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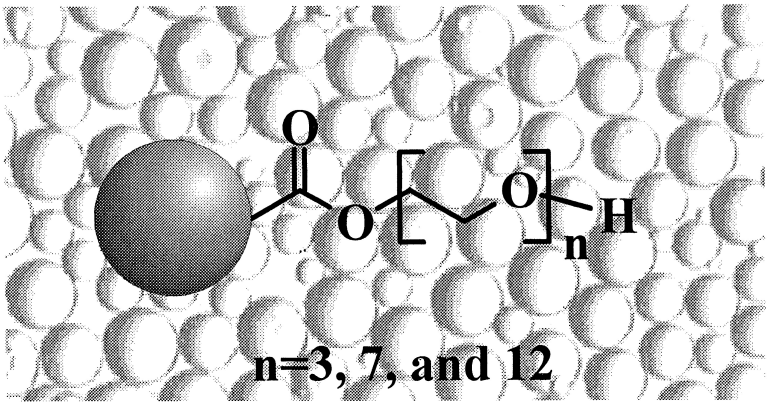

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Hydrophilic Polymer Supports for Solid-Phase Synthesis: Preparation of Poly(ethylene glycol) Methacrylate Polymer Beads Using "Classical" Suspension Polymerization in Aqueous Medium and Their Application in the Solid-Phase Synthesis of Hydantoins

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Lightly cross-linked hydrophilic polymer beads representing new types of supports for solid-phase synthesis have been prepared from commercial oligoethylene glycol monomethacrylates using an aqueous suspension polymerization process and specifically designed polymerization mixtures. These beads swell extensively in solvents with a wide range of polarities from dichloromethane, tetrahydrofuran, and water to dimethylformamide, and they enable high functional loadings of 1.2−1.8 mmol g^{-1}. Their ability to serve as supports was demonstrated in the model solid-phase synthesis of a small library of hydantoins. This four-step synthesis using primary amines readily affords yields of over 70%.

Introduction

Polymer-supported organic chemistry emerged about 4 decades ago as a result of Merrifield's pioneering work.[1] However, this field did not find any significant application outside the academic community for a number of years. The new wave of interest in this research area has been triggered by the advent of combinatorial chemistry in the early 1990s.[2,3] The vast majority of studies has been carried out using styrene−divinylbenzene beads as the support.[4−9] Only recently, some new supports have been developed and commercialized, which, in some respects, better address the requirements of contemporary solid-phase synthesis.[10,11]

The physical and chemical properties of solid supports play a decisive role in their use for a specific synthesis. The reactivity of a resin depends on both its chemistry and the environment in which the support is used.[2,3,12−15] Similarly, the access of reagents to reactive sites within the polymer matrix is critical for success of a synthesis.[16,17] For example, the pores of macroporous polymer beads, which are highly cross-linked materials characterized by a rigid structure that remains porous even in the dry state, enable access of reagents to the sites located within the bead in virtually any solvent.[18,19] However, the accessible reaction sites of these resins are located only on the surface of pores, thus making their loading capacity strongly dependent on their surface area and pore size. In contrast, the reactive sites of lightly cross-linked gels are accessible only after the beads are swollen in a suitable solvent. The typical Merrifield resin (polystyrene (PS) cross-linked with 1% divinylbenzene) swells best in low-polarity aromatic solvents such as benzene, toluene, halogenated hydrocarbons, and tetrahydrofuran (THF). For example, this resin absorbs about 8 mL/g THF,[16] thereby "opening" the matrix and enabling a number of reactions to be carried out. However, these beads do not swell in aliphatic hydrocarbons or polar solvents such as methanol (MeOH) and water. Therefore, the majority of reactive sites within the unswollen beads remains "hidden" and cannot be accessed easily. Since "on-bead" screening for biological activity is typically carried out in aqueous solutions, the use of polar solvents in solid-phase synthesis is a requirement for many of today's supports.

The problem of accessibility of reaction sites in a broad range of solvents is typically addressed by grafting hydrophilic poly(ethylene glycol) (PEG) chains onto hydrophobic polystyrene beads using anionic polymerization[20,21] or a reaction with preformed PEG chains.[22] For example, the commercial resins TentaGel and ArgoGel incorporate 60−80% PEG chains. These supports swell to a certain extent in solvents of any polarity. Another approach involves the polymerization of monomers more polar than styrene and divinylbenzene. Dimethylacrylamide,[23] 2-acrylamidoprop-1-yl(2-amidoprop-1-yl)-PEG_{300},[24] 1,4-bis(vinylphenoxy)butane,[25] and PEG based divinyl monomers such as trimethylolpropane ethoxylate triacrylate[26] are a few examples of these polar monomers and cross-linkers. Obviously, the swelling of a specific support also depends on the moieties that are attached to the reactive sites during the synthetic route leading to the desired product. The various effects that the resin exerts in solid-phase organic synthesis have been reviewed recently.[27]

