unreacted monomers, the beads were extracted in a Soxhlet apparatus with methanol (150 mL) for 12 h and dried under vacuum. Yield: 5.5−7.5 g (60−83%). Particle size: 160−250 μm.

General Procedure for the Removal of Isopropylidene Groups. The polymer beads were suspended in 60% acetic acid (5 mL/g resin) and shaken at 60 °C for 4 h. The filtered beads were washed with water (300 mL) and methanol (300 mL), extracted in a Soxhlet apparatus with methanol (150 mL) for 12 h, and dried under vacuum.

Determination of Accessible Hydroxy Groups. Three polymer samples (100, 150, and 200 mg) were suspended in a mixture of trimethylsilylimidazole (0.38 mL) and pyridine (1.12 mL) containing fluorene (50 mg) as internal standard. The vials were sealed and heated to 80 °C for 12 h. After cooling to room temperature, the mixtures were analyzed by GC, and the developed imidazole was quantified using a calibration curve derived from methanol.

Mitsunobu Reaction. Phthalimide (530 mg, 3.6 mmol) was added under argon to a suspension of SUBPOL VIII resin (7.2 mmol OH/g) (500 mg, 3.6 mmol) in 1:1 dry DCM/THF (18 mL) containing PPh_3 (944 mg, 3.6 mmol). The mixture was cooled to 0 °C, and a solution of DEAD (567 μL, 3.6 mmol) in 1:1 dry DCM/THF (8 mL) was added over 1 min. The reaction mixture was stirred for 20 h at room temperature, and the resin was filtered, washed with DCM, DMF, NMP, methanol, and diethyl ether, and dried under high vacuum. Remaining hydroxy groups were protected by suspending the resin in dry THF (8 mL) with TBDMSCl (1.6 g, 10.8 mmol) and imidazole (1.5 g, 21.6 mmol). After shaking for 12 h at room temperature, the resin was washed with water, methanol, DMF, DCM, and diethyl ether and dried under vacuum. The phthalimide-protecting group was finally removed by treating the resin swollen in NMP (3 mL) with hydrazine hydrate (3 mL). After shaking for 12 h at room temperature, the resin was extensively washed with methanol, DMF, DMSO, water, DCM, and diethyl ether and dried under vacuum.

CNBr Activation and Diamine Coupling. A solution of CNBr (1 g) in dry acetonitrile (0.5 mL) was added to SUBPOL resins (I, III, IV, and VIII) (400 mg, ∼2.9 mmol OH) swollen in 1 M sodium carbonate solution (5 mL). After vigorously being shaken for 2 min, the resin was immediately filtered and extensively washed with water. The activated polymer was suspended in a solution of 1,6-diaminohexane (120 mg, 1 mmol) in 0.1 M bicarbonate buffer (5 mL, pH 9.5), and shaken at room temperature for 20 h. The amino-functionalized resin was filtered, washed with methanol, DMF, DCM, and diethyl ether (10 mL), and dried under vacuum. Remaining hydroxy groups were protected by treating the resin with a solution of TMSCl (2 mL) in pyridine (4 mL). After being shaken for 24 h, the resin was washed with DMF, DCM, and diethyl ether (10 mL) and dried under vacuum.

Determination of Cyanate Ester Groups. To freshly distilled pyridine (8 mL), a solution of pure HCl (1.6 mL, p.a.) was added under stirring at 0 °C within 2 min, and the solution was diluted with distilled water (0.4 mL). Barbituric acid (50 mg, 0.4 mmol) was dissolved in the pyridinium hydrochloride solution at room temperature and the CNBr-activated resin (50 mg) was added. The mixture was shaken at 40 °C for 30 min. An analytical sample of the supernatant (100 μL) was diluted with distilled water (5 mL), and the adsorption of the developed color complex was measured at 580 nm (ϵ = 20 200 M^{-1} cm^{-1}).

Periodate Oxidation and Reductive Amination. SUBPOL VIII resin (50 mg) was suspended in 0.4 M sodium periodate solution (1.5 mL), and the mixture was shaken in the dark at room temperature for 1 h. The resin was washed with water (5 × 2 mL) and suspended in 1 M ethanolamine buffer (2 mL, pH 9.9). After the mixture was shaken for 2 h, resulting imine groups were reduced by addition of a sodium cyanoborohydride solution (100 μL, 5 M in 1 M NaOH). The reaction mixture was shaken at room temperature for an additional 30 min, and the resin was washed with 2 N HCl, 10% NaOH, water, methanol, THF, and pyridine (2 mL). Remaining hydroxy groups were protected by treating the resin with TMSCl (0.5 mL) in pyridine (1 mL). After being shaken for 24 h, the resin was washed with DMF, DCM, and diethyl ether (2 mL) and dried under vacuum.

