Figure S1: Sticks representation of the Alisporivir molecule. Residues 3 and 4 that have the only chemical variations between Alisporivir and CsA are represented with a larger stick radius and the chemical differences are encircled in orange. EthV4Cs is identical to Alisporivir except for the absence of a methyl group at position 3 (Sar3 instead of D-Ala3).

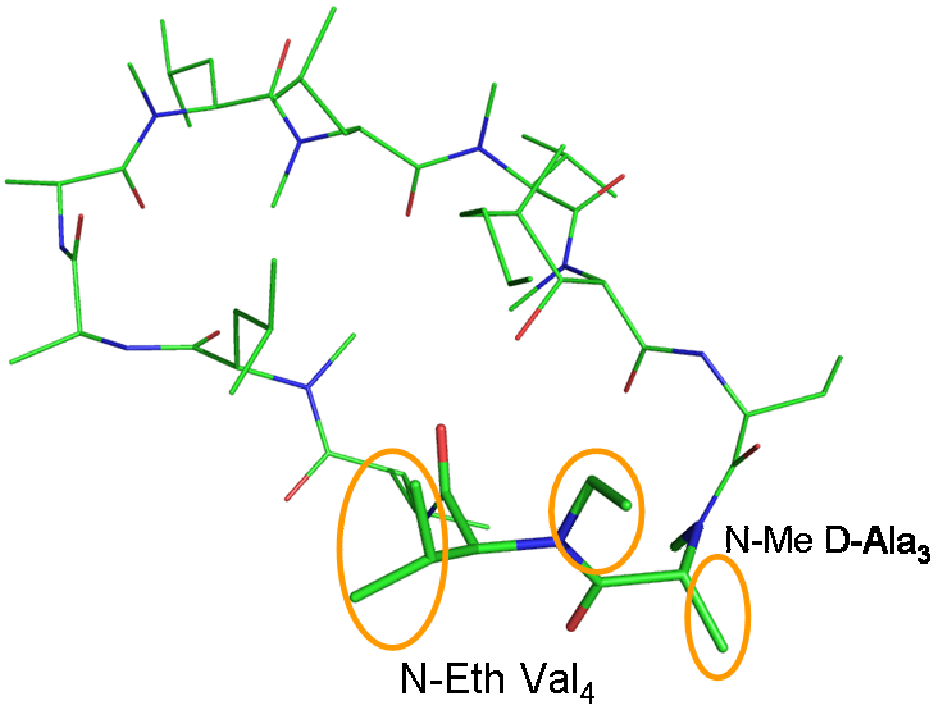

Figure. S2: Overlayed [^{1}H,^{15}N]-HSQC spectra of [^{15}N]-CypA (blue), [^{15}N]-CypA-Alisporivir (black) and [^{15}N]-CypA-CsA (red). Residues of CypA that have distinct resonances in these three spectra are boxed and labeled. Spectra were acquired on a 800 MHz Bruker spectrometer equipped with a 3 mm dual resonance (BBI) probe head with 16 scans and ^{1}H and ^{15}N dimension of 2048 and 256 points, respectively. Protein concentration was 240 µM. These residues are reported on the CypA-CsA structure (1CWA, Mikol, V.; Kallen, J.; Walkinshaw, M. D. *Proc Natl Acad Sci U S A* **1994**, *91*, 5183) as red sticks. CsA in the complex is represented as green sticks with residues 3 and 4 as orange spheres labeled in blue.

