Synthesis of Poly(oxyethylene)−Poly(Z-L-lysine) Hybrid Graft Copolymers

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Introduction

Conjugation of biological and synthetic polymers into hybrid polymeric materials with various architectures is a useful way to overcome some limitations and to combine the advantageous properties of the individual components.[1] Hybrid block and graft copolymers have found applications in different biomedical fields as tissue engineering, implantation of medical devices and artificial organs, bone repair, and drug delivery.[2] Graft copolymers have some advantages over hybrid block copolymers in terms of the ability to tailor their properties for potential biomedical applications.[3] Hybrid graft copolymers usually comprise polypeptide backbone whose pendant functional groups are used to attach the side chains.[4] Nevertheless, there are examples of copolymers with polypeptide grafts prepared by polymerization of macromonomers ("grafting through"),[5] by polymerization initiated from a multifunctional macroinitiator ("grafting from"),[6] or by grafting of preformed polypeptide side chains onto a multifuntional backbone ("grafting onto").[7] The secondary structure of the polypeptide chains may affect the main-chain conformation, introducing more interesting properties (pH and temperature response).[8] When the copolymer grafts consist of polycationic peptides such as polylysine, this may lead to applications in gene delivery systems.[9]

To our best knowledge, no experimental work has been reported so far on the synthesis of hybrid graft copolymers with poly(oxyethylene) (PEO) backbone and polypeptide side chains due to the absence of functionalities on the polyether chain. Recently, we reported the synthesis of PEOs bearing a number of tertiary amine groups regularly distributed along the chain. These groups were subsequently quaternized with different reagents in order to obtain various pendant functionalities.[10]

This paper presents the synthesis and characterization of PEO-*graft*-poly(Z-L-lysine) hybrid copolymers by ring-opening polymerization of N^{ϵ}-(carbobenzoxy)-L-lysine *N*-carboxyanhydride (ZLLys-NCA) from multifunctional PEO bearing regularly distributed pendant primary amine hydrochloride groups. This system allows the design of comb-type copolymers with various number and distance between the polypeptide side chains.

The synthesis of well-defined Y-shaped (AB_2-type) block copolymers [PZLLys-$(PEO)_2$] as model compounds representing building blocks of the hybrid graft copolymers is also described.

Experimental Section

Materials. Reagent chemicals were purchased from Fluka unless otherwise indicated. Ethyl acetate (>99%) was distilled from CaH_2. *N*,*N*-Dimethylformamide (DMF, >99.5%) was dried over phosphorus pentoxide and distilled under reduced pressure. Z-L-Lysine (99%), triphosgene (>99%), and 3-chloropropylamine hydrochloride (CPA·HCl, 98%, Aldrich) were used as received. ZLLys-NCA was prepared from Z-L-lysine and triphosgene in ethyl acetate at 70 °C. The purification procedure involved rapid washings of the reaction mixture with chilled water and aqueous bicarbonate, which was very efficient in obtaining high-purity monomer.[11] Yield: 90%. IR (KBr pellets): N−H (NCA, Z), ν 3342 cm^{-1}; CH_2, ν_{as} 2936 cm^{-1}; CH_2, ν_s 2863 cm^{-1}; C═O (NCA), ν 1814, 1774 cm^{-1}; C═O(Z), ν 1687 cm^{-1}; N−H(Z), δ 1533 cm^{-1}; C(═O)−O, ν 1258 cm^{-1}.

Preparation of PEO Macroinitiators Bearing Pendant Primary Amine Hydrochloride Groups (PmE_nNE_n-$(NH_2{\cdot}HCl)_m$ or E_nNE_n-$(NH_2{\cdot}HCl)$). The synthesis of PEO bearing a tertiary amine group in the middle of the polyether chain (E_nNE_n) and polyfunctional PEOs with controlled number (m) and distance between the in-chain functionalities (PmE_nNE_n) was previously described.[10] ^{1}H NMR (DMSO-d_6): δ 4.6 (O−CH_2−O); δ 3.5 (O−CH_2CH_2−O); δ 2.48 (CH_2−N−CH_2); δ 2.19 (CH_3−N). Aqueous GPC (vs PEO standards): $E_{17}NE_{17}$: M_n = 1500, M_w/M_n = 1.12; $E_{50}NE_{50}$: M_n = 4400, M_w/M_n = 1.09; $P25E_{17}NE_{17}$: M_n = 37 100, M_w/M_n = 1.95; $P11E_{25}NE_{25}$: M_n = 23 800, M_w/M_n = 1.50.

