Polymer Bulletin 37, 565–572 (1996)

Polymer Bulletin

Polystyrene-immobilized poly(ethylene imine) chains – a new class of graft copolymers

I. Synthesis and characterization

E. Bayer *, X. N. Liu, U. Tallarek, A. Ellwanger, K. Albert, M. Kutubuddin

Institute of Organic Chemistry, University of Tübingen, Auf der Morgenstelle 18, D-72076 Tübingen, Germany

Received: 20 May 1996/Revised version: 11 July 1996/Accepted: 11 July 1996

SUMMARY

This work reports the synthesis and characterization of a new polystyrene-based graft copolymer. By immobilization of transition metal complexes it shows high potential for selective heterogeneous hydrogenation under outstanding mild conditions. Here, a branched poly(ethylene imine) with average M_r around 3 x 10^4 was used to be grafted on acetylated polystyrene beads. ^{13}C solid state NMR investigations demonstrate a high mobility and flexibility of the chains of this spacer due to branching as compared to, e.g., linear poly(ethylene glycol) with M_r around 3000.

INTRODUCTION

Previous work over the last two decades has shed much light into the optimized synthesis and dynamics of polystyrene-immobilized poly(ethylene glycol) chains (PS-PEG) and their valuable application in different areas as peptide synthesis, immunology, catalysis and chromatography (1). For the grafting reaction, functionalized polystyrene beads of any degree of porosity can be used. In the case of nonporous ones the PEG chains extend outward from the matrix like tentacles. Due to their pressure stability and equal swelling factors in different solvents these tentacle PS-PEG graft copolymers are interesting as separation materials (stationary phases) in liquid chromatography (2) or packed beds for catalytic reactions. On the other hand, spacer polymers based on gelatinous and porous polystyrene are more suitable for and successfully applied in peptide as well as nucleotide synthesis using the *continuous flow* method (3,4).

Via anionic polymerization of ethylene oxide, PEG chains of molecular masses of up to 20 kilodaltons can be immobilized on crosslinked polystyrene containing hydroxyl functional groups (5-8). These spacers are relatively mobile and show a quasi-homogeneous behaviour on the otherwise insoluble matrix (9), thereby combining the advantages of solid- and liquid-phase methods. Furthermore, PS-PEG beads show a high chemical stability also and can be prepared in spherical form and monodispersed bead size for a range of particle sizes (10,11).

This is the first and rapid report of a new class of polystyrene-based graft copolymers where poly(ethylene imine) or PEI instead of PEG is used as the spacer. PEI can exist in linear and branched forms thereby providing (finally directed) access to

* Corresponding author

different properties of the graft copolymer when immobilized on PS. It is expected that the substitution of oxygen by nitrogen as the basic and nucleophilic center in the spacer part also leads to drastic changes regarding the coordination chemistry and affinity of the graft copolymer with respect to metal centers of, e.g., transition metal complexes used in catalysis (heterogeneous hydrogenation) or even heavy metal ions (for -possibly- their enrichment). The PS-PEI spacer polymer was synthesized according to classical methods and characterized by means of FTIR and ^{13}C solid state NMR spectroscopy. Here, it could be revealed by static as well as dynamic ^{13}C solid state NMR investigations that the grafted PEI shows a high mobility most probably due to the branched nature of this spacer.