Beaded polymeric materials are typically produced by suspension polymerization.[28,29] The "classical" approach involves stirring a mixture of an organic phase, most often monomers and solvents immisible with water, and a continuous phase, typically an aqueous solution of a suspension stabilizer. This approach is best suited for the preparation of polymers from monomers that do not significantly dissolve in water. This condition is met with monomers such as

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styrene and methyl methacrylate. In contrast, difficulties are usually encountered with hydrophilic monomers. Therefore, beads with hydrophilic functionalities are often prepared by polymerization of hydrophobic monomers followed by chemical modification (grafting) of the preformed beads (vide supra) rather than by direct polymerization. The "inverse" suspension polymerization process is an alternative approach in which an organic liquid, such as aromatic and aliphatic hydrocarbons[30] and perfluorinated solvents[31] that are immiscible with the dispersed hydrophilic polymerization mixture, is used as the continuous phase. This technique has been used for the preparation of beads from acrylamide and its derivatives[23] that cannot be polymerized using the "classical" process. However, implementation of this process tends to be difficult.

Polymeric supports with a well-balanced combination of both hydrophobic and hydrophilic functionalities are very desirable for solid-phase organic synthesis. However, the current multistep preparation techniques are less convenient than a direct preparation using a single-step suspension polymerization. Therefore, we report herein our approach to the preparation of lightly cross-linked polar polymer supports by "classical" suspension polymerization of commercial *hydrophilic* poly(ethylene glycol) methacrylates (PEG-MA) in an *aqueous* continuous phase. The potential of these beads is demonstrated in a solid-phase synthesis of a small library of aromatic hydantoins.

Results and Discussion

Preparation of Supports. The solubility of hydrophilic monomers in the aqueous phase is controlled by the partition coefficient, which is approximately defined as the ratio of the concentration of the monomer in the organic phase to the concentration in the aqueous phase. If this coefficient is close to zero, the monomer partitions predominantly in the organic phase, and because the concentration of the monomer in the aqueous phase is low, the classical suspension polymerization process in aqueous medium can safely be used. The PEG-MA monomers are rather soluble in water, and suspension copolymerization of these monomers with 2% ethylene dimethacrylate (EDMA) did not afford the desired beads. The actual value of the distribution coefficient depends on a number of factors including the composition of the organic phase and can be modulated by addition of a hydrophobic compound to the monomer mixture.[32] Therefore, we added 60% of cyclohexanol to polymerization mixtures to change the partition of monomers between the aqueous and organic phases. The addition of this solvent to the organic phase considerably decreases the concentration of PEG-MA in the aqueous phase and practically eliminates the concurrent and undesired solution polymerization of the monomers within the aqueous phase. Once the polymerization reaction is completed, cyclohexanol and other unpolymerized components of the reaction mixture are removed from beads by extraction using a series of solvents. This approach affords beads in yields that usually exceed 80% after the complete workup.

The glass transition temperature of these 2% cross-linked hydrophilic resins is below room temperature. Therefore, the beads containing PEG-MA monomers are tacky, thus resembling the commercial resins such as ArgoGel. The tackiness appears to increase as the length of PEG linkers increases. This also makes size fractionation of the beads difficult. Therefore, the PEG-MA supports were used without any additional sieving. Figure 1 shows an optical micrograph of the nonfractionated products and demonstrates their relatively narrow particle size distribution in a range of 50−100 μm. The IR spectra of the PEG-MA beads exhibit the expected absorptions at 1724 cm^{-1} for the carbonyl groups of the methacrylate ester units and at ~3500 cm^{-1} for the PEG terminal hydroxyl groups.

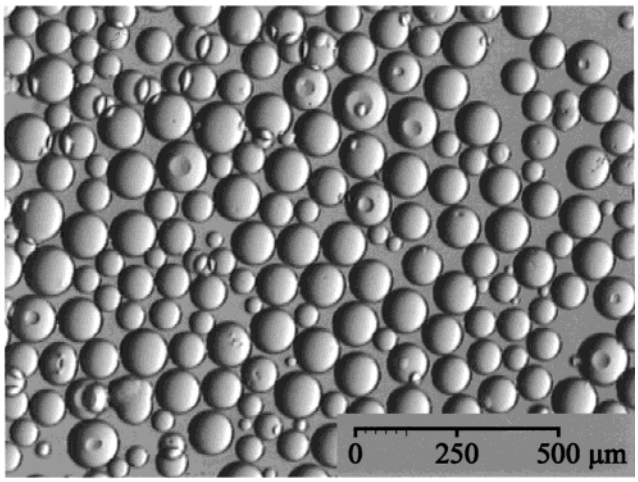


Figure 1. Optical micrograph of the nonfractionated PEG-MA beads.