Excess CPA·HCl (1.2 mol) was used to quaternize the tertiary amine groups in E_nNE_n or PmE_nNE_n. In a typical reaction $P25E_{17}NE_{17}$ (0.8 g, 0.534 mmol of tertiary amine groups) and CPA·HCl (0.08 g, 0.641 mmol) were dissolved in 5 mL of H_2O. The reaction was performed at 60 °C and was completed within 2 days. The solvent was evaporated. The residue was dissolved in dichloromethane and was precipitated in diethyl ether. The excess of the quaternizing reagent was removed through a Soxhlet extraction with dichloromethane. Yield: 0.79 g, 98%. ^{1}H NMR (DMSO-d_6): δ 4.6 (O−CH_2−O); δ 3.65 (CH_2−N$^+$−CH_2); δ 3.5 (O−CH_2CH_2−O + N$^+$−CH_2CH$_2$CH_2−N$^+$); δ 2.98 (CH_3−N$^+$); δ 2.55 (N$^+$−CH$_2$CH_2CH$_2$−N$^+$).

Synthesis of Hybrid Graft Copolymers. Typically, the macroinitiator $P25E_{17}NE_{17}$-$(NH_2{\cdot}HCl)_{25}$ (0.15 g, 0.1 mmol of amine hydrochloride groups) was "freeze-dried" from benzene, while ZLLys-NCA (0.92 g, 3 mmol) was dried at room temperature in a high vacuum. Two separate DMF solutions were prepared and subsequently combined via transfer needle under argon to give ~9 wt % (12 mL) solution ($[ZLLys\text{-}NCA]_0/[NH_2{\cdot}HCl]_0$ = 30). Polymerization was conducted at 60 °C in an inert atmosphere for 7 days. The product was precipitated in diethyl ether, extracted with methanol, and dried in a vacuum. Yield: 0.66 g, 70%. GPC in *N*,*N*-dimethylacetamide (vs PEO standards): M_w/M_n = 1.38. ^{1}H NMR (DMSO-d_6): δ 8.1 (αCH−NH); δ 7.3−6.8 (C_6H_5 + αCH(CH$_2$)$_4$−NH + CH$_3$−N$^+$-(CH$_2$)$_3$−NH); δ 5.0 (Z−CH_2); δ 4.6 (O−CH_2−O); δ 3.8−4.3 (NH-αCH); δ 3.5 (O−CH_2CH_2−O + CH$_3$−N$^+$−CH_2); δ 3.0 (αCH(CH$_2$)$_3$−CH_2 + CH_3−N$^+$ + CH$_3$−N$^+$-(CH$_2$)$_2$−CH_2); δ 1.1−2.1 (αCH−(CH_2)$_3$). IR (KBr pellets): N−H (NCA, Z), ν 3290 cm^{-1}; C═O(Z), ν 1698 cm^{-1}; C═O (amide I), ν 1652 cm^{-1}; N−H(Z) (amide II), δ 1541 cm^{-1}.

Y-shaped copolymers were synthesized similarly to the hybrid graft copolymers. The only difference was the use of a macroinitiator bearing central pendant primary amine hydrochloride group (E_nNE_n-$(NH_2{\cdot}HCl)$).

Characterization. ^{1}H NMR spectra were recorded on a Bruker 250 MHz and JEOL GX 400 MHz instrument. Gel permeation chromatography (GPC) was performed in *N*,*N*-dimethylacetamide (DMA) + 0.5 wt % LiCl at a flow rate of 1.0 mL/min on a set of GRAM Linear and GRAM 100 Å, 300 × 8 mm, 10 μm columns (Polymer Standards Service GmbH, Mainz, Germany), calibrated vs PEO narrow molar mass standards; the column and DRI detector temperature was 50 °C. Aqueous GPC was performed on a set of CATSEC (Eprogen Inc. and Eichrom Techn. Inc.) columns 100, 300, 1000, and 4000 Å, calibrated versus PEO narrow molar mass

