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SYNTHESIS OF POLYMERICALLY BOUND CATECHOLCARBOXAMIDE CHELATORS FOR IRON(III)

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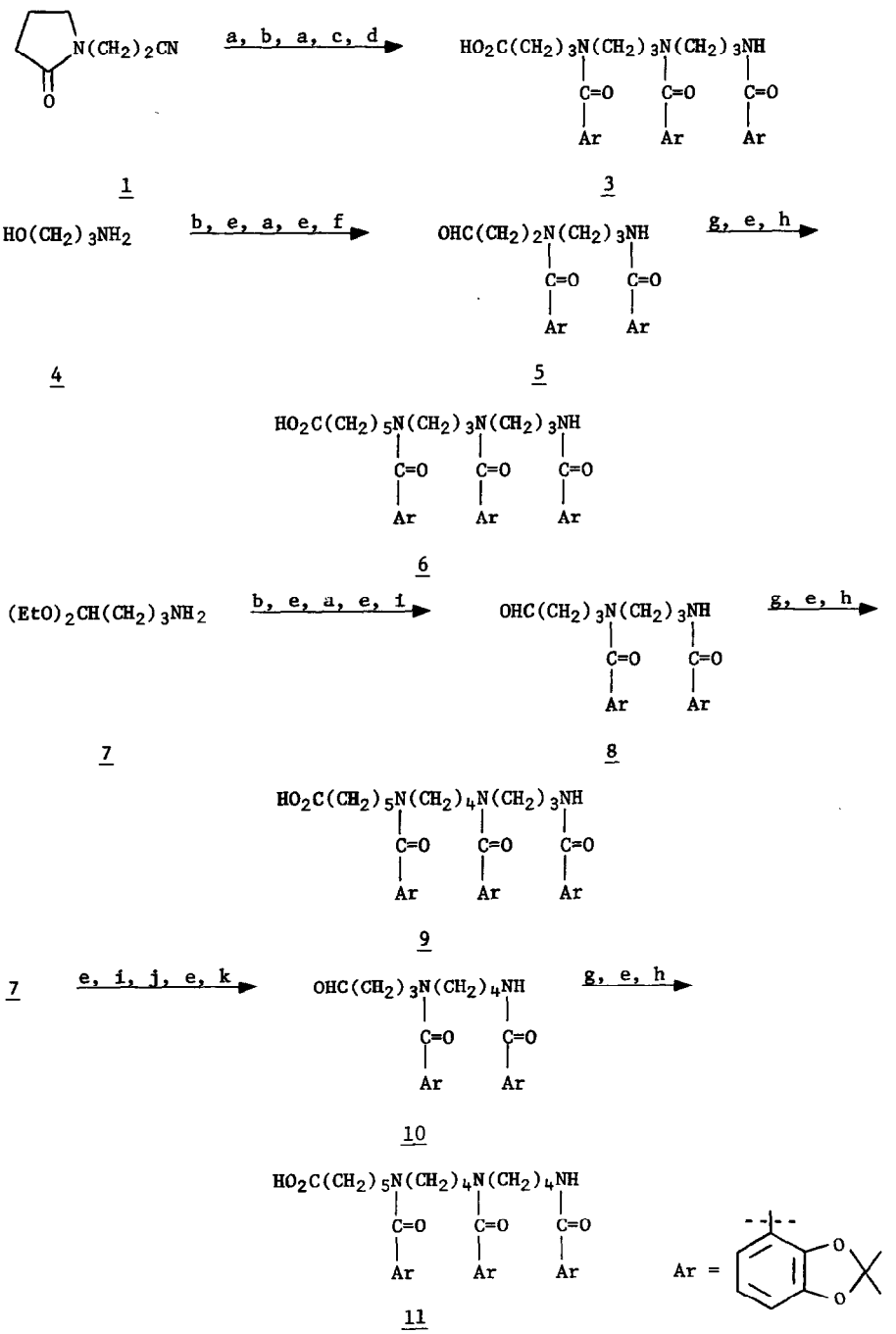
Summary Tris(catecholcarboxamide) ligands were covalently linked to poly(vinyl amine--vinyl sulfonate sodium salt), and the iron binding capacity of the resultant polymers was found to exceed that of transferrin.