Swelling in Solvents. The swellability of supports is an important feature that determines their applicability for solid-phase synthesis. Swellability is most often measured by comparing the visual readings for the volume of the beads in a graduated cylinder before and after swelling.[14,16,17,23,25,33,34] In our hands, this method afforded inconsistent results. Therefore, we used a more accurate approach based on the weight difference between dry and swollen beads, recalculating the data to express it as volume per weight values.[36] The bar diagram in Figure 2 shows that the PEG-MA beads swell in dichloromethane (CH_2Cl_2), THF, H_2O, and dimethylformamide (DMF), solvents that cover a broad range of polarities. A comparison of the swelling properties of the commercial PS resins with grafted PEG chains (Figure 2) indicates that PEG_x-MA and ArgoGel beads swell to a similar extent in all of the solvents, although the affinity for each individual solvent is different. For example, PEG-MA beads swell more in DMF while ArgoGel swells more in THF. The swelling properties of the PEG_{12}-MA beads are superior to those of all other supports tested. Since the overall content of ethylene glycol repeat units in the PEG_3-MA, PEG_7-MA, and PEG_{12}-MA beads is 11, 15, and 18 mmol/g, respectively, it is clear that the extent of swelling can be correlated to the overall content of PEG in the beads.

Solid-Phase Synthesis of Hydantoins. Solid-phase synthesis of hydantoins has been demonstrated several times using various approaches.[35] For testing the ability of our resin to perform as a support, we adapted the method developed by Kaldor et al.[35b] The four-step reaction sequence leading

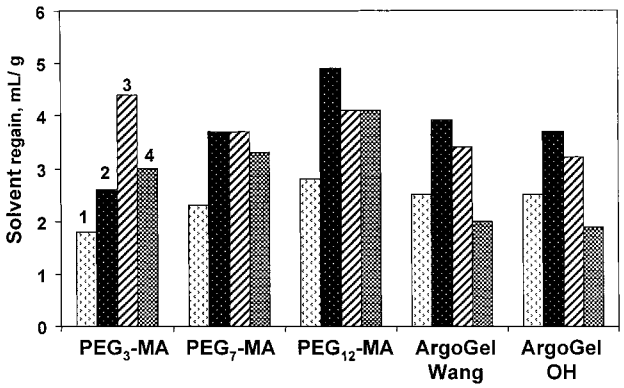


Figure 2. Swelling of PEG_x-MA and PEG-grafted ArgoGel beads in DMF (1), H_2O (2), THF (3), and CH_2Cl_2 (4).

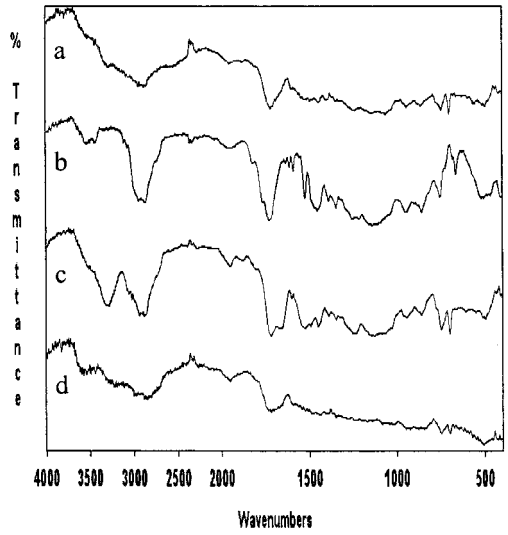
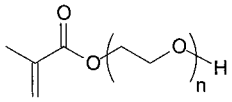


Figure 3. IR spectra of the PEG_7-MA resin activated using 4-nitrophenylchloroformate (a), reacted with amino acid (b), and reacted with amine (c) and of the resin after cleavage reaction (d).

to hydantoins (Scheme 1) was selected because it involves a variety of reactions and reagents in different solvents.

