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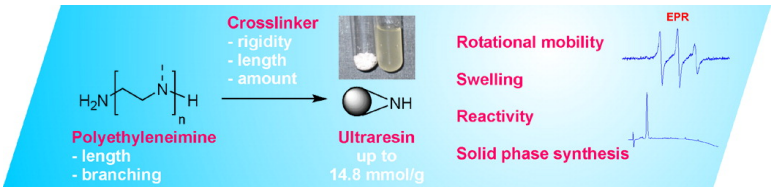

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Tailoring Ultraresins Based on the Cross-Linking of Polyethylene Imines. Comparative Investigation of the Chemical Composition, the Swelling, the Mobility, the Chemical Accessibility, and the Performance in Solid-Phase Synthesis of Very High Loaded Resins

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Ultraresins have been prepared from polyethyleneimines and cross-linking molecules and have been provided with various degrees of cross-linking. The total nitrogen loading and the loading with secondary and with tertiary amines have been determined in all products. Nitrogen loadings of the novel resins were up to 15 mmol/g, reactive secondary amines up to 13.8 mmol/g. In addition to the exceptionally high loading, the novel resins displayed efficient swelling volumes in polar and nonpolar solvents. The mobility of resin-bound species as determined by EPR-spectroscopy, depending on the amount of cross-linker, indicated good flexibility and reactivity of this resin type. The novel, high-loaded resins have been investigated subsequently in solid-phase synthesis. The Rink amide linker and two different hydroxy linkers (hydroxyacetamide, HMPB) have been attached to the resin. Despite the high loadings, the secondary amines were easily accessible and could be functionalized exhaustively. Reactivity of the linker-coupled resins was found to be closely related to the resin composition. Increased resin cross-linking led to reduced swelling, reduced mobility, and reduced reactivity in the synthesis of a medium-sized model peptide. As the result of the systematic investigation of structure−property relations in Ultraresins, a support material was identified that combined high reactivity and a mobility in the range of the extremely flexible Tentagel supports. In the optimized Ultraresin, >95% of all available secondary nitrogens could be coupled with Rink linker or with the small 2-hydroxyacetamide anchor, resulting in loadings from 2.7 to 6.8 mmol/g, respectively. A resin with an attached HMPB linker and spacer delivered analytically pure peptides in solid-phase synthesis, fully exploiting the exceptionally high loadings.

Introduction

Polymer-supported methods have contributed significantly to efficiency, speed, and flexibility in organic chemistry[1−3] and biochemistry. Heterogeneous-phase systems, such as polymer supports, other than homogeneous solutions, allow for the facile isolation of reagents, products, or intermediates by filtration. This facilitated phase separation between a supported and a solution phase built the foundation for most advanced applications in combinatorial chemistry and biochemistry.

When, however, one is taking a closer look at the "solid phase", the supported reaction systems become increasingly complex. The polymer−solvent−reagent system possesses various chemical and physical parameters that determines its performance in reactions and other applications and are usually the reason for success as well as for failure.[4,5] As a consequence, today's limitations in polymer-supported chemistry might be overcome at least in part by novel carrier materials tailored with an alternative property profile better suited for a defined purpose.

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Unfortunately, to date, most polymer-supported methods have been developed only for the prevailing carrier polymer, which is cross-linked polystyrene. Many alternative polymers have been prepared, but usually the literature describing their practical use is rather slim.[6,7] Cross-linked polystyrene by itself displays several well-described limitations. It tolerates only few solvents for swelling, tends to adsorb nonpolar reagents, and can be chemically modified or even destroyed, for example, under strongly acidic reaction conditions that are thereby excluded from their use in synthesis. As a consequence, some of the failures reported or not reported in polymer-supported reactions might actually be specific for the polystyrene system. Thus, extending the potential of solid-phase methods by introducing new carrier materials remains to be a promising endeavor.

Classically, polymer supports are prepared by a copolymerization of monovalent monomers together with a small percentage of bivalent monomers, which lead to the cross-linking of the polymer (Scheme 1A). Properties of the polymers can be tailored by varying the amount and the length of the cross-linking unit. Examples of this type of resin include cross-linked polystyrenes,[8−11] acrylamides,[12,13] acrylic esters,[14] epoxides,[15] and oxetanes.[16]

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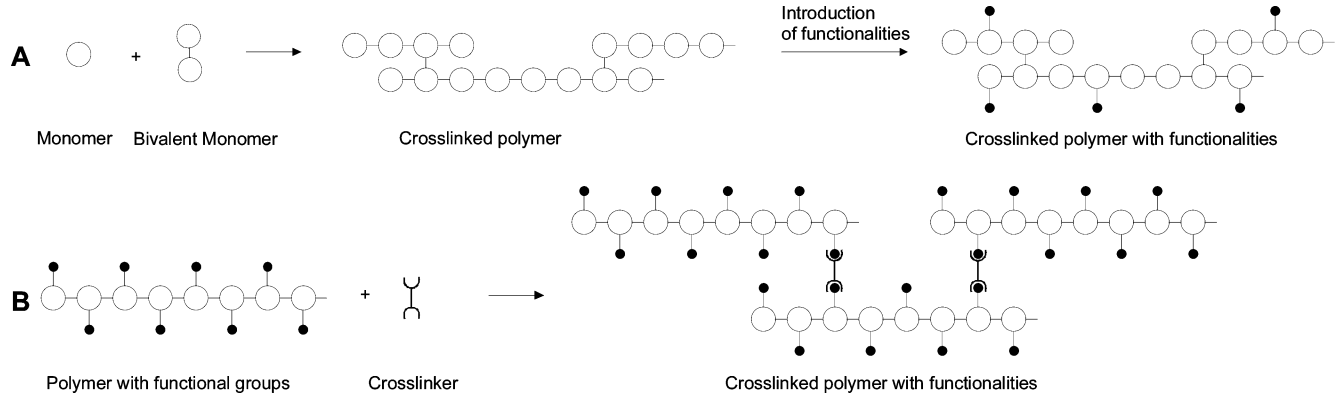
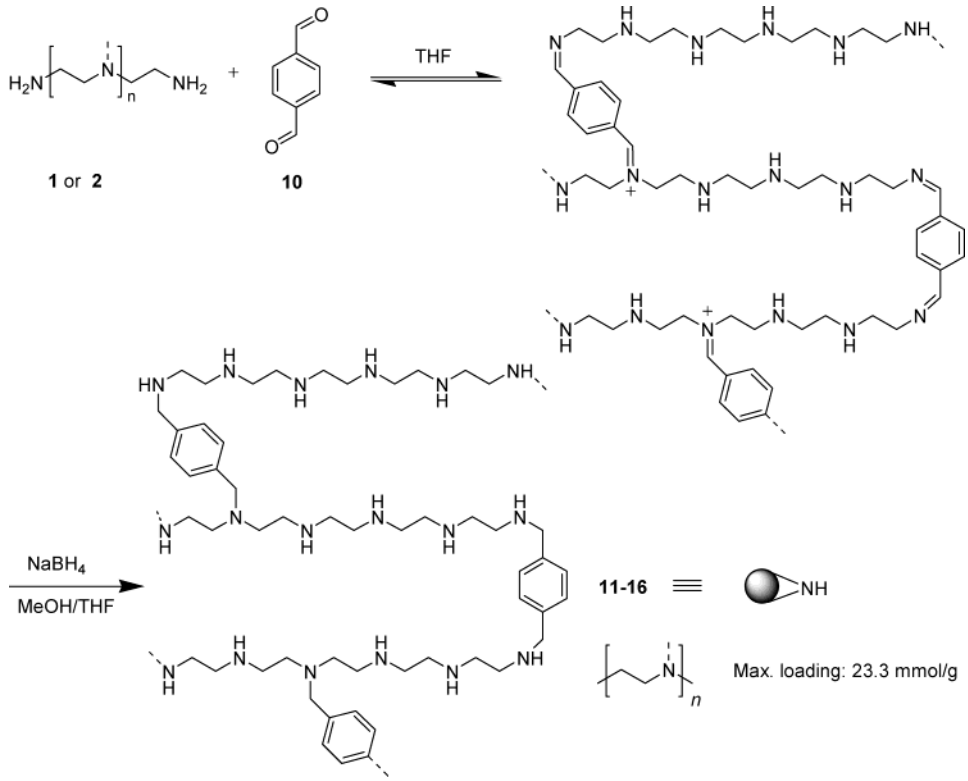
Scheme 1. Alternative Concepts for the Preparation of Cross-Linked Polymers[a]



