

Underquaternized Anion Exchange Resins as Covalent Scavengers

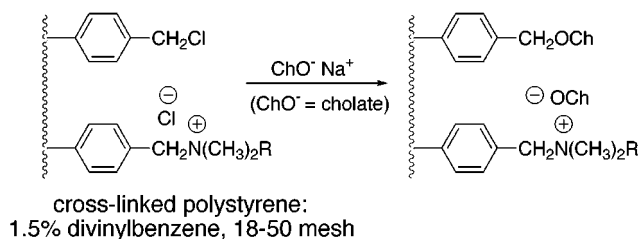
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ABSTRACT



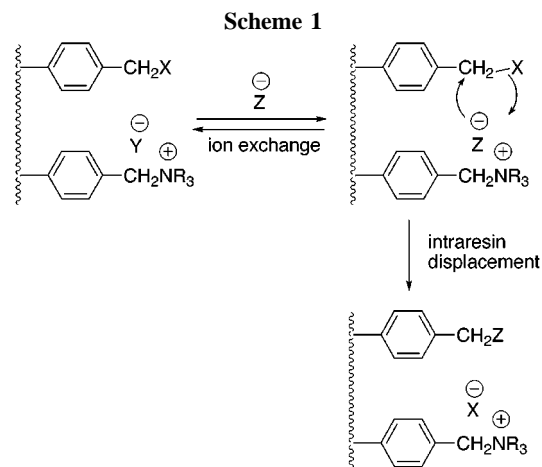
The use of partially quaternized, chloromethylated polystyrene as a covalent scavenger of cholate ion in aqueous media has been demonstrated. The ability of such polymers to scavenge organic anions by covalent as well as by ionic means has important implications in the areas of medicinal and environmental chemistry, which are briefly discussed.

Anion-exchange resins are widely used to remove acidic molecules from solution. In the area of organic synthesis, for example, such resins provide a convenient means for “scavenging” excess reagents and byproducts; a feature that has recently been exploited in the solution-phase synthesis of chemical libraries.² Anion-exchange resins are also of considerable interest as scavengers in the areas of environmental science and medicinal chemistry, e.g., in removing organic contaminants from water, and bile acids from the intestines.^{3,4} While the reversibility of ion-exchange processes is generally regarded as an attractive feature, such lability can represent a significant limitation for molecular scavenging purposes.^{4,5}

In this paper, we show how *underquaternized* anion-exchange resins (i.e., resins derived from chloromethylated polystyrene, having residual chloromethyl groups along the polymer backbone) can eliminate the lability of a significant fraction of scavenged molecules by forming *covalent* bonds.

To the best of our knowledge, there exists no prior description of this approach to limit desorption from an ion-exchange resin.

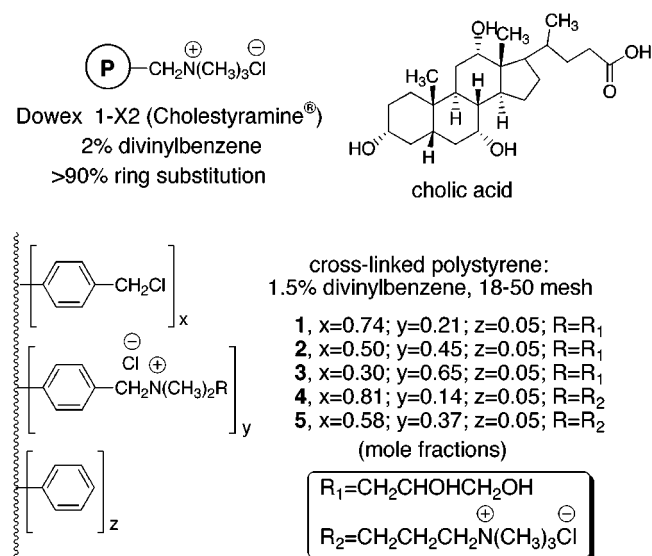
Scheme 1 illustrates our general concept. In essence, we hypothesized that the pendant quaternary ammonium groups



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 (2) Gayo, L. M.; Suto, M. J. *Tetrahedron Lett.* **1997**, 38, 513.
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of an underquaternized anion-exchange resin would concentrate organic anions and assist the nucleophilic displacement at the chloromethyl sites.^{6,7} At the same time, these ammonium groups would allow for swelling in aqueous media, thereby making the chloromethyl groups accessible.

To test our hypothesis, we examined the ability of five resins (**1–5**), bearing mono- or bis-quaternary ammonium pendant groups, to capture cholic acid via *covalent as well as ionic bonds*. The purpose of the hydroxyl groups in **1–3** was to improve their swelling behavior in water; bis-quaternary ammonium groups were also expected to enhance resin swelling. Cholic acid was chosen as a prototype for scavenging, since it is known that the efficiency of commercial anion-exchange resins in promoting the excretion of bile acids *in vivo* is very low, i.e., less than 2% of the exchangeable sites in Dowex 1-X2 (Cholestyramine) remain populated.⁴ In addition, it is currently believed that this low efficiency is a direct consequence of the lability of the captured bile acid with respect to ionic desorption.^{4,8}



Using standard synthetic procedures, 1.5% cross-linked polystyrene was chloromethylated, and subsequently quat-

(6) *Phase Transfer Catalysis*; Starks, C. M., Liotta, C., Eds.; Academic Press: New York, 1978; pp 13–56.