EXPERIMENTAL

Synthesis of PS-PEI graft copolymers

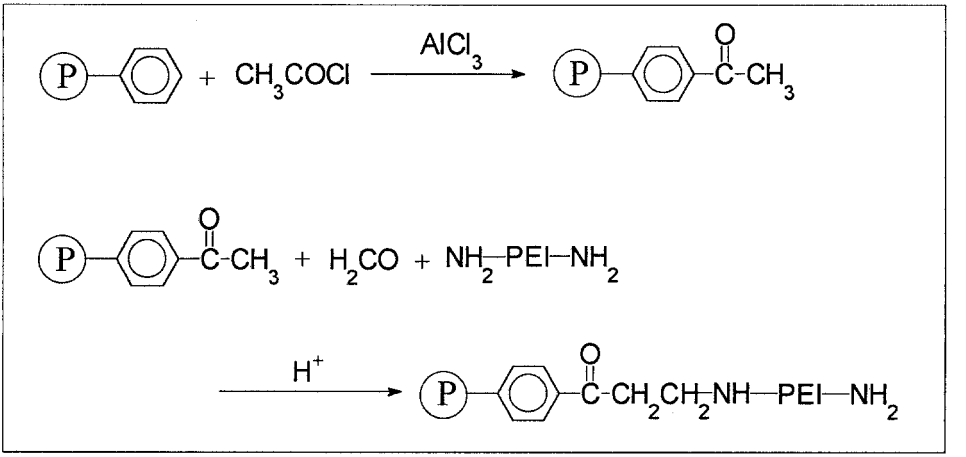
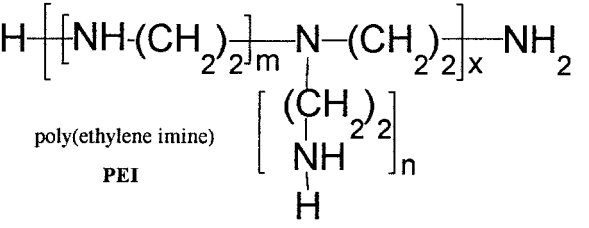


Figure 1: Synthetic route to polystyrene-poly(ethylene imine) graft copolymers.

The poly(ethylene imine) chains were immobilized on a functionalized polystyrene matrix consisting of acetylated, crosslinked polystyrene (1-2% divinylbenzene, DVB). Acetylation of the monodispersed polystyrene beads (average particle size 7-9 µm) was carried out using classical Friedel-Crafts methods, usually with $AlCl_3/CH_3COCl$ and under standard conditions. The final grafting reaction was run by a Mannich reaction (Figure 1). The poly(ethylene imine) used in the experiments reported here was a branched one with molecular weights around 3 x 10^4, a colourless, highly viscous liquid.



poly(ethylene imine)

PEI

It was supplied by the BASF AG (Ludwigshafen, FRG) and used without further treatment prior to reaction. The ratio of primary to secondary and tertiary nitrogen in this PEI as determined by titration was 1:2:1. The final graft copolymer was washed thoroughly under demanding conditions to remove any components just adsorbed on the polystyrene matrix and dried under vacuum.

Characterization of PS-PEI graft copolymers

The functionalized (acetylated) polystyrene beads as well as the final spacer polymers were characterized via FTIR and ^{13}C solid state NMR spectroscopy. High resolution solid state NMR spectra were recorded in the CP/MAS mode (12,13) as well as the single-pulse excitation ^{13}C MAS mode with proton high power decoupling (14) on a Bruker (Karlsruhe, FRG) ASX 300 spectrometer operating at 7.046 Tesla, corresponding to a resonance frequency of 75.467 MHz for the ^{13}C nucleus. ^{1}H and ^{13}C $\pi/2$ pulse times of 4.4 to 4.7 µs and contact pulse times of 3 ms were used. Samples were packed in 4 mm rotors (ZrO_2) and were spun at 10 kHz. All chemical shifts were measured from external tetramethylsilane. For the acquisition of the ^{13}C MAS spectra a recycle delay of 60 s was used. The Hartmann-Hahn condition for the ^{13}C CP/MAS experiment was calibrated with glycine. Peak deconvolution in the NMR spectra was performed with WIN-NMR (Bruker-Franzen GmbH, Bremen, FRG). Elemental analysis was carried out with a Carlo Erba elemental analyzer (Model 1106, software Eager 100), and FTIR spectra were recorded on a Bruker Vector 22 infrared Fourier spectrometer (Bruker, Karlsruhe, FRG).

