Catalytic Functionalization of Polymers: A Novel Approach to Site Specific Delivery of Misoprostol to the Stomach

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The application of functionalized polymers to site-directed delivery of the antiulcer prostaglandin, misoprostol, is described. By use of homogeneous catalysis, the simple polymer, polybutadiene, was modified to incorporate the specialized requirements for controlled delivery of misoprostol to the stomach. An acid labile silyl ether bond to the C-11 hydroxyl of misoprostol was installed as the primary rate determining step for drug release, and a series of analogs, in which the steric hindrance about the silicon atom was varied, was prepared and evaluated for *in vitro* release rates, efficacy against indomethacin induced gastric damage and diarrheagenic activity. The diisopropylsilyl analog, the slowest releasing system studied, showed efficacy equal to misoprostol against indomethacin-induced gastric damage and no diarrhea at the highest dose tested.

Introduction

The requirements for selectively delivering a particular drug to its site of action present a need to prepare highly specialized systems which are specifically tailored to the drug and its site of action.[1] Traditionally, the functionalized polymer matrixes used as delivery systems are prepared by polymerization of functionalized monomers or by grafting of synthetic or natural polymers. Control of the features that would allow the preparation of a highly specialized system are often difficult or impossible by these traditional approaches.[1a] One of the goals of our research is to develop synthetic methods that permit flexible assembly of customized polymeric delivery systems from a base or template polymer in order to accommodate delivery requirements of a drug.

This paper describes a new, flexible method of preparing site specific, polymeric delivery systems. By use of homogeneous catalysis, functional groups are added to a base polymer to serve a variety of purposes. One type of functionality is used to covalently attach the active drug to the polymer and to selectively release the drug at its site of action at an adjustable rate. In particular, this paper examines the usefulness of silyl ether functional groups in selectively delivering a drug to the stomach at a controllable and adjustable hydrolysis rate. Other functional groups are employed to fine-tune the hydrophilicity of the polymer matrix and to create a pH dependent mechanical gate for opening the polymer.[2]

The above methodology was used to tailor a delivery system for the prostaglandin, misoprostol.[3] Misoprostol (Figure 1) is a synthetic 16-hydroxy analog of natural prostaglandin E_1 and possesses both gastric antisecretory and mucosal protective properties and is indicated for the prevention of nonsteroidal antiinflammatory drug (NSAID) induced gastric ulcers. Although misoprostol is, in general, a safe and well tolerated drug, it is contraindicated in pregnant women because of its uterine effects and causes mild diarrhea and abdominal discomfort in a small but

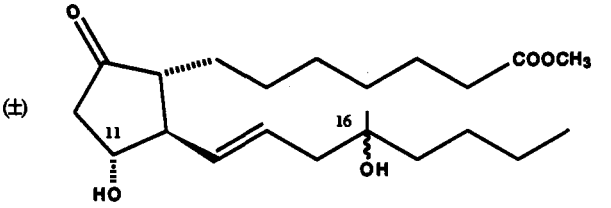

Figure 1. Misoprostol.

significant number of patients (10% in clinical trials[3]). Misoprostol can exert its therapeutic effects locally in the stomach,[4] but side effects are caused either by blood borne drug (uterotonic activity) or a combination of locally exerted effects in the lower GI tract and systemic exposure (diarrhea). As a consequence, misoprostol is uniquely suited to an orally delivered site-specific (stomach) protocol. Thus, controlled delivery of misoprostol to the stomach could provide local therapeutic action and limit both systemic and lower GI exposure. Prolonged gastric availability might also permit a reduction in dosing frequency. The inherent acidity difference between the stomach (pH = 1–3) and the lower GI tract (pH = 4–8) led to the consideration of a pH selective delivery system from which the drug is selectively released at stomach pH by covalent bond cleavage.[5]

These pH requirements prompted an investigation of a silyl ether bond for attachment of misoprostol to a delivery vehicle. The key features of this attachment strategy fit nicely with the following requirements: (1) the silyl ether drug bond is formed under mild conditions;[6] (2) the silyl ether bond is cleaved and releases misoprostol under acidic conditions; and (3) the release rate is fine-tuneable to the pH of the stomach by proper choice of organosilyl groups.[7]

Besides utilizing functional groups on a polymer to covalently attach the drug to the polymer, functional groups are needed to customize the physicochemical properties of the entire macromolecule[8] to the environment of the stomach. These physicochemical properties must be fashioned in such a way to allow (1) acid to enter the matrix, (2) hydrolysis of the silyl ether drug bond, and (3) diffusion of the detached drug from the matrix. The

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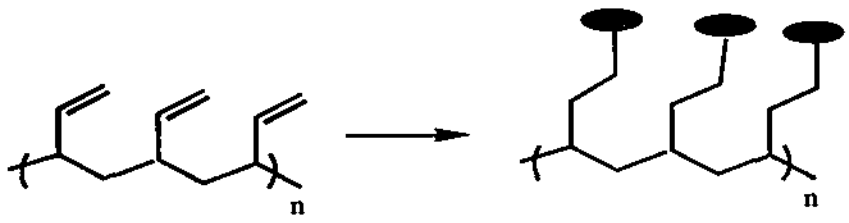


Figure 2. Functionalization of polybutadiene.

properties of the polymer must also ensure that the polymer has a low potential of being absorbed from the GI tract; it would be highly advantageous if the polymeric vehicle is physiologically inert.

In searching for a method that would overcome the limitations of some of the traditional polymer systems stated above and that could be used to build a tuneable, tailored system for delivery of misoprostol to the stomach, catalytic functionalization of polymers was evaluated. This method for preparing functionalized polymers is relatively unexplored[9,10] and has never been applied to the development of a tailored drug delivery system. The features that made this approach attractive were as follows: (**1**) the synthesis begins with a choice of a base or template polymer with a specific narrow molecular weight range;[11] (2) the concentration of functional group density for a specific polymer chain length can be precisely controlled;[10] (3) the kinds of functional groups can be controlled by sequential catalytic reactions; (4) in particular, key functional groups can be attached that would otherwise be very difficult to place on a polymer backbone via monomer polymerization (i.e., silyl chloride groups); and (5) the system can be readily optimized by fine-tuning of functional group density.

Chemistry

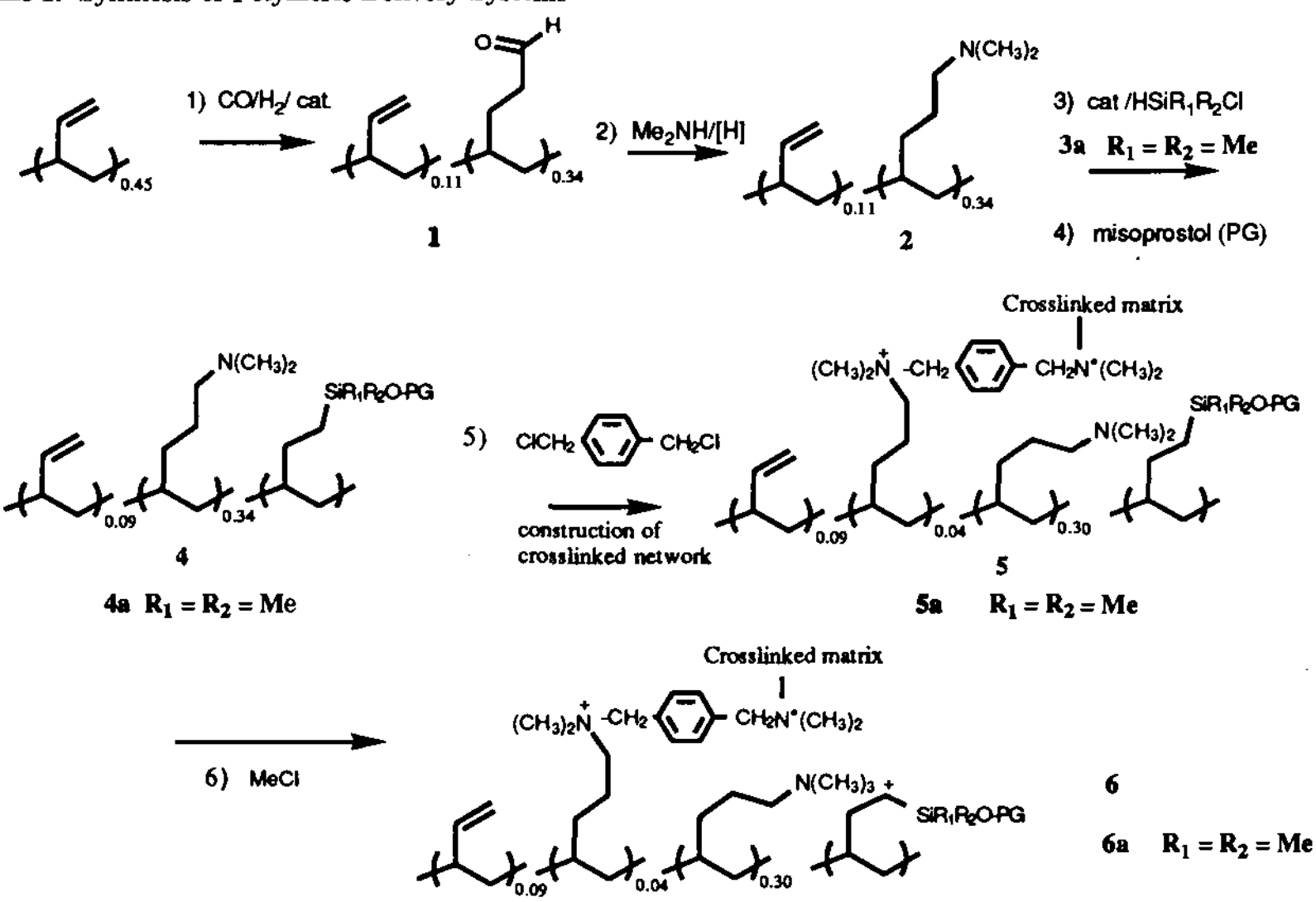
The Template Polymer—Polybutadiene. The polymer chosen as the template or base of the delivery system was polybutadiene, P(bd). P(bd) is available at various molecular weight ranges and with various vinyl contents by anionic polymerization of **1**,4-butadiene.[11] By catalytic reactions on P(bd), a functional group could potentially be placed on each butadiene unit (Figure **2**).