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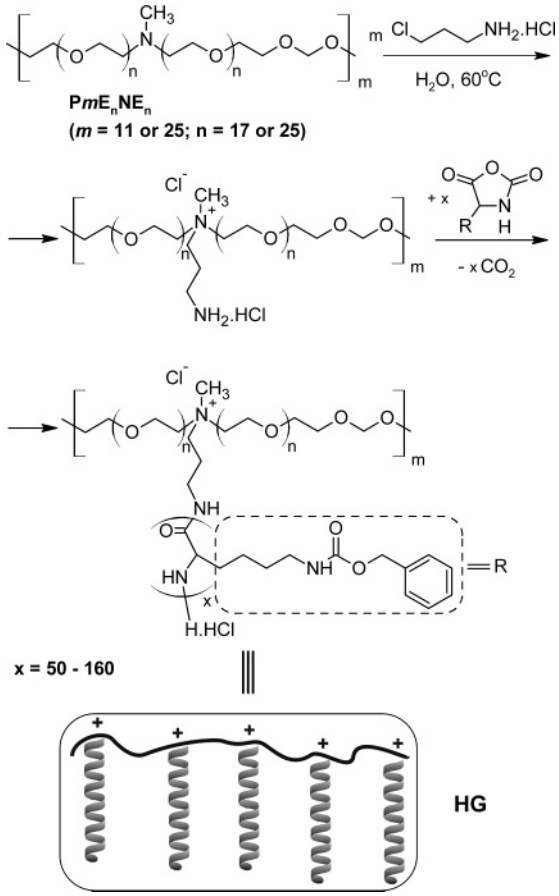
Scheme 1. Synthesis of Hybrid Graft Copolymers



standards; the mobile phase was 0.2 M CH_3COOLi, 0.2 M CH_3COOH with pH ≈ 4.6 and a flow rate of 0.25 mL/min; the column and DRI detector temperature was 40 °C. Solution viscosities were determined with an Ubbelohde viscometer in DMA at 50 °C. Infrared spectra were recorded at room temperature in KBr pellets on a Bruker Vector 22 spectrometer.

Results and Discussion

Hybrid Graft Copolymers. For the synthesis of hybrid graft copolymers the ring-opening polymerization of amino acid *N*-carboxyanhydrides initiated by a multifunctional macroinitiator was applied. In general, primary amino-initiated polymerization of NCA proceeds through a nucleophilic ring-opening process known as the "amine mechanism".[12] When primary-amine functionalized macroinitiators are used, this should result in formation of polypeptide-based block and graft copolymers. However, the NCA polymerization suffers from side reactions. Usually the so-called "activated monomer mechanism" also takes place and leads to broad molar mass distributions.[13] Lately, we introduced a simple and efficient method that involves the use of hydrochloric salt of primary amino-functional macroinitiators.[14] As a result, the "activated monomer mechanism" was suppressed, and nearly monodisperse hybrid block copolymers were obtained.

Here, we prepared hybrid graft copolymers containing polypeptide side chains applying a two-step procedure. In the first step multifunctional PEO macroinitiators with pendant primary amine hydrochloride groups were synthesized. The process depicted in Scheme 1 involved quaternization of P*m*E$_n$NE$_n$ containing *m* acetal-linked E$_n$NE$_n$ precursors. The quaternizing reagent was 3-chloropropylamine hydrochloride. The reaction proceeded smoothly in water. The product was

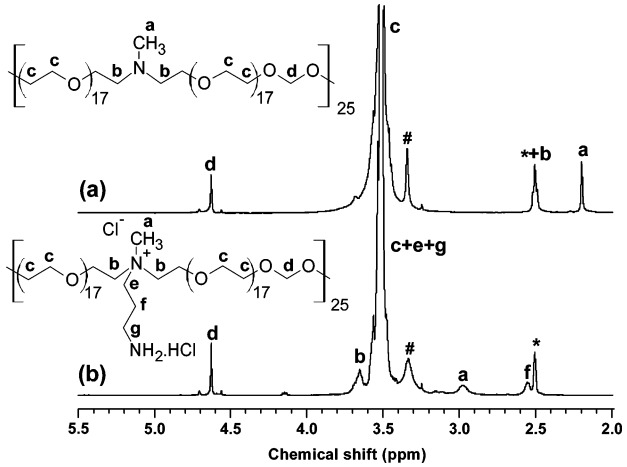


Figure 1. ^{1}H NMR (400 MHz) spectrum in dimethyl-d_6 sulfoxide at 25 °C of (a) the polyfunctional backbone polymer P25E$_{17}$NE$_{17}$ and (b) the corresponding macroinitiator (*, dimethyl sulfoxide; #, water).