Preparation of acetylated polystyrene

The polystyrene beads (ca. 1 g) are suspended in 10 ml of abs. CH_2Cl_2, together with the calculated amount of acetyl chloride (for the acetylation degree wanted). To homogenize, this suspension is hold in an ultrasonic bath for a moment. After ca. 15 min. one part of the $AlCl_3$ is given to this suspension and stirred at room temperature for 1 h, then the rest is slowly added (up to a 3-fold excess). This mixture is stirred over night. The product then is filtered and washed with CH_2Cl_2, ether/CH_2Cl_2, tetrahydrofuran and methanol (3 times each). Finally, it is dried under vacuum. The increase in sample weight (dry) is 39.9%. This corresponds to an acetylation of 99.2%. Elemental analysis calculated (found) for *quantitatively* acetylated polystyrene (crosslinked with 1% DVB): C, 82.2 (81.6); H, 6.9 (7.4). FTIR (dry, KBr): 3050-2990 (arom. C-H), 2921, 2848 (aliph. C-H), 1678 (C=O), 1600, 1565 (C=C), 1359 (CO-**CH_3**), 829 (arom. C-H *out of plane*, 1,4-bisubstituted ring) cm^{-1}.

Preparation of PS-PEI

Acetylated polystyrene (10 mmol), fine-powdered paraformaldehyde (100 mmol) and PEI-salt (0.8 mmol PEI, 25 mmol HCl) are refluxed in 30 ml of abs. ethanol. After 1 h 0.5 ml of conc. HCl is added to this solution to completely dissolve the paraformaldehyde. Then the mixture is filtered (hot) and thoroughly washed with HCl (1 mol/l), H_2O, NaOH (1 mol/l), H_2O, tetrahydrofuran, ethanol, methanol and ether (5 times each). The PS-PEI is finally dried under vacuum. Increase in sample weight (dry): 98.8%. This corresponds to 11.6 (found: 9.9) mmol/g of N. Elemental analysis (found): C, 63.1; H, 8.9; N, 13.9. FTIR, see Figure 5.

RESULTS AND DISCUSSION

Due to their insoluble nature regarding organic solvents characterization of crosslinked polystyrene, its acetylated derivative and the final PS-PEI was performed by ^{13}C solid state NMR, because in this case it easily provides information about the polymer structure. Figure 2a shows a conventional ^{13}C CP/MAS spectrum of polystyrene. As becomes evident the methylene and methine resonances are not resolved (around 40 ppm). It is well known that the relatively sharp part at the peak maximum belongs to the methine carbons, whereas the shoulder is caused by the methylene carbons.