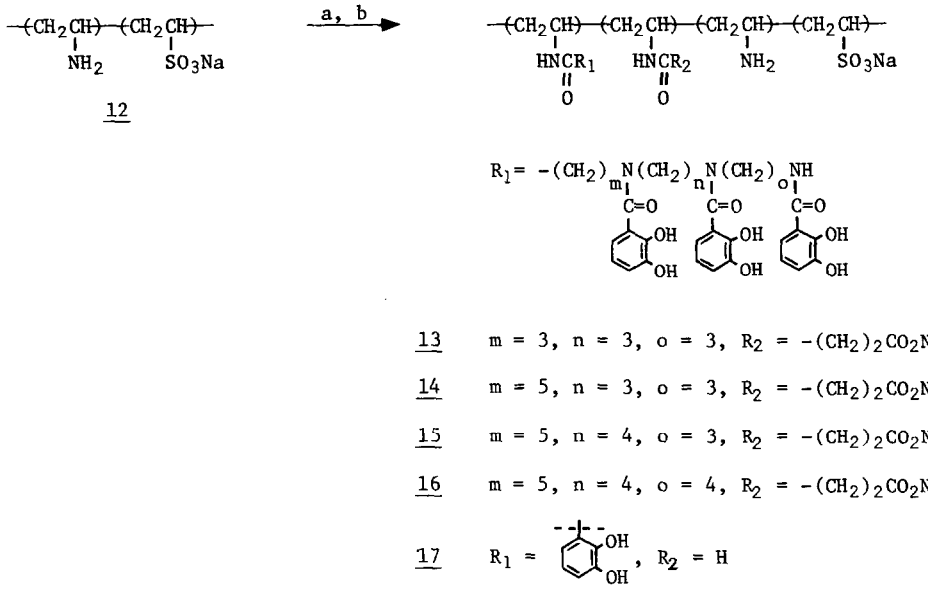
Since the human body has no effective physiological mechanism for the excretion of excess iron, diseases requiring chronic erythrocyte transfusion therapy eventually lead to hemosiderosis, a condition in which the tissues become overloaded with iron. Death usually results in the second or third decade of life.[1] As part of a program to develop new iron chelators for the treatment of iron overload disease, we have undertaken the synthesis of catecholcarboxamides covalently bound to a water-soluble polymer. The selection of catechols was based on work by Raymond,[2] who has used an excellent biomimetic approach to synthesize linear ligands in which the 2,3-dihydroxybenzoyl group has been conjugated to spermidine [$H_2N(CH_2)_3NH(CH_2)_4NH_2$] affording compounds that are analogs of catechol-containing siderophores. In addition, Raymond[3] has demonstrated that the tris(catecholcarboxamide) enterobactin[4] is a powerful chelator of iron(III) and Cerami[5] has shown that 2,3-dihydroxybenzoic acid can be used to remove iron(III) from Chang cell cultures.

We sought to extend this concept to the preparation of polymeric chelating agents having linear tris(catecholcarboxamides) attached by a polymethylenecarboxamido tether group to the random copolymer poly(vinyl amine--vinyl sulfonate sodium salt) (12).[6] A tri- or penta-methylene group was used on the tether linkage to determine the effect of tether length on iron(III) binding capability (polymeric adducts 13 and 14), and the distance between adjacent nitrogen groups was varied from three to four methylene units to optimize binding (polymeric adducts 14, 15, and 16). The polymeric monocatecholcarboxamide 17 was also prepared. If 2,3-dihydroxybenzoyl substitution onto 12 is sufficiently high, the minimal spacing between adjacent ligand groups would be three methylene units. However, unlike polymers 13 to 16, in which the correct geometry for iron(III) chelation is already present in the pendant ligand, polymer 17 may have to undergo conformational alterations of its backbone to assume the correct geometry for chelation.

