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Dendrimeric Organic Reagents for Macromolecular Cross-Linking: Synthetic Approaches to 2,6-Bis[3,6-dioxa-9,9'-bis[bis[2-((*p*-carboxy phenoxy)carbonyl) ethyl]phosphinyl]nonyl]heptanedioic Acid [Bis(*p*-BCCEP-NHPDA)]

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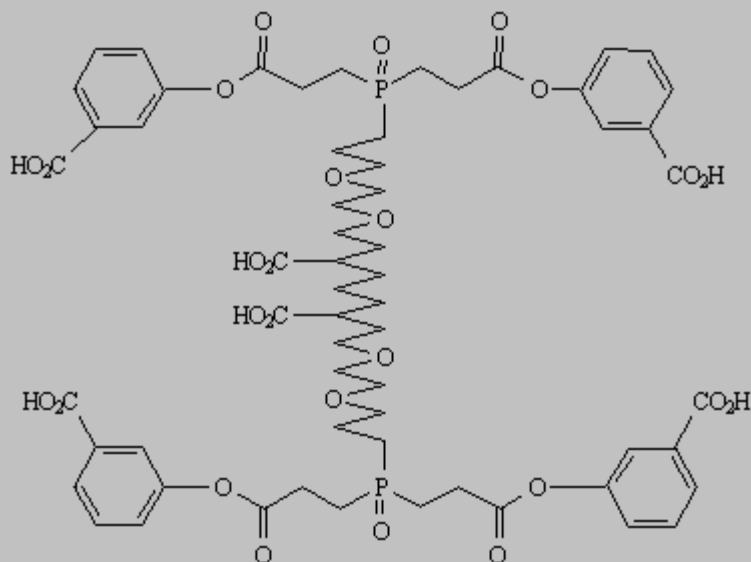
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INTRODUCTION

Efforts to develop alternatives to blood as an oxygen carrier date back well over half a century with increased efforts in recent years being partly motivated by fears of potential contamination with the viruses responsible for AIDS and hepatitis (1-4). An additional impetus rests in the recognition that wide availability of properly banked blood is absent in many parts of the world. An efficient acellular oxygen carrier could have several distinct advantages over intact red blood cells, including elimination of the need for typing and cross-matching before transfusion and a useful shelf-life greater than the current six weeks or less for packed red blood cells (1). While a number of other alternatives have been explored over the years (1-4), the use of cell-free hemoglobins for an oxygen-carrying resuscitation fluid has excellent prospects since (a) hemoglobin solutions are completely metabolizable and are well tolerated by the body, and (b) hemoglobin is available in virtually unlimited amounts and is relatively inexpensive, (c) hemoglobin is fully saturated with oxygen under ambient conditions, has oncotic activity, and exhibits cooperative oxygen binding behavior. However, there are two major problems associated with using cell-free hemoglobins for transfusions. First, the retention time of cell-free hemoglobins in circulation after infusion is very short (4), and most of the infused hemoglobin is rapidly filtered and eliminated by kidneys in 1-4 hours; second, cell-free hemoglobins have too high oxygen affinity that prevents them from adequately unloading the oxygen acquired from lungs to tissues. Both of these problems have their roots in 2,3-diphosphoglycerate (DPG) and other polyanionic species which are conspicuously absent in cell-free hemoglobins, but are known to be essential co-factors in intact red blood cells. Efforts have since been focused on covalently cross-linking hemoglobin between the two like subunits, *e.g.*, alpha-1 to alpha-2 or beta-1 to beta-2. Such a cross-link is believed to substitute for the native allosteric modifier DPG to lower the oxygen affinity, while at the same time preventing dissociation of the tetramer (1).