Considering the strategy of adding various functional groups on a polymeric substrate by sequential catalytic reactions, there is a potential of transforming base polymers into specialized systems with a reasonable degree of flexibility. The initial goal of this research, which is described in this paper, was to construct a flexible nonabsorbable system that releases misoprostol at an adjustable rate and to identify a pH selective silyl ether linker that releases the drug at an optimal rate at stomach pH **1**–3.

The System. Utilizing catalytic reactions on commercially available P(bd), the delivery system (Scheme I) was constructed in a six step synthesis. The steps were as follows: (**1**) controlled addition of hydrophilic groups by hydroformylation of polybutadiene; (2) reductive amination of the polyaldehyde to the polyamine; (3) attachment of the pH sensitive linker by hydrosilylation of the polyamine; (4) coupling of misoprostol to the silyl chloride polymer; (5) construction of an interconnected network by cross-linking of the misoprostol-polymer via the amine functional groups; and (6) methylation of the cross-linked polyamine.

Addition of Hydrophilic Groups (Steps 1 and 2 in Scheme I). Experimentally the desired concentration of hydrophilic groups can be readily controlled by catalytic transformation of the olefinic groups in P(bd). Taking advantage of the high concentration of evenly distributed olefins throughout P(bd), the amount of functional groups can be varied over a wide range in order to optimize hydrophilicity. The first step, the hydroformylation of polybutadiene, controls the functional density of hydrophilic groups on the polymer. The functional density of the aldehyde can be readily "dialed in" by monitoring gas

Scheme I. Synthesis of Polymeric Delivery Systems



uptake. We have varied the aldehyde concentration from 1% to 85%.[10] The polyaldehyde can be converted to a polyamine by reaction of a secondary amine and a suitable reductive amination catalyst such as borohydride reagents[12] or ruthenium carbonyls.[13] In the present work, a 5000 M_n (number-average molecular weight) polybutadiene (45% vinyl) was hydroformylated until 34% of the butadiene vinyl groups were converted to formyl units (Scheme I, step 1). Next the aldehyde units were reductively aminated with dimethylamine and H_2 to produce a polyamine in which 34% of the units contain amine groups (Scheme I, step 2). The polyamine M_n shifted to a slightly higher number average due to the increase in weight of the aminomethyl groups.

Attachment of the pH Selective Polymer-Drug Bond (Steps 3 and 4). The polyamine, **2**, was smoothly hydrosilylated at 100 °C with dimethylchlorosilane to produce a terminal chlorosilylated functionalized polyamine (**3a**, Scheme I, step 3). The hydrosilylation reaction was performed with a catalytic amount of Wilkinson catalyst and was selective for the vinyl groups of P(bd).[14] The reaction of the resulting chlorosilylated polymer with methanol is a key method for determining the quantity of active chlorosilane on the polymer. The methoxysilane resonances of **3a** are integrated relative to the olefinic peaks in the polymer backbone in order to calculate the percent of the butadiene units that have been silylated.

This water sensitive chlorosilylated polymer reacts with misoprostol to yield a polymer bound drug system (**4a**, Scheme I, step 4). The reaction was usually carried out with the prostaglandin as the limiting reagent using imidazole as the coupling catalyst and a DMF/THF mixture as solvent. The unreacted chlorosilyl groups on the polymer were then capped with methanol. The coupling yield (approximately 90%) for **4a** was obtained by cleavage of the silyl ether drug bond in pH 1 aqueous hydrochloric acid. Due to the high potency of misoprostol (the clinical dose is 100–200 μg qid), the amount of this drug covalently attached to the polymer was typically between 0.6 and 1 wt %.

Coupling Characterization. In contrast to the low loading of drug needed for the delivery system, it was necessary to increase the loading in order to fully characterize misoprostol's attachment to the polymer. The coupling of misoprostol to the 8000 M_n P(bd) in which 0.90% of the vinyl groups were chlorodimethylsilylated was performed in 1:1 DMF/THF using imidazole as the catalyst. After 2 h, the disappearance of the Si–Cl bond (464 cm^{-1}), and the appearance of a Si–OR bond at 1088 cm^{-1} was observed by *infrared* analysis. Thin-layer chromatography (SiO_2) also indicated that all of the misoprostol had reacted. The polymer–misoprostol system was then isolated and purified from the reaction matrix by precipitation from methanol which allowed traces of unreacted drug as well as catalyst to be removed in the methanol phase. The precipitated polymer displayed a strong carbonyl stretch at 1743.9 cm^{-1} which was assigned to bound misoprostol by comparison to free misoprostol. The ^1H NMR spectrum of the polymer indicated the presence of bound misoprostol [δ 3.60 (s, methyl ester), 4.0 (quartet, C-11-H)]. The ^{13}C NMR of the precipitated polymer demonstrated that the attachment position of misoprostol to the dimethylsilyl chloride functionalized polymer is at the C-11 hydroxyl of misoprostol. The evidence for the attachment site is based on comparison of the ^{13}C NMR signals of the C-11 and C-16 carbons of polymer bound misoprostol with those of free misoprostol, the bistriethylsilyl derivative, the mono C-11 *tert*-butyldimethyl- and *n*-butyldiisopropylsilyl derivatives (Table I). The C-11 position and the C-16 position of the polymer–misoprostol were assigned the 72.63 and 72.03 ppm resonances, respectively, by a ^{13}C attached proton (^{13}C APT) experiment. There was no evidence of coupling to the C-16 position; if the C-16 position of misoprostol was silylated, the predicted ^{13}C NMR chemical shift for C-16 would be in the 75-ppm region (Table I). It was determined that no unbound misoprostol was present by "spiking" the polymer sample with misoprostol; the C-11 and C-16 signals for free misoprostol could be observed in the spiked experiment. Silicon NMR was also used to verify the structure of **4a**. The ^{29}Si chemical shift of **4a** (19.5 ppm) was consistent with the ^{29}Si chemical shift of the 11-dimethyloctylsilyl derivative of misoprostol (18.7 ppm).

Table I. Comparison of ^{13}C Chemical Shifts of Various Silylated C-11 and C-16 Positions of Misoprostol

system	C-11 position ^{13}C, ppm	C-16 position ^{13}C, ppm
misoprostol	71.83	72.59
C-11 monosilylated misoprostol	73.08	72.24
t-Bu$(CH_3)_2$Si– disilylated C-11,C-16 triethylsilyl–	72.5	75.53
C-11 silylated Bu(*i*Pr)$_2$Si–	73.1	72.2
P(bd)–Si$(CH_3)_2$–	72.63	72.03

Construction of a Three-Dimensional Network and Swelling Properties at the pH of the Gastrointestinal Tract. At this point a polymer has been synthesized containing a fixed amount of hydrophilic groups (tertiary amines) and a specific amount of covalently bound misoprostol (**4a**). Next the polymer was transformed into a three-dimensional network by reaction of a portion of the amine groups with α,α-dichloro-*p*-xylene. The resulting cross-link sites are positively charged quaternary amine groups which are useful in creating a water swellable matrix. The cross-linking reaction also creates an insoluble interconnected polymer network that should have very low potential of being absorbed from the GI tract. The degree or density of cross-linking is controlled by the amount of cross-linker employed in the reaction. For **5a**, 10 mol % of the amine groups of **4a** were reacted at room temperature with α,α-dichloro-*p*-xylene to produce a white solid (Scheme I, step 5) that swells in solvents like THF and could be readily purified by washing with THF.

These cross-linked polyamines, in general, have some very interesting swelling (hydration) features in aqueous media that is dependent on the cross-linking density and the concentration of amine groups.[2] Figure 3 depicts the effect of pH on swelling of **5a**. (Swelling was measured both by volume and weight increases; the figures show only the weight changes.) Under acidic conditions the amine groups are protonated creating a positively charged matrix which will hydrate (swell) to relieve positive charge repulsion. Swelling of the polymer will allow acid to enter the matrix and hydrolyze the silyl ether drug bond and will also allow the prostaglandin to diffuse out of the polymer. Under neutral and basic pH conditions the polymer does not swell (Figure 3).

Our synthetic methods allow control of the amine concentration on the polymer and the cross-linking density, and the effect these variables have on the mechanical properties are summarized in Figures 4 and 5. Figure 4 illustrates the effect on swelling at pH 1 (stomach pH) as a function of amine concentration on the polymer (34%

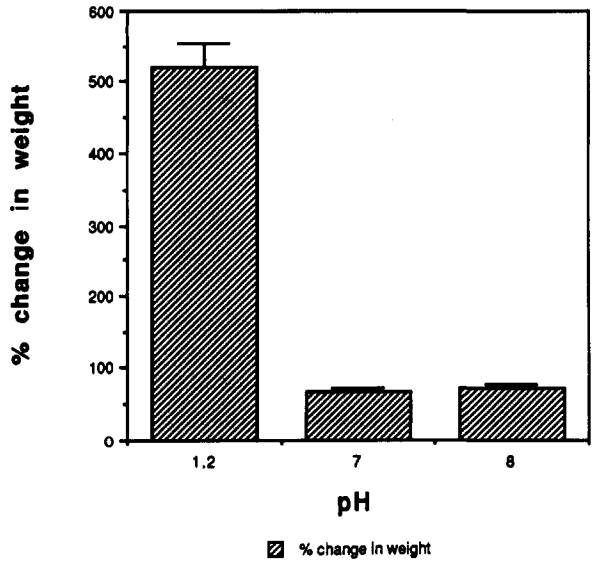


Figure 3. Effect of pH on 10% cross-linked polyamine.

