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Registry No. 1, 30614-73-4; 2, 71041-45-7; 3, 71060-27-0; 4, 71041-46-8; 5, 71041-47-9; 6 methyl ester, 71041-48-0; 7, 71060-26-9; 8 methyl ester, 71041-49-1; 9, 6141-57-7; 10, 71041-50-4; 11 methyl ester, 71041-51-5; 12, 51080-20-7; 13, 71041-52-6; 14 methyl ester, 71041-53-7; valeric acid anhydride, 2082-59-9; 1-chloro-9-iodononane, 57152-87-1.

An Entirely Beaded Poly(dimethylacrylamide) Support for Peptide Synthesis

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During the last decade extensive experience with polystyrene-based resins used in the Merrifield method of peptide synthesis[1] has shown that the insoluble support has a dynamic influence on the synthesis of the peptide attached to it. Unfortunately, the physiochemical incompatibility of polystyrene with the attached peptide negatively influences mass transport of reagents, solvation of polymer matrix and attached peptide, and reaction rates (acylation as well as deprotection).[2] In extreme cases this incompatibility has even led to disintegration of the polymer beads at some stage of the synthesis.[2,3] Consequently, there is a need to improve the technique of peptide synthesis through the development of insoluble polymeric supports which are physicochemically compatible with the backbone structure of a peptide.

In order to offer a serious alternative to the overwhelmingly popular polystyrene supports, any new polymeric support must have distinctly improved properties and be easy to obtain and to use. As a general type, polyacrylamide resins have properties that should make them highly suited for use as supports for peptide synthesis.[4,5] We have been working on procedures to make acrylamide polymers readily available in a beaded form suitable for use in equipment presently employed for solid-phase peptide synthesis, and we recently reported the preparation by a reverse-phase suspension technique of an entirely beaded poly(acrylylpyrrolidine) resin.[6,7] Another acrylamide polymer that has been prepared and used in peptide synthesis is one based on dimethylacrylamide.[8-10] This resin was synthesized in a partially beaded form, and attempts to scale up the procedure from 5 to 50 g resulted in totally amorphous material.[9] While the physical form of a polymer does not change the chemical properties, in our experience the handling of amorphous polymer poses technical problems and requires special procedures. *For routine use a beaded resin is essential. Of equal importance is the ability to precisely control the degree of functionalization.* In the reported preparation of the poly(dimethylacrylamide) resin only 66% of the functionalizing agent *N*-(*tert*-butyloxycarbonyl)-β-alanyl-*N'*-acrylyl-1,6-diaminohexane was incorporated into the polymer.[8] We have reported a high-yield synthesis of *N*-acrylyl-1,6-diaminohexane·HCl, a monomer which we have found useful for the introduction into the polymer of a derivatizable primary amine.[11] Because of its high polarity and resulting water solubility, this monomer does not partition into the organic phase and the quantity of functional group is controlled simply by the amount of *N*-acrylyl-1,6-diaminohexane·HCl in the monomer solution.

This report describes the application of our reverse-phase suspension procedure[6,7] to the synthesis of completely beaded poly(*N,N*-dimethylacrylamide) cross-linked with *N,N'*-bisacrylyl-1,2-diaminoethane to the extent of 10 mol %, which was the composition reported by Atherton et al.[8] By using *N*-acrylyl-1,6-diaminohexane·HCl rather than *N*-(*tert*-butyloxycarbonyl)-β-alanyl-*N'*-acrylyl-1,6-diaminohexane,[8] we were able to incorporate into the polymer the theoretical quantity of amino function. Entirely beaded resin was prepared on both a small (7 g) and large (50 g) scale by reverse-phase suspension copolymerization of the constituent monomers with oxidation–reduction initiation. An aqueous solution of the monomers and the first half of the redox initiator ammonium peroxydisulfate was suspended in a heptane–CCl_4 mixture and the composition was adjusted so that the density of the two phases was approximately equal.

