TABLE

Oxygen Affinity of Human and Bovine Hemoglobins Cross-linked with MMSBA

Sample	$P\frac{1}{2}$ (mmHg)	n	% Delivery to Tissue of the Oxygen Bound Hemoglobin in the Lungs
Human red blood cells	27.02	2.8	40
Human stroma-free hemoglobin, (untreated)	18.01	2.64	10
Human stroma-free hemoglobin, (oxy-reacted)	26.90	2.11	39
Human stroma-free hemoglobin, (deoxy-reacted)	26.95	2.41	39
Bovine red blood cells	33.01	2.3	52
Bovine stroma-free hemoglobin, (untreated)	32.66	2.16	46
Bovine stroma-free hemoglobin, (oxy-reacted)	38.17	1.52	40
Bovine stroma-free hemoglobin, (deoxy-reacted)	40.17	1.80	46

Note:
The last column in the Table shows values computed with the Hill equation (Wyman, J., Adv. Prot. Chem., 19:223 (1964) assuming that the partial pressure of oxygen is 100 mmHg in the lungs and 30 mmHg in the tissues.

In the Table above, $P\frac{1}{2}$ indicates the partial pressure of oxygen necessary for saturating hemoglobin at 50% (i.e., $P\frac{1}{2}$ measures the oxygen affinity). Also, the value n is the expression of oxygen-binding cooperativity, which in normal human red cells is very close to $n=3$.

As shown in the Table above, the cross-linked stroma-free hemoglobin obtained as described above had an oxygen affinity lower than that of the corresponding untreated stroma-free hemoglobin. In addition, all the cross-linked stroma-free human hemoglobins obtained had a value of n in the Hill plot near 2.3, demonstrating the persistance of a very good oxygen-binding cooperativity in the cross-linked hemoglobins.

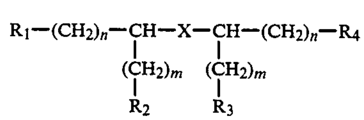
Cross-linked bovine hemoglobins had a relatively low value of n, but it was still higher than 1.5.

As shown in the Table, and as above discussed, the oxygen binding characteristics of all of the cross-linked hemoglobins assured acceptable levels of oxygen delivery to tissues.

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

We claim:

1. Cross-linked stroma-free hemoglobin obtained by cross-linking stroma-free hemoglobin with at least one cross-linking reagent selected from the group consisting of compounds of the general formulae (I) and (II):



(I)

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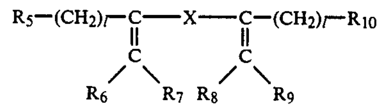

(II)

wherein one of R_1 or R_2 represents an electron-withdrawing atom or group, and the other of R_1 or R_2 represents a leaving atom or group or an electron-withdrawing group containing a leaving atom or group; one of R_3 or R_4 represents an electron-withdrawing atom or group, and the other of R_3 or R_4 represents a leaving atom or group or an electron-withdrawing group containing a leaving atom or group; R_5 and R_{10}, which may be the same or different, each represents an electron-withdrawing atom or group or an electron-withdrawing group containing a leaving atom or group; one of R_6 or R_7 represents a hydrogen atom, an alkyl group, an aryl group, or a heterocyclic group, and the other of R_6 or R_7 represents a leaving atom or group; one of R_8 or R_9 represents a hydrogen atom, an alkyl group, an aryl group or a heterocylic group, and the other of R_8 or R_9 represents a leaving atom or group; X represents a linking atom or group, $n=0$ to 4, $m=0$ to 4 and $l=0$ to 4.

2. The cross-linked stroma-free hemoglobin as claimed in claim **1**, wherein the electron-withdrawing group represented by R_1, R_2, R_3, R_4, R_5 and R_{10} is a member selected from the group consisting of CN, NO_2, halogen, $S(O)R_{11}$, $S(O_2)R_{11}$, $C(O)R_{11}$, wherein R_{11} is selected from the group consisting of a hydrogen atom, a substituted or unsubstituted C_1–C_{12} alkyl group, a substituted or unsubstituted mono- or bicyclic aryl group, and a substituted or unsubstituted heterocyclic group.

3. The cross-linked stroma-free hemoglobin as claimed in claim **2**, wherein said substituents on said alkyl group, aryl group, and heterocyclic group are selected from the group consisting of a halogen atom, CN, a C_1–C_{12} alkyl group, a substituted or unsubstituted phenyl group, NO_2, OH, and a C_1–C_{12} alkoxy group.

4. The cross-linked stroma-free hemoglobin as claimed in claim **2**, wherein said heterocyclic group is selected from the group consisting of substituted or unsubstituted imidazolyl and substituted or unsubstituted pyrazinyl.

5. The cross-linked stroma-free hemoglobin as claimed in claim **1**, wherein R_1, R_2, R_3, R_4, R_5, and R_{10} are selected from the group consisting of CN and SO_2R_{11}.

6. The cross-linked stroma-free hemoglobin as claimed in claim **1**, wherein the electron-withdrawing group containing a leaving group represented by R_1, R_2, R_3, R_4, R_5 and R_{10} is a member selected from the group consisting of $R_{12}P(O)OR_{12}$, $P(O)(OR_{12})_2$, $OP(O)(OR_{12})_2$ and CO_2R_{12}, wherein R_{12} is selected from the group consisting of a hydrogen atom, a substituted or unsubstituted C_1–C_{12} alkyl group, a substituted or unsubstituted mono- or bicyclic aryl group, a substituted or unsubstituted heterocyclic group and a halogen atom and $P(O)(R_{13})_2$, $S(O)R_{13}$, $S(O_2)R_{13}$ and $C(O)R_{13}$, wherein R_{13} is a halogen atom.

7. The cross-linked stroma-free hemoglobin as claimed in claim **1**, wherein the group other than a leaving group represented by R_6, R_7, R_8 and R_9 is selected from the group consisting of a hydrogen atom, a C_1–C_{12} alkyl group, an aryl group, and a heterocyclic group.