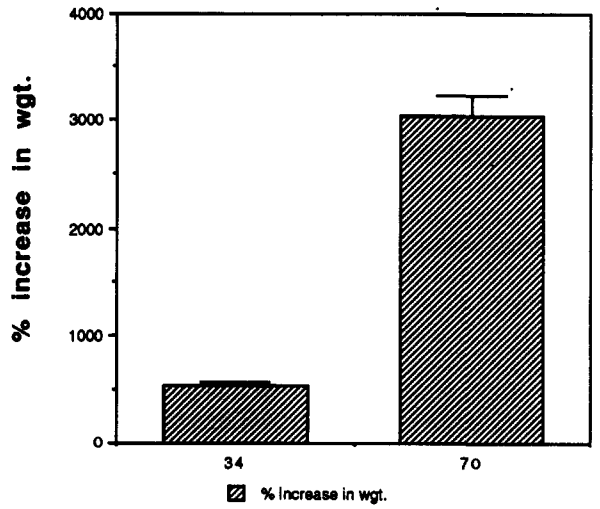


Figure 4. Cross-linked polyamines. Effect of amine functional density on hydration at stomach pH.

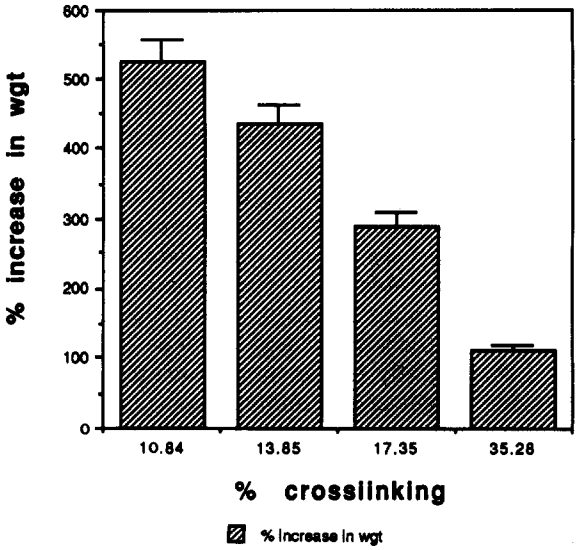


Figure 5. Cross-linked polyamines. Effect of cross-linking on swelling at stomach pH.

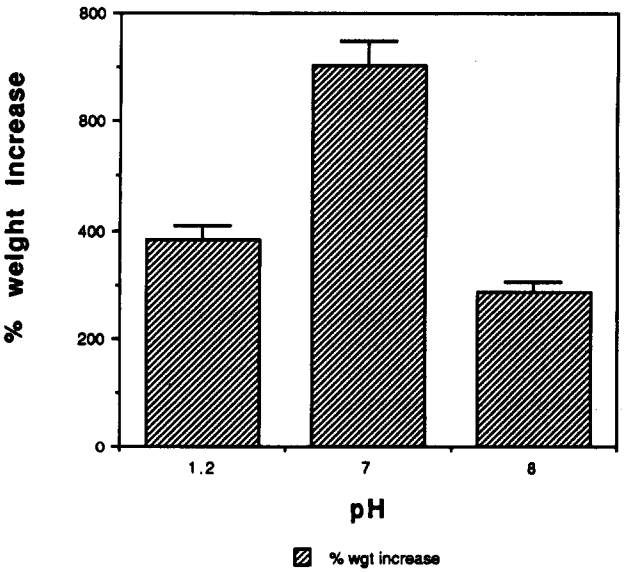


Figure 6. Effect of pH on swelling of 6a.

and 70% of the butadiene units) at a constant 10% cross-linking density. The greater the concentration of amine groups, the more positive charge is created at pH 1 due to amine protonation and the more the polymer matrix will swell.[2] Figure 5 illustrates the swelling at pH 1 as a function of cross-linking density at a constant amine concentration; polyamine 2 was used for this study. The higher the cross-linking density, the less the polymer swells at pH 1.

Methylation of the Cross-Linked Polyamine. By reacting the 10% cross-linked polyamine system (5a) with methyl chloride the system was converted into a positively charged quaternized system 6a (Scheme I, step 6). The reaction was performed by swelling the insoluble polymer matrix (5a) in THF and reacting the swollen polymer with excess methyl chloride in a Fischer & Porter pressure bottle at room temperature for 60 h. The resulting crystalline polymer was milled in a cryogenic grinder, seived (250 microns), and mixed with an equal weight of hydroxypropylmethylcellulose (HPMC) to give 6a as a white, free-flowing powder having a 68 ± 3 μm mean volume diameter. This quaternized system swells under aqueous conditions (pH 1–8), and its pH hydration profile is shown in Figure 6.

The above swelling studies demonstrate the role that the hydrophilic quaternary ammonium groups and cross-linking density have on the mechanical properties of the polymeric delivery system at the pH of gastrointestinal tract (pH 1–8). These results also illustrate the ability to create a mechanical pH-gated system that can be fine-tuned to swell under acidic conditions (Figure 3) and where further modification of the hydrophilic groups can lead to a matrix (6a) that swells throughout the GI tract (Figure 6). The degree of swelling can be further adjusted by changing the functional density of the hydrophilic groups and cross-linker (Figures 4 and 5).

A critical component in 6a is the hydrophilic quaternary ammonium groups. In initial systems not containing this functionality, the polymer was extremely hydrophobic and did not release misoprostol in aqueous acidic medium (gastric juice). The quaternary ammonium groups, in addition to instilling swelling properties and increasing the hydrophilicity of the polymer, also improve the crystallinity of the final polymer product. It may be possible that these groups also provide a bioadhesive property[15] by virtue of charge interaction with the mucus layer of the stomach.

Hydrolysis Studies of the Polymer-Silyl Ether

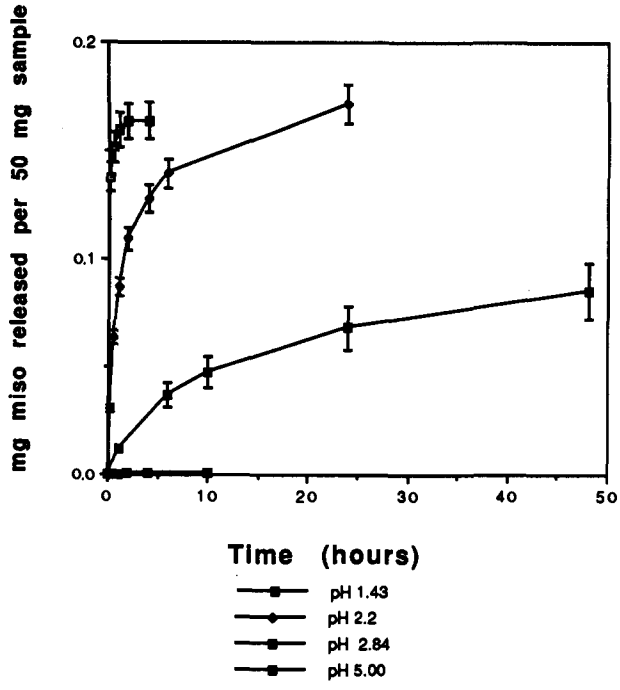


Figure 7. pH Regulated release of misoprostol from 6a.

Drug System 6a. One of the primary aims of this research was to evaluate the possibility of selectively releasing misoprostol at stomach pH. Although the hydration of the polymer can be adjusted to selectively swell the polymer under acidic conditions and accelerate the diffusion of cleaved misoprostol from the matrix, the upper intestinal tract can also be acidic (duodenum-pH 4–6) and might allow misoprostol to be released after the delivery system exits the stomach. By incorporating a silyl ether hydrolysis mechanism as the primary method of releasing misoprostol from the matrix and using matrix swelling as a secondary mechanism, selective release of the drug at stomach pH at an adjustable rate should be achievable. In order to evaluate the role of the silyl ether group, a silyl ether hydrolysis study was performed with the methylated cross-linked polyamine **6a** which swells throughout the GI tract (pH 1–8). Because the matrix will remain open from pH 1 to 8, this study allowed focus on the hydrolysis reaction. The release rate of misoprostol was studied with time by stirring **6a** in a 1:1 mixture of methanol and pH 1.18 HCl aqueous solution. The pH profiles are shown in Figure 7. This system readily releases misoprostol at pH 1, but the release of misoprostol decreases as the pH increases with less than 3% release of misoprostol above pH 5.

Fine-Tuning the Hydrolysis Rate of Misoprostol from the Polymer Matrix. The next goal was to develop a system that delivered misoprostol at an adjustable rate. Inspection of Figure 7 for **6a** (dimethylsilyl analog) indicates that at pH 1.4 greater than 90% of the drug is released in the first one-half hour. Besides modifying the swelling properties of the matrix by varying the cross-linking density and amine concentration, the release rate should be adjustable at the molecular level by controlling the hydrolysis rate of the silyl ether drug bond. This implies, of course, that the hydrolysis reaction is a pivotal factor in the rate determining step in drug release from the polymer matrix. To confirm this hypothesis a series of polymer systems, in which the quaternary ammonium groups and cross-linking density were held constant and the organosilyl group varied over a steric range, was prepared. This analog study in which only one polymer variable was changed (the organosilyl linker) could easily be accomplished using the catalytic reactions on polymer methodology. For the analog study, the concentration of quaternary ammonium groups and the cross-linking density of the polymer matrix were kept the same as employed for the dimethylsilyl system (**6a**), and the organic groups on silicon were varied according to Table II; the percent of butadiene units that were silylated varied from 1–2%. As stated above the silylation of the vinyl groups of polyamine **2** with dimethylchlorosilane produced the expected saturated polymer–silicon bond ($-CH_2CH_2Si(CH_3)_2Cl$). Interestingly, the silylation of **2** with diisopropylchlorosilane produced either a saturated polymer–silicon bond (**3f**) when platinum divinyltetramethyldisiloxane was used as a catalyst or an unsaturated polymer–silicon bond ($-CH{=}CHSi(i\text{-}Pr)_2Cl$, **3e**) when Wilkinson catalyst was employed.

