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Synthesis and Anti-HIV Activity of Poly(cysteic acid)

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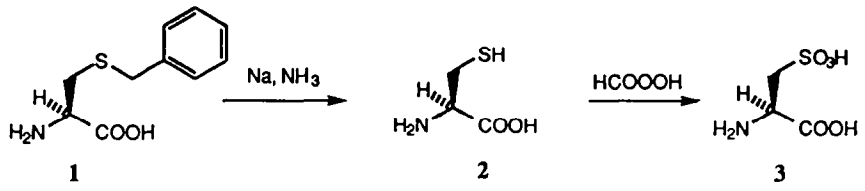
Abstract: *Poly(cysteic acid) was synthesized and found to inhibit the cytopathic effect of HIV-1 in CEM cell cultures at concentrations that were not cytotoxic to uninfected CEM cells.*

A variety of polyanionic polymers are known to inhibit the cytopathic effects of HIV-1 and HIV-2, the causative agents of AIDS. Included are the sulfated polysaccharides dextran sulfate,[1] heparin,[2] pentosan sulfate,[3] polyxylan sulfate (HOE/BAY 946),[4] and other related polysulfated polysaccharides.[5-11] In addition, various high molecular weight sulfonic acid polymers, including poly(4-styrenesulfonic acid), poly(anetholesulfonic acid), poly(vinylsulfonic acid), and poly(2-acrylamido-2-methyl-1-propanesulfonic acid) have been reported to inhibit HIV-1 and HIV-2 cytopathic effects in MT-4 cells at concentrations that are not cytotoxic to uninfected cells.[12] On the other hand, polyanionic substances have traditionally been associated with several features that have limited their potential usefulness as anti-AIDS agents, including poor oral absorption, undesirable anticoagulant activity, metabolic instability, and lack of demonstrated efficacy in animal models.[13-17] In view of the present AIDS crisis, the promising *in vitro* anti-HIV properties of these substances warrants the further investigation of strategies aimed at overcoming these difficulties. This situation, in conjunction with our prior interest in the anti-HIV polyanion aurintricarboxylic acid (ATA),[18-20] prompted the present investigation of the preparation and anti-HIV activity of poly(cysteic acid).

The strategy envisioned for the preparation of poly(cysteic acid) involved debenzylation of commercially available poly-*S*-benzyl-L-cysteine with sodium in liquid ammonia[21] followed by oxidation of the cysteine residues to cysteic acid residues with performic acid.[22] As a model study, sodium metal was added to a suspension of *S*-benzyl-L-cysteine (**1**) in liquid ammonia until a permanent blue color persisted, indicating complete removal of the benzyl group[21] and formation of L-cysteine (**2**). The blue color was then discharged by addition of a minimum amount of ammonium chloride, the ammonia evaporated, and the product dried *in vacuo*. A solution of performic acid was prepared by mixing 95% formic acid (9.2 mL), 30% hydrogen peroxide (1 mL), and water (0.46 mL) and allowing the mixture to stand at room temperature for 30 min. The solution was cooled to -15 °C, crude L-cysteine (50 mg, 0.413 mmol) was added, and the resulting solution was allowed to stand at 0 °C for 1 h. The reaction mixture was lyophilized to give solid L-cysteic acid (**3**).

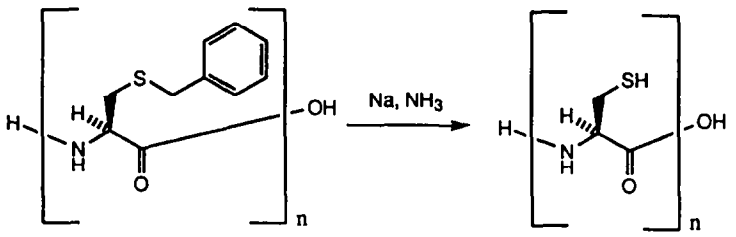
The reaction sequence outlined in Scheme 1 was then attempted with poly-*S*-benzyl-L-cysteine (**4**) (Scheme 2). Accordingly, a suspension of poly-*S*-benzyl-L-cysteine (**4**) (350 mg, MW 2,000-10,000, ICN Biomedicals, Inc.) in anhydrous liquid ammonia (70 mL) was stirred under nitrogen and sodium metal was added until the blue color persisted. However, the ^1H NMR spectrum of the crude product obtained after the sodium in liquid ammonia reduction indicated incomplete debenzylation, most likely reflecting the limited solubility of the polymeric material in liquid ammonia. However, complete debenzylation was achieved by stirring the mixture for 1 h after a persistent blue color had been obtained in the sodium in liquid ammonia reduction reaction. The crude poly-L-cysteine (**5**) was obtained after discharge of the blue color with a minimum amount of ammonium chloride, evaporation of the ammonia, and drying the crude product *in vacuo*. Performic acid oxidation of crude poly-L-cysteine (**5**) (50 mg) was performed as described for the conversion of **2** to **3** using performic acid (16.1 mL), 30% hydrogen peroxide (1.75 mL), and water (0.81 mL). The resulting poly(cysteic acid) (**6**) was purified by dialysis.