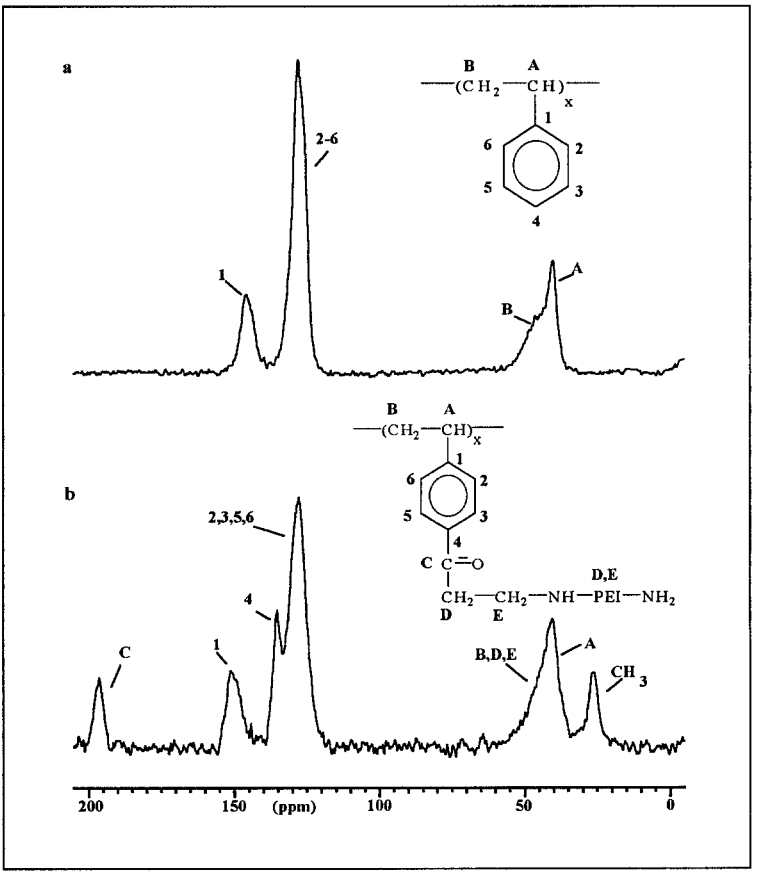


Figure 2: ^{13}C CP/MAS spectra of polystyrene (a) and PS-PEI (b).

Stepping forward in synthesis Figure 3 shows the spectrum of acetylated polystyrene, obtained by the Friedel-Crafts method. Due to the keto group the resonance of carbon 1 shifts to lower field (from 145.7 to 150.9 ppm) and the one of carbon 4 (now 135.3 ppm) moves out of the main peak at 128.2 ppm. The characteristic and unambigous features of acetylation are the methyl and carbonyl resonances at 26.3 and 196.7 ppm, respectively. The final PS-PEI graft copolymer was obtained from quantitatively acetylated polystyrene by means of a Mannich reaction with formaldehyde.