Table II. Relative Initial Hydrolysis Rates

substrate	pH 2[a]	pH 2.2[b]
Bu(*i*Pr)$_2$SiOMiso	18.0	
polymer–(*i*Pr)$_2$SiOMiso **6e**	1.0	1.0
polymer–(Ph)$_2$SiOMiso **6d**		2.1
polymer–PhMeSiOMiso **6c**		4.4
polymer–(Et)$_2$SiOMiso **6b**		5.1
polymer–(Me)$_2$SiOMiso **6a**		62.5

[a] Hydrolysis conditions: 0.21 mmol of substrate in 2 mL of acetonitrile and 4 mL of 0.01 N aqueous hydrochloric acid. [b] Hydrolysis conditions: 50 mg of polymer (**6a–e**) in 3 mL of methanol and 3 mL of 0.01 N aqueous hydrochloric acid.

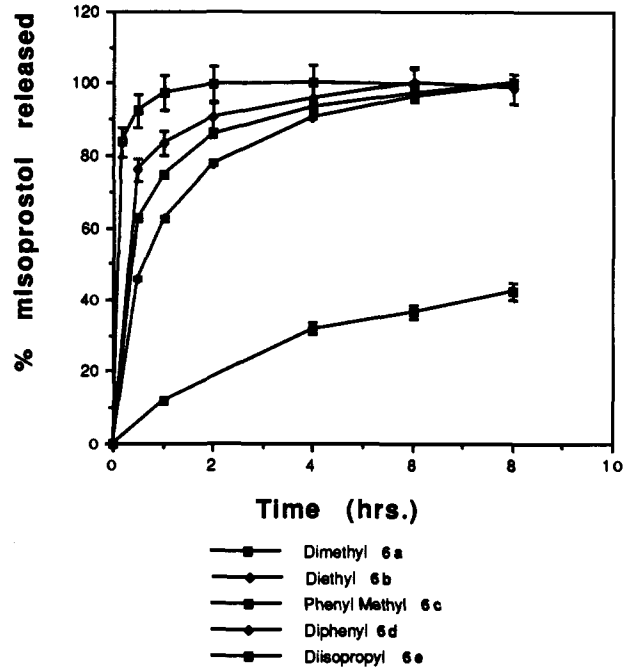


Figure 8. In vitro release rate of misoprostol from polymer delivery systems at pH 1.4.

If the hydrolysis rate is the important factor in the drug release rate, then a change in release rate as a function of organosilyl groups should be observed. Figures 8 and 9 show this relationship. As the pH is increased (Figure 8 vs 9), the hydrolysis rate of misoprostol from all the analogs decreases, and as the size of the organosilyl group increases, the rate of release decreases.

The hydrolysis studies were also used to determine the maximum amount of misoprostol on the polymer. Although the maximum quantity of misoprostol from the faster release systems (i.e., the dimethylsilyl analog **6a**) could be determined by acid cleavage, the maximum amount from the slow release diisopropylsilyl analogs (**6e**

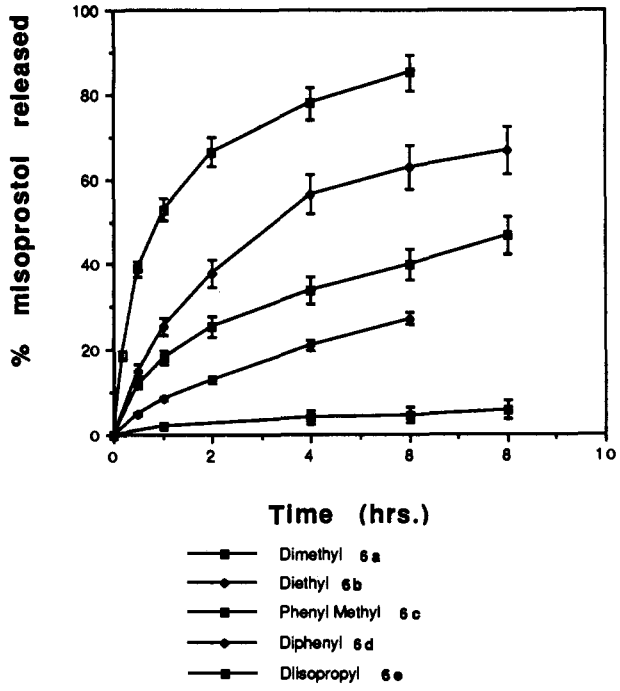


Figure 9. In vitro release rate of misoprostol from polymer delivery systems at pH 2.2.

and **f**) had to be determined indirectly by reaction of the polymer–drug matrix with sodium methoxide/methanol. The reason for this is that misoprostol slowly degrades under the acidic analysis conditions, and the long (>48 h) analysis time required for **6e** and **f** compromised the accuracy of this method. The sodium methoxide reaction rapidly and quantitatively converts all of the bound misoprostol to the corresponding PGB derivative (prosta-8(12),13-dien-1-oic acid, 16-hydroxy-16-methyl-9-oxo-, methyl ester (13*E*)).[16] As a control, the C-11-*tert*-butyldimethylsilyl derivative of misoprostol was subjected to the sodium methoxide/methanol assay; a 98.5% yield of the PGB derivative was obtained by HPLC.

The concentration of misoprostol on **6a–f** was assumed to be the maximum amount released in the hydrolysis studies. The concentration varied between polymer systems but generally was in the range of 1.2–5 μg of misoprostol per milligram of weight. Confirmation of these concentrations will be obtained by studies with radiolabeled misoprostol. The initial rate of the hydrolysis reaction for the analogs was determined at pH 2.2 (Table II); the rate of release decreases by a factor of 63 by substituting a diisopropylsilyl group for a dimethylsilyl group. The initial rate can be fine-tuned by proper choice of organosilyl group as illustrated in Table II.

The release of misoprostol from the polymer as a function of organosilyl groups is consistent with the hydrolysis studies of Kawazoe[7] and the solvolysis studies of Sommer.[17] Kawazoe determined the rate constants for acid and base hydrolysis of monomeric trialkylsilyl ethers. Increase in steric size of the organosilyl group effectively reduces the rate of acid hydrolysis. In his studies of the hydrolysis of benzyl-*O*-SiR(Me)$_2$ the rate of acid hydrolysis increases by 6.5×10^3 when R is changed from *t*-Bu to Me. Kawazoe also has demonstrated that increase in steric size around the silicon atom has a larger impact in rate reduction of neutral and base hydrolysis of the silyl ethers than acid hydrolysis. In Kawazoe's base catalyzed hydrolysis studies with benzyl-*O*-SiR(Me)$_2$ the increase in the steric size of the R group reduces the rate remarkably.

Table III. Comparison of Initial Hydrolysis Rates

substrate	k_{rel}[a]	k_{rel}[b]	substrate
Me_3SiOmenthyl	1	1	polymer–$(Me)_2SiO$Miso
Et_3SiOmenthyl	0.16	0.08	polymer–$(Et)_2SiO$Miso
$PhMe_2SiO$menthyl	0.86	0.07	polymer–(PhMe)SiOMiso
		0.034	polymer–$(Ph)_2SiO$Miso
Ph_3SiOmenthyl	0.0025		

[a] Acid catalyzed methanolysis of R_3SiOMe using equimolar pyridine–pyridine hydrochloride. Data obtained from ref 17. [b] Equimolar methanol/pH 1.9 aqueous hydrochloric acid.

Kawazoe's results are consistent with our data obtained with the bulky diisopropylsilyl system which releases very little misoprostol above pH 3. Table III compares our hydrolysis study with Sommer's solvolysis study of steric and polar effects of some R_3SiO-menthyl (secondary alcohol) systems. The general trend of increase in steric size with decrease in acid catalyzed release rate can be observed.

The fact that we do not observe as large a change in the rate of hydrolysis when going from a dimethyl analog to a diisopropyl analog when compared to Kawazoe's study of the hydrolysis of benzyl-*O*-SiR(Me)$_2$ (R = Me and *t*-Bu) is probably partially due to the effect of the polymer backbone in controlling the rate of hydrolysis.[18] It was found that the initial rate of hydrolysis of the polymeric diisopropylsilyl analog **6e** was 18 times slower than the rate of hydrolysis of a monomeric C-11 *n*-butyldiisopropylsilyl derivative of misoprostol (Table II). Although the hydrolysis medium was the same (acetonitrile/0.01 N HCl) for both the polymer and the monomer, the hydrolytic cleavage of misoprostol from **6e** occurs in the unique acid swelled polymer phase, whereas the hydrolysis of the monomer analog occurs under homogeneous conditions. This comparison suggests that the unique microenvironment of the hydrated polymer also has an impact on the rate of release of the drug from the matrix. When misoprostol is physically infused (intimately mixed but not covalently attached) into the polymer matrix (methylated cross-linked matrix **6** containing no covalently attached misoprostol), the release rate of misoprostol in pH 1–2 methanol/aqueous hydrochloric acid is rapid and quantitative and occurs in less than 5 min; this observation suggests that diffusion of misoprostol from the matrix is not part of the rate determining step. Further studies are needed to understand the differences in hydrolysis of the polymer and monomer analogs.

Pharmacology

The five delivery systems (Table VI) were compared individually to misoprostol/HPMC[19] for antilesion activity against indomethacin-induced gastric damage and for diarrheagenic activity in rats. Misoprostol/HPMC was run as a control in each experiment because of the variability encountered with it in these assays. HPMC and blank polymers (containing no misoprostol) were also run as controls, and neither produced diarrhea or exhibited mucosal protective activity at the doses tested. Interestingly, although the *in vitro* release rate of drug from the first system prepared, **6a**, was relatively rapid at pH 2.2 (Figure 9), the equal antilesion activity and the reduced diarrheagenic activity observed with **6a** relative to misoprostol/HPMC suggested that our strategy of controlled, site-directed delivery of this drug was successful in reducing intestinal side effects while maintaining antilesion efficacy. Consequently, additional experiments were run with this material (Table IV). Diarrhea was not observed following

Table IV. Diarrheagenic and Mucosal Protective Activity of Misoprostol/HPMC and Polymer System 6a

test	route[a]	ED_{50} (95% C.I.) (μg misoprostol/kg) misoprostol/HPMC	6a
		Diarrheagenic Activity	
	I.G.	265 (188,388)	715[b] (387, 1352)
	I.J.	534 (377, 757)	>1470[c]
	I.C.	97 (50–190)	>1470[d]
		Mucosal Protective Activity	
4 h EtOH model		525 (410, 655)	161[e] (120, 217)
indo/gastric		14 (9, 19)	10 (5, 17)
indo/intestinal		294 (212, 407)	265 (205, 342)

[a] IG, intragastric, IJ intrajejunal, IC intracolonic. [b] Significantly greater than misoprostol/HPMC ED50, p = 0.006 (likelihood ratio test). [c] Diarrhea not observed at any dose. [d] One of six rats had diarrhea at this dose; no diarrhea was observed at lower doses. [e] Significantly less than misoprostol/HPMC, $p < 0.001$.