Several bifunctional and polyfunctional organic reagents (**BORs** and **PORs**) have been employed in the past to carry out this task (5-12). Varying degrees of success have been achieved with a number of these reagents, and some of the modified hemoglobin products are currently undergoing clinical trials (1a,1b). The current thrust in hemoglobin based blood substitutes is to significantly increase the intravascular retention time from the current level of several hours to the optimally desired several days. To this end, our present focus is on design and synthesis of novel dendrimeric organic reagents (**DORs**) that are capable of simultaneously forming specific intra- as well as intermolecular cross-links in cell-free human hemoglobins. We report here our progress and current status on the synthesis of the title **DOR**, [Bis(*p*-BCCEP-NHPDA)]



BIS(*p*-BCCEP-NHPDA)

RESULTS AND DISCUSSION

(A) Design of the Title Dendrimeric Organic Reagent (DOR) by Molecular Modeling: The title DOR was designed employing molecular modeling techniques. Molecular modeling was performed on a Silicon Graphics workstation, using the software INSIGHT/DISCOVER (Molecular Simulations, Inc., San Diego, California). The X-ray coordinates of human OxyHbA₀ (13), imported from the Brookhaven National Laboratory, Upton, New York, were employed for molecular modeling. The reagent was energy-minimized (see **Figure 1**), and the two cross-linking sites on each end of the bifurcate reagent were docked into the *two* -clefts (DPG pockets) of two independent hemoglobin tetramers, followed by energy minimization of the reagent-Hb complex. All atoms that were 15 Å or farther from the ligand were fixed with a temperature constant of 300 K. No constraints were applied to the remaining residues in and around the ligand site. The complex was minimized to convergence using consecutive Steepest Descent and Conjugate Gradient (VAO9A) minimization protocols (see **Figures 2** and **3**). There were no morse or cross terms.

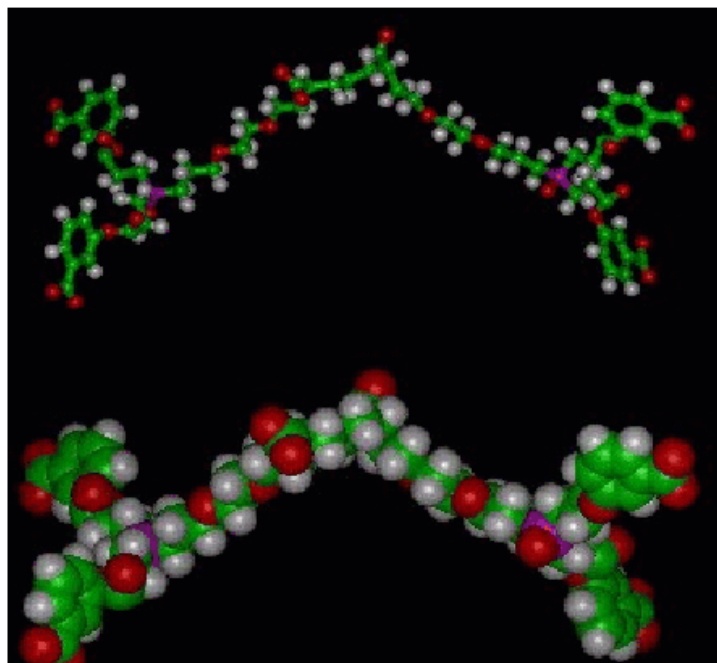


Figure 1: Energy-minimized structure of Reagent Bis(*p*-BCCEP-NHPDA)