Importantly, the use of this organic phase prevents partitioning of the monomers from the aqueous phase and therefore allows for the precise control of polymer composition. The bead size was adjusted by the stirring rate and the addition of sorbitan sesquioleate; the reaction was initiated by addition of the second half of the redox system *N,N,N',N'*-tetramethyl-1,2-diaminoethane. After approximately 30 min the beaded product was filtered and washed with 2-propanol, $CHCl_3$, EtOH, H_2O, EtOH, and ethyl acetate. Beads are obtained of such uniform size that they are used without additional sizing other than flotation in $CHCl_3$ to remove the fines.

Verification of the amount of functionalizing group present in the resin was accomplished by completely acylating it with Boc-norvaline and then analyzing, after hydrolysis, for norvaline; found 0.47 mmol of norvaline per

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g of resin (theory, 0.47 mmol/g for 0.5 mmol of NH_2 per g of resin).

These results demonstrate that by reverse-phase suspension polymerization, entirely beaded poly(dimethylacrylamide) resins of predetermined composition can be prepared on a large or small scale.

Experimental Section

Poly(dimethylacrylamide-*co*-*N*,*N*′-bisacrylyl-1,2-diaminoethane-*co*-*N*-acrylyl-1,6-diaminohexane·HCl) (1). (a). Into a three-necked 500-mL, round-bottomed flask, maintained under a nitrogen atmosphere and equipped with a mechanical stirrer, were added *n*-heptane (148 mL) and CCl_4 (83 mL). Freshly distilled dimethylacrylamide (Kohjin, Tokyo) (5.47 g, 55.2 mmol), *N*,*N*′-bisacrylyl-1,2-diaminoethane[12] prepared according to ref 7 (1.1 g, 6.5 mmol), and *N*-acrylyl-1,6-diaminohexane·HCl[11] (0.765 g, 3.7 mmol) were dissolved in water (50 mL). After dissolution was complete, $(NH_4)_2S_2O_8$ (0.15 g) was added and this aqueous solution was added to the organic phase. While the mixture was stirred at 300 rpm, sorbitan sesquioleate (0.3 mL) was added followed by *N*,*N*,*N*′*N*′-tetramethyl-1,2-diaminoethane (0.3 mL). After 40 min the reaction was filtered and the beaded product washed three times with 200 mL each of 2-propanol, $CHCl_3$ (the fines were then removed by flotation in $CHCl_3$), ethanol, water, and ethanol and five times with ethyl acetate. The resin was dried in vacuo for 3 days at 45 °C: yield 6.79 g, 92.5% (7.31 g, 99.6% with fines). Chloride analysis of the hygroscopic resin performed by a modified Volhard method[13] as outlined by Stewart and Young[14] showed 0.47 mmol of Cl^- per g of resin.

(b). A large-scale preparation of 1 using 37.3 g of dimethylacrylamide, 7.5 g of *N*,*N*′-bisacrylyl-1,2-diaminoethane, 5.2 g of *N*-acrylyl-1,6-diaminohexane hydrochloride, and 1 g of $(NH_4)_2S_2O_8$ was performed as in (a). The organic phase consisted of heptane (1043 mL), CCl_4 (565 mL), and sorbitan sesquioleate (2 mL). The reaction was stirred at 600 rpm in a three-necked, 2-L vessel and was initiated by the addition of 2 mL of *N*,*N*,*N*′,*N*′-tetramethyl-1,2-diaminoethane. The product was washed with 1-L aliquots as described above: yield 49.1 g, 98.2% (49.5 g, 98.9% with fines).