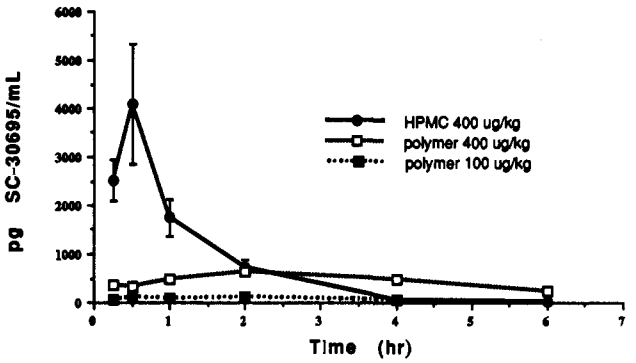


Figure 10. Plasma concentrations of SC-30695 after oral administration of misoprostol/Polymer **6a** (n = 1–3/sampling time).

Table V. Plasma Pharmacokinetic Parameters for SC-30695 after Oral Administration of Misoprostol/HPMC (400 μg miso/kg) or 6a (100 and 400 μg/kg) to Male Rats

dose group	time to peak (h)	peak concn (Pg/mL)	AUC[a] (Pg/mL/h)	% systemic exposure[b]
miso/HPMC	0.5	4100	4770	
polymer 100 μg/kg	2.0	131	402	8.4
polymer 400 μg/kg	2.0	646	2790	58.5

[a] Area under the plasma concentration-time curve from time 0–6 h. [b] Relative to the HPMC 400 μg miso/kg dose which is considered 100%.

injection of **6a** into the lumen of either the upper small intestine or the colon in doses as high as 1470 μg of misoprostol/kg, indicating that cleavage of the prostaglandin from the polymer does not occur in the intestinal tract. The material (ED_{50} = 161 μg of misoprostol/kg) was significantly more potent than misoprostol/HPMC (ED_{50} = 525 μg of misoprostol/HPMC) in the 4 h ethanol rat model. This observation suggests sustained availability of the prostaglandin in the stomach. **6a**, like misoprostol/HPMC, protected against indomethacin-induced intestinal damage in the rat on oral administration; the two materials exhibited similar potency, the ED_{50} values being, respectively, 294 and 265 μg of misoprostol/kg.

As a followup to the pharmacological findings, a pharmacokinetic study was done in rats to compare the concentration of the free acid metabolite of misoprostol (SC-30695) after bolus intragastric doses of misoprostol/HPMC (400 μg of misoprostol/kg) and **6a** (100 and 400 μg of misoprostol/kg) (Figure 10, Table V). Analysis was conducted for the free acid metabolite because misoprostol is rapidly deesterified *in vivo*. The polymeric delivery system substantially reduced the peak plasma level and, at the 400 μg/kg dose, provided a sustained, low level of prostaglandin for the duration of the experiment (6 h). At equal doses of 400 μg/kg of misoprostol, the total amount of systemic exposure to SC-30695 (AUC) from **6a** was approximately 58% of the misoprostol/HPMC dosage. These findings suggest both a sustained availability of misoprostol from the delivery system in the stomach as well as a reduced potential for systemic side effects such as uterotonic activity.

Table VI. Comparative Pharmacology of Misoprostol Polymer Systems

system	silicon substitution	ED_{50} (μg of misoprostol/kg i.g.) antiulcer activity (indo/gastric)	diarrheagenic activity
6a	$R_1 = R_2$ = Me	10.0	715[a]
	miso/HPMC	14.0	265
6b	$R_1 = R_2$ = Et	7.7	910[a]
	miso/HPMC	9.5	290
6c	R_1 = phenyl, R_2 = Me	7.0	858[a]
	miso/HPMC	7.6	325
6d	$R_1 = R_2$ = phenyl	16.0	2031[a]
	miso/HPMC	27.1	485
6e	$R_1 = R_2$ = isopropyl	32.6	no diarrhea at 1865
	miso/HPMC	20.5	430

[a] Significantly greater than control (misoprostol/HPMC) $p < 0.05$.

Systems **6b–e** were designed to progressively increase the steric hindrance around the silyl ether and thus incrementally reduce the rate of drug release. Figure 9 shows the progressive reduction in drug release rate at pH 2.2. While systems **6b** and **6c** did not show improvement over **6a** in separation of desired and undesired pharmacological effects, the diphenyl system **6d** did show an improvement over **6a** by virtue of reduced diarrheagenic activity. Thus it appears that progressively slowing the release rate of drug reduces the intestinal side effects without affecting antilesion activity. This conclusion was dramatically confirmed with the diisopropyl system **6e**[20] which exhibited the slowest *in vitro* release rate of drug, equal antilesion efficacy as misoprostol/HPMC, and no evidence of diarrhea at the highest dose tested. This system is currently under detailed investigation.

Conclusions

This study demonstrates that metal catalyzed reactions on a template polymer can be used to design tailored drug delivery systems. This study also demonstrates the usefulness of a polymer-bound organosilyl linker in achieving a selective stomach directed delivery system. From the release rate profiles the hydrolysis of the silyl ether drug polymer bond is the primary rate determining step. By comparison of the monomeric and polymeric systems we also observed that the polymer matrix has a role in determining the release characteristics which can be fine-tuned by choice of the organosilyl functional group. The pharmacological properties of these delivery systems confirmed our theory that controlled release of misoprostol at its site of action provides effective antilesion activity while reducing or eliminating intestinal side effects. Blood level studies with **6a** also suggest that systemic side effects can be reduced or perhaps eliminated with slower release systems such as **6e**. Studies are in progress to further characterize these interconnected polymer networks as well as the mechanistic implications of the hydrosilylation reactions on polymers. We are also applying this delivery system technology to other classes of drugs.

Experimental Section

General Procedures. ^1H and ^{13}C NMR spectrum were recorded on Varian 300 and 400 spectrometers in deuteriochloroform unless stated otherwise. ^1H–^{29}Si HMBC, ^1H–^{13}C HMQC,

and ^1H–^{13}C HMQC indirect detection experiments were performed on a Varian Unity 400 MHz spectrometer. All chemical shifts are reported with respect to TMS. THF and toluene were distilled from sodium/benzophenone, and anhydrous DMF (Aldrich) was used as received. Polybutadiene for this study was obtained from Revertex, Catalog no. N4500. Hydroxypropylmethylcellulose was USP 3 CPS grade. HPLC analytical: HPLC analysis of misoprostol was performed on a Spectra-Physics HPLC fitted with an Altex/Beckman Ultrasphere ODS 5 μm 80Å poresize 4.6 mm × 25 cm reverse phase column (part no. 235329). Samples were injected using a Spectra-Physics autosampler set to a 100-μL injection size. Samples were eluted with a ternary solvent system of water, methanol, and acetonitrile using an SP8500 dynamic mixer for good solvent mixing. All solvents used were Burdick and Jackson HPLC grade. Samples were detected with a UV detector set to λ = 210 nm, AUFS = 0.100, and rise time = 1.00 unless otherwise noted. Additional information on the HPLC method for detecting prostaglandins can be found in the supplementary material.

Particle size analyses were performed on the methylated crosslinked polyamines (**6a–f**) that were each mixed to 50 wt % with hydroxypropylmethylcellulose. The particles were measured on a Brinkmann Model 2010 particle size analyzer. The particles were dispersed in a mixture of freon–acetone.

Swelling Studies. A 50-mg sample of polymer was placed in a graduated vial, and the weight and volume were recorded. The volume of the dry polymer could be verified from its weight and density. Next 2 mL of water at a specified pH (pH 1, 7, or 8) was added to the vial. After the mixture was placed on a shaker bath overnight at 25 °C, the weight and volume were again recorded. The percent weight increase was calculated from the following equation:

$$\%\ \text{weight increase} = 100 \times [W_{\text{final}} - W_{\text{initial}}]/W_{\text{intial}}$$

Analysis of Drug Content. A 50-mg sample of the polymer–misoprostol system was weighed into a centrifuge tube with a stir bar. Next 3 mL of a 0.01 N solution of HCl/H_2O and 3 mL of HPLC MeOH were transferred into the reaction vessel. The reaction was allowed to proceed for a sufficient time period to obtain the maximum release of drug from the matrix. This procedure was used to determine the maximum amount of misoprostol on polymers **6a–d**. The percent release was then obtained at each time point from the following equation:

$$\%\ \text{release at time } t = 100 \times \frac{[\text{conc of misoprostol}]_t}{[\text{conc of misoprostol}]_{\text{max}}}$$

Indirect assay for the total amount of misoprostol bound to the diisopropylsilyl systems (**6e** and **f**): The polymer–misoprostol system (50 mg) was stirred in 5 mL of methanol and 1 mL of 25% methanolic sodium methoxide for 10 min at 25 °C. A 250-μL aliquot was analyzed by reverse phase HPLC using the same column conditions and mobile phase as above but with the UV detector set at 280 nm. The only product observed was the PGB derivative of misoprostol. A 98.5% yield of the PGB derivative was obtained for prost-13-en-1-oic acid, 11-[[butylbis(1-methylethyl)silyl]oxy]-16-hydroxy-16-methyl-9-oxo-, methyl ester (11α, 13*E*) by HPLC.