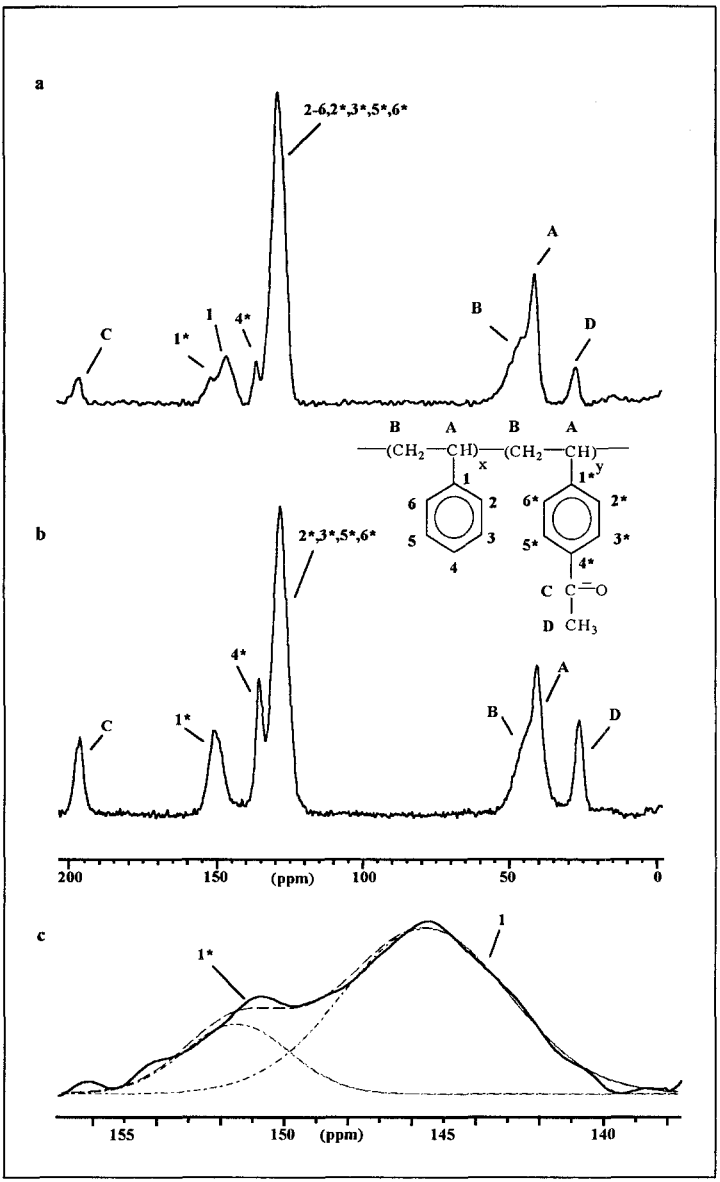


Figure 3: ^{13}C CP/MAS spectra of acetylated polystyrene (a,b). In (c) the expanded part of (a) is shown where peak deconvolution is used to give an estimate of the degree of the acetylation step. In (a) it is about 25% whereas in (b) acetylation essentially was carried out quantitatively.

Due to the branched nature of this PEI spacer with M_r around 3 x 10^4 and consequently to the high mobility of its chains the $^1H/^{13}C$ cross polarization technique fails to clearly visualize the aliphatic carbon resonances of the PEI spacer via magnetization transfer from the protons. The CP/MAS method is particularly effective for rigid solids only. This could also be demonstrated in a contact time variation CP/MAS experiment with

contact times of up to 40 ms where the poly(ethylene imine) component was only slightly visible at higher contact times. Thus, the ^{13}C CP/MAS spectra of PS-PEI (see Figure 2b) and its acetylated polystyrene precursor (Figure 3b) are essentially identical.

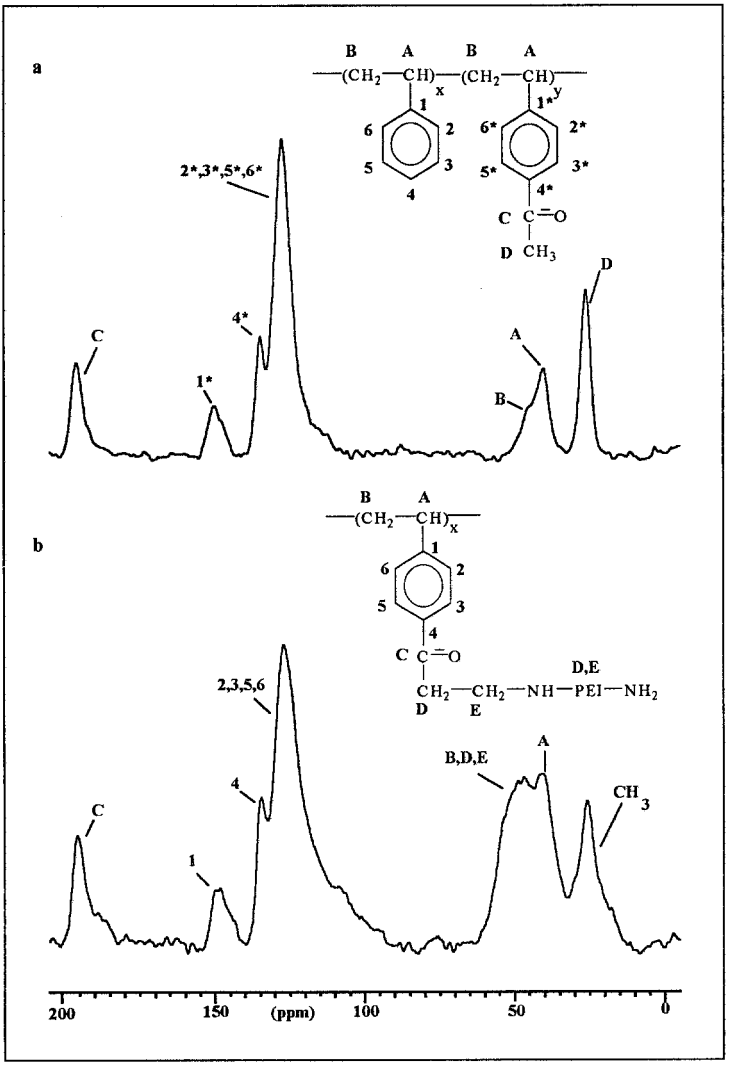


Figure 4: ^{13}C SPE/MAS spectra; PS-PEI (b), obtained from the quantitatively acetylated polystyrene (a).