Poly(dimethylacrylamide-*co*-*N*,*N*′-bisacrylyl-1,2-diaminoethane-*co*-*N*-acrylyl-*N*′-Boc-norvalyl-1,6-diaminohexane) (2). A sample of 1 (2 g, 1 mmol of the amino group) was washed for 2 min with 25-mL aliquots of EtOH (thrice), water (thrice), water plus 1 N NaOH (1.0 mL) (10 min), water (five times), EtOH (four times), and CH_2Cl_2 (four times). Boc-norvaline symmetrical anhydride[15] (3 equiv), prepared from Boc-Nva (1.3 g, 6 mmol) and dicyclohexylcarbodiimide (DCC) (0.62 g, 3 mmol) in CH_2Cl_2 (25 mL), was added to the resin followed by Et_3N (0.1 mL, 0.7 mmol). After 30 min DCC (0.41 g) was added and after 5 min the beads gave a negative Kaiser test.[16] The resin was washed with 25-mL aliquots of EtOH (thrice) and ethyl acetate (5 times) and then dried in vacuo at 45 °C for 3 days: yield 2 g. Following hydrolysis in 6 M HCl for 22 h at 110 °C, amino acid analysis[17] showed 0.47 mmol of norvaline per g of resin.

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ASIS Effect for Tetrahydroselenophene

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Aromatic solvent induced shifts (ASIS) have been determined for tetrahydroselenophene (THS); they are very different from those previously reported by Strom et al. in a study of the ASIS effect on five-membered rings of the type $(CH_2)_4X$, X = CH_2, O, S, Se, CO, and SO_2.[1] These authors used eq 1 to analyze the data with $\gamma_{CCl_4}{}^H$

$$\Delta^H = \gamma_{CCl_4}{}^H - \gamma_{C_6D_6}{}^H \quad (1)$$

the center of resonance with respect to Me_4Si for a particular kind of proton at infinite dilution in CCl_4 and $\gamma_{C_6D_6}$ the corresponding center of resonance in C_6D_6. The γ values in eq 1 will approach the corresponding chemical shift values (δ) as the system approaches first-order behavior. Except for THS, they found a linear relationship between the solvent shifts (Δ^β) of the β protons and the dipole moments ($\mu_{C_6H_6}$) in benzene. A similar relationship between the solvent shifts (Δ^α) of the α protons and the $\mu_{C_6H_6}$ was not apparent in their work. Since a correlation of the Δ's with dipole moments can be expected in the absence of steric effects in the benzene–solute complex, Strom et al. suggest that steric hindrance perhaps is the cause of the anomaly for the β-proton shift of THS, as well as of the nonlinearity of the α-proton shifts. The same interpretation was given to explain that, contrary to expectation, their value of the dipole moment of THS in CCl_4 is smaller than that in C_6H_6.

The proton chemical shifts and the solvent shifts of the $(CH_2)_4X$ molecules are listed in Table I. The γ_{CS_2} values were obtained by us from the complete analysis of the proton spectra from solutions in CS_2 (molar fraction $\simeq$ 0.1).[2] For tetrahydrofuran (THF) and tetrahydrothiophene (THT) they are only 2.0–2.9 Hz smaller than the γ_{CCl_4} values of Strom et al., while for THS the differences, $\gamma_{CCl_4}{}^\alpha - \gamma_{CS_2}{}^\alpha = 60.8$ Hz and $\gamma_{CCl_4}{}^\beta - \gamma_{CS_2}{}^\beta = 7.0$ Hz, are so large they cannot be explained as either solvent or concentration effects and give rise to the suggestion that different substances were used as solutes. This was later confirmed when we measured the γ_{CCl_4} values of THS that resulted close to those for γ_{CS_2}.

With our Δ values, instead of those of Strom et al., the Δ^β values could be fitted to the straight line

$$\Delta^\beta = 20.0\mu_{C_6H_6} + 5.6 \quad (2)$$

the average deviation being 1.4 Hz and the largest deviation being 2.2 for cyclopentanone. The Δ^α values could be fitted, except for THF, to the straight line

$$\Delta^\alpha = 12.5\mu_{C_6H_6} + 1.5 \quad (3)$$

the average deviation being in this case 3.0 Hz. If a second-order function in $\mu_{C_6H_6}$ is used, one obtains eq 4,

$$\Delta^\alpha = 1.2\mu_{C_6H_6}{}^2 + 6.8\mu_{C_6H_6} + 5.5 \quad (4)$$

the average deviation being reduced to 0.9 Hz. The Δ^α values for THF calculated with (3) and (4) are respectively 18.2 and 16.0 Hz greater than the experimental values.

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