[a] In the classical cross-linking polymerization (A), monovalent monomers react with bivalent monomers to yield the cross-linked polymer product. In most cases following the polymerization steps, functional groups are attached for use in solid-phase chemistry. In a second approach demonstrated in this article, the polymers were obtained from high-loaded soluble polymers that were connected with a bifunctional cross-linking molecule (B).

Scheme 2. Synthesis of Ultraresins **11**−**16**[a]



[a] Polyethyleneimines of various length and degree of branching (1, n = 5.27, 0.93 tertiary amines per chain; 2, n = 8.13, 1.52 tertiary amines) were cross-linked (see Scheme 3), preferably with terephthaldialdehyde **10**. The intermediary imine-network was reduced to yield the final resin product which was provided by sieving with defined size distribution.

Alternatively, polymer supports were obtained via the cross-linking of soluble polymers with a high density of functional groups, allowing for later derivatization (Scheme 1B). Whereas the second concept has been investigated for ion exchange[17−20] and enzyme immobilization,[21,22] only recently have polymers of this type been employed in the solid-phase synthesis and as polymer reagents.[23] Products of this polymer synthesis were named Ultragels or Ultraresins, respectively, referring to their extremely high loadings demonstrated for the cross-linking of polyethyleneimine (Scheme 2).

This preceding work indicated that several of the physical and chemical properties of Ultraresins should be variable over a broad range. Now in this article, the structure−property relationships of the novel class of polymer supports are investigated. For this purpose, the length of the starting polymer, the degree of cross-linking, and the nature of the cross-linker have been varied, and the resulting changes in swelling, rotational mobility, and chemical reactivity have been studied. Ideally, as a result of the obtained structure−property relationships in cross-linked polymers, criteria to select an optimized resin for a defined chemical or biochemical purpose are to be expected.

Results and Discussion

Polymer carriers with a high loading of reactive functional groups are desirable for several important reasons. High-loaded resins increase the efficiency and the atom economy[24]

of polymer-supported methods. The concentration of polymer-supported reactants is elevated, reducing the amounts of solvents and polymer backbone. Especially for scaling-up purposes, the increased space−time−yield is crucial for efficiency. In addition, higher concentrations of the supported reactants might accelerate reaction rates and increase yields.

In the case of the standard cross-linked chloromethyl polystyrene, the loading is limited by the molecular weight of the starting monomer. Furthermore, the practical loading is limited by Lewis-acid-catalyzed methylene cross-linking of chloromethyl groups within the resin. The loading of polystyrene resins has been enhanced by the attachment of branched structures carrying multiple functionalities (dendrimers).[25] However, the strategy requires additional multistep procedures and, thus, is comparably tedious. Living radical polymerization of immobilized benzyl ethers of tetramethylpiperidinyl hydroxide can be used to increase the loading of polystyrenes by grafting with styrene derivatives.[26]

In principle, the maximal loading of carrier materials is obtained in polymers constructed from low-molecular-weight functional monomers, such as poly(vinyl alcohol), polyethyleneimine, or polyvinylamine. Realizing this fact, polyethyleneimine was selected as the starting material for Ultraresins.

Characterization of Polyethyleneimines. The thorough characterization of the starting polymers was crucial for the reproducible synthesis of Ultragels. Polyethyleneimine (PEI) is the polymerization product of aziridine. PEIs are obtained as mixtures of polymers, characterized by distributions of the degree of polymerization and the degree of branching. Physical properties in solution and neat are influenced strongly by the pH and the salt concentration, as expected for a polymeric salt.

For the physical properties of a cross-linked polymer, the degree of branching of the starting polymer is a crucial parameter; however, by determining the ratio of primary, secondary, and tertiary amines, it will shape the chemical properties, as well.

The actual degree of branching in a PEI sample can be compared to the special case of statistical branching. In statistical branching, the aziridine monomers react with primary and secondary amines at equal rates. This kinetic of the reaction was simulated numerically, yielding after prolonged reaction time a polymer product with the ratio of primary, secondary, and tertiary amines approaching the limits of 1.61:1:1.61, corresponding to 38, 24, and 38% of the respective amines.[27] As a consequence, PEIs with a relatively high degree of reactive primary and secondary amines are to be expected among the low-molecular-weight polymers.

For the maximum loading of a cross-linked polymer with reactive groups and for a broad variability of the cross-linking degree, starting PEIs have to be selected with a low degree of branching. Linear PEIs as starting materials would be ideal; however, the preparation of purely linear PEI requires considerable synthetic effort and is not economical for resin synthesis. Therefore, low-molecular-weight PEIs displaying low branching at low costs were selected.