Rate Measurements for Hydrolysis of the Silyl Ethers (Table II). The polymer systems (50-mg sample) were stirred in 6 mL of methanol/aqueous hydrochloric acid (1/1) at the desired pH at 25 °C. The pH of the system remained the same throughout the experiment and was recorded with a pH meter (Corning Semi-Micro Combination Electrode catalog no. 476540). At specified time intervals the solution was centrifuged and a 250-μL aliquot was removed and analyzed by reverse phase HPLC at 210 nm. Each k_{obs} was the mean from at least three separate experiments with correlations >0.96 for the slope k_{obs}. The monomer prost-13-en-1-oic acid, 11-[[butylbis(1-methylethyl)silyl]oxy]-16-hydroxy-16-methyl-9-oxo-, methyl ester (11α, 13*E*) and polymer **6e** were hydrolyzed by reacting 0.21 mmol of the monomer or 50 mg of **6e** in 2 mL of acetonitrile and 4 mL of 0.01 N aqueous hydrochloric acid at 25 °C.

Synthesis: Prost-13-en-1-oic Acid, 11-[[Butylbis(1-methylethyl)silyl]oxy]-16-hydroxy-16-methyl-9-oxo-, Methyl Ester (11α, 13*E*). A solution of 83.3 mg of *n*-butyldiisopropylchlorosilane (0.40 mmol), 0.049 g of (dimethylamino)pyridine (0.40 mmol), and 0.140 g of misoprostol (0.366 mmol) were reacted in 2.6 g of methylene chloride for 96 h at 27 °C. The product was isolated by elution on silica gel using ethyl acetate: Si NMR 15.4 ppm; high-resolution mass spectrum calc 509.3662, obs 509.3855; ^{13}C NMR C-1, 174.01; C-2, 33.99; C-3, 24.833; C-5, 29.38; C-6, 26.98; C-7 28.025 & 28.054; C-8 54.64, 54.66; C-9, 215.95 ← 215.96; C-10 47.75; C-11, 73.1; C-12, 53.79; C-13, 134.09 & 134.12; C-14, 128.6; C-15, 45.09 & 45.12; C-16, 72.21; C-17, 41.477 & 41.66; C-18, 26.689; C-19, 23.27; C-20, 14.11; CH_3 13.71; CH_2Si 14.12; CH_2CH_2Si, 25.58; $CH_2CH_2CH_2Si$, 26.029; CH, 12.6 and 12.63; isopropyl CH_3, 17.56 and 17.59.

Prost-13-en-1-oic Acid, 11-[[Dimethyloctyl)silyl]oxy]-16-hydroxy-16-methyl-9-oxo-, Methyl Ester (11α, 13*E*). A solution of 0.03 g of *n*-octyldimethylchlorosilane (0.145 mmol), 0.01 g of imidazole (0.145 mmol), and 0.056 g of misoprostol (0.145 mmol) was reacted in 3 g of DMF at 27 °C for 2 h. After extracting the product from the DMF solution by addition of water and ether, the crude product was isolated from the ether phase. The product was purified by elution on silica gel using 80/20 hexane/ethyl acetate: Si NMR 18.7 ppm.

Prost-13-en-1-oic Acid, 11,16-[(Triethylsilyl)oxy]-16-methyl-9-oxo-, Methyl Ester (11α, 13*E*). A solution of 300 mg (0.78 mmol) of misoprostol, 204 mg (3 mmol) of imidazole, and 272 mg (1.8 mmol) of triethylchlorosilane in 5 mL of DMF was stirred at room temperature for 16 h. The solution was partitioned between ether and water, the aqueous layer extracted with additional ether 4 times, and the combined organic layers washed with water, dried (Na_2SO_4), and evaporated. The resulting oil was purified by chromatography or silica gel (5% EtOAc in hexane): ^1H NMR δ 1.15 (d, 3H, 16-CH3), 4.0 (q, 1H, C-11 H).

Polyaldehyde (1). A 289-g sample of polybutadiene (Revertex N4500; 45% vinyl M_n = 4500) was dissolved in 308 mL of toluene. Under a N_2 atmosphere, this polymer solution was poured into a 2-L autoclave. Next 12.7 g of triphenylphosphine and 0.27 g of hydridocarbonyltris(triphenylphosphine)rhodium(I) were added to the clave under N_2. The clave was sealed and further degassed with N_2 by pressuring the clave with 200 psi of N_2 and then venting the N_2 gas. The clave was then heated to 80 °C under 400 psi of N_2. After removing the N_2, the clave was charged with 300 psi 1:1 CO/H_2 at 80 °C. The reaction was stirred at 1000 rpm until 3.5 mol of 1:1 CO/H_2 was reacted from a calibrated reservoir. After venting the clave, the polymer product was removed. The clave was washed with 100 mL of toluene, and the washings were added to the polymer product. The product solution was concentrated to a 500-mL volume by rotary evaporation. This polymer solution was then slowly dripped into a solution containing 1600 mL of methanol and 400 mL of water. The solution was allowed to separate into two phases and the top phase was decanted. The bottom polymer phase was dissolved in 600 mL of toluene, and the above precipitation procedure was repeated. From this procedure, 308.4 g of polymer product was isolated. An infrared spectrum of a thin film of the polymer product on a KBr plate indicated aldehyde absorption at 1730 cm^{-1}. ^1H NMR indicated that 33.4% of the double bonds in the P(bd) polymer were hydroformylated. The aldehyde protons are integrated relative to the olefinic peaks in the polymer backbone in order to calculate the percentage of butadiene units that have been hydroformylated. Selected ^1H NMR resonances: δ 9.75 major (> 90%); 9.55 and 9.65 minor, aldehyde; 4.8–5.1, m, CH_2=C; 5.15–5.65, m.

Polyamine (2). Method A. A toluene solution of polyaldehyde 1 was concentrated *in vacuo*. A 2-L clave was then charged with 150 g of 1 in 392.5 mL of DMF, 330 mL of cyclohexane, 112 g of dimethylamine, and 1.5 g of ruthenium carbonyl. The autoclave was sealed and purged with 100 psig of CO/H_2. The autoclave was charged with 100 psig of CO/H_2 and 900 psig of H_2 and was heated to 120 °C with a stirring speed of 1000 rpm. Gas uptake began when the reaction temperature reached approximately 90 °C. The reaction was allowed to proceed until gas uptake ceased (approximately 2 h). After cooling to room temperature, the contents of the autoclave were removed and placed in a 1-L separatory funnel and allowed to phase out. The total volume level was marked on the funnel. The lower reddish-brown DMF level was removed and discarded. Three hundred milliliters of DMF were added to the separatory funnel, and cyclohexane was also added to increase the liquid level in the flask to the original volume. The funnel was shaken to extract

the cyclohexane layer with the DMF, and the separation procedure was repeated. This DMF extraction procedure was performed a total of three times. The cyclohexane layer was filtered with a medium porosity glass-fritted buchner funnel and rotovapped at 50 °C with toluene to remove residual DMF. The polymer was dried on a vacuum line for 2 h. The concentration of amine groups was determined by ^{1}H NMR using acetic acid-d_4 as the solvent. In acetic acid-d_4 the protonated amine signals were shifted downfield and away from the proton signals for the polymer backbone allowing for integration of the amine protons and comparison of these integrals to the fixed amount of olefinic proton signals in the polymer backbone. ^{1}H NMR in acetic acid-d_4 indicated total conversion of aldehyde and indicated that 35% of the butadiene units were aminomethylated. The protons on the carbon groups that are α to the amine functional groups are integrated relative to the olefinic peaks in the polymer backbone in order to calculate the percentage of the butadiene units that have been functionalized with tertiary amines. The aminated polymer was then stored in toluene in the dark. Selected proton NMR resonances (CD_3CO_2D): olefinic peaks δ 5.45–5.1, m, and 4.9–4.65, m, CH_2=C; δ 2.65, s, $(CH_3)_2NH^+$; δ 2.9, s, polymer-CH_2NH $(CH_3)_2$. No aldehyde peaks were observed. Anal. Calc. for 34% functionalization: C, 81.0; H, 11.76; N, 6.40. Found: C, 81.02; H, 12.26; N, 6.51.

Method B. A solution of formyl-functionalized polymer (63.14 g) in 100 mL of 4Å molecular sieve dried toluene and 20 mL of methanol was placed in a 2-L, three-necked, round-bottomed flask that was equipped with a thermometer, addition funnel, and a magnetic stir bar. The solution was further diluted with 500 mL of THF and 40 mL of methanol. After cooling to 5 °C under N_2, dimethylamine (50 g) was added with stirring. After 15 min, 85.8 mL of a 4.5 M HCl in dioxane solution was slowly added to the solution by means of an addition funnel. Finally, 17 g of sodium cyanoborohydride was added by means of a funnel and rinsed into the flask with 20 mL of THF. The solution was stirred for 40 h and allowed to warm slowly to room temperature. The polymer solution was stirred with 60 mL of water for 1 h and then filtered. The filtrate was concentrated to a 200-mL volume, and after settling for 2 h the top polymer phase was slowly dripped into a mixture of 400 mL of methanol and 100 mL of water. The bottom polymer phase was decanted and isolated. This precipitation procedure was repeated: 64.6 g of polyamine was isolated. Anal. Found: C, 80.62; H, 11.66; N, 6.62. The NMR spectrum was identical to that obtained by method A.