thoroughly purified in order to remove any traces of quaternizing reagent which, if present, could also initiate the NCA polymerization. The quaternization reaction was quantitative as evidenced by the ^{1}H NMR spectra of the derivatives in DMSO-d_6 (Figure 1). The signal at δ 2.19 ppm characteristic of the CH_3N protons completely disappeared, and a signal appeared at δ 2.98 ppm attributed to CH_3N^+ protons. The relative intensities of the $-OCH_2O-$ protons from acetal links at 4.6 ppm and those of CH_3N^+ protons were equal to 2:3, indicating that no degradation took place during the reaction. By using precursor polymers with different length of the acetal-linked PEO blocks, we were able to control the number and the distance between the pendant primary amine hydrochloride groups in the macroinitiators obtained.

The second step of the synthetic procedure was the ring-opening polymerization of ZLLys-NCA initiated by the amine hydrochloride pendant groups of multifunctional PEO (Scheme 1). Polymerizations were conducted in DMF as a solvent at 60 °C. In the IR spectra of the products (not shown), the characteristic peaks of $-CO-O-CO-$ anhydride group at 1814 and 1774 cm^{-1} have disappeared, indicating complete consumption of ZLLys-NCA. They were replaced by absorption bands at 1652 and 1541 cm^{-1}, characteristic of newly formed polypeptide grafts. The crude products were extracted with methanol. As a result, a methanol-soluble fraction containing macroinitiator grafted with a few polypeptide units was separated. The purified graft copolymers were characterized by IR, ^{1}H NMR, and GPC analyses. The position of the amide I and amide II bands at 1652 and 1541 cm^{-1}, respectively, is consistent with the predominantly α-helical structure of polypeptide side chains in the solid state.[15] The copolymer composition was estimated from the ^{1}H NMR analyses conducted in DMSO-d_6. A typical proton NMR spectrum of PEO−PZLLys hybrid graft copolymer is shown in Figure 2.

Since the molar mass and the functionality of the PEO macroinitiator are known, the average degree of peptide grafting on every active center (DP$_n$) was calculated from the ratio of the integral areas of methylene signal of the Z-protecting group from ZLLys units at 5.0 ppm to the oxyethylene protons signals from the backbone at 3.5 ppm. Generally, the calculated value of DP$_n$ was 35−40% higher than targeted one. Some deactivation of the active centers leading to the formation of the methanol soluble fraction might be a reason for these results. The molar mass characteristics of the macroinitiators and hybrid copolymers obtained are summarized in Table 1.

Table 1. Characteristics of the Macroinitiators and the Hybrid Copolymers

	macroinitiator				hybrid copolymer			
entry	code	M_n[a] (g/mol)	M_w/M_n[a]	M_w/M_n[b]	code	target DP_n[c]	DP_n[d]	M_w/M_n[b]
1	$E_{17}NE_{17}$-NH_2·HCl	1600	1.12	1.25	HY1	50	82	1.43
2	$E_{50}NE_{50}$-NH_2·HCl	4500	1.09	1.29	HY2	150	240	1.47
3	$P25E_{17}NE_{17}$-$(NH_2$·HCl$)_{25}$	38700	1.95	1.97	HG1	30	50	1.38
4	$P25E_{17}NE_{17}$-$(NH_2$·HCl$)_{25}$				HG2	50	78	1.42
5	$P11E_{25}NE_{25}$-$(NH_2$·HCl$)_{11}$	24800	1.50	1.60	HG3	70	118	1.45
6	$P11E_{25}NE_{25}$-$(NH_2$·HCl$)_{11}$				HG4	100	164	1.47

[a] Determined by aqueous GPC (vs PEO standards). [b] Determined by GPC in *N*,*N*-dimethylacetamide (vs PEO standards). [c] The target degree of polymerization of each side chain, target $DP_n = [ZLLys\text{-}NCA]_0/[NH_2\cdot HCl]_0$, where $[NH_2\cdot HCl]_0$ is the active centers concentration. [d] Number-average degree of ZLLys polymerization, as determined by ^1H NMR analysis; see Results and Discussion.