(7) For other examples of intraresein nucleophilic displacement, see: (a) Kim, B.; Kirszensztejn, P.; Bolikal, D.; Regen, S. L. *J. Am. Chem. Soc.* **1983**, *105*, 1567. (b) Regen, S. L., Bolikal, D., *J. Am. Chem. Soc.* **1981**, *103*, 5248.

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ernized with limited quantities of 3-(dimethylamino)-1,2-propanediol or 3-*N,N*-(dimethylamino)-1-propyl-*N,N,N'*-trimethylammonium chloride to give the requisite polymers. A 50.0 mg sample of each polymer was then suspended in 10.0 mL of an aqueous solution that was 15 mM in cholic acid, 150 mM in NaCl, and 10 mM in phosphate buffer (pH 7). Each suspension was agitated by use of a wrist-action shaker at 23 °C, and the extent of scavenging of cholic acid analyzed, polarimetrically, as a function of time. As shown in Figure 1, increased levels of quaternization, on going from

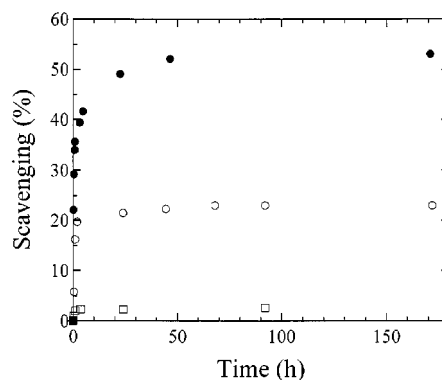


Figure 1. Percentage of scavenging of cholic acid (ionic- plus covalent-attachment) as a function of time at 23 °C, in the presence of **1** (□), **2** (○), and **3** (●). In each case, 100% scavenging corresponds to a state of the resin that has all of its ionically and covalently attached chloride replaced by cholate.

1 to **2** to **3** resulted in increased scavenging efficiency; similar results were found for **4** and **5** (not shown).

To confirm that covalent attachment contributes to the scavenging of cholate ion, each resin was rinsed, sequentially, with 50 mL of a saturated NaCl solution and 50 mL of water to remove ionically bound sterol. After drying (23 °C, 24 h, 0.01 Torr), examination of the polymers by IR (KBr pellet) showed increasing levels of ester formation, on going from **1** to **2** (not shown) to **3**, as judged by relative band intensities of the ester carbonyl (1726 cm⁻¹), the aromatic (1601 cm⁻¹), and the chloromethyl (1263 cm⁻¹) groups (Figure 2). Similarly, the intensity of the ester band in **5** was greater than in **4** (not shown).

A quantitative analysis of the extent of covalent attachment of cholic acid to **3** was made by subjecting the resin to saponification. Thus, a 54.3 mg sample (which was washed with saturated sodium chloride in order to remove ionically bound cholate) was suspended in an aqueous solution that was 0.5 M in NaOH and 2.85 M in NaCl. After 75 h of shaking at 23 °C, 0.022 mmol of the sterol was released into the aqueous phase. This quantity of cholic acid corresponds to ca. 12% ring substitution, i.e., 12% of the phenyl groups along the polymer backbone contain the cholate ester.⁹ Extending the saponification time to 315 h did not lead to any further release of cholate. Extensive rinsing with deionized water, drying (23 °C, 24 h, 0.01 Torr), and examination by IR showed the complete loss of the ester

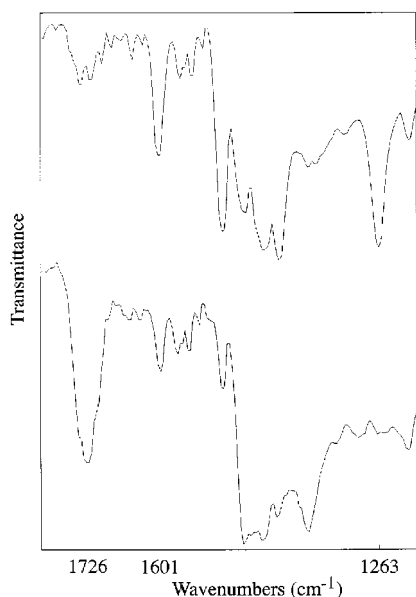


Figure 2. Relative band intensities for **1** (upper spectrum) and **3** (lower spectrum) after removal of ionically bound cholate.

carbonyl band. Based on the total amount of cholic acid that was scavenged by **3** (Figure 1), and the quantity of sterol that was covalently attached, the contribution from ionic scavenging is estimated to be ca. 38% ring substitution.

