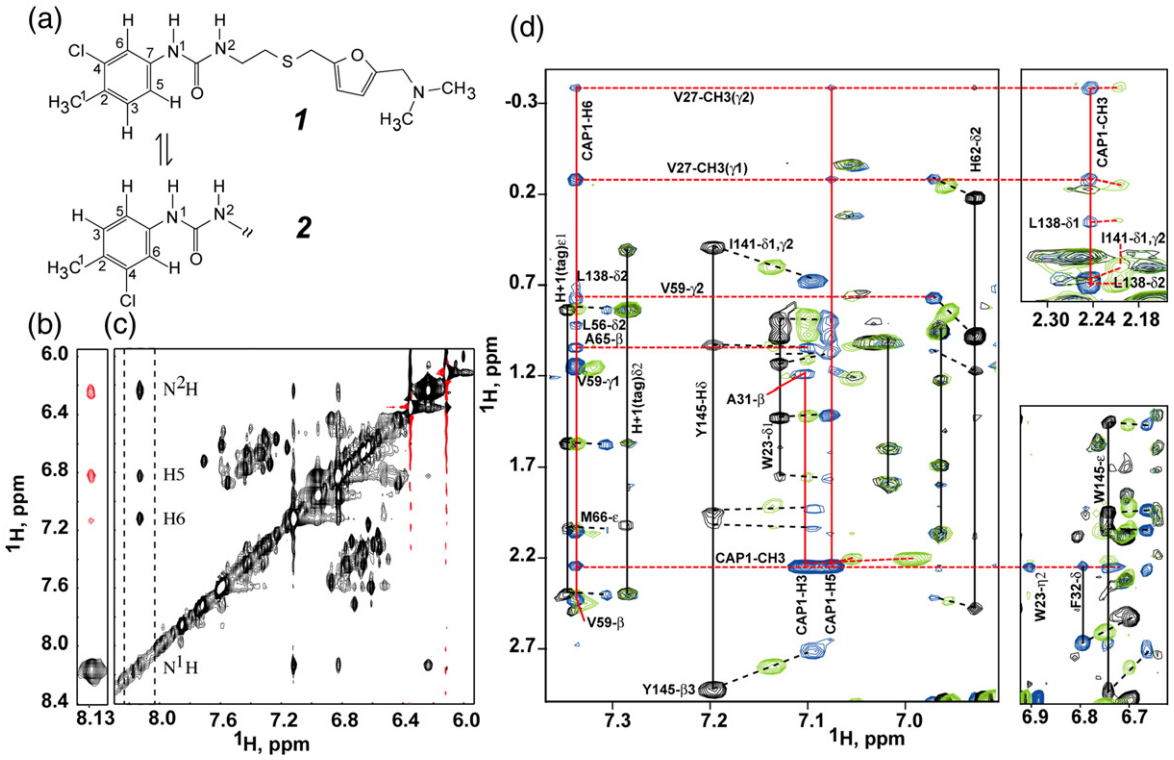

Figure 2. Portions of the 2D NOESY spectra (11 °C, 95% H_2O/5% DMSO-d_6) used to determine the intramolecular orientation of the amide protons in CAP-1. (a) Structures of two possible CAP-1 conformations. (b) Portion of the 2D NOESY spectrum of free CAP-1 showing the positive (red) intramolecular ^{1}H-^{1}H NOEs of HN1 to HN2, H5 and H6. HN1 in the free state is preferentially orientated closer to H5. (c) Row and corresponding 2D NOESY spectrum of CAP-1 (2 mM) in the presence of CA^N (0.1 mM) showing the negative (black) ^{1}H-^{1}H transfer NOEs. (d) Portions of the 2D NOESY data obtained for CA^N (0.7 mM) in the presence of increasing amounts of CAP-1 (CA^N:CAP-1 = 1:0 (black), 1:1 (green), 1:4 (blue)). Chemical shift changes observed upon titration are denoted by broken lines, and intermolecular NOEs are labeled.

sequestered within the hydrophobic pocket vacated by Phe32.

Structure of the CAP-1:CA^N complex

As indicated above, the NMR and X-ray crystallographic data provided complementary information and were therefore used jointly to determine the structure of the CA^N:CAP-1 complex. A starting model for refinement trajectories was built by manually docking CAP-1 into the Phe32 cavity of the CA^N crystal structure obtained in the presence of CAP-1. It was not possible to generate reasonable models consistent with the NOE data using CA^N crystal structures obtained in the absence of CAP-1, in which the Phe32 side-chain was buried. Atoms with well-defined electron density were restrained to the coordinate positions of the crystal structure, and atoms that lacked well-defined density were allowed to move during the calculations. After heating and equilibration at 350 K, a total of 20 structures obtained at 0.2 ps intervals were independently cooled for 2 ps to ~0 K and subjected to energy minimization, which afforded 20 final CAP-1:CA^N structures (Table 1 and Figure 3).

The position and orientation of the CAP-1 phenyl group is well defined by the NMR data within the binding pocket. The C1 methyl group packs against the side-chains of Leu138 and Ile141, the aromatic H6 proton and chlorine atom pack against the side-chain of Val59, and the H3 and H5 protons on the opposite side of the phenyl ring pack against Ala31 and the aromatic ring of Phe32. This specific packing arrangement is consistent with the observation of Val59 and Met66 side chain NOEs with CAP-1 H6 (but not H3 or H5), and Ala31 and Phe32 side-chain NOEs with CAP-1 H3 and H5 (but not H6) (Figure 4). In all the energy-minimized structures, the urea N^1H and N^2H protons are within hydrogen bonding distance of the backbone carbonyl of Val59 (Figure 4). The remaining atoms of CAP-1 are exposed to solvent and appear generally disordered, except that the dimethylamonium group resides near the carboxylate of Glu28 (Figure 4).

Evidence that CAP-1 binding is promoted by Ala31–Phe32 main-chain strain

Because the burial of phenylalanine side-chains is highly favorable, it was surprising to find that the Phe32 side-chain is displaced from the core of the protein upon CAP-1 binding. We therefore inspected Phe32 in unbiased (simulated annealing omit) density for all crystal structures of HIV-1 CA^N crystallized in the absence of CAP-1 that have been published at high resolution.[31,32] Of these 22