Chlorodimethylsilylated Polyamine (3a). An 84.5-g sample of a 29.6 wt % solution of the polyamine **2** (25 g of **2**) in toluene was added to a Fischer & Porter bottle that was equipped with a stir bar. The solution was concentrated to 50 g *in vacuo*, and the evacuated vessel was brought into the drybox. Enough dry toluene was added in order to make a 50 wt % solution. Next 0.125 g of tris(triphenylphosphine)rhodium(I) chloride and 12.5 g of dimethylchlorosilane were added to this solution. After capping and removing the Fischer & Porter bottle from the drybox, the solution was heated to 100 °C for 17 h. The reaction was again placed in a drybox and transferred to a dry 250-mL round-bottomed flask. The solution was concentrated to 37.6 g (to remove monomer silane) and then diluted with 100 mL of dry THF. ^{1}H NMR indicated 2.9% chlorosilane incorporation. Selected ^{1}H NMR resonances: δ 0.38, $(CH_3)_2$SiCl–polymer; 5.45–5.1, m; 4.9–4.65, m, CH_2=C. The methoxy derivative was formed by reaction with methanol to further characterize the silylated polyamine: selected ^{1}H NMR resonances δ 0.0, $(CH_3)_2$SiOMe–polymer; 3.38, $(CH_3)_2$SiOCH_3–polymer; Si NMR δ 19.5 $(CH3)_2$-*Si*OMe–polymer. The silicon NMR chemical shift is in agreement with that of 18.77 ppm for prost-13-en-1-oic acid, 11-[[(dimethyloctyl)silyl]oxy]-16-hydroxy-16-methyl-9-oxo-, methyl ester (11α, 13*E*).

Chlorodiethylsilylated Polyamine (3b). A solution of 10.0 g of **2** (30 wt % solution in toluene, 33.5% amine functionality, mol of **2** = 0.136) was added to a 6 oz. Fischer & Porter glass pressure reaction vessel with a magnetic stir bar. The toluene was removed from **2** *in vacuo*. The reactor was taken into a drybox under vacuum, 50 mg of chlorotris(triphenylphosphine)-rhodium(I) (Strem catalog no. 45-0650) was added, and then enough dry toluene was added to make a 50 wt % polymer solution. Diethylchlorosilane (0.0204 mol) was added, and the reactor vessel was sealed and removed from the drybox. The reaction was heated in a temperature controlled oil bath at 100 °C with stirring for 17 h. The solution gelled upon heating, but became fluid within 1 h. The reactor vessel was removed from the oil bath and placed in a drybox for cleanup and coupling. The polymer was transferred from the reactor vessel to a 250-mL round-bottomed flask with stir bar. Forty grams of dry THF and 30 g of dry DMF were added and stirred. The silylated polyamine was isolated by removing the THF and toluene *in vacuo* and then allowing the polymer product to phase separate from the DMF layer in a 125-mL separatory funnel. The DMF layer was drained, and then the polymer was redissolved with 50 g of dry THF. Forty grams of dry DMF were added and stirred. The polymer isolation procedure was repeated. The polymer was redissolved in dry THF. The methoxy derivative was prepared by reacting a dry sample of **3b** with imidazole and methanol. A sample was dried *in vacuo* for ^{1}H NMR. ^{1}H NMR integration of the olefinic and the Et_2SiO*Me* polymer regions indicated that 1.95% of the double bonds had been silylated. Selected ^{1}H NMR resonances: δ 3.6, s, polymer Si(Et)$_2$ OCH_3; 5.2–5, m, CH_2=C; 5.85–5.4, m; 1.15, t, $(CH_3CH_2)_2$Si(CH_2polymer)-(OMe); 0.75, q, $(CH_3CH_2)_2$Si(CH_2polymer)(OMe); 1.0, m, $(CH_3$-$CH_2)_2$Si(CH_2polymer)(OMe).

Chloromethylphenylsilylated Polyamine (3c) was prepared in an analogous manner to **3b**. ^{1}H NMR integration of the olefmic and the PhCH_3SiCl polymer regions indicated that 1.95% of the double bonds had been silylated. Selected ^{1}H NMR resonances: δ 0.6, PhCH_3SiCl polymer; 7.6 and 7.25, m, *Ph*CH$_3$-SiCl polymer; 5.0–4.8, m, CH_2=C; 5.7–5.2, m; ^{29}Si 9.5.

Chlorodiphenylsilylated Polyamine (3d) was prepared in an analogous manner to **3b**. ^{1}H NMR integration of the olefinic and the Ph_2SiO*Me* polymer regions indicated that 1.9% of the double bonds had been silylated. Selected ^{1}H NMR resonances: δ, 7.25–7.7, m, *Ph*$_2$SiOCH$_3$ polymer; 3.52 major, 3.45 and 3.59 minor, Ph_2SiOCH_3 polymer; 5.0–4.8, m, CH_2=C; 5.6–5.2, m.

Chlorodiisopropylsilylated Polyamine with $(Ph_3P)_3$RhCl (3e) Unsaturated Carbon–Silicon Bond. The procedure for **3b** was employed with the following exceptions: To neat **2** (10 g) was added 0.100 g of chlorotris(triphenylphosphine)rhodium-(I) (Strem catalog no. 45–0650). After bringing the wt % of the polyamine to 50% by adding dry toluene, diisopropylchlorosilane (5.44 mmol, Petrach D4850) was added. The reactor vessel was placed in an oil bath controlled at 100 °C for 24 h. ^{1}H NMR integration of the olefinic and the $[(CH_3)_2CH]_2$SiO*Me* polymer regions indicated that 1.2% of the double bonds had been silylated. Selected ^{1}H NMR resonances: δ 3.42, s, $[(CH_3)_2$-$CH]_2$SiO*Me* polymer; 5.0–4.8, m, CH_2=C; 5.6–5.2, m; 6.0–5.6, m; ^{29}Si 7.0.

The attachment site was characterized by NMR spectroscopy. Proton-silicon and proton-carbon indirect detection experiments (HMQC, HMBC) gave strong evidence for the identity of the attached functional groups. The ^{29}Si chemical shift was 6–7 ppm. Evidence was seen for long range bonding relationships between the silicon (1) the diisopropyl protons (0.9–1.0 ppm), (2) methoxy protons (3.4–3.6 ppm), and (3) olefinic protons (5.4 and 5.8–5.9 ppm). One of the olefinic protons exhibits a proton-carbon correlation peak at 5.8–5.9 ppm (1H) and 154–155 ppm (13C). No evidence was seen for a saturated methylene linkage to the silicon in either the proton-silicon or the proton-carbon indirect detection experiments. With the diisopropyl and methoxy groups accounting for three of the four possible binding sites to the silicon, the olefinic correlations represent an unsaturated linkage to the polymer.

Chlorodiisopropylsilylated Polyamine with Platinum Catalyst (3f) Saturated Polymer–Silicon Bond. The procedure for preparation of **3b** was employed with the following exceptions: To neat **2** (10 g) was added 0.48 g of platinum divinyltetramethyldisiloxane complex (Petrach PC072 2–3% Pt complex in xylene). After bringing the wt % of the polyamine to 50% by adding dry toluene, diisopropylchlorosilane (5.44 mmol) was added. The reactor vessel was placed in an oil bath controlled at 100 °C for 24 h. ^{1}H NMR integration of the olefinic and the $[(CH_3)_2CH]_2$SiO*Me* polymer regions indicated that 1.0% of the double bonds had been silylated. Selected ^{1}H NMR resonances: δ 3.43, s, $[(CH_3)_2CH]_2CH]_2$SiO*Me* polymer; 5.0–4.8, m, CH_2=C; 5.6–5.2, m.

Proton-silicon and proton-carbon indirect detection experiments (HMQC and HMBC) gave strong evidence for the identity

of the attached functional groups. The ^{29}Si chemical shift was approximately 17.5 ppm. Evidence was seen for long range bonding relationships between the silicon and (1) the diisopropyl protons (0.9–1.0 ppm), (2) the methoxy protons (3.4–3.6 ppm), and (3) the methylene protons (0.6 ppm). The methylene group exhibited a proton-carbon correlation peak at 0.6 ppm (1H) and 6–7 ppm (13C). No evidence was seen for an olefinic linkage in either the proton-silicon or the proton-carbon indirect detection experiments. With the diisopropyl and methoxy groups accounting for three of the four possible binding sites to the silicon, the methylene group represents a saturated linkage to the polymer.

Misoprostol Coupled to the Dimethylsilyl Chloride Polymer (4a). The above chlorosilylated polyamine (**3a**) in THF was diluted with 100 mL of DMF (dried over alumina). After 1 h, 0.090 g of imidazole (1.3 mmol) in 5 mL of THF was added slowly and dropwise. After 15 min, 0.5 g of misoprostol (1.3 mmol) in 5 mL of THF was added and rinsed into the solution with 2 mL of THF. After stirring for 6 h, 0.583 g of imidazole (8.56 mmol) in 15 mL of THF was added dropwise with stirring. Next 0.411 g of methanol in 2 mL of THF was added and allowed to stir for 16 h. After adding an additional 1 mL of methanol, the product solution was evaporated to remove all of the THF. The remaining DMF/polymer solution was allowed to stand in a 250-mL separatory funnel for 1 h in order to phase separate the polymer from the DMF solvent. The top polymer layer was separated and further dried by vacuum to remove trace DMF. From this procedure 20.6 g of polymer product was isolated.

Misoprostol Coupled to the Diethylchlorosilane Polymer (4b). The polymer solution of **3b** was concentrated *in vacuo* to 80 wt %. Dry THF was added to adjust the concentration of the solution to 4 mL of THF/g of polymer. The polymer solution was stirred well. The solution was then diluted by 50% with anhydrous DMF and mixed well. Next 100 mg (0.982 mmol) of triethylamine, 33 mg (0.491 mmol) of imidazole, and 188 mg (0.491 mmol) of misoprostol were added. The solution was capped and stirred 17 h at room temperature in a drybox. Finally 7.53 mmol of triethylamine, 7.53 mmol of imidazole, and 37.65 mmol of methanol were added to cap unreacted chlorosilane groups. The solution was stirred for 1 h before removing from drybox. The misoprostol–polymer was isolated by removing the THF *in vacuo* and then pouring the remaining mixture into a separatory funnel. After approximately 0.5 h, the DMF layer phase separated from the polymer layer. The DMF layer was removed and the polymer redissolved with THF. DMF was added, and the isolation procedure was repeated. All solvents were then removed *in vacuo*, and the polymer–misoprostol compound was dried until constant weight.