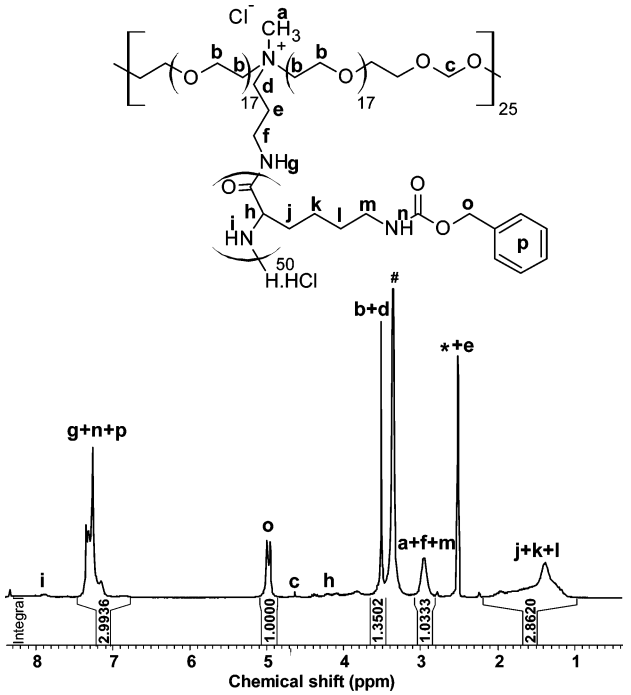


Figure 2. ^1H NMR (250 MHz) spectrum of the hybrid graft copolymer HG1 in dimethyl-d_6 sulfoxide at 25 °C (*, dimethyl sulfoxide; #, water).

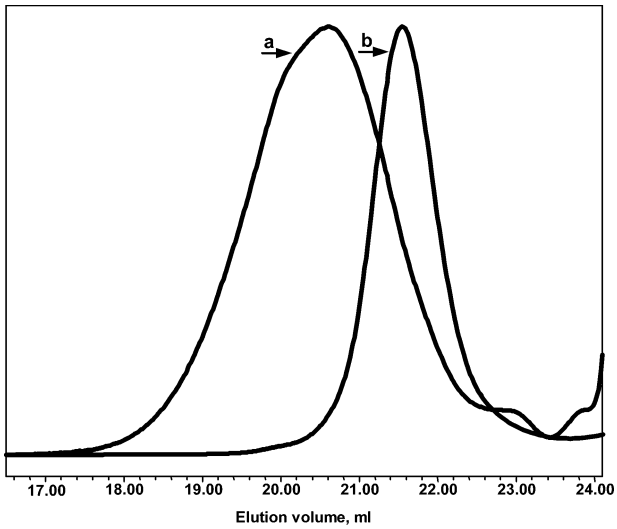
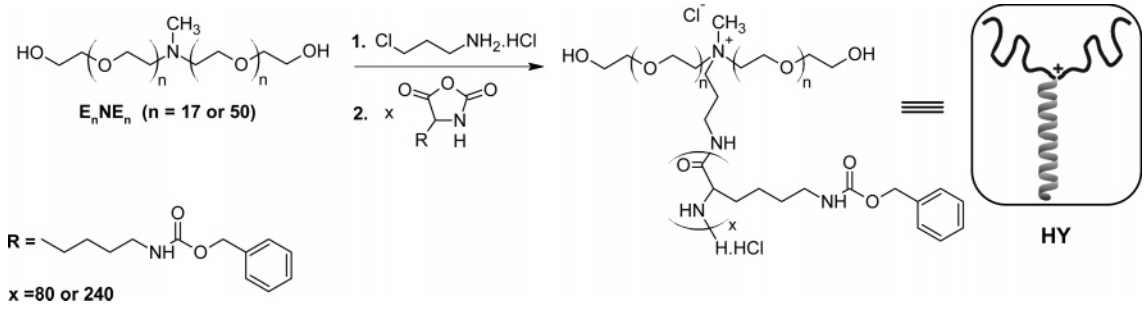


Figure 3. Gel permeation chromatograms in *N*,*N*-dimethylacetamide (+0.5 wt % LiCl) at 50 °C of (a) the polyfunctional backbone polymer $P25E_{17}NE_{17}$ (M_n = 14 000, M_w/M_n = 1.97) and (b) the hybrid graft copolymer HG1 (M_n = 5800, M_w/M_n = 1.38). The GPC data were obtained relative to PEO standards.