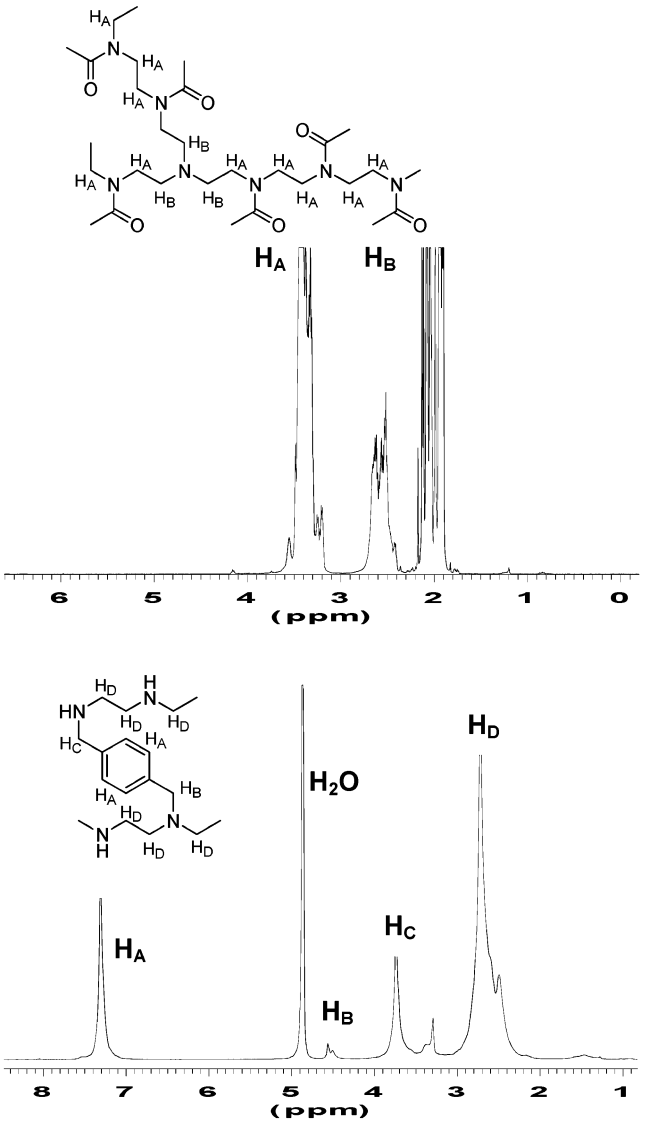


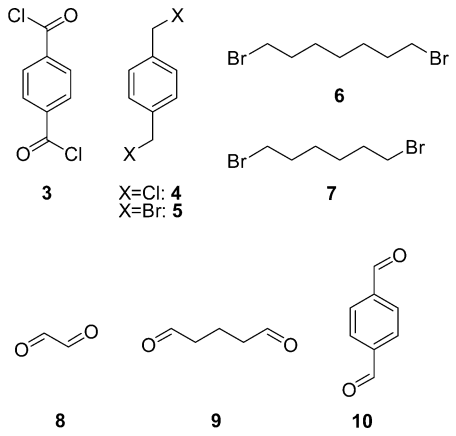
Figure 1. NMR spectroscopy of peracetylated PEI for determination of branching (top). The composition of Ultragels can be determined by HR-MAS NMR spectroscopy (below).

Conventionally, the size of PEI was determined by viscosimetry, light scattering, and size-exclusion chromatography.[28] In addition, in this work, the average molecular mass of PEIs was confirmed by elemental analysis, as described in a preceding publication.[23] The validity of the formula is not affected by a variation in the branching.

In a second step, the degree of branching was determined. For large PEIs, this has been reported by employing ^{13}C NMR spectroscopy.[29] For small PEIs, a method based on ^{1}H NMR was elaborated and was found to be more precise and convenient. For this, a sample of PEI was peracetylated employing acetic anhydride in pyridine. In ^{1}H NMR spectra, the signals of the PEI−CH_2 groups vicinal to an amide nitrogen are shifted downfield with respect to those vicinal to a tertiary amine (Figure 1, top). From the ratio of both types of PEI signals, the percentage of tertiary amines in the cross-linked PEI (Ultraresin) could be calculated.

Employing these tools of polymer characterization, two PEIs were selected for resin synthesis: PEI-1(**1**), with $n =$ 5.27 (244 Da) and 0.93 tertiary amines per chain, and PEI-2

Scheme 3. Dialdehydes, Dihalogenides, and Diacyl Chlorides Investigated for PEI-Cross-Linking[a]



[a] Dialdehydes address primary amines first, thus yielding a higher loading with reactive secondary amines. Flexible cross-linkers provided comparably soft polymers, whereas the rigid terephthaldialdehyde **10** produced mechanically and chemically robust resins.

(**2**), with $n = 8.13$ (367 Da) and 1.52 tertiary amines per chain. With statistical polymerization of aziridine (see above) 38% of tertiary amines would have been expected, resulting in 2.4 and 3.5 tertiary amines for PEIs of the respective molecular weight. Thus, **1** and **2**, although not fully linear polymers, display a significant reduction in branching that will be advantageous for subsequent functionalization and use as a highly loaded polymer in polymer-assisted solution-phase synthesis.

Synthesis of Ultraresins. Cross-linking PEIs was conducted by alkylation with dihalogenides **3**−**7** and by reductive amination with dialdehydes **8**−**10** (Scheme 3). Dialdehydes address primary amines selectively in the presence of secondary and tertiary amines. Thus, reductive amination yields the maximum loading with secondary amines, whereas primary amines can be completely converted. Therefore, this report will focus on dialdehydes as cross-linkers. Three dialdehydes were under investigation. The rigid terephthalic dialdehyde **10** provided the mechanically most stable resins by a reliable and robust protocol. Glyoxaldehyde provided no cross-linked product at all, and glutaric dialdehyde yielded soft, gelatinous materials, which was disadvantageous with respect to handling and pelleting.

Ultraresins **11**−**16** were synthesized with various ratios of cross-linker **10** and PEI, ranging from 1.2 to 2.8 molecules of cross-linker per PEI chain (Table 1).

^{1}H Gel-Phase MAS NMR Studies. The free amine resins **11**−**16** were investigated by high-resolution ^{1}H MAS NMR spectroscopy in MeOD. The signals of the PEI-methylene, benzyl-methylene, and aromatic protons were resolved and integrated in order to determine the ratio of cross-linker and PEI in the polymer networks (Figure 1, bottom). Benzylic protons are resolved into those vicinal to a secondary amine (Figure 1, bottom, protons C at 3.6−3.9 ppm) and those vicinal to a tertiary amine (Figure 1, bottom, protons B at 4.4−4.6 ppm).

The NMR analysis of the resins could be exploited to determine the amount of tertiary and secondary amines in the free amine Ultraresins.

Table 1. Synthesis of Ultragels **11**−**16** from **1** and **2** Employing Various Amounts of Cross-Linker **10**

resin	PEI	*n* (PEI) [mmol]	*n* (**10**) [mmol]	cross-linker/ PEI ratio	reaction time[a] [min]
11	**1**	17.2	25.6	1.5	13
12	**1**	17.2	29.1	1.7	10
13	**1**	17.2	36.2	2.1	15
	1	17.2	43.2	2.5	*b*
	2	11.5	14.1	1.2	*b*
14	**2**	11.5	17.6	1.5	10
15	**2**	11.5	24.8	2.2	10
16	**2**	11.5	32.0	2.8	60

[a] Time until magnetic stirring was terminated. [b] No polymer resin formed.

The ratios of PEI versus cross-linker measured by NMR were in good agreement with the amount of starting materials employed in the reaction, indicating full incorporation in the products (see Tables 1 and 2). Furthermore, the NMR data allowed the experimental verification of the amount of secondary and tertiary amines (Table 2).

Linker Attachment. For solid-phase synthesis, suitable linker molecules had to be attached to the Ultraresins. Moreover, the coupling of various linkers revealed valuable information about the synthetic accessibility of the high-loaded Ultraresins. Three types of linkers have been employed, varied with respect to their molecular weight, the steric demand, and the functional groups provided for solid-phase synthesis. The 2-hydroxyacetamide resins[30] **18**−**23** were constructed from 2-acetoxyacetyl chloride **17** at 0 °C in DCM in the presence of catalytic 4-(dimethylamino)-pyridine (DMAP). Subsequently to capping with acetic anhydride, deprotection of the *O*-acetyl groups was effected with catalytic sodium methoxide in MeOH. Loadings of the hydroxyacetamide resins were determined subsequently to acylation with Fmoc-glycine using *N*,*N*′-diisopropylcarbodiimide (DIC) with *N*-hydroxybenzotriazole (HOBt) as condensing reagent. Taking into account the mass increase of the resin, the loadings of the starting hydroxy resins were calculated. Loadings were between 4.6 and 6.8 mmol/g and were significantly higher than in standard hydroxy resins. The yields starting from the secondary-amine resins (four steps: acylation, deprotection, acylation, deprotection) ranged between 58% and quantitative.

Carrying an alternative, acid-cleavable hydroxy linker, the 4-(4-hydroxymethyl-3-methoxyphenoxy)butanoyl amide resins **25**−**30** were prepared.[31] Starting from 4-(4-acetoxymethyl-3-methoxyphenoxy)butanoic acid **24** with DIC and 7-aza-1-hydroxybenzotriazole (HOAt) for activation, followed by capping and deacetylation with sodium methoxide, the hydroxyresins **25**−**30** were obtained with loadings from 2.5 to 3 mmol/g. Again, these loadings were calculated after the coupling and deprotection of Fmoc-glycine. The corresponding yields (four steps) were between 71% and quantitative.

