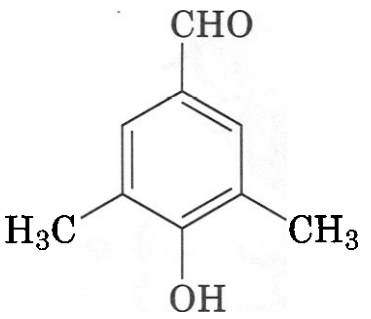
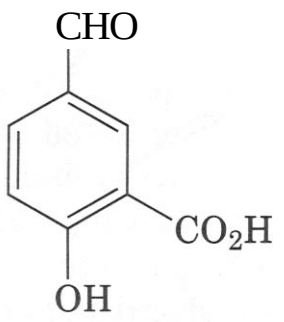
Over the course of these studies, an interesting anomaly was solved. Allosteric effectors (such as **8a** and **8b**) can bind to a similar site



(8a) DMHB



(8b) 5-FSA

and yet effect opposite shifts in the oxygen-binding curve. Agents such as 5-FSA bind to the N-terminal Val and provide groups for hydrogen bonding with the opposite dimer (across the twofold axis) right shift the oxygen-binding curve. In contrast, agents that disrupt the water-mediated linkage between the N-terminal αVal with the C-terminal αArg141 left shift the curve (47) (Fig. 10.4). Structure-based stereospecific allosteric effectors for Hb have also been developed and possess activities and profiles appropriate for clinical efficacy (48, 49).

2.2.4 Crosslinking Agents. The first crosslinking agent that possessed potential as a Hb-based blood substitute was described by Walder et al. (50). Bis(4-formylbenzyl)ethane and bisulfite adducts of similar symmetrical aromatic dialdehydes, previously studied by Goodford and colleagues, provided the starting points that led to these compounds. Chatterjee et al. identified the binding site to deoxy-Hb, and found that the two Lys99 side chains were crosslinked (51). One of the derivatives was proposed as a blood substitute (52), and has been explored commercially (see Vol. 3, Chapter 8. Oxygen Delivery and Blood *Substitutes* and Blood Products, by Andeas Mozzarelli et al.). Another crosslinked Hb engineered by Nagai and colleagues, at the MRC-LMB in Cambridge, was developed into a blood substitute that was clinically investigated at Somatogen, now Baxter (53). Boyiri et al. synthesized a number of crosslinking agents (molecular ratchets, such as 9) whose

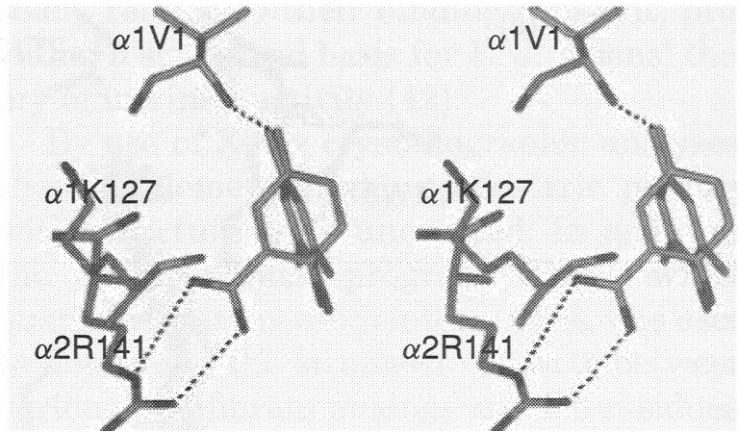
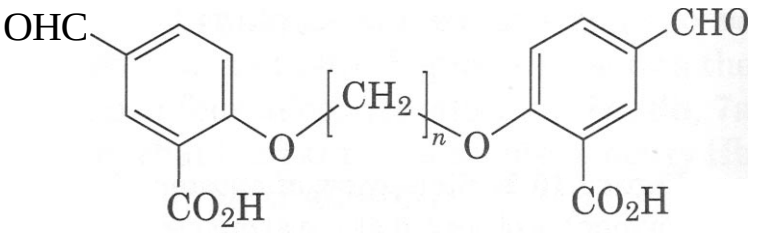


Figure 10.4. Stereoview of superimposed binding sites for (**8b**) (5-FSA, yellow) and (**8a**) (DMHB, magenta) in deoxy hemoglobin. A similar compound environment is observed at the symmetry-related site and therefore not shown here. Both compounds form a Schiff base adduct with the α1Val1 *N*-terminal nitrogen. Whereas the m-carboxylate of 5-FSA forms a salt bridge with the α2Arg141 (opposite subunit), this intersubunit bond is missing in DMHB. The added constraint to the T-state by 5-FSA that ties two subunits together shifts the allosteric equilibrium to the right. On the other hand, the binding of DMHB does not add to the T-state constraint. Instead, it disrupts any T-state salt- or water-bridge interactions between the opposite a-subunits. The result is a left shift of the oxygen equilibrium curve by DMHB. See color insert.



(9) n = 1–10 TB36, n = 3

potency was directly related to the length of the crosslink: the shorter the crosslink (three atoms), the stronger the shift of the oxygen binding curve to the right (54) (Fig. 10.5).

Perutz's hypothesis (55) and the MWC model (56) for allostery, that the more tension is added to the tense (deoxy) state of Hb, the greater the shift to the right of the oxygen-