Underquaternized Anion Exchange Resins as Covalent Scavengers

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Supporting Information Available

Chloromethylated Polystyrene. To a stirred mixture of 4.40 g of cross-linked polystyrene (1.5% divinylbenzene, 18-50 mesh), 11.0 mL of CCl_4, 9.8 mL of $ClCH_2OC_2H_5$ was added a solution of 2.57 mL of $SnCl_4$, 14.7 mL $ClCH_2OC_2H_5$ and 7.3 mL of CCl_4, (prepared at 0°C by adding $SnCl_4$ to both components) over a period of 30 min such that the temperature was maintained at 20-23°C. After stirring the mixture for 24 h at rt, the polymer was separated and washed, sequentially, with 100 mL of dioxane/water (1/1, v/v), 100 mL of 1 M HCl/dioxane (1/1,v/v), 100 mL of water, and 400 mL of THF. The polymer was then extracted with THF (Soxhlet) for 24 h, dried (23°C, 48 h, 0.01 Torr). Examination by IR showed the characteristic chloromethyl group appearing at 1263 cm^{-1}. Based on chlorine analysis (22.63%), the extent of chloromethylated is calculated to be 95% ring substitution.

Underquaternized Resin 3. To a heterogeneous mixture of 0.304 g of chloromethylated polystyrene (1.5% divinylbenzene, 6.38 mmol Cl/g, 1.95 mmol Cl; 18-50 mesh) and 0.2 mL of anhydrous N,N-dimethylacetamide was added a solution of 0.176 g (1.48 mmol) of 3-(dimethylamino)-1,2-propanediol in 0.3 mL of anydrous N,N-dimethylacetamide. The flask was filled with an argon atmosphere, sealed and gently stirred at 70°C for 100 h. The external solution was then removed by filtration, and the polymer beads washed, sequentially, with 50 mL of THF, acetone, dichloromethane, acetone and THF. Subsequent extraction (Soxhlet) with THF for 24 h, followed by drying (23°C, 72 h, 0.01 Torr) afforded 0.454 g of resin having a chlorine (14.42%) and nitrogen (3.79%) content indicating that 65% of the chloromethyl groups were quaternized. Resins **1** and **2** were prepared using similar procedures, except that the quantity of 3-(dimethylamino)-1,2-propanediol was reduced, accordingly.

Scavenging of Cholate. A 50 mg sample of **3** was preswollen with 5 mL of PBS (10 mM phosphate buffer plus 150 mM NaCl, pH 7.0) in a test tube that was equipped with a screw cap. After 1 h, the external aqueous phase was separated (centrifuge, 1 min), and 10 mL of PBS, which was also 15 mM in sodium cholate, was introduced. The mixture was agitated by use of a wrist-action shaker, and the external aqueous phase analyzed as a function of time. For this purpose, 5 mL of the solution was withdrawn and analyzed, polarimetrically; sodium cholate has a specific rotation of $[\alpha]_D$ +31.3° (c=0.5, H_2O). After 170 h, the polymer was transferred to a column (4 mm, i.d.), washed with 50 mL of saturated NaCl (4 h) and 50 mL of water (4 h), and freeze-dried (24 h, 0.01 Torr). A portion of the resin (8 mg) was then analyzed by IR (KBr, 80 mg).

Determination of the Extent of Ester Formation. To 54.3 mg of **3**, which had been used to scavenge cholic acid (see above) was added 3 mL of 5.7 M NaCl and 3 mL of 1 M NaOH. The mixture was gently stirred, and the external aqueous phase monitored for the release of sodium cholate by polarimetry. The extent of release reached a constant value after 75 h, which corresponded to 22 μmol of the sterol. This corresponds to 0.41 mmol cholate/g of dry resin, or 12% ring substitution. At the end of the saponification, the polymer was transferred to a column (4 mm, i.d.), washed with 50 mL of water, and dried (23°C, 24 h, 0.01 Torr), and its IR spectrum recorded using 8 mg of polymer and 70 mg of KBr.

Inhibition of Scavenging by Sodium *p*-Toluene Sulfonate. A 47.2 mg sample of **3** was added to 9.0 mL of PBS (10 mM phosphate buffer plus 150 mM NaCl, pH 7.0), which contained 150 mM sodium p-toluene sulfonate. After equilibration for 2 h, 1.0 mL of PBS that was also 15 mM in sodium cholate was then added. The uptake of cholate was monitored, polarimetrically, for 96 h.

Hydroxamic Acid Formation.[13] To 3.99 mg of **3**, which had previously been used to scavenge cholate ion, was added 50 μL of methanol in order to swell the polymer. After swelling for 5 min, 100 μL of 2.0 M NH_2OH in CH_3OH was then added (prepared by addition of 2.0 mL (14 mmol) of 7 M KOH in CH_3OH to 5.0 mL of a stirred solution of 2.8 M $NH_2OH{\cdot}HCl$ in CH_3OH at 10°C under an argon atmosphere). The reaction mixture was then stirred for 6 h at 60°C, cooled to room temperature, diluted with 200 μL of CH_3OH, and acidified using 200 μL of 1 M HCl. After adding 70 μL of a 200 mM aqueous solution of $FeCl_3$, the mixture was further diluted by an equivalent volume of CH_3OH for colormetric analysis at 530 nm. Based on the absorbance observed for an analogous reaction of 0.93 mg (1.86 μmol) of benzylcholate with NH_2OH under identical conditions, the ester content in the spent resin is estimated to be 0.390 mmol/g, which corresponds to 11% ring substitution, or 1.53 μmol of ester within the 3.99 mg sample of polymer. Extending the reaction time with benzylcholate or with the polymer-bound analog to 16 h did not alter the absorbance readings.

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(13) (a) Thouin, E.; Lubell, W.D., *Tetrahedron Lett*., **2000**, *41*, 457. (b) Belcheva, R.; Stoichkova, K.; *Bulg. Neft Khim*., **1971**, *10*, 357.