1. Activation of Support. The first step involves the formation of reactive 4-nitrophenyl carbonate moieties by reaction of the hydroxyl groups of the resins with a solution of *p*-nitrophenyl chloroformate in the presence of base. Obviously, the goal is to achieve as high a loading of the reactive functionalities as possible. Because the *N*-methylmorpholine suggested as the base for the reaction of PS-based resins[35b] afforded only very low yields with PEG-MA supports, the effect of different bases such as pyridine, (dimethylamino)pyridine (DMAP), and triethylamine (Et_3N), as well as solvents such as CH_2Cl_2 and THF, was explored. The pyridine hydrochloride salt formed during the reaction with pyridine is insoluble in all of the solvents and disqualifies this base for this synthesis. The DMAP salt also precipitates in THF but is soluble in CH_2Cl_2 at low concentrations. In contrast, the use of Et_3N in THF does not lead to the formation of any additional solid in the system and therefore constitutes the method of choice.

Figure 3a shows that the activation of beads **1** can be



1

PEG-MA, n = 3, 7, 12

followed by IR analysis. The carbonate absorption at 1768 cm^{-1} is clearly seen as a shoulder on the band of the ester carbonyl absorption. The strong nitro group absorptions at 860, 1348, and 1526 cm^{-1} are the primary indicators for a successful reaction. Though the hydroxyl absorption at about 3500 cm^{-1} decreased relative to the ester carbonyl, there are still hydroxyl groups left in the beads, which indicates that the extent of functionalization we can achieve is less than the theoretical amount calculated from the content of PEG-MA units in the resin.

2. Loading Capacities. Loading capacities of solid supports can be determined by reacting the beads with an excess of molecules containing a chromophore, removing the unreacted compounds by washing, and cleaving the chromophores, which are then detected using UV spectrophotometry. Obviously, the cleavage reaction should be quantitative to afford reliable values. For example, reaction with fluorenylmethoxycarbonyl (Fmoc) protected amino acid followed by cleavage with piperidine is used for the

Scheme 1

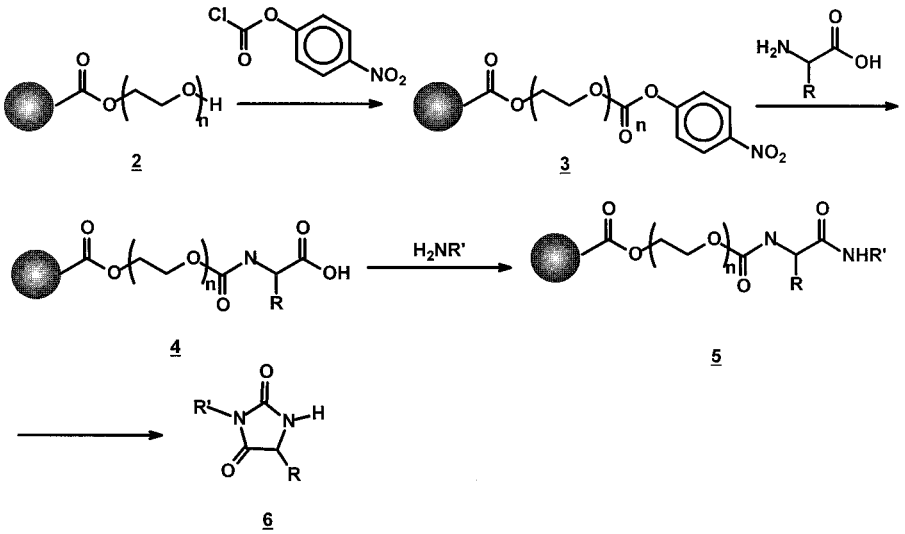


Table 1. Average Loading Capacities of Resins Determined by Using the Reaction with 4-Nitrophenyl Chloroformate Followed by Cleavage in Water or DMF[a]

	loading capacity of resin, mmol g^{-1}				
	PEG_3-MA	PEG_7-MA	PEG_{12}-MA	ArgoGel OH	ArgoGel Wang
cleavage in water	0.13	1.15	0.93	*b*	*b*
cleavage in DMF	1.88	1.27	1.03	0.47	0.40
calculated[c]	3.70	2.20	1.50		

[a] The results are an average of three separate experiments. [b] Polystyrene beads modified with chloroformate do not swell in H_2O. [c] Theoretical loading capacity calculated from the percentage of PEG_x-MA monomer in the polymerization mixture.

quantitation of loading capacities of typical Merrifield resin based supports.[3] This approach is popular because the addition of Fmoc protected amino acid is often the first step in the solid-phase synthesis of peptides.