Thus, ^{13}C single-pulse excitation/MAS (SPE/MAS, without cross polarization, but still with proton high power decoupling) was used in this case to characterize the PS-PEI spacer polymer. Figure 4 compares the corresponding ^{13}C SPE/MAS spectra of these two specimen. Due to its methylene carbons bound to primary, secondary or tertiary nitrogen the grafted PEI shows up as a broad splitted signal (around 40 and 47 ppm), partially overlapping with the methylene and methine resonances of polystyrene. There is practically

no difference between the SPE (Figure 4a) and CP/MAS spectrum (Figure 3b) of acetylated polystyrene.

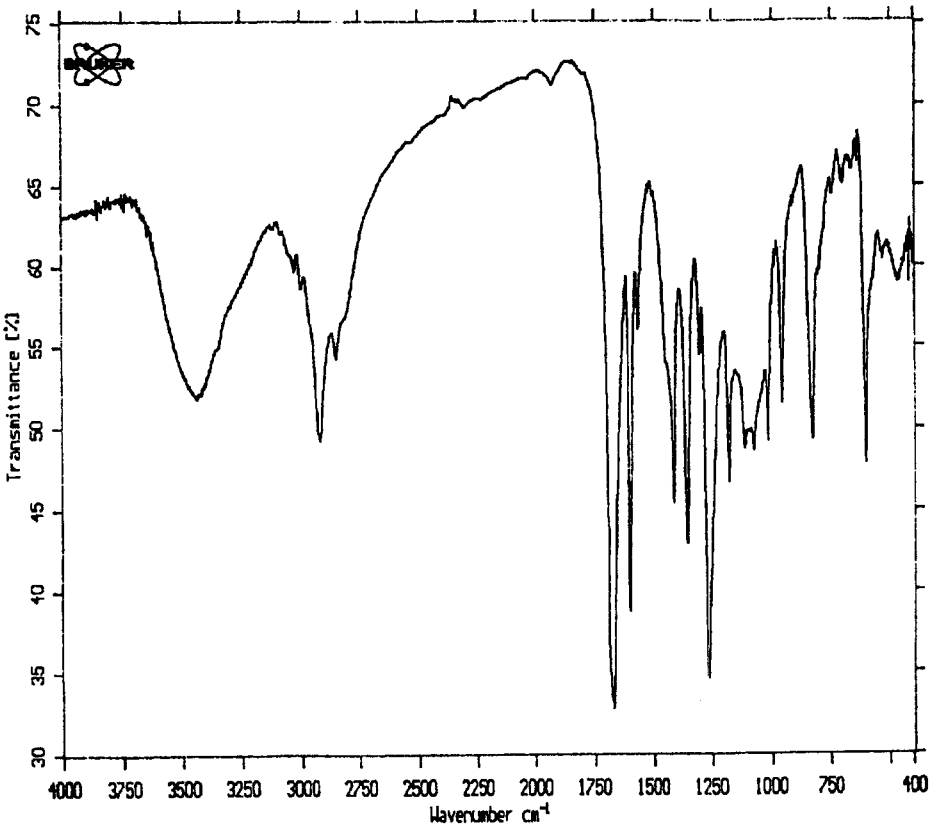


Figure 5: FTIR spectrum of polystyrene-poly(ethylene imine), PS-PEI.

The still existing methyl peak in the PS-PEI spectrum indicates that the Mannich reaction was not quantitative, which, of course, is expected because of the bulky and branched nature of this spacer that occupies quite a large surface area of the polystyrene matrix, together with the fact that the polystyrene is quantitatively acetylated. Thus, this relatively high density of potential reaction centers (acetyl groups) is mostly unused because of the shape and size of the spacer. In the end, only a small portion of the methyl groups probably is responsible for the (chemical) bonding between spacer and matrix. In effect, the poly(ethylene imine) is highly mobile, even in the solid state.