Quantification of the Amino Groups. The loading was calculated by reaction of a weighed amount of amino-functionalized, silylated resin (50 mg) with Fmoc-Gly-OH (74 mg, 0.25 mmol) using BOP (111 mg, 0.25 mmol), HOBt (38 mg, 0.25 mmol), and DIPEA (130 μL, 0.75 mmol) as activating agents in DMF (2 mL) at room temperature for 30 min. After repeating the reaction twice, Fmoc was removed by treating the resin twice with 25% (v/v) piperidine in DMF (2 mL) at room temperature for 15 min. The solutions were recovered and diluted with DCM for the determination of the UV absorbance at 300 nm (ϵ = 7800 M^{-1} cm^{-1}).

Batchwise Manual SPPS of Peptides kwiivw (5) and hlfffw (6). Peptide syntheses were carried out in parallel on two batches of amino-functionalized, silylated SUBPOL VIII-A resin and on Rink amide resin (each 50 mg, ∼25 μmol), respectively. Prior to peptide synthesis, Fmoc-Gly-OH and Fmoc-Ser(*t*Bu)-OH were coupled as an additional hydrophilic spacer. To enable analysis of peptides prepared on sucrose-based supports, the Fmoc-Rink linker (*p*-[(*R*,*S*)-α-[1-(9H-fluoren-9-yl)-methoxyformamido]-2,4-dimethoxybenzyl]-phenoxyacetic acid) was coupled on one batch of SUBPOL VIII-A resin directly after the Gly-Ser spacer. For each subsequent coupling cycle, a solution of the corresponding *N*-α-Fmoc-amino acid (125 μmol), activated by addition of BOP (55 mg, 125 μmol), HOBt (20 mg, 125 μmol), and DIPEA (65 μL, 375 μmol) in DMF (1.5 mL) was added to resin. The reaction mixture was shaken at room temperature for 30 min. After repeating the reaction twice, Fmoc removal was achieved by treating the resin twice with 25% (v/v) piperidine in DMF (2 mL) at room temperature for 15 min. Between each coupling and deprotection step, washings of the resin were performed with DMF and DCM (4 × 2 mL each). During peptide synthesis of kwiivwSG, each coupling step was monitored by determination of the UV absorbance of cleaved Fmoc groups to calculate the amount of coupled amino acid. Peptides immobilized on SUBPOL VIII-A resin via the Rink-linker and on the Rink amide resin were cleaved by treatment with reagent K[48] (2 mL) at room temperature for 3 h, precipitated in cold diethyl ether (25 mL) and separated by centrifugation at 3000 rpm. The carboxylic group remaining from the Boc-protecting group on the indole ring of tryptophan was removed by stirring the peptide in a mixture of water, methanol, and AcOH (4 mL, 2:5:1) at room temperature for 3.5 h. After dilution with water (15 mL), the crude products were characterized by MALDI-TOF. Peptide **5** (calcd mass: 843.09): found, 843.48 (SUBPOL VIII-A); found, 844.23 (Rink amide resins). Peptide **6** (calcd mass: 895.08): found, 895.62 (SUBPOL VIII-A resin); found, 895.48 (Rink amide resin). Purity of the crude products as determined by RP-HPLC was 79% for peptide **5** prepared on SUBPOL VIII, 77% for peptide **5** prepared on Rink amide resin, 82% for peptide **6** prepared on SUBPOL VIII-A resin, and 81% for peptide **6** prepared on Rink amide resin. Cleavage of the side-chain-protecting groups of permanently attached peptides was achieved by treating the resin with reagent K (2 mL) at room temperature for 3 h, followed by a washing cycle using DMF, DCM, and diethyl ether (2 × 2 mL each). The loading of the resulting peptide resin was calculated after Fmoc removal of the N-terminus by treating the resin twice with 25% (v/v) piperidine in DMF (2 mL) at room temperature for 15 min from the UV absorbance at 300 nm (ϵ = 7800 M^{-1} cm^{-1}). Loading for **5**-SUBPOL VIII-A resin and **6**-SUBPOL VIII-A resin was 440 and 470 μmol/g, respectively.