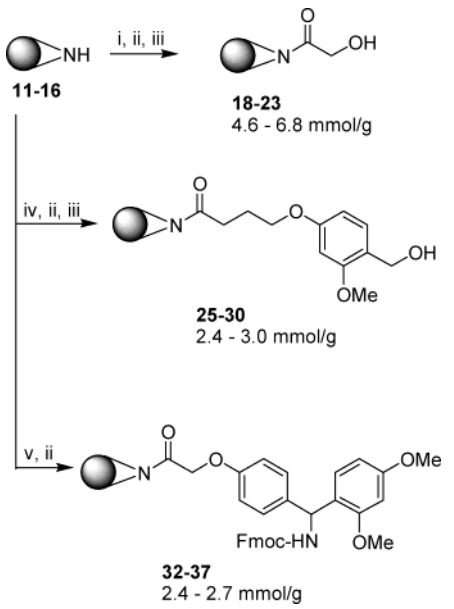
The Rink amide linker[32] as a diaromatic system had the highest steric demand within this comparative study. *p*-[α-[9*H*-Fluoren-9-yl)methoxyformamido]-2,4-dimethoxybenzyl]-phenoxyacetic acid **31** was coupled by DIC/HOAt activation, it was capped, and the loading could be determined by Fmoc cleavage without further derivatization. Loadings of the resins

Table 2. Composition of the Free-Amine Ultragels **11**−**16** Based on ^{1}H-MAS NMR Spectroscopy[a]

	integration		cross-linker per PEI			secondary amines	tertiary amines (mmol/g)	
resin	aromatic H	PEI−CH_2	exp	theor	ratio (exp/theor)	(theor) (mmol/g)	theor	exptl
11	28.3	100	1.49	1.48	1.01	12.8	2.2	3.1
12	31.3	100	1.65	1.69	0.97	11.1	2.9	3.3
13	36.2	100	1.91	2.10	0.91	8.4	4.6	4.8
14	20.3	100	1.65	1.53	1.08	13.8	2.5	2.7
15	25.3	100	2.06	2.16	0.95	10.8	3.8	3.1
16	32.2	100	2.61	2.78	0.94	7.9	5.5	3.9

[a] MeOD, 400 MHz.

Scheme 4. Accessibility of Ultragels for Derivatization, as Demonstrated by the Attachment of Linker Molecules[a]



[a] 2-Hydroxy acetamide resins **18**−**23**: (i) 2-acetoxyacetyl chloride (17), cat. 4-dimethylamino pyridine, DCM, 0 °C; (ii) acetic anhydride/pyridine/DMF 1:1:4; (iii) sodium methoxide 0.5 mmol/mL, THF/MeOH. HMPB resins 25-30: (iv) 4-(4-acetoxymethyl-3-methoxyphenoxy)butyric acid (24), *N*,*N*′-diisopropylcarbodiimide (DIC), 1-hydroxy-7-azabenzotriazole (HOAt), DMF; (iii). Rink amide resins **32**−**37**. (V) *p*-[α-[9*H*−Fluoren-9-yl)methoxy-formaido]-2,4-dimethoxybenzyl]phenoxyacetic acid (**31**), DIC, HOAt, DMF.

Table 3. Accessibility of Resins **11**−**16** Investigated by the Attachment of Linkers

ultra-sec-amine resin	2-hydroxyacetamide resins (mmol/g)[a]	HMPB resins (mmol/g)[a]	Rink-amide resins (mmol/g)[a]
11	**18:** 5.5 (74%)	**25:** 2.8 (86%)	**32:** 2.4 (91%)
12	**19:** 5.8 (86%)	**26:** 2.6 (81%)	**33:** 2.3 (90%)
13	**20:** 4.9 (87%)	**27:** 2.9 (quant)	**34:** 2.4 (quant)
14	**21:** 4.6 (58%)	**28:** 2.4 (71%)	**35:** 2.7 (quant)
15	**22:** 6.8 (quant)	**29:** 3.0 (94%)	**36:** 2.6 (quant)
16	**23:** 5.6 (quant)	**30:** 2.9 (quant)	**37:** 2.4 (quant)

[a] Calculated for the free hydroxy/free amine resin.

32−**37** based on the free amine were between 2.3 and 2.7 mmol/g and were significantly elevated in comparison to commercially available products. (Scheme 4, Table 3) With Rink linkers, coupling yields were close to quantitative in all experiments.

In summary, the results indicate that the influence of the degree of cross-linking on the accessibility of the resins' secondary amines is little. As expected, the loading with the different linker moieties was affected strongly by their molecular weight. The reduced theoretical loadings obtained

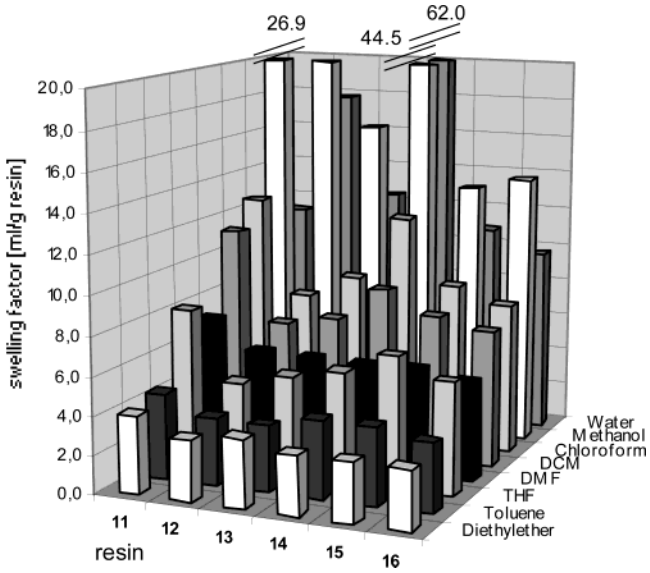


Figure 2. Swelling of the free-amine Ultragels have been measured in various solvents. Ultraresins displayed efficient swelling in polar and nonpolar solvents. Increasing the cross-linking led to reduced swelling. Increasing the size of the starting PEI (resins **14**−**16** vs **11**−**13**) had no strong impact on the swelling behavior.

for some of the hydroxy resins might be explained by inefficient esterifications carried out as the subsequent reaction step.

Swelling Volumes. Swelling volumes were determined for the free amine resins **11**−**16**, the hydroxyacetyl resins **18**−**23**, and the Rink resins **32**−**37** in various solvents (Figure 2, Table 4).

The swelling was strongly affected by the cross-linking; higher cross-linking strongly reduced the swelling in all solvents. The length of the starting PEIs, however, had no significant impact on the swelling.

Despite of their high polarity, the free-amine Ultraresins **11**−**16** swelled well in relatively nonpolar organic solvents, such as dichloromethane and even in diethyl ether, a solvent leading to complete collapse of polystyrene and PEG-containing resins. Swelling in protic solvents is very high for the free amine resins **11**−**16**, especially in water, and is further increased at lower pH.

Linker attachment had a strong influence on the swelling behavior of the Ultraresins. The small hydroxyacetamide linker on **18**−**23** displayed reduced swelling in polar and nonpolar solvents with the exception of DMF. The hydrophobic Rink amide linker on **32**−**37** led to strongly improved swelling volumes in nonpolar solvents.