Scheme 1

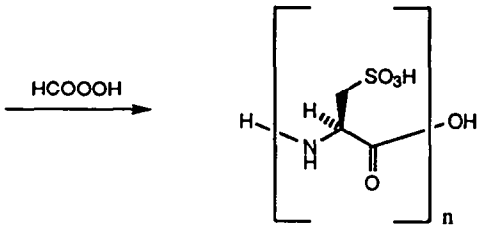


1 2 3

Scheme 2



4 5



6

The poly(cysteic acid) (6) prepared in this study was converted to its ammonium salt and then tested *in vitro* for prevention of the cytopathic effect of HIV-1 in CEM lymphocyte cell cultures. As shown in Figure 1, it prevented the cytopathic effect of HIV-1 at concentrations that were not cytotoxic to uninfected CEM cells. The EC_{50} value for prevention of cytopathicity was 11.5 μg/mL, which is similar to the potencies reported for related sulfonic acid polymers.[12] We assume that the anti-HIV effect of poly(cysteic acid) (6), like that of other related polysulfonates, polysulfates, and ATA, results from inhibition of the gp120-CD4 interaction.[1,2,9,12,23]

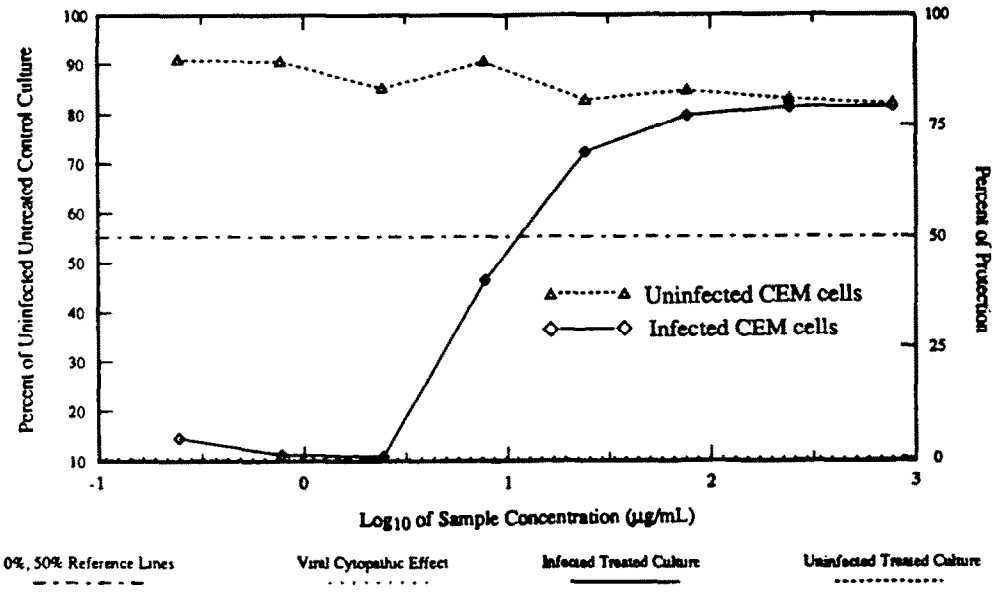


Figure 1. Anti-HIV screening results on poly(cysteic acid) ammonium salt. Cell viability in HIV-1-infected and uninfected CEM cells was determined by a tetrazolium (XTT) assay.[24]

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