Similar experiments that were carried out with polymers **1**, **2**, **4**, and **5** gave scavenging results that are summarized in Table 1. Since we do not know whether cholate is

Table 1. Scavenging of Cholate Ion by Underquaternized Anion Exchange Resins

polymer	swelling ^a (%)	covalent binding		ionic binding (mmol/g) ^d
		mmol/g ^b	prs ^c	
1	6	nd ^e	nd ^e	0.14
2	21	0.30	7	0.72
3	55	0.41	12	1.65
4	25	0.13	2.6	0.53
5	44	0.27	4.2	1.20

^a Percent increase in resin volume relative to the dry state, in the presence of 150 mM NaCl plus 10 mM phosphate buffer (pH 7), estimated by changes in height within a capillary tube. ^b Millimoles of covalently bound cholate per gram of starting polymer. ^c Percentage of phenyl rings bearing covalently bound cholate. ^d Millimoles of ionically bound cholate per gram of starting polymer. ^e Not determined, since the IR spectrum did not show the presence of an ester carbonyl band.

randomly distributed among the pendant ammonium groups in **4** and **5**, we report ionic binding in terms of millimoles

(9) In a separate experiment, a portion of **3**, which had been used to scavenge cholate, was treated with excess NH_2OH . This was done in order to confirm the presence of ester formation, via the release of the sterol from the polymer as a hydroxamic acid. Subsequent addition of FeCl_3 and colorimetric analysis (530 nm) indicated the presence of 0.39 mmol of ester/g of polymer, which corresponds to 11% ring substitution.

of cholate that is ionically bound per gram of starting polymer. For both series of resins that were investigated, a higher degree of quaternization resulted in a higher degree of scavenging. Such a finding, we believe, is a likely consequence of greater swelling and greater accessibility to the chloromethyl sites. The fact that a 3-fold increase in the degree of quaternization (on going from **1** to **3**) leads to a 15-fold increase in the percentage of scavenging (Figure 1) is fully consistent with this interpretation, since it indicates that resin efficiency is not directly proportional to the number of ion exchange sites along the polymer backbone.

To confirm that ion exchange assists the covalent attachment of cholic acid to **3**, we tested for inhibition using a nonnucleophilic anion that effectively competes in the ion exchange process, i.e., *p*-toluenesulfonate. Thus, after suspending a 47.2 mg sample of **3** in 9 mL of an aqueous solution that was 150 mM in *p*-toluenesulfonate, 150 mM in NaCl, and 10 mM in phosphate buffer (pH 7) for 2 h, the polymer's ability to scavenge cholic acid was evaluated. For this purpose, sodium cholate was added to the suspension, which corresponded to 15 mM in the absence of scavenging. Analysis of the aqueous phase as a function of time showed substantially reduced scavenging of the sterol, i.e., only 6% of the available sites (ionic plus covalent) captured cholate ion after 96 h. Examination of this resin by IR, after extensive washing (saturated NaCl and water) and drying (23 °C, 24 h, 0.01 Torr) showed negligible ester formation. Thus, *p*-toluenesulfonate strongly inhibits both ionic- and covalent-scavenging by **3**.¹⁰ This finding is fully consistent with a direct connection between the ion exchange and the nucleophilic displacement processes.¹¹

The use of underquaternized anion-exchange resins as covalent scavengers of a bile acid represents a fundamentally new approach to a problem that has daunted medicinal and polymer chemists for more than four decades.¹² Whether or not such a strategy can lead to improved therapeutic agents by minimizing desorption in vivo will depend on other important factors that remain to be examined; e.g., (i) the effectiveness of intraresin nucleophilic displacement in vivo, and (ii) the stability of sterol-resin, ester bonds with respect to hydrolysis.

In principle, the capture of environmental contaminants using underquaternized anion-exchange resins (especially ones that can be regenerated by chemical means after use) is an attractive concept. Efforts aimed at creating such resins, and also at increasing the level of covalent attachment by use of second-generation analogues, are currently in progress.

Acknowledgment. This research was based, in part, on studies that were carried out in a segment of an undergraduate

(10) In the presence of 150 mM NaCl plus 10 mM phosphate buffer (pH 7), containing 150 mM *p*-toluenesulfonate, the increase in volume of the polymer was 50% relative to the dry state of the starting resin.

(11) A similar degree of inhibition was found in phosphate-buffered saline (PBS) when the NaCl concentration was increased to 3.8 M; the increase in the volume of the resin due to swelling was 44%.

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laboratory course (Chemistry 353) at Lehigh University during the Spring of 2000. We are grateful to the National Science Foundation (Grant CHE-9612702) and to Lehigh University for support of this research. We are also grateful to Dr. Ken Foster (Dow Chemical Co., Midland, MI) for a sample of cross-linked polystyrene.

Supporting Information Available: Experimental procedures for the synthesis of chloromethylated polystyrene

and **3**, the scavenging of cholic acid, the saponification of the esterified form of **3**, the conversion to the corresponding hydroxamic acid, and inhibition studies using *p*-toluenesulfonate. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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