The loss and broadening of peaks corresponding to α-CH and $(CH_2)_3$ protons of the poly(Z-L-lysine) segments observed in the copolymer ^1H NMR spectra (Figure 2) point to a decreased mobility of these groups, indicating the presence of intra- and/or interchain interactions between the closely situated grafts. GPC analyses were performed in *N*,*N*-dimethylacetamide and confirmed that there are no homopolypeptide contaminants in the copolymer samples. Characteristic GPC curves of the backbone polymer and the corresponding hybrid graft copolymer are presented in Figure 3. The elution volume of the backbone polymer is smaller than that of the graft copolymer. This indicates that the graft copolymers form very compact structures with hydrodynamic volumes lower than that of the backbone polymer. This is a common feature for the densely grafted copolymers and is most likely due to the interchain interactions between the grafts.[16] The results are consistent with those obtained from the ^1H NMR analyses.

Y-Shaped [PZLLys-$(PEO)_2$] Block Copolymers. Following the synthetic procedure illustrated in Scheme 2, asymmetric AB_2-type hybrid block copolymers were synthesized. Well-defined PEOs with tertiary amine groups in the middle of the polyether chain (E_nNE_n) were quaternized with CPA·HCl and subsequently used as macroinitiators for the ring-opening polymerization of ZLLys-NCA. Control experiments with E_n-NE_n quaternized with allyl bromide were performed, and it was found that the two hydroxyl end groups of the macroinitiator do not initiate the ring-opening polymerization of ZLLys-NCA. The copolymers obtained were characterized similarly to the hybrid graft copolymers (Table 1; Figure S1, Supporting Information) and represent their building blocks. GPC analyses showed a shift to a higher elution volume for the Y-shaped

Scheme 2. Synthesis of Y-Shaped Hybrid Block Copolymers



copolymer (HY2) compared to the corresponding precursor (Figure S2, Supporting Information). This could be explained by the copolymers' asymmetric architecture and the considerably lower hydrodynamic volume of the polypeptide chain compared to PEO macroinitiator.[17] The results are consistent with the lower intrinsic viscosity of the Y-shaped copolymer ($[\eta]_0 = 0.077$ dL/g) measured in DMA as compared to that of the macroinitiator ($[\eta]_0 = 0.118$ dL/g). The asymmetric AB_2 type block copolymers are interesting materials to study since it is known that they exhibit different behavior compared to the corresponding linear AB diblocks.[17,18] Moreover, Y-shaped hybrid copolymers obtained possess three functional end groups (one primary amine and two hydroxyl groups) allowing further modifications.

Conclusions

Well-defined hybrid graft copolymers comprising PEO backbone and poly(Z-L-lysine) side chains were synthesized applying the "grafting from" technique. Polyfunctional PEOs bearing equal numbers of acetal and tertiary amine groups regularly distributed along the polyether chain were quaternized with CPA·HCl. These polymers were used as macroinitiators for the ring-opening polymerization of ZLLys-NCA. The grafting density was controlled by the number and distance between the primary amine hydrochloride pendant groups. Varying the monomer to macroinitiator molar ratio, graft copolymers with different length of the polypeptide side chains were obtained. Regularly distributed along the backbone acetal groups impart biodegradability to the hybrid graft copolymers and make them promising candidates for numerous biomedical applications (after deprotection of ϵ-NH_2 groups of the polypeptide side chains).

Y-shaped block copolymers [PZLLys-$(PEO)_2$] with different lengths of PEO and polypeptide segments were also prepared. Their asymmetric architecture and chain-end functionalities could lead to different properties compared to their linear analogues and to further chemical modification.

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Supporting Information Available: ^{1}H NMR spectrum of Y-shaped copolymer; GPC traces of macroinitiator and the corresponding Y-shaped copolymer. This material is available free of charge via the Internet at http://pubs.acs.org.

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