Chemical Stability of Ultraresins. The chemical stability of the Ultraresins was investigated under various reaction

Table 4. Swelling of Amine-Free (**11**−**16**) and Linker-Coupled Ultragels (**18**−**23** 2-Hydroxy Acetamide Resins, **32**−**37** Rink Amide Resins) in Various Solvents[a]

resin	Et_2O	toluene	THF	DMF	DCM	$CHCl_3$	MeOH	water
11	4.0	4.4	8.1	7.1	11.3	12.6	26.9	11.2
12	3.1	3.5	4.6	5.6	6.5	7.5	20.3	17.8
13	3.5	3.4	5.2	5.5	7.0	8.7	16.5	12.4
14	3.0	4.0	5.7	5.4	8.8	12.1	44.5	62.0
15	3.0	4.0	6.8	5.5	7.6	8.7	13.5	10.8
16	3.0	3.5	5.8	5.1	7.1	7.9	14.1	9.6
18	2.0		2.7	3.4	3.0		3.4	4.0
19	3.0		3.9	5.9	4.4		4.9	4.9
20	2.1		3.3	6.4	4.2		4.6	3.3
21	2.8		3.8	9.2	4.8		4.8	5.6
22	2.8		3.3	5.6	5.6		4.5	4.5
23	3.1		3.7	7.0	4.4		4.4	4.2
32	3.8		6.6	10.3	8.0		5.6	5.6
33	4.1		4.6	6.9	6.4		4.1	3.7
34	3.4		4.9	5.9	5.9		3.4	2.9
35	2.9		6.9	11.5	10.0		4.6	3.4
36	4.4		6.6	9.3	7.1		4.4	4.9
37	3.9		6.8	8.2	7.3		3.9	3.4

[a] All swelling volumes are displayed in mL/g.

conditions. The free amine resin **14** was treated with butyllithium (2.6 M in heptane, 16 h, rt) and potassium *tert*-butoxide (3 M, 16 h, rt) without any change with respect to the resin's mass, swelling, and IR spectrum following extensive washing and drying. With methyl iodide/triethylamine, the resin was quaternized, yielding a polymer gel with an iodine content of 49%. Acid treatment (3 M HCl, 70 °C, 16 h) yielded the Ultraresin in its protonated form, displaying extended swelling in polar solvents. The protonated resin was easily reconverted to the free amine by treatment with sodium hydroxide. Lewis acids did not lead to any detectable resin destruction, as well. After $SOCl_2$ treatment (neat, 16 h, rt) resin **14** was swelling as before; the IR displayed the protonated polymer species with a 1.7-fold mass increase. TMS-triflate/acetic anhydride (v/v 1:2, no solvent, 16 h, rt) led to extensive acetylation of the amines (IR), a 3-fold mass increase, and good swelling behavior.

The HMPB resin **29** was treated with strong base (2.6 M butyllithium in heptane, 16 h, rt; 3 M KOH, 16 h, rt) and with methyl iodide (with triethylamine, 16 h, rt) without any change in the resin's mass, swelling, and IR-spectrum. By protic acid (3 M HCl, 70 °C, 16 h) and Lewis acids (neat SO_2Cl, 16 h, rt; TMS-triflate/acetic acid 1:2, 16 h, rt), the resin was colored red, presumably due to the formation of the dialkoxy-substituted benzyl. The swelling was decreased, whereas the resin mass was unaffected.

As a conclusion, the chemical stability of the Ultraresins is higher than that reported for resins constructed from ether linkages (PEG)[16] or for polystyrene.[39]

Mobility of Spin Labels Inside Ultraresins. Mobility of the reaction center has been identified as one important parameter determining the reactivity of polymer-supported species. Electron paramagnetic resonance (EPR) spectroscopy is an excellent method to measure mobility inside of swollen and nonswollen cross-linked polymers.[33,34] Paramagnetic probes attached to a polymer can be observed with high sensitivity, because the nonparamagnetic backbone does not contribute any signal to the EPR spectrum. Therefore, in contrast to NMR spectroscopy, no specific equipment is

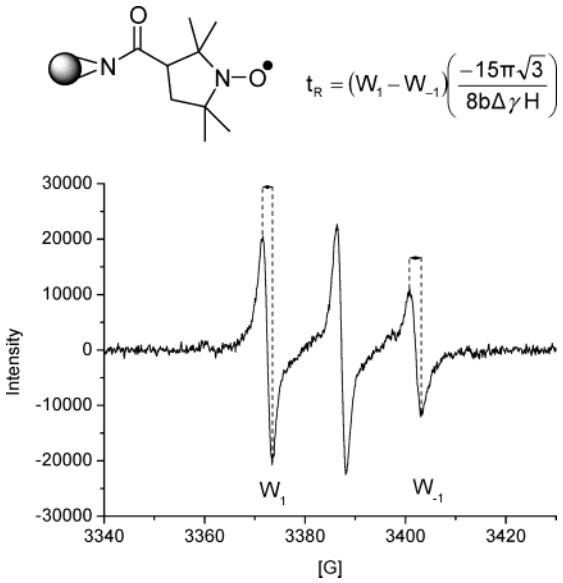


Figure 3. EPR spectroscopy of spin-labeled Ultragels was conducted to determine the mobility of reaction centers inside of the polymer. Rotational correlation times for the persistent radicals can be derived from the broadening of recorded EPR signals.[36] $H = 3383.6$ G (laboratory field); $b = 3.06 \times 10^{-8}$ s^{-1}; $\Delta\gamma = 4.22 \times 10^{-4}$ s^{-1} G^{-1}.

Table 5. Rotational Correlation Times for Spin-Labeled Resins **39**−**43**[a]

starting resin	resin	t_R ($\times 10^{-10}$ s)
11	**39**	14.4
14	**40**	7.4
15	**41**	14.4
polystyrene[b]	**42**	13.2
Tentagel[c]	**43**	5.8
solution		2.0

[a] See ref 36. [b] Aminomethyl polystyrene cross-linked with 1% divinylbenzene, 200−400 mesh, 1 mmol/g. [c] Polystyrene (cross-linked with 1% DVB) and grafted with ω-amino-poly(ethylene glycol), 0.2 mmol/g, 200−400 mesh. All measurements were recorded in chloroform.

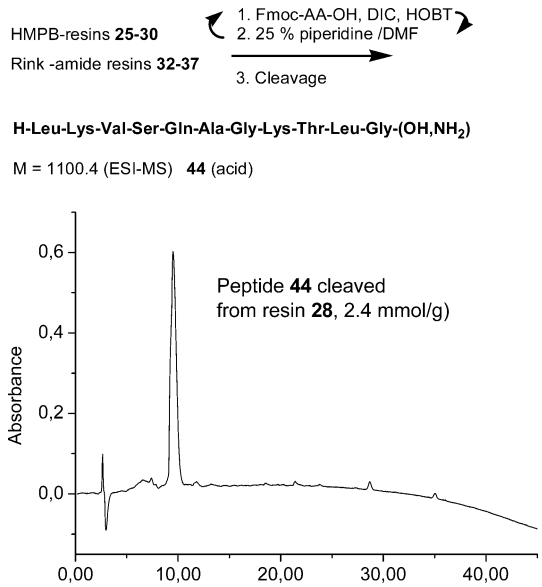
necessary to record spectra of polymer-supported spin labels. For peptide synthesis, it has been demonstrated that the rotational mobility detected by EPR correlated well with the reactivity of resin-bound species.[35]

According to the theory of Waggoner et al., EPR spectroscopy allows for the direct determination of rotational correlation times from the peak-broading in the EPR spectrum.[36] The method can be applied for swollen, cross-linked polymer gels.[37,38] A low concentration of spin labels is required in order to exclude signal broadening by dipolar interactions of the unpaired electrons.