Misoprostol Coupled to the Phenylmethylchlorosilane Polymer 4c and Diphenylchlorosilane Polymer 4d. The procedure for preparation of **4b** was used for these systems.

Misoprostol Coupled to the Diisopropylchlorosilyl Polymers 4e and 4f. To the concentrated polymer (**3e or 3f**) were added 22 mg (0.214 mmol) of triethylamine, 271 mg (2.22 mmol) of DMAP (4-(*N*,*N*-dimethylamino)pyridine), and 41 mg (0.107 mmol) of misoprostol. The concentration of the solution was adjusted to 50 wt % with dry THF. The reaction was stirred for 24 h at room temperature in a drybox. The polymer mixture was then diluted to approximately 25 wt % with equal volumes of dry THF and dry DMF. Methanol (3.9 mmol) was added and stirred for 1 h before removing the reaction vessel from the drybox. THF was removed *in vacuo*, and the remaining mixture was poured into a separatory funnel. After approximately 30 min, the DMF layer was removed, and the polymer was redissolved with THF. DMF was added, and the clean up procedure was repeated. The isolated polymer was dried *in vacuo* overnight.

Cross-Linked Polyamine System (5a). The polymer **4a** (14.2 g, 64.5 mmol of amine) was dissolved in 33.2 g of THF, then 1.13 g (6.45 mmol) of α,α-dichloro-*p*-xylene was added, and the solution was stirred until the reaction mixture gelled. The reaction was allowed to stand for 60 h. The cross-linked polymer was cut with a spatula and ground in an analytical mill. The polymer was then stirred with 1400 mL of THF for 30 min and then filtered through a coarse glass fritted Buchner funnel. Next the polymer was stirred with 1400 mL of water for 30 min and then filtered. Finally the polymer was stirred with four consecutive portions of THF (1400 mL) and collected by filtration after each wash. Anal. Cl/N ratio = 0.25. The theoretical Cl/N ratio is 0.20. Experimental values imply that not all of the polyamine is utilized in the cross-linking reaction. The noncross-linked polyamine was removed in the THF purification step.

Table VII. Analysis for Total Drug Content

polymer system 50% HPMC; <250 μ	Si substitution	concn of misoprostol on polymer (μg/mg)
6a	dimethyl	3.3
6b	diethyl	3.9
6c	phenylmethyl	5.2
6d	diphenyl	4.4
6e	unsaturated diisopropyl	1.2
6f	saturated diisopropyl	1.5

Systems **5b–f** were prepared in a similar manner but with a modified cleanup step: The cross-linked polymer was cut with a spatula and ground in an analytical mill. The polymer was then stirred with 1400 mL of THF and then filtered through a coarse glass fritted Buchner funnel. The washing procedure was repeated five times. Anal. Cl/N ratios: **5b**, 0.28; **5c**, 0.26; **5d**, 0.27; **5e**, 0.27; **5f**, 0.28. Anal. Cl/N ratios: **5b**, 0.28; **5c**, 0.26; **5d**, 0.27; **5e**, 0.27; **5f**, 0.28.

Cross-Linked Quaternized System (6a). A mixture of 8 g of cross-linked polyamine (**5a**) and 160 mL of dry THF was stirred in a 12 oz. Fisher & Porter bottle for 4 h. Then 9 mL of dry methyl chloride was added at −78 °C, and the mixture was allowed to warm to room temperature. The slurry was stirred for 64 h, and the polymer product was isolated by filtration after removing the methyl chloride by N_2 purge. After drying by vacuum, 9.7 g of methylated product was isolated. A small sample was dried overnight at 50 °C for elemental analysis: Cl/N = 0.872 (Theoretical Cl/N is 1.0 when all nitrogens are quaternized with ether cross-linker or MeCl). A 9.53-g sample of the cross-linked methylated polymer was then milled at liquid nitrogen temperature for 5 min to obtain 9.33 g of a fine powder. The milled material was placed in a mortar, and 9.33 g of hydroxypropylmethylcellulose was added. The solids were mixed well with a pestle. The fine white powder was then transferred to an analytical mill at room temperature and ground for 3 min. After removal from the analytical mill, the product was mixed in a ball mill for 9 h and sieved with a 250 micron sieve. From this procedure 17.4 g of polymer product (<250 micron) was recovered. Systems **6b–f** were prepared in a similar manner. Anal. Cl/N ratio: **6b**, 0.913; **6c**, 1.0; **6d**, 1.0; **6e**, 1.05; **6f**, 1.05.

Diarrheagenic Activity. Male Charles River rats [Crl: COBS,CD(SD)BR], weighing 180–210 g and having fasted for 20–24 h with water available *ad libitum*, were used in these studies. Compounds were suspended or dissolved in distilled water; dose volume was 10 mL/kg. Logistic regression was used to define 8-h ED_{50} (i.e., dose causing diarrhea in 50% of rats) values. Ninety-five percent confidence intervals (C.I.) were estimated using a maximum likelihood approach.[21]

Intragastric Administration. Doses of misoprostol/HPMC (68–681 μg of misoprostol/kg), hydroxypropylmethylcellulose (HPMC; 68 mg/kg), **6a–e** (100–1470 μg of misoprostol/kg i.g.), or blank polymer (408 mg/kg) were administered intragastrically using a 2-in., 16 gauge curved feeding needle to groups of six rats. Immediately after being dosed, the rats were placed in individual wire mesh cages, and the collection trays were lined with Kraft (Inlander-Steindler Co., Chicago, IL) paper. Diarrhea (i.e., unformed or watery stools that wet the paper liner) was assessed on an all-or-none basis at hourly intervals for 8 h after compound administration. The experiment was performed "blinded".

Intraintestinal Administration. These experiments were done as described above with the following exceptions. The rats were anesthetized with methoxyfluorane, the proximal small intestine or colon was exteriorized through a midline abdominal incision, and compounds were injected into the intestinal lumen through a 23 gauge needle 10 cm from the pylorus (misoprostol/HPMC, 215–1000 μg misoprostol/kg; HPMC, 101 mg/kg; **6a**, 316–1470 μg of misoprostol/kg; blank polymer, 408 mg/kg) or into the proximal ascending colon (misoprostol/HPMC, 30–1000 μg of misoprostol/kg; HPMC, 101 mg/kg; **6a**, 316–1470 μg of misoprostol/kg; blank polymer, 408 mg/kg). Incisions were closed

with wound clips and collodian, and the animals were allowed to recover from the anesthetic. Diarrhea was assessed hourly for 8 h.

Mucosal Protective Activity. Male Charles River rats [Crl: COBS,CD(SD)BR], weighing 180–210 g, were used in these experiments. Twenty to 24 h fasted rats were used in the ethanol- and indomethacin-induced gastric lesion experiments. Rats used in the indomethacin-induced intestinal lesion study received food and water *ad libitum*. Compounds were suspended or dissolved in distilled water; dose volume was 10 mL/kg.

Four-Hour Ethanol Model. Misoprostol/HPMC (100–600 μg of misoprostol/kg), HPMC (61 mg/kg), **6a** (50–400 μg of misoprostol/kg), or blank polymer (121 mg/kg) was administered intragastrically to groups of six rats 4 h before each rat received an intragastric 1-mL dose of absolute ethyl alcohol. One hour after ethanol administration, the animals were killed by CO_2 asphyxiation, and the stomach of each rat was removed, opened, and rinsed with tap water. Gastric glandular mucosal lesions were visualized using a stereomicroscope at 10× and counted by one of us (J.J.C.) who had no knowledge of the treatment. The data from two replicate experiments were pooled. ED_{50} values and 95% confidence intervals were determined using a quadratic model and inverse prediction from confidence bounds, respectively.

Indomethacin-Induced Gastric Damage. Misoprostol/HPMC (3–100 μg of misoprostol/kg), HPMC (101 mg/kg), **6a–e** (1–30 μg of misoprostol/kg), or blank polymer (9.1 mg/kg) was administered intragastrically to groups of six rats immediately before each rat received intraperitoneally 16 mg/kg of indomethacin suspended in 0.5% aqueous methylcellulose (4000 cps) solution (20 mL/kg). Five hours later, the animals were killed by CO_2 asphyxiation, and gastric glandular mucosal lesions were counted as described above. Data from two replicate experiments were pooled, and ED_{50} values were calculated using a quadratic model. The 95% confidence intervals were determined using inverse prediction from confidence bounds around the regimen curve.[22]

Indomethacin-Induced Intestinal Damage. Misoprostol/HPMC (50–400 μg of misoprostol/kg), HPMC (40 mg/kg), **6a** (50–400 μg of misoprostol/kg), or blank polymer (121 mg/kg) was administered intragastrically 30 min before and 8, 24, and 48 h after intragastric administration of indomethacin (16 mg/kg, 10 mL/kg) suspended in 0.5% aqueous methylcellulose (4000 cps). The rats were killed by CO_2 asphyxiation 72 h after receiving indomethacin. The abdominal cavity of each rat was opened, and the presence or absence of adhesions (intestinal injury) was determined by an investigator "blinded" as to treatment. ED_{50} values and 95% confidence intervals were estimated using logistic regression[23] and a maximum likelihood approach, respectively.

Oral Bioavailability Study. Twenty-four hour fasted male Charles River rats [Crl:COBS,CD(SD)BR], weighing 180–220 g, were dosed intragastrically with either misoprostol/HPMC (400 μg of misoprostol/HPMC), **6a** (100 μg of misoprostol/kg), or **6a** (400 μg of misoprostol/kg) suspended or dissolved in distilled water (10 mL/kg). Blood was collected by cardiac puncture from methoxyfluorane anesthetized rats (three rats/time/treatment) at 0.25, 0.5, 1, 2, 4, and 6 h postdosing. Plasma was assayed for the free acid of misoprostol, SC-30695, by an automated HPLC/RIA method upgraded for PyTechnology.[24]

Supplementary Material Available: HPLC gradient and conditions for hydrolysis studies; pH 2.2 initial rate date of polymer analogs (1 page). Ordering information is given on any current masthead page.

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