



Figure 1.11. MC/FEP results for $\Delta\Delta G_b$ (kcal/mol) established a strong preference for a single methyl group oriented “in” toward Tyr188 in the NNRTI binding site. The precision of the calculations is reflected in the cycle’s small hysteresis, 0.4 kcal/mol.

design given the computer resources available before ca. 2000. In 1985, the ethane-to-methanol FEP calculation in a periodic cube with 125 water molecules required two weeks on a Harris-80 minicomputer,³⁸ and the Wong/McCammon MD simulation for the trypsin-benzamidine complex covered only 29 ps but was performed on a Cyber 205 “supercomputer.”³⁹

Thus, until recently the application of FEP or TI calculations on protein-ligand systems predominantly featured retrospective calculations to reproduce known experimental inhibition data and generally addressed small numbers of compounds. Kollman was a strong advocate of the potential of free-energy calculations for molecular design, and he and Merz reported a rare, prospective FEP result on the binding of a phosphoramidate versus phosphinate inhibitor with thermolysin.^{41,42} Pearlman also advanced the technology, though publications in 2001 and 2005 were still retrospective and confined to a simple congeneric series of 16 p38 kinase inhibitors.^{43,44} In addition, Reddy and Erion have been steady contributors; they have used FEP calculations to evaluate contributions of heteroatoms and small groups to the binding of inhibitors to gain insights on directions for improvement.^{45,46} Our own computations on protein/ligand binding began to appear in 1997 using MC/FEP methodology.^{47,48} Many issues and systems were subsequently addressed, including substituent optimization for celecoxib analogs,⁴⁹ COX-2/COX-1 selectivity,⁵⁰ and heterocycle optimization for inhibitors of fatty acid amide hydrolase.⁵¹ An additional series of publications used MC/FEP calculations to compute the effects of HIV-RT mutations on the activity of NNRTIs.^{52–55} The latter work included predictions for the structures of the complexes of efavirenz and etravirine with HIV-RT, which were subsequently confirmed by x-ray crystallography.^{52,54,56} Confidence in the predicted structures came from agreement between the FEP results and experimental activity data.

FEP-guided optimization of azines as NNRTIs

With this preparation, large increases in computer resources, the hiring of synthetic chemists, and collab-

oration with biologists, FEP-guided lead optimization projects were initiated in 2004. Early successes in the optimization of potent NNRTIs are reflected in Figures 1.2 and 1.3 for the Het-NH-3-Ph-U series.^{11–13} MC/FEP calculations were used to optimize the heterocycle and the substituent in the 4-position of the phenyl ring. The calculations are run with MCPRO and all use the OPLS/CM1A force field for the ligands and OPLS-AA for the protein.^{10,16} This quickly led to selection of 2-pyrimidinyl and 2-(1,3,5)-triazinyl for the heterocycle and chlorine or a cyano group at the 4-position. These combinations yielded NNRTIs with EC_{50} values near 200 nM.

Extensive FEP calculations then focused on optimization of substituents for the heterocycle.¹³ For the 2-pyrimidines, the immediate question concerned whether 4,6-disubstitution would be favorable or if mono substitution at the 4- or 6-position is preferred. In complexes with HIV-RT, the 4- and 6-positions are not equivalent; for example, in Figure 1.2, the methoxy group could be directed toward the viewer (“out”) or away (“in”), as shown. From display of structures of the complexes, the preferences for in or out were not obvious. This was clarified by MC/FEP results, which showed a strong preference for having a single small substituent on the pyrimidine ring and that the substituent would be oriented “in” (Figure 1.11). Synthesis of a variety of such mono-substituted pyrimidines and triazines yielded ten NNRTIs with EC_{50} s below 20 nM.^{11–13} There was good correlation between the FEP results and the observed activities.^{11,13} The methoxypyrimidine **2** in Figure 1.3 (2 nM) was the most potent, although it was also relatively cytotoxic ($CC_{50} = 230$ nM). The corresponding 1,3,5-triazine is also a potent anti-HIV agent (11 nM) and has a large safety margin ($CC_{50} = 42$ μ M).

Heterocycle scans

FEP results also established the orientation of the methoxy methyl group in the pyrimidine and triazine derivatives shown in Figure 1.2, that is, pointing toward Phe227 rather than Tyr181. This suggested the possibility of cyclizing the methoxy group back into the azine ring to form 6–5 and 6–6 fused heterocycles in the manner indicated in Scheme 2.