Our procedure also uses the product of the first step of the reaction sequence shown in Scheme 1. The resin-containing hydroxyl groups is swollen in THF and allowed to react with a 10-fold excess of 4-nitrophenyl chloroformate in the presence of Et_3N. The polymeric nitrophenyl carbonate is then cleaved using basic conditions to release 4-nitrophenolate, and UV spectrometry is used to quantitate the release of the chromophore. In contrast to quantitation by elemental analysis of nitrogen, this method determines only the amount of 4-nitrophenol released from the resin and is not affected by the presence of other nitrogen-containing functionalities or trapped molecules such as Et_3N that may have remained within the beads. A solution of sodium hydroxide in DMF was used to promote the cleavage reaction and to release the nitrophenolate. Table 1 shows the data obtained for the loading capacities of PEG-MA supports as well as those determined for commercial resins. The latter data are in agreement with the values published by the manufacturer and indicate that our method is well suited for the determination of hydroxyl groups.

The actual loading capacities determined for the PEG-MA supports are only 51−67% of those expected from the percentage of polymerized PEG-MA monomer. This may result from a variety of effects including sterics, changes in reactivity due to reacted neighboring groups, and solvation ability of the polymer chains.[37] Despite the lower than theoretical loading capacities, all of the PEG-MA beads contain twice as many reactive hydroxyl functionalities as those of the commercial PEG-grafted PS resins.

3. Attachment of Amino Acid. The next reaction step is the coupling of L-phenylalanine to the activated resins. The amino acid is modified with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) to enhance its solubility in organic solvents such as THF, DMF, and *N*-methylpyrrolidone (NMP).[35b] Highest yields for the coupling reactions were obtained in DMF as a solvent.

The IR spectrum of the beads after the reaction is shown in Figure 3b. This spectrum does not exhibit the carbonate absorption at 1768 cm^{-1}, suggesting that reaction with the amino acid was complete. Small bands corresponding to the phenyl group with the distinctive aromatic overtones are observed at 1881 and 1953 cm^{-1}. A broad absorption in the range 3000−3700 cm^{-1} is characteristic of N−H and CO_2−H stretching vibrations.

4. Addition of Amine. The addition of benzylamine to the resin-bound amino acid is achieved using a standard diisopropyl carbodiimide (DIC) coupling procedure. We found that the order of addition of the reagents is an important factor that helps to reduce the formation of undesired side products such as the guanidyl derivative formed by reaction of DIC with benzylamine. This is a significant feature because the guanidines, when formed, cannot be removed from beads even after multiple washing with a variety of solvents. Our procedure involves a sequential addition of the reagents and works well for both the PEG-MA and commercial resins. The reaction with benzylamine was repeated twice to ensure complete functionalization and high yields.

The success of this reaction is confirmed by the appearance of a shoulder amide absorption at ∼1660 cm^{-1} in the IR spectrum of the beads (Figure 3c). Also, the broad absorption of the carboxylic acid groups was replaced by a well-defined N−H stretching vibration at 3288 cm^{-1}. The addition of this second aromatic moiety also increases the intensity of the aromatic overtones at 1881 and 1953 cm^{-1}.

5. Cyclization Cleavage of Hydantoin. The final step is the base-catalyzed cyclization of the amino acid−benzylamine adduct to form a hydantoin. Since this is accompanied by cleavage from the resin, the process can be monitored by ESI-MS in negative ion mode, NMR, and reversed-phase HPLC from which the yields can easily be evaluated. In contrast to the often used calculation of yields by relating the peak area of the desired compounds in the chromatogram to the sum of areas of all peaks, we calibrated the HPLC method measuring peak areas for different injected amounts of pure hydantoin. This method is not affected by differences in the magnitude of absorption coefficients of other compounds in the mixture and therefore is more accurate.

We confirmed that the cleavage from the commercial PS-based resins affords very good yields, using Et_3N in methanol (MeOH) at 65 °C.[35b] However, this approach proved to be ineffective with the PEG-MA resins because the yields did not exceed 10%. Even the use of other bases such as diisopropylamine, diisopropylethylamine, and piperidine did not improve the yields. Obviously, a stronger base is required for the reaction to occur with the alkyl carbamates originating from the PEG-MA resins compared to those of benzyl carbamates typical of the PS Wang resin. This is also the case for commercial resins because the ArgoGel Wang resin consistently affords higher yields of hydantoin than the ArgoGel OH support. This confirms the importance of the linker, which becomes the leaving group in the final reaction step and therefore has a considerable effect on the yields of the entire synthesis.