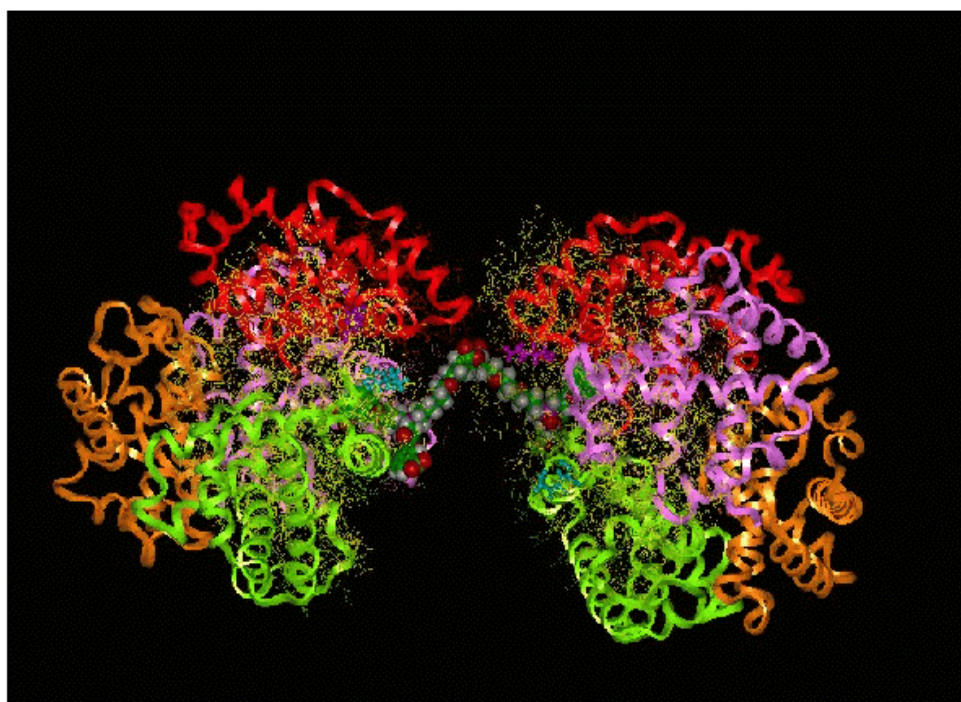


Figure 2: Energy-Minimized Reagent-Hb Complex

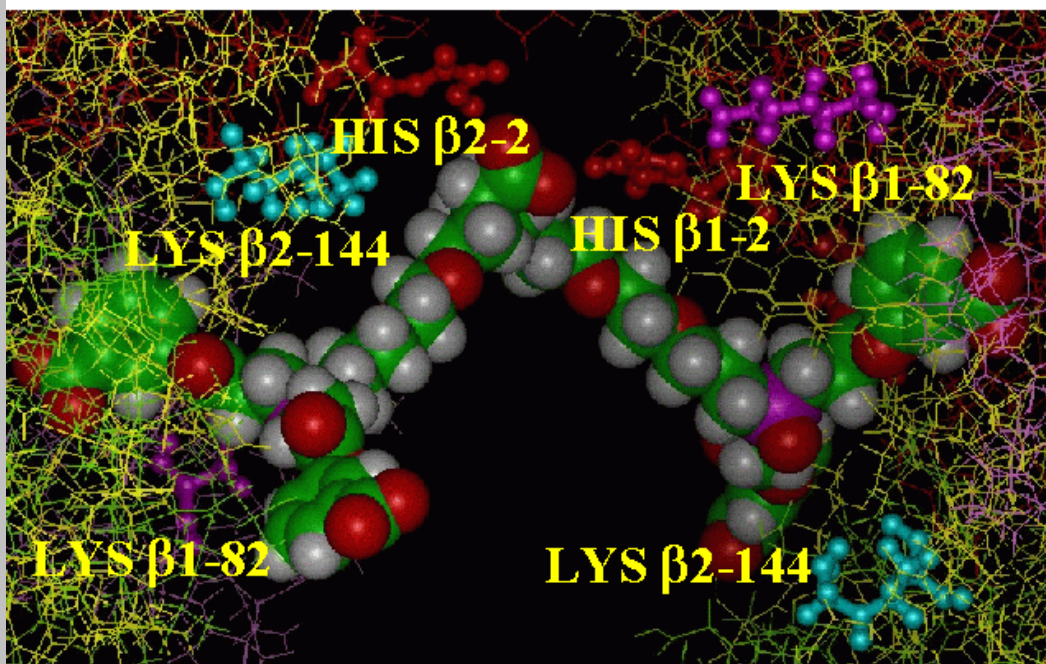
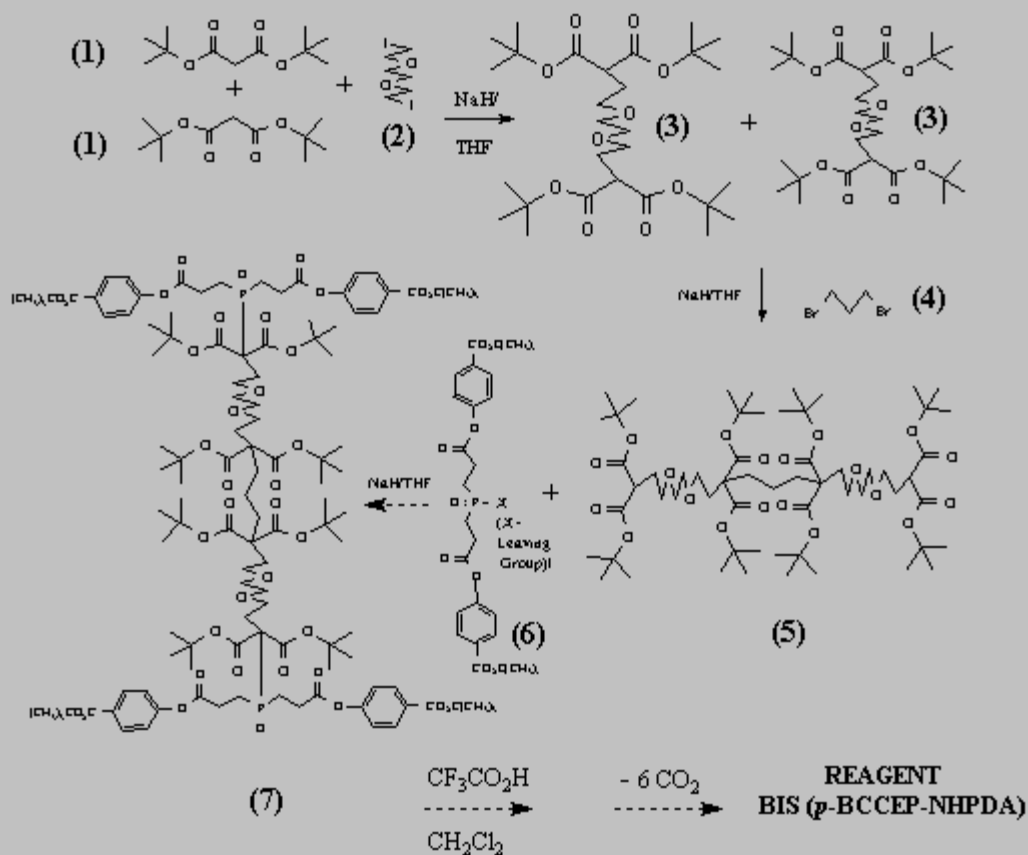