Free-amine Ultraresins **11**, **14**, and **15** were coupled with 0.001 mmol/g 3-carboxy-2,2,5,5-tetramethylpyrrolidin-1-yloxy **38**, yielding the spin-labeled resins **39**−**41**. For comparison, the spin label was measured in solution and attached to aminomethyl(1% divinylbenzene/polystyrene) **42** and to amino-Tentagel resin **43**. EPR spectroscopy was conducted in $CHCl_3$, and the peak width of the triplet was determined and employed to calculate rotational correlation times as described (Figure 3, Table 5).

The increase of cross-linker between resins **14** and **15** was reflected in the rotational mobility of the spin label attached.

Scheme 5. Reactivity and Accessibility of Ultragels Carrying a Linker Moiety Investigated by the Synthesis of Peptide 44 and Peptide Amide 45, an Undecapeptide Sequence of Human Caspase[a]



[a] The low-cross-linked HMPB resin **28** was superior with respect to the purity of the crude product (HPLC, 214 nm).

In the case of the lower cross-linked resin **40**, a rotational correlation time of 7.4×10^{-10} s was recorded, which was in the range of the very mobile amino-Tentagel **43** (5.8×10^{-10} s). Increasing the amount of cross-linker in resin **15** led to a decrease in rotational mobility. For resin **15**, a rotational correlation time of 14.4×10^{-10} s was measured, close to the value found in 1% cross-linked aminomethyl polystyrene **42** (13.2×10^{-10} s). For comparison, the rotational correlation time of the spin label was determined in solution with 2.0×10^{-10} s.

Ultraresins in Solid-Phase Synthesis. The accessibility of Ultraresins was investigated in peptide synthesis on resins **25**−**30** and **32**−**37**. The 11-mer sequence LKVSQAGKTLG of the human caspase (acid **44**, $M = 1100.4$ Da; amide **45**, $M = 1099.4$) was prepared by Fmoc peptide synthesis employing 10 μmol of each resin. Coupling of amino acids was conducted for 90 min with a concentration of 0.1 mmol/mL Fmoc-amino acid activated with diisopropyl carbodiimide and *N*-hydroxybenzotriazole (HOBt) in dimethylformamide (DMF). Cleavage was conducted with 95% TFA, and 2.5% of both triisopropylsilane and water. Peptide isolation was conducted by triple trituration with diethyl ether, followed by lyophilization (tert-BuOH/water). Products were analyzed by HPLC, and purities were determined by UV at 214 nm.

Results (Scheme 5, Table 6) of the peptide synthesis indicated that the cross-linking and the choice of the linker moiety were crucial for the success of peptide synthesis. The main byproducts in the synthesis of peptides **44** or **45**, respectively, were the 10mer and 9mer formed by failed couplings to the valine in position 8. On the bulky Rink linker (resins **32**−**37**), significantly more byproducts were detected than on the more flexible HMPB resins **25**−**30**. The highest purity of the peptide **44** and the complete disappearance of the deletion products were observed for the HMPB linkers on the lowest cross-linked resins (**25** and **28**).

Table 6. Results of Peptide Synthesis on Various Ultragels (**25**−**30** HMPB Resins, **32**−**37** Rink-Amide Resins) and on Wang Polystyrene (PS)

		loading			crude	purity
entry	resin	(mmol/g)	(mg)	(μmol)	(mg)	(%)
1	**25**	2.8	3.6	10.1	11.3	100
2	**26**	2.6	3.9	10.1	11.9	98.4
3	**27**	2.9	3.4	9.9	9.9	97.1
4	**28**	2.4	4.2	10.1	13.4	100
5	**29**	3.0	3.7	11.1	11.3	92.2
6	**30**	2.9	3.5	10.2	11.0	85.1
7	**32**	2.4	4.2	10.1	9.2	92.7
8	**33**	2.3	4.3	9.9	9.4	90.7
9	**34**	2.4	4.3	10.3	12.2	90.8
10	**35**	2.7	3.9	10.5	14.5	88.3
11	**36**	2.6	3.8	9.9	11.1	85.3
12	**37**	2.4	4.3	10.3	12.5	82.1
13	PS	0.5	22.8	11.4	15.0	100

Conclusions

In this article, polyethyleneimines of varying length and linearity have been cross-linked with varying degrees of different cross-linkers. Physical and chemical properties of six selected Ultraresins (**11**−**16**) were investigated with respect to swelling behavior, rotational mobility, chemical accessibility, and their use in peptide synthesis.

The study suggests preferable resin compositions offering an optimized combination of chemical accessibility and reactivity with physical and chemical stability.

The optimized Ultraresin **14** displayed a loading of 14.8 mmol/g nitrogen in the free amine form. From the ^{1}H-MAS NMR, it could be concluded that 1.65 cross-linker per PEI was incorporated, and the resin loading with secondary amines was 13.8 mmol/g. The increase of cross-linker between resins **14** and **15** was reflected in the rotational mobility of the spin label attached. In the case of the lower cross-linked resin **40**, a rotational correlation time of 7.4×10^{-10} s was recorded, which was in the range of the very mobile amino-Tentagel resin (5.8×10^{-10} s). Increasing the amount of cross-linker in resin **15** led to a decrease in rotational mobility. For resin **15**, a rotational correlation time of 14.4×10^{-10} s was measured, close to the value found in 1% cross-linked aminomethyl polystyrene (13.2×10^{-10} s).

The mobility detected in resins **14** and **15** corresponded with excellent chemical reactivity of these supports. The free amine resin was readily accessible to chemical derivatization, and the Fmoc-Rink linker was attached quantitatively as determined by spectrophotometric Fmoc-determination (resin **14**, 2.7 mmol/g; **15**, 2.6 mmol/g). Even higher loadings were realized with the smaller HMPB resin **29** (3.0 mmol/g) or even on the hydroxyacetamide resin **22** (6.8 mmol/g).

For the synthesis of a medium-sized peptide **44** or **45**, resin **28** carrying the HMPB linker attached to resin **14** was superior to all other resins used in this study. Obviously, in this resin, the low cross-linking of the longer PEI, together with the butyric acid spacer, offered the potential swelling volume and the flexibility to host the fully protected peptides without the deletion of any of the amino acids during peptide

synthesis. Remarkably, the swelling volume of resin **14** increased largely during peptide synthesis, and the final resin product carrying the linker and the fully protected peptide displayed more than 10-fold the weight of the starting resin.

Thus, it can be concluded that Ultraresins based on polyethyleneimine cross-linking that combine very high loading with good chemical reactivity and accessibility can be prepared. Ultraresins offer exceptional properties that might render them favorable polymers for many established and perhaps future applications. Most prominently, the high loading of Ultraresins will be extremely advantageous in applications of polymer-supported chemistry, for solid-phase synthesis, as scavenger, and as a carrier for polymer reagents. The resins could be an important contribution to more economic solid-phase chemistry that might allow for scale-up, possibly even to the technical scale in the future.

Moreover, the novel resin type described and employed in this article might enable further applications based on their polar character in the biochemical and biological context. One obvious possibility is the use of Ultraresins as carriers of multivalent ligands, which is currently under investigation in our laboratory.