To achieve cyclization and cleavage, we used a stronger, nonnucleophilic base, 1,1,3,3-tetramethylguanidine. This

Table 2. Solid-Phase Synthesis of Hydantoin Using Various Supports[a]

resin	content of OH groups, mmol	yield mg	yield %
PEG_3-MA	0.19	42	79
PEG_7-MA	0.14	31	79
PEG_{12}-MA	0.13	27	74
hydroxymethyl PS	0.11	31	100
PS Wang	0.10	21	75
ArgoGel OH	0.05	3.5	28
ArgoGel Wang	0.04	8.5	75

[a] 100 mg of each support was used for the synthesis. For reaction conditions see Experimental Section.

compound has a pK_b value in aqueous solution close to 1, whereas the aliphatic amines tested earlier have pK_b in the range 3−4. Indeed, amounts as low as 0.5 equiv of guanidine afford yields of at least 70% for the PEG-MA resins. The overall yields shown in Table 2 were achieved with 1 equiv of guanidine. Only a small improvement was observed when the reaction was done using 5 or 10 equiv of guanidine. Therefore, no excess guanidine was used, thereby facilitating isolation because the removal of a large amount of nonvolatile base is difficult.

The changes in intensity in several peaks of the IR spectrum of the polymer beads after the cyclization reaction clearly reflect the cleavage that has occurred, but several bands that remain even after 48 h of reaction with guanidine confirm that some species have remained uncyclized and therefore polymer-bound (Figure 3d).

6. Purification of Product. Although the presence of the guanidine does not affect the results of the HPLC analysis, this base must be removed from the system to get a pure product. We tested three different methods for the removal of the tetramethylguanidine from the reaction mixtures.

(i) Use of Ion Exchangers and Scavenger Resins. The strongly acidic Dowex 50W X2 resin can be used in solvents such as H_2O, ACN, and MeOH. However, its use is not optimal because a large excess of resin is required to remove completely the guanidine. Similarly, the macroporous scavenging resin Combizorb containing isocyanate functionalities is not effective in removing the guanidine from the mixtures.

(ii) Solid-Phase Extraction. The Sep-Pak Plus solid-phase extraction cartridge packed with 400 mg of C18 silica beads and used in a way similar to that of reversed-phase HPLC proved to be effective for the product purification step. The practical capacity of the cartridge was determined by loading different volumes of the mixture onto the column followed by elution of the product. A very good separation of guanidine from hydantoin is achieved with a loading of up to 5 mg of the crude product mixture. The mixture is loaded onto the cartridge using 0.3 mL of a 50:50 ACN/H_2O mixture, then the guanidine is eluted first with 2 mL of 10% aqueous ACN, followed by the hydantoin using a 50:50 ACN/H_2O mixture.

(iii) Flash Chromatography. A column packed with about 20 mL of silica gel enables the separation of up to 50 mg of crude product containing both hydantoin and guanidine. The most effective solvent system is a 1:1 ethyl acetate/hexanes mixture. Since the guanidine is very polar, it is fully retained on the silica, and this purification approach also proved to be satisfactory.

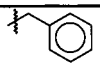
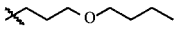
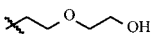
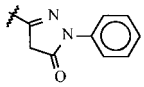
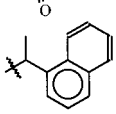
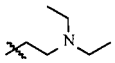
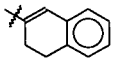
Comparison of Supports. Our experiments with the model synthesis of hydantoins have demonstrated that PEG_x-MA beads are well suited for application in some solid-phase syntheses. In our model system, the new beads afforded significantly higher quantities of product per unit weight of resin than can be achieved with the commercial PEG-grafted polystyrene supports we tested. Although yields of up to ∼77% can also be achieved using the ArgoGel OH resin, the total amount of the product was always lower than that obtained using the same weight of PEG-MA beads. This can be attributed to the difference in contents of hydroxyl groups in the beads, which, in turn, is a function of the length of the PEG linker. While ArgoGels typically contain 60 PEG units per hydroxyl end group, the PEG-MA beads contain linkers that are only 3−12 ethylene glycol units long. Thus, the long PEG linker of the ArgoGel decreases the loading capacity per unit weight because they mainly contribute to the mass and swelling characteristics of the support and much less to its functionality. In contrast to the highly hydrophobic polystyrene matrix of typical supports, the PEG-MA beads are formed from more hydrophilic methacrylates, and therefore, the PEG chains can be shorter while still affording the desired swelling properties. As a result, the PEG-MA resins have significantly higher loading capacities.