Figure 3: A Close-up view of energy-minimized Reagent-Hb complex

Progrss Toward Synthesis of the Title Reagent Bis(p-BCCEP-NHPDA): The synthetic route for the title reagent is outlined in **Scheme 1**. The synthesis commenced with di-*tert*-butyl malonate (**1**) (Aldrich) which was condensed with 1,2-bis(2-iodoethoxy)ethane (or 1,8-

SCHEME 1



diiodo-3,6-dioxaoctane) (Aldrich) to obtain [2,2'-(3,6-dioxaoctanediyl)dimalonic acid tetra-*tert*-butyl ester] [bis(*t*-butyl malonate)-DOD] (**3**). It is to be noted that the corresponding tetraethyl ester of **3** (Beilstein Registry No. 1809812; CAS Reg. No. 78649-90-8) and a series of its homologues are known in the literature. Two molecules of Compound **3** were further condensed with 1,3-dibromopropane to obtain **5**. Compound **5** contains a central tether that has a middle three-carbon unit joined by two DOD fragments. This is where the synthesis stands at the present time. Reaction of **5** with two molecules of **6** (which will be prepared from the cross-linking reagent **BCCEP** whose synthesis and hemoglobin cross-linking studies we reported recently) (14) will yield **7**. The removal of the protecting groups of the latter by treatment with trifluoroacetic acid in methylene chloride is anticipated to be accompanied by decarboxylation (of six CO_2 molecules) to afford the title Reagent **Bis(p-BCCEP-NHPDA)**.

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