Using ^{13}C CP/MAS spectroscopy it is (in this case) also possible to give an estimate of the degree of the acetylation step. Here, the relative intensities of the resonances of carbon atoms 1 and 1* (belonging to styrene and acetylated styrene units, respectively) were taken for that purpose, assuming still identical relaxation times. Figure 3a shows a polystyrene spectrum with about 25% acetylation (as has been verified by peak

deconvolution of the overlapping peaks of interest, see Figure 3c), whereas it was carried out quantitatively in the spectrum shown below (Figure 3b).

The acetylation step of practically quantitative nature in Figure 3b could also be verified by elemental analysis (see experimental section) and FTIR results. In the latter case this could be achieved due to the characteristic C-H *out of plane* resonance patterns of mono- and 1,4-bisubstituted benzene derivatives below 900 cm^{-1}. It is clearly seen in the case of PS-PEI obtained from this quantitatively acetylated polystyrene (Figure 5). Here, the resonance at 830 cm^{-1} is the characteristic one for p-bisubstituted benzene. In addition, there is a broad band ranging from ca. 3550 to 3350 cm^{-1}, caused by primary and secondary amine functions of the PEI chains.

ACKNOWLEDGEMENT

The Deutsche Forschungsgemeinschaft (DFG, Bonn-Bad Godesberg, FRG) is gratefully acknowledged for financial support and the BASF AG (Ludwigshafen, FRG) for the gift of the PEI sample.

REFERENCES

1. Bayer E, Rapp W (1992) *Polystyrene-Immobilized PEG Chains.* In: Harris JM (ed) *Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications.* Plenum Press, New York (pp 325-345)
2. Bayer E, Rapp W (1990) 14th International Symposium on Column Liquid Chromatography. May 20-25th, Boston
3. Bayer E (1991) Angew Chem **103**: 117; Angew Chem Int Ed Engl **30**: 113
4. Zhang L, Rapp W, Bayer E (1991) *Continuous Flow Peptide Synthesis: Dependence of the Kinetics upon the Nature of Polymeric Support, Method of Activation and Reaction Conditions.* In: Giralt E, Andreu D (eds) *Peptides 1990.* Epson, Amsterdam (pp 196-197)
5. Bayer E, Rapp W (1988) German Patent DOS 3714258
6. Bayer E, Dengler M, Hemmasi B (1985) Int J Pept Protein Res **25**: 178
7. Bayer E, Rapp W (1986) *New Polymer Supports for Solid-Liquid-Phase Peptide Synthesis.* In: Voelter W, Bayer E, Ovchinnikov YA, Ivanov VI (eds) *Chemistry of Peptides and Proteins.* Walter de Gruyter, Berlin (pp 3-8)
8. Rapp W, Zhang L, Häbich R, Bayer E (1989) *Polystyrene-Polyoxyethylene Graft Copolymers for High Speed Peptide Synthesis.* In: Jung G, Bayer E (eds) *Peptides 1988.* Walter de Gruyter, Berlin (pp 199-201)
9. Bayer E, Albert K, Willisch H, Rapp W, Hemmasi B (1990) Macromolecules **23**: 1937
10. Ugelstadt J, Mork PC (1980) Adv Colloid Interface Sci **13**: 101
11. Hansen FK, Ugelstadt J (1978) J Polym Sci **16**: 1953; (1979) J Polym Sci **17**: 3033
12. Yannoni CS (1982) Acc Chem Res **15**: 201
13. Mehring M (1983) *Principles of High Resolution NMR in Solids.* Springer, Berlin Heidelberg New York
14. Fyfe CA, Gobbi GC, Kennedy GJ (1985) J Phys Chem **89**: 277