Library of Hydantoins. We also prepared a small parallel library of hydantoins using various amines to demonstrate the suitability of the PEG-MA resins for combinatorial solid-phase synthesis. The 11 different amines shown in Table 3 were used in step 3. Each product was analyzed by HPLC, and the products that appeared to contain hydantoins were resynthesized on a larger scale. Individual hydantoins were then isolated by flash chromatography, and their structures were verified by NMR spectroscopy. Although HPLC can be used to determine the presence of products, an exact quantitation of yields would require construction of a calibration curve for each hydantoin. Table 3 also indicates that the success of the hydantoin synthesis largely depends on the amine structure. For example, hydantoins were not obtained when reactions of either aromatic or secondary amines were attempted. In contrast, the desired hydantoins were easily prepared in high yields from most of the primary amines unless another nucleophilic moiety such as a hydroxyl group was also part of the molecule.

Conclusions

This work demonstrates that hydrophilic supports can be prepared by "classical" suspension polymerization using an aqueous continuous phase with polymerization mixtures that contain commercial oligoethylene glycol monomethacrylates, ethylene dimethacrylate, and an organic solvent. Although hard to compare exactly, these PEG-MA beads appear to be less tacky compared to the commercial resins we tested. They are well suited for some types of solid-phase-supported organic synthesis. In a model reaction these beads afforded significantly higher quantities of product per unit weight of resin compared to those achieved with commercial PEG-grafted polystyrene supports. Obviously, the ester chemistry

Table 3. Solid-Phase Synthesis of a Library of Hydantoins Using PEG_3-MA Support and Different Amines[a]

R:	product, mg	% yield
	28	68
	20	44
	35	88
	23	62
	0.8[a]	2[a]
	1[a]	2[b]
	1[a]	2[b]
	0.8[a]	2[b]
	0.8[a]	2[b]
	0.8[a]	2[b]
	0.9[a]	2[b]

[a] For reaction conditions see Experimental Section. [b] Estimated from HPLC analysis.

of our PEG-MA beads has intrinsic limitations because it may not be compatible with some reagents. However, the polymethacrylates are known to be very resistant to both acid and base hydrolysis.[38]

Although the vastly simplified concept of the direct preparation of hydrophilic supports featuring a high loading capacity was successfully demonstrated with only three different monomers, suspension polymerization of a large number of other hydrophilic methacrylate monomers incorporating a wide variety of functionalities is conceivable. Extension of our technique to these new monomers will allow for the preparation of supports tailored for specific applications such as solid-phase synthesis, solid-phase-assisted solution-phase synthesis, solid-phase extraction, and catalysis.

Experimental Section

General. The poly(ethylene glycol) monomethacrylate monomers, with an average length of the poly(ethylene glycol) chains of 3.5, 7, and 12.5 (abbreviated as PEG_3-MA, PEG_7-MA, and PEG_{12}-MA, respectively) were purchased from Sartomer (Exton, PA) and Aldrich and used as delivered. The average numbers of ethylene glycol units were determined using NMR spectroscopy (Bruker AMX300). The commercial resins were obtained from Argonaut Technologies (San Carlos, CA) and NovaBiochem (San Diego, CA). All other reagents were purchased from Aldrich and NovaBiochem and used as received unless otherwise noted.

Table 4. Typical Polymerization Mixture Used for the Preparation of PEG-MA Beads by Means of "Classical" Suspension Polymerization Charged to the 250 mL Reactor

component	weight, g
Aqueous Phase	
demineralized water	90.0
poly(vinylpyrrolidone) (MW 360 000)	0.9
sodium dodecyl sulfate	0.06
sodium sulfate	0.04
potassium nitrite	0.05
Organic Phase	
PEG-MA monomer	24.5
ethylene dimethacrylate (2% cross-linking)	0.5
cyclohexanol	36.0
azobis(isobutyronitrile)	0.27

Suspension Polymerization. The aqueous phase, which was used as the suspension medium and consisting of purified H_2O, poly(vinylpyrrolidone) (MW 360 000), sodium dodecyl sulfate, sodium sulfate, and potassium nitrite, was placed in a 250 mL jacketed glass autoclave reactor (Buchi BEP 280, Switzerland). The organic phase including the monomers, solvent, and initiator (azobis(isobutyronitrile)) was then added, and the complete polymerization mixture was deaerated by purging with nitrogen for 10 min. A typical composition of the reaction mixture is shown in Table 4. The reactor was sealed, and the stirring rate was adjusted to 350 rpm. The polymerization reaction was then carried out at 70 °C for 20 h. The resulting beads were repeatedly decanted in hot water to remove the surfactant and stabilizer until the supernatant liquid was clear. The unreacted components were removed from the beads by extraction with MeOH, ACN, and THF for several days, and the beads were dried at room temperature in a vacuum.