We wish to report the successful completion of the syntheses of these polymeric adducts by the routes shown in Schemes 1 and 2. The syntheses[7] of the acetonides 3, 6, 9, and 11 of the tris(catecholcarboxamide) polymethylenecarboxylic acids are presented in Scheme 1. These syntheses are based on a series of cyanoethylations of amines, hydrogenations of the resultant cyano groups to the amines,[8] and reductive-aminations of aldehydes.[9] Benzoylation[10] of the amino groups with the acetonide of 2,3-dihydroxybenzoyl chloride (2) produced the protected catecholcarboxamides. The carboxylic acid groups were converted to their acyl chlorides with excess oxalyl chloride in toluene. The anhydride was also present, and its formation appeared to be favored by the presence of the benzamido groups. Acylation of polymer 12 was effected by the route shown in Scheme 2. For example, a 3:2 mixture of anhydride/acyl chloride prepared from 3.43 mmol of 9 in 30 mL of THF was added to a vigorously stirred solution of 2.3 g (3.0 mmol



Scheme 1: a) H_2, 5% Rh/Al_2O_3, $NH_3/EtOH$; b) $H_2C{=}CHCN$, 0–20°C; c) 20% KOH/H_2O, reflux; d) ArCOCl (2),[10] pH 10; e) 2, Et_3N, THF; f) Me_2SO, $(COCl)_2$, Et_3N, -60° to 20°C;[12] g) $MeO_2C(CH_2)_5NH_2$, $MeO_2C(CH_2)_5NH_2{\cdot}HCl$,[13] THF; $NaBH_3CN$; h) 5% aq. KOH/MeOH, reflux; 3 N HCl; i) 50% $HOAc/H_2O$; j) 7, THF; HCl, THF, $NaBH_3CN$; k) 3 N HCl, THF/EtOH.



Scheme 2: a) R_1COCl or 2, THF/H_2O, pH 10; $(CH_2CO)_2O$ for 13, 14, 15, 16; b) pH 2.5, reflux.

NH_2 groups) of 12 in 160 mL of H_2O and 40 mL of THF. During the addition and for 1 h afterwards a pH of 10 was maintained by the addition of 5% NaOH. Next 3.0 mmol of succinic anhydride in 10 mL of THF was added at pH 10. Neutralization with 3 N HCl, filtration, ultrafiltration with an Amicon PM10 membrane, and lyophilization afforded 650 mg of white powder: UV (0.1 M Tris, pH 9) 286 nm. The acetonide protecting groups were removed by dissolving the polymer in 200 mL of deoxygenated water, lowering the pH to 2.5 with 3 N HCl, and heating at reflux for 24 h. Cooling, filtration, ultrafiltration, and lyophilization afforded 350 mg of 15: UV 282 nm. UV analysis[11] indicated that 20% of the amino groups on the polymer backbone were acylated by the ligand. Succinoylation of the unacylated amino groups was necessary to preserve solubility for polymeric ligands 13 to 16. This procedure was unnecessary for 17, in which 20% of the amino groups were benzoylated with 2: UV 317 nm.

The pM values[2] for these polymeric adducts are shown in Table 1. The pM value indicates

TABLE 1

pM Values for Polymerically Bound Catecholcarboxamides Compared to Other Iron Chelators

Compound	pM[a]
Enterobactin	35.6[3]
Desferrioxamine B	26.6[3]
17	25.4
16	25.1
15	25.1
14	24.7
13	24.5
Transferrin	23.6[3]

[a]Calculated for 10 μM ligand, 1 μM iron(III), 0.1 M Tris, pH 7.4

the ability of a ligand to sequester iron(III) at physiological pH. Our measurements indicated that all five polymeric ligands can bind iron(III) more tightly that does the iron transport protein transferrin. These polymers are not as effective as desferrioxamine B, the currently used clinical drug, and the siderophore enterobactin. The variation in the tether length did not significantly affect binding capacity. Binding capacity was reduced when three methylene groups bridged the three adjacent nitrogens in the polymeric tris(catecholcarboxamides). Interestingly, the polymeric monocatecholcarboxamide 17 possessed the highest binding affinity. Competitive binding studies with EDTA indicated that formation of the tetradentate complex was favored over that of the hexadentate complex at high iron(III) concentrations. Biological testing of this polymer is now in progress.

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