Experimental Section

General Procedures. Polyethyleneimines **1** and **2** were obtained from Sigma Aldrich. Solvents were purchased in HPLC grade. All the reactions were carried out in plastic syringes equipped with Teflon filters. The NMR measurements for the characterization of polyethyleneimine were made on a Bruker Avance 400 MHz spectrometer. High-resolution MAS NMR was recorded on a Bruker ARX 400. The FT-ATR-IR measurements were performed on a Bruker Vector 22 containing a split-pea unit.

Characterization of Polyethyleneimine 1 and 2. The degree of polymerization, *n*, was calculated from the N/C ratio obtained from elemental analysis [$n = 0.583/(\mathrm{N/C} - 0.583)$]. (**1**) C, 51.60; H, 11.72; N, 35.82%; ratio N/C = 0.694, $n = 5.27$, averaged molecular mass 243.65 g/mol. (**2**) C, 52.56; H, 12.16; N, 34.43%; ratio N/C = 0.655, $n = 8.132$, corresponding to an average molecular mass of 366.7 g/mol.

The number of protons in **1** and **2** was calculated from the degree of polymerization and the general formula of PEI and was compared with the results obtained from the integration of ^{1}H NMR spectra measured in $CDCl_3$. (**1**) Theory for $n = 5.27$: CH 21.08, NH 8.27. Results from ^{1}H NMR measurements: CH protons (multiplet between 2.53 and 2.14) 21.08, NH protons (singulet at 1.02 ppm) 7.45. (**2**) Theory for $n = 8.132$: CH 32.528, NH 11.132. Results from ^{1}H NMR measurements: CH protons (multiplet between 2.72 and 2.22) 32.528, NH protons (singulet at 1.16 ppm) 10.51.

Peracetylation of 1 and 2. (**1**) In a 20-mL flask equipped with a magnetic stirrer, **1** (224 mg, 5.74 mmol amines) was dissolved in DCM (5 mL) and cooled to 0 °C. After addition of acetic anhydride (662 μL, 7 mmol) and pyridine (563 μL, 7 mmol), the solution was stirred for 5 h at room temperature. After evaporation to dryness, a yellow to brown oil was obtained.

(**2**) In a 20-mL flask equipped with a magnetic stirrer, **2** (138 mg, 3.44 mmol amines) was dissolved in DCM (3 mL) and cooled to 0 °C. After addition of acetic anhydride (425 μL, 4.5 mmol) and pyridine (362 μL, 4.5 mmol), the solution was stirred for 5 h at room temperature. After evaporation to dryness, a yellow to brown oil was obtained.

NMR Measurements of Peracetylated 1 and 2. Peracetylated **1** and **2** (50 mg) was dissolved in $CDCl_3$ (0.7 mL). The integration area for the primary and secondary amines was between 3.52 and 3.12 for **1** and between 3.55 and 3.20 ppm for **2**. For the evaluation of the amount of the tertiary amines, the multiplets between 2.62 and 2.46 (for **1**) and 2.95 and 2.56 ppm (for **2**) were integrated. The ratio between tertiary amines and primary and secondary amines was 0.36 for **1** and 0.39 for **2**.

MALDI-TOF MS Measurements. The MALDI-TOF MS spectrum of **2** showed a mass distribution between 192.8 and 430.1 Da. For **1**, we obtained a better defined spectrum with mass peaks at 184.0, 192.1, 218.1, 314.9, 402.9, 430.5. 641.7, 668.9, and 696.8 Da.

Syntheses of Ultraresins

General Procedure. In a 50-mL flask equipped with a magnetic stirrer, polyethyleneimine **1** or **2** (PEI, 4.2 g) was dissolved in dry THF (10 mL). The corresponding amount of terephthalic dialdehyde **10** was dissolved in THF at a concentration of 0.83 mmol/mL and added rapidly to the PEI solution. The mixing generated heat up to 45 °C. After a recorded time (10 min up to 1 h), the stirbar ceased rotating. After 4 h, the polymer was crushed into small pieces (>1 cm) and reduced with $NaBH_4$ (3 equiv, calculated relative to the amount of **10**, in 100 mL of THF/MeOH, 2:1) for 16 h. To remove the excess of $NaBH_4$, the polymer was transformed to its hydrochloride salt by treatment with 1 N HCl for 1 h. The swollen polymer was filtered over a glass frit, washed with water, and stirred in 2 N NaOH for 15 min to convert the polymer to the free amine form. After washing with water, THF, and MeOH, the polymer was extruded through a metal sieve (pore size 400 μm). Again it was filtered over a glass frit, and the polymer was washed with THF and DCM and, finally, dried in vacuo over phosphorus pentoxide. The polymers were characterized with FT-ATR-IR spectroscopy, HR-MAS, and by elemental analysis. FT-ATR-IR: 1116, 1451, 1510, 2812, 2890, 3300 cm^{-1}.

11. Compound **10** (3.43 g, 25.57 mmol) was dissolved in THF (30 mL) and added to a solution of **1** (4.2 g, 17.24 mmol) in THF (10 mL). After 13 min, the stirring bar ceased rotating. Elemental analysis: C, 66.19; H, 9.14; N, 20.01; Cl, 0.07%. Yield 4.3 g (59.9%). ^{1}H-MAS suspension NMR (400 MHz, MAS with 4500 Hz, MeOD): $\delta = 2.2-3.0$ (m, PEI$-CH_2$, rel integration 100), 3.72 (bs, sec-N$-CH_2$-aryl, 25.9), 4.56 (bs, tert-N$-CH_2$-aryl, 0.94), 7.35 ppm (bs, aryl-H, 28.4).

12. Compound **10** (3.9 g, 29.07 mmol) was dissolved in THF (35 mL) and added to a solution of **1** (4.2 g, 17.24 mmol) in 10 mL THF. After 10 min, the stirring bar ceased rotating. Elemental analysis: C, 64.44; H, 12.3; N, 19.09; Cl, 0%. Yield 4.97 g (65.6%). ^{1}H-MAS suspension NMR

(400 MHz, MAS with 4500 Hz, MeOD): $\delta = 2.1-3.1$ (m, PEI−CH_2, rel integration 100), 3.72 (bs, sec-N−CH_2-aryl, 28.3), 4.52 (bs, tert-N−CH_2-aryl, 1.9), 7.0−7.5 ppm (bs, aryl-H, 31.3).

13. Compound **10** (4.85 g, 36.16 mmol) was dissolved in THF (45 mL) and added to a solution of **1** (4.2 g, 17.24 mmol) in THF (10 mL). After 15 min, the stirring bar ceased rotating. Elemental analysis: C, 67.59; H, 11.8; N, 18.53; Cl, 0%. Yield 4.51 g (53.7%). ^{1}H-MAS suspension NMR (400 MHz, MAS with 4500 Hz, MeOD): $\delta = 2.0-3.0$ (m, PEI−CH_2, rel integration 100), 3.72 (bs, sec-N−CH_2-aryl, 30.1), 4.54 (bs, tert-N−CH_2-aryl, 4.7), 6.9−7.4 ppm (bs, aryl-H, 36.2).

14. Compound **10** (2.36 g, 17.59 mmol) was dissolved in THF (25 mL) and added to a solution of **2** (4.2 g, 11.48 mmol) in THF (10 mL). After 10 min, the stirring bar ceased rotating. Elemental analysis: C, 61.55; H, 9.03; N, 20.73; Cl, 0%. Yield 4.72 g (75.6%). ^{1}H-MAS suspension NMR (400 MHz, MAS with 4500 Hz, MeOD): $\delta = 2.0-3.0$ (m, PEI−CH_2, rel integration 100), 3.72 (bs, sec-N−CH_2-aryl, 18.7), 4.54 (bs, tert-N−CH_2-aryl, 1.0), 7.0−7.5 ppm (bs, aryl-H, 20.3).