Determination of Swelling. About 200−300 mg of resin was weighed into a polypropylene tube (syringe barrel) fitted with a frit at the bottom. These tubes were attached to a manifold enabling the application of vacuum through the frit to remove liquids from the bead beds. Then 4 mL of DMF, H_2O, THF, and CH_2Cl_2 each were added to the beads. The solvent was allowed to equilibrate with the beads for about 20 min and then was removed using vacuum from the manifold. This process was repeated three times. After the last swelling, the vacuum was applied for another 30 s to remove any remaining solvent that may have been trapped at surface of the beads and within the frit. The syringe containing the swollen beads was weighed again, and the weight difference was converted into the volume of solvent retained per gram of beads.

Hydantoin Synthesis. The Quest synthesizer (Argonaut Technologies, San Carlos, CA) was used for all reactions. The optimized synthetic path shown in Scheme 1 involves four reaction steps.

1. Activation of the Support. First, Teflon reaction vessels were charged with 100−200 mg of resin and attached to the synthesizer. A 10-fold excess of *p*-nitrophenyl chloroformate with respect to the theoretical content of hydroxyl groups was added to the resin in each reaction vessel

followed by addition of 4 mL of CH_2Cl_2, and the contents were stirred for about 1 h under a nitrogen atmosphere. After the chloroformate dissolved, Et_3N (half of the molar amount of chloroformate) was added. The reactions were allowed to proceed overnight under nitrogen. The liquid was removed from reaction vessels, and the resins were washed three times with each of CH_2Cl_2, THF, CH_2Cl_2, and DMF.

2. Attachment of Amino Acid. L-Phenylalanine was dissolved in a mixture of BSA (4 equiv) and DMF following a procedure published elsewhere.[35] The mixture was allowed to react for 1 h and was then added to the resins. DMAP (2 equiv) was added last, and the reaction was carried out for 48 h. This order of addition of the components is a critical factor for the success of this procedure. After reaction, the liquid was removed from the reactors and the resins were washed three times with each of CH_2Cl_2, MeOH, THF, and DMF.

3. Addition of Amines. Various amines were added to the carboxylic acid functionality of the polymer-supported phenylalanine using a standard carbodiimide coupling procedure. First, hydroxybenzotriazole (HOBt, 4 equiv) was dissolved in DMF, and this solution was transferred to the vessel containing the resin. After 1 h, DIC (4 equiv) was added, and the mixture was stirred for another hour. Finally, an amine (4 equiv) was added, and the reaction was allowed to proceed overnight. Once the reaction was completed, the liquid was removed and the resin was washed three times with each of CH_2Cl_2, MeOH, THF, and DMF. To enhance functionalization of the resin, the reactions with amines were repeated twice.

4. Liberation of Hydantoin. An organic base (0.25 mL) and a solvent (1.5 mL) were added to each reaction vessel containing the beads from previous steps. The reaction was allowed to continue for 48 h at 65 °C. After the reactions were completed, the supernatant solutions were drained into preweighed vials. The beads remaining in the reactor were extracted twice with 2−3 mL of MeOH, and the extracts were also collected in the vials. The solvent was removed under vacuum, affording the crude product. The weight of the product was used for calculation of the overall yields. The hydantoin yields were determined either using HPLC analysis of the crude product mixture or after purification by flash chromatography.

Determination of Accessible Hydroxyl Groups. The chloroformate-modified resin from step 1 (40−60 mg) was placed into a 7 mL glass vial, and 0.5 mL of aqueous 1 M NaOH solution and 1.89 g (2 mL) of DMF were added. The hydrolytic reaction was carried out over 24 h using gentle mixing by rotation. Then 10−50 μL of the supernatant solution in the vials was transferred to 10 mL volumetric flasks partly filled with DMF, and the total volume was adjusted to 10 mL with DMF. The UV spectrum was recorded with a Cary50 UV−vis spectrophotometer, and the concentration of nitrophenol in solution was calculated from the peak height at 435 nm using a calibration curve.

Reversed-Phase Chromatography. The HPLC system consisted of a 510 HPLC pump, a 150 mm × 4.6 mm i.d. Symmetry column packed with 3.5 μm C18 silica beads, and a 486 UV detector (λ = 230 nm) controlled by Millennium 2010 software (all Waters Co.) All separations were carried out using a mobile phase consisting of 40% water containing 0.1% trifluoroacetic acid and 60% ACN at a flow rate of 1 mL/min. Pure hydantoin, which was prepared separately and purified by flash chromatography, was used to construct a calibration curve used for quantification of the HPLC measurements.

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