Semi-Automated SPPS of FMDV Peptide 7. The synthesis of the 19-mer FMDV peptide **7** corresponding to the sequence GSGVRGDFGSLAPRVARQL was prepared in parallel on two batches of SUBPOL VIII-A resin (50 mg, ∼25 μmol) using a multiple semi-automated peptide synthesizer.[51] To enable analysis of peptides by MALDI-TOF and RP-HPLC, the Fmoc-Rink linker was coupled on one of these two batches. For each coupling cycle, a solution of the appropriate *N*-α-Fmoc-L-amino acid (125 μmol) activated by addition of BOP (55 mg, 125 μmol), HOBt (20 mg, 125 μmol), and DIPEA (65 μL, 375 μmol) in DMF (2 mL) was added manually, while Fmoc deprotection with 25% (v/v) piperidine in DMF (2 mL) (2 × 15 min) and all washings between each coupling and deprotection steps were performed automatically. The N-terminus of the peptide was acetylated by shaking the peptide resin with acetic anhydride (300 μL) in pyridine (1.5 mL). Peptide immobilized on SUBPOL VIII-A resin via the Rink linker was cleaved by treating the polymer beads with a mixture of TFA/TIPS/DTT/water, 88:5:5:2 (2 mL) at room temperature for 3 h, precipitated in cold diethyl ether (25 mL), and separated by centrifugation at 3000 rpm. The peptide was redissolved in 100 μL AcOH and characterized after dilution with water (20 mL) by MALDI-TOF (calcd mass: 1984.27): found, 1984.80. The purity of the crude peptide was higher than 75%, as determined by RP-HPLC. The residue composition of the permanently attached peptide was confirmed by amino acid analysis: aspartic acid, 1.23; glutamine, 1.01; serine, 1.80; glycine, 4.60; arginine, 2.48; alanine, 2.27; proline, 1.09; valine, 2.18; leucine, 2.17; phenylalanine, 1.19.

Preparation of an Affinity Support by SPPS of the Fibrinogen-Binding Peptide GPRPK 8. The fibrinogen-binding pentapeptide GPRPK **8** was synthesized manually in the same manner as described for peptides **5** and **6** on SUBPOL resins I-A, III-A, IV-A, VIII-A, and VIII-B (50 mg, ∼25 μmol) and on amino-functionalized POEPOP resin. In the case of SUBPOL resin VIII-B and POEPOP−NH_2, Fmoc-6-aminohexanoic acid was coupled prior to SPPS of GPRPK as additional spacer. Removal of the side-chain-protecting groups of permanently attached peptides as well as the cleavage of peptides attached via the Rink linker (on SUBPOL VIII-A and VIII-B) was achieved by treating the resins with a mixture of TFA, water, and TIPS (90:5:5 v/v) at room temperature for 3 h. Workup and analysis of cleaved peptides were performed as described above. Masses determined by MALDI-TOF for peptides prepared on SUBPOL VIII-A and VIII-B were 553.47 and 553.98, respectively (calcd mass: 552.68). The purity of the crude products assessed by RP-HPLC was 84% for SUBPOL VIII-A and 77% for SUBPOL VIII-B. The amount of permanently attached peptides was calculated after removal of Fmoc-protecting groups via spectrophotometric determination at 300 nm: **8**-SUBPOL I-A: 280, **8**-SUBPOL III-A: 530, **8**-SUBPOL IV-A: 250, **8**-SUBPOL VIII-A: 470, **8**-SUBPOL VIII-B: 470, and **8**-POEPOP: 390 μmol GPRPK/g of resin.

Test for Fibrinogen Adsorption. The different adsorbents **8**-SUBPOL I-A, III-A, IV-A, VIII-A, VIII-B and **8**-POEPOP as well as the corresponding matrixes (SUBPOL I, III, IV, VIII, and POEPOP) were washed in 0.9% NaCl. Aliquots of 50 mg of individual adsorbents were weighed into Eppendorf tubes and to each aliquot, 450 μL of human plasma spiked with fibrinogen (final concentration 7.5 mg/mL) was added. After incubation for 60 min at room temperature, polymer particles were removed by centrifugation, and fibrinogen concentration was determined by nephelometry. Fibrinogen adsorption (mg/g adsorbent wet weight) was calculated from the difference in concentration found for the control (spiked plasma without adsorbent) and the samples (spiked plasma with adsorbent).

Biocompatibility of SUBPOL Resins. The polymers were tested for cytotoxicity with the EZ4U cell proliferation and cytotoxicity test kit (MTT test, Biomedica, Vienna, Austria). This test is based on the ability of living cells to reduce uncolored tetrazolium salts (MTT:

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into intensely colored formazan derivatives. As this ability is lost within a few minutes after cell death, the method provides an excellent tool to discriminate between dead and living cells. For the test, 10% (v/v) suspensions of the adsorbents in pyrogen-free 0.9% NaCl were incubated at 37 °C overnight. Adsorbents were removed by centrifugation, and 20 μL of incubation supernatant was added to L929 mouse fibroblasts grown in a 96-well plate. Each well of the plate contained 2×10^5 cells in 200 μL culture medium. Samples were incubated overnight at 37 °C in a humidified atmosphere containing 5% CO_2. The MTT assay was performed according to the instructions of the manufacturer. All assays were performed in triplicate.

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Supporting Information Available: Additional figures showing gas chromatograms of monomer mixtures **2**, **3**, and **4**, homo- and heteronuclear COSY NMR spectra of 3,3′,4′,6′-tetra-*O*-methacryoyl-2,1′:4,6-di-*O*-isopropylidene sucrose, FT-IR spectra of SUBPOL VIII resin (before and after treatment with TFA, piperidine, and hydrazine), RP-HPLC chromatogram of crude peptide **7**, and the determination of hydroxy groups by silylation (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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