15. Compound **10** (3.33 g, 24.82 mmol) was dissolved in THF (30 mL) and added to a solution of **2** (4.2 g, 11.48 mmol) in THF (10 mL). After 10 min, the stirring bar ceased rotating. Elemental analysis: C, 64.87; H, 10.63; N, 20.35; Cl, 0.1%. Yield 4.25 g (60.0%). ^{1}H-MAS suspension NMR (400 MHz, MAS with 4500 Hz, MeOD): $\delta = 2.0-3.1$ (m, PEI−CH_2, rel integration 100), 3.72 (bs, sec-N−CH_2-aryl, 22.7), 4.53 (bs, tert-N−CH_2-aryl, 1.9), 7.0−7.4 ppm (bs, aryl-H, 25.3).

16. Compound **10** (4.29 g, 31.98 mmol) was dissolved in THF (40 mL) and added to a solution of **2** (4.2 g, 11.48 mmol) in THF (10 mL). After 60 min, the stirring bar ceased rotating. Elemental analysis: C, 66.4; H, 10.51; N, 19.21; Cl, 0%. Yield 3.8 g (48.1%). ^{1}H-MAS suspension NMR (400 MHz, MAS with 4500 Hz, MeOD): $\delta = 2.0-3.0$ (m, PEI−CH_2, rel integration 100), 3.72 (bs, sec-N−CH_2-aryl, 24.0), 4.52 (bs, N−CH_2-aryl, 6.2), 6.9−7.4 ppm (bs, aryl-H, 32.2).

Functionalization of Ultraresins 11−16. General Procedure. Three different linker systems were coupled to each Ultraresin, and the loading of the resin was determined by Fmoc analyis.

2-Hydroxyacetamide Resins (18−23). Ultraresin (100 mg) was swollen in DCM and cooled to 0 °C. 2-Acetoxyacetyl chloride **17** (484 μL, 4.5 mmol) and a catalytic amount of 4-(dimethylamino)pyridine (DMAP) was added. After 2 min, pyridine (483 μL, 6 mmol) was added slowly. The reaction was run for 30 min at 0 °C and then warmed to room temperature. After 3 h, the resin was washed with DCM, DMF, and DCM and dried in vacuo. The resin was capped with a solution of DMF/acetic anhydride/pyridine 4:1:1 for 16 h. After washing and drying again, no secondary and primary amines could be detected, as indicated by the Chloranil test and the Kaiser test, respectively. To remove the acetyl protecting group of the linker, the resin was treated with 0.5 mmol/mL sodium methoxide solution in THF/MeOH for 2 h. To determine the loading of the resins, **18**−**23** (50 mg) were coupled with Fmoc-glycine (297,3 mg, 1 mmol), *N*,*N*′-diisopropylcarbodiimide (DIC) (155 μL, 1 mmol), and *N*-hydroxybenzotriazole (HOBt) (153 mg, 1 mmol) in DMF. After 2 h, the resin was washed and coupled again with the same amount of reagents for 16 h. After washing with DMF and DCM, the resin was dried in vacuo. The loading was determined by cleaving the Fmoc group with 25% piperidine in DMF for 2 h and measuring a UV spectrum in the range between 250 and 320 nm.

4-(4-Hydroxymethyl-3-methoxyphenoxy)butanoyl amide resins (**25**−**30**). Ultraresin (100 mg) was coupled with 4-(4-acetoxymethyl-3-methoxyphenoxy)butyric acid **24** (424 mg, 1.5 mmol), *N*,*N*′-diisopropylcarbodiimide (DIC) (232 μL, 1.5 mmol), and 1-hydroxy-7-azabenzotriazole (HOAt) (0.5 mmol/mL in DMF, 3 mL, 1.5 mmol) in DMF for 16 h. After washing with DMF and DCM, the resin was dried in vacuo. The capping of the remaining amines, the deprotection of the acetyl group, and the determination of the loading were performed as described above.

Rink-Amide Resins (32−37). The procedure for the coupling of *p*-[α-[9*H*−fluoren-9-yl)methoxyformamido]-2,4-dimethoxybenzyl]phenoxyacetic acid **31** was the same as described above. The remaining amines of the resins were also capped with acetic anhydride. The loadings could be detected directly by cleaving the Fmoc group from the resins.

Determination of Swelling Factors for Ultraresins. A weighed sample of the resin was placed in a 2-mL syringe equipped with a Teflon filter. Solvent was added in excess, and the syringe was shaken (1 h). After removal of the supernatant, the volume of the swollen resin was determined. After each measurement, the resin was dried in vacuo. This procedure was repeated three times to determine the swelling factor in average.

EPR Measurements for Determination the Mobility in Ultraresins. General Procedure. 3-Carboxy-2,2,5,5-tetramethylpyrrolidin-1-yloxy **38** (Carboxy-PROXYL) (0.018 mg, 0.0001 mmol) was coupled with tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate (TBTU) (0.032 mg, 0.0001 mmol) and *N*-diisopropylethylamine (0.017 μL, 0.0001 mmol) in DMF (2 mL) to the free Ultraresin **11**, **14**, and **15** (100 mg); aminomethyl (1% divinylbenzene/polystyrene) **42**; and to amino-Tentagel resin **43**. After 16 h, the resin was washed with DMF and DCM and dried in vacuo. The spin-labeled resins **39**−**43** (30 mg) were transferred to the sample tube and swollen with $CHCl_3$. The EPR measurements were recorded on a Bruker ESP 300 E with an X-band of 9.8 GHz. To control the loading of the resins, the solution of the reaction was also investigated with EPR measurements, and in no sample was there found more than 5% of the initial amount of the spin label Carboxy-PROXYL.

Solid-Phase Syntheses on Ultraresins. For the synthesis of the peptide sequence LKVSQAGKTLG (**44**, $M = 1100.4$ Da as the acid, **45**, $M = 1099.4$ Da as the amide) HMPB (**25**−**30**) and Rink amide (**32**−**37**) Ultraresins (10 μmol) were used. Each amino acid (0.1 mmol) was coupled using DIC (15.5 μL, 0.1 mmol) and HOBt (15.3 mg, 0.1 mmol) in DMF (1 mL, $c = 0.1$ mmol/mL) for 1.5 h. Deprotection of the Fmoc group was performed for 30 min using 25% piperidine in DMF. Cleavage of the peptide from the resin was done with 95% trifluoroacetic acid (TFA), 2.5% triiso-

propyl silane, and 2.5% water for 3 h. After being precipitated with cold diethyl ether three times, the peptides were lyophilized from *tert*-butyl alcohol/water (4:1, v/v).

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(27) Simulation of PEI composition. The polymerization of aziridine was described by two reactions: Primary amines react with aziridine under formation of one primary and one secondary amine. Secondary amines react with aziridine under formation of one tertiary and one secondary amine. This reaction system was simulated starting from only primary amines under the assumptions that aziridine is always present in high excess, and the rates of both reactions are equal. Over time, the percentage of primary, secondary, and tertiary amines approached a limit of 38, 24, and 38%, respectively. The simulation was conducted by H.-J. Egelhaaf, University of Tübingen, employing the Origin program package. The simulated ratio was in accordance with the experimental findings for large, branched polymer (ref 29).
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