figurations of the same ligand docked with a receptor, or to rank selected configurations of different ligands docked to the same receptor.

These approaches implicitly assume that the observed receptor cavity has some physical stability (i.e., a static view) and is not significantly altered by binding of different ligands. Although there is no guarantee that this is true for any particular case under study, the specificity seen in biological systems argues that a receptor site has some functional significance in imposing its specific steric and electrostatic characteristics in the molecular recognition and selection process. One must always be prepared, however, for binding to sites other than that targeted, and possible exposure of cryptic sites that are not observed in the absence of the ligand (181). The current computational limits in molecular dynamics simulations restrict the chance of uncovering such alternative binding modes in our studies. If we can assume the binding mode of our candidate drug is nearly identical to that of a known compound, however, then we have a legitimate basis for thermodynamic perturbation calculations. Multiple or alternate binding modes remain a major fly in the ointment. Naruto et al. (284) demonstrated a systematic approach to the determination of productive binding modes for mechanism-based inhibitors (Fig. 3.22) that could select starting structures for complexes for molecular dynamics simulations. Combinations of methods, such as Monte Carlo or systematic search, to generate multiple starting configurations for simulations to improve sampling and thermodynamic reliability will increase as adequate computational power to support these hybrid approaches becomes more readily available.

Many technical limitations remain to be overcome before ligand design becomes reliable and routine. Many deficiencies in molecular mechanics previously cited remain that limit reliability. Adequate modeling of electrostatics remains elusive in many experimental systems of interest such as membranes. Newer derivations of force fields, such as MM3 (27, 296 and references therein), CHARMM (297, 298), AMBER/OPLS (157), ECEPP (299), and others (156, 300), are attempting to more accurately represent the experimental data, whereas others include a broader spectrum of chemistry such as metals (29–31,301–305). Combinations of molecular mechanics with quantum chemistry (159, 160, 162, 306) are clearly necessary for problems in which chemical transformations are involved. Rather amazing agreement between calculation and experiment has been reported (165, 307) on the relative stabilities of transition-state structures, although there is some controversy (308) regarding this approach. In any case, this is another area of rapid growth as adequate computational resources become available. Riley et al. (309, 310) found an excellent correlation between the relative stabilities of conformers in manganese complexes of pentaazacrowns and their ability to catalyze the dismutation of superoxide.

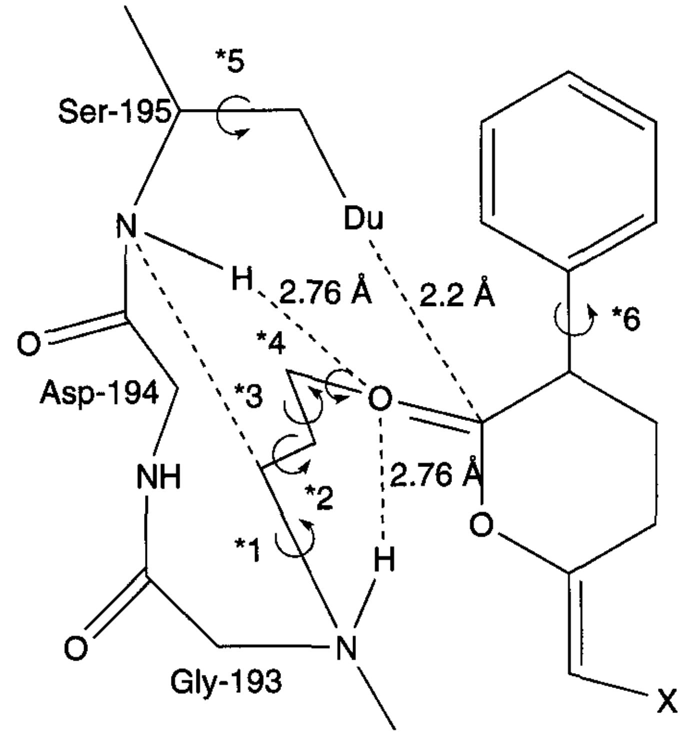

Figure 3.22. Use of systematic search to explore possible binding modes of mechanism-based inhibitors of chymotrypsin (284) by rotation of six bonds (*), which orient carbonyl of substrate relative to hydroxyl (Du) of Ser-195.

## 3.4 Calculation of Affinity (260)

**3.4.1 Components of Binding Affinity (255).** The ability to calculate the affinity of prospective ligands based on the known three-dimensional structure of the therapeutic target would allow prioritization of synthetic targets. It would bring quantitation to the qualitative visualization of a potential ligand in the receptor site. Although this problem has been solved in principle, in practice, direct application of molecular mechanics has not yet