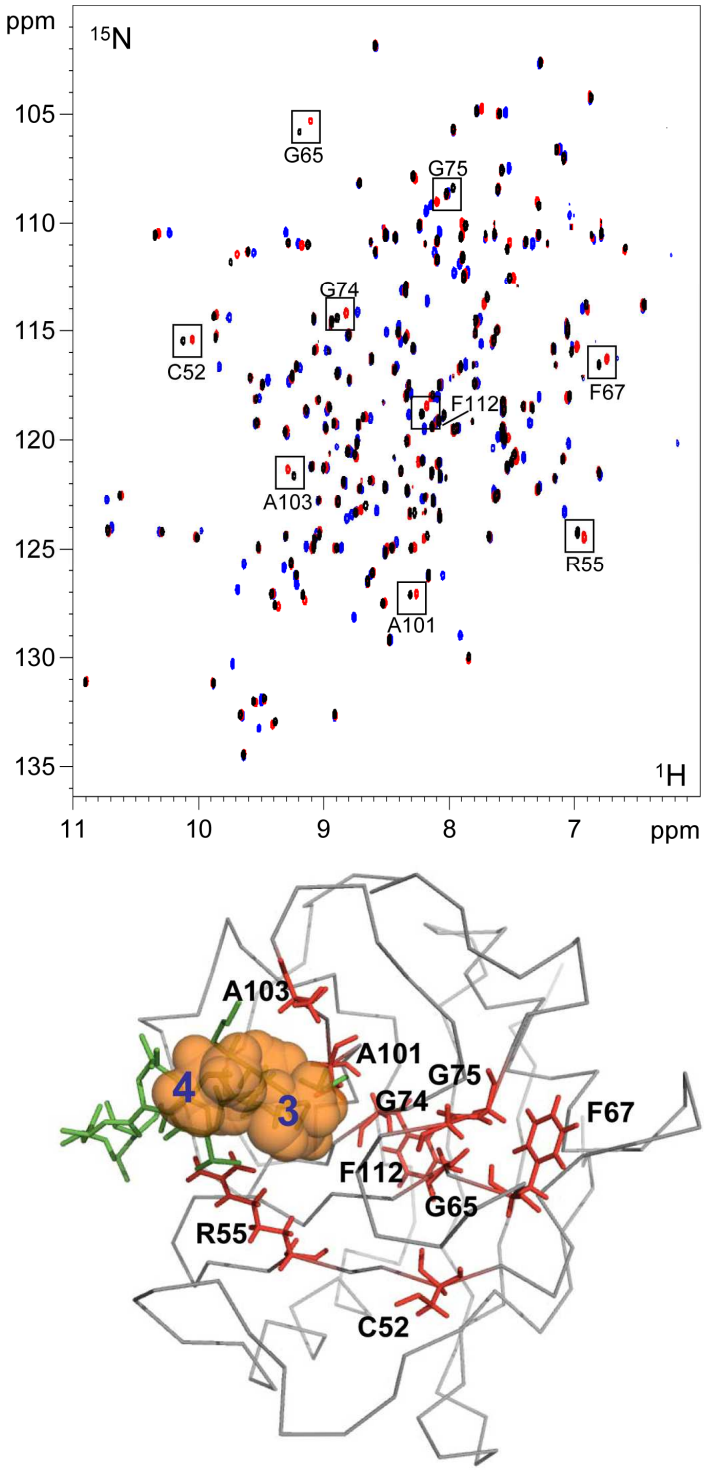

Figure S3: Represented in the graph are the evolution in time of the integrals of the resonance of Arg55 in the CypA-CsA (red triangles) or CypA-Alisporivir (open black circles) complexes or in CypA (blue crosses), normalized on the sum of the signals (green open diamonds), during loading of the free proteins by a 2:1 Alisporivir:CsA mixture of the ligands dried on the tube walls from a chloroform solution. Time points are every 33 minutes, corresponding to the duration of an HSQC spectrum. Experiment was performed at 900 MHz, with 5 mm tubes and a concentration of CypA of 240 µM.

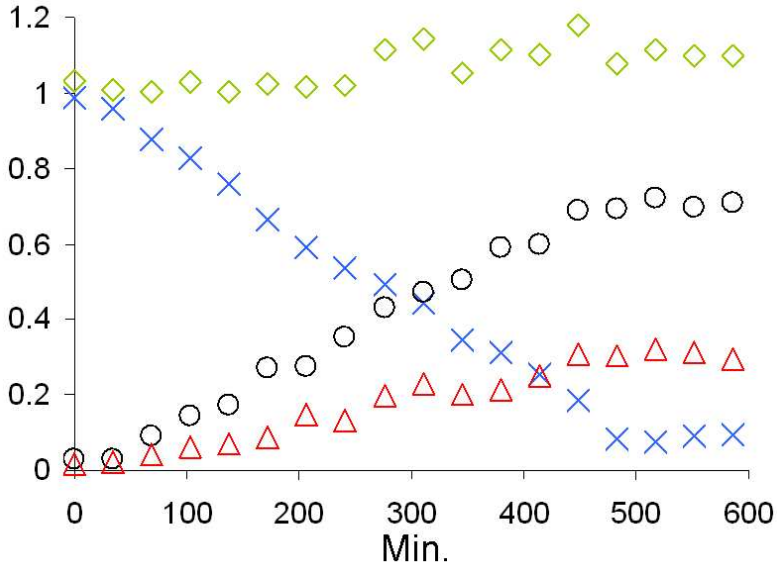

Figure S4: Comparison of 2 independent experiments (in black and red) with different batches of [^{15}N]-CypA protein preparation, independent weighing of the ligands and with NMR spectra acquired on different spectrometers.

Represented in the graph are the evolution over time of the integrals of the resonance of Arg55 in the CypA-EthV4Cs (squares) or CypA-Alisporivir (open circles) complexes or in CypA (crosses), during loading of the free proteins by a Alisporivir and EthV4Cs (1:1 ratio) mixture of the ligands dried on the tube walls from a chloroform solution.

In black, kinetics on the 900 MHz equipped with a cryogenic probe head (8 scans with 3072 and 256 complex points in the direct and indirect dimensions, respectively and a repetition delay of 0.8 sec). Acquisition time of an [^{1}H,^{15}N]-HSQC was 34 min. and 29 sec. *In red*, kinetics on the 800 MHz equipped with a standard TXI probehead (8 scans with 2048 and 256 complex points in the direct and indirect dimensions, respectively and a repetition delay of 1.0 sec). Acquisition time of an [^{1}H,^{15}N]-HSQC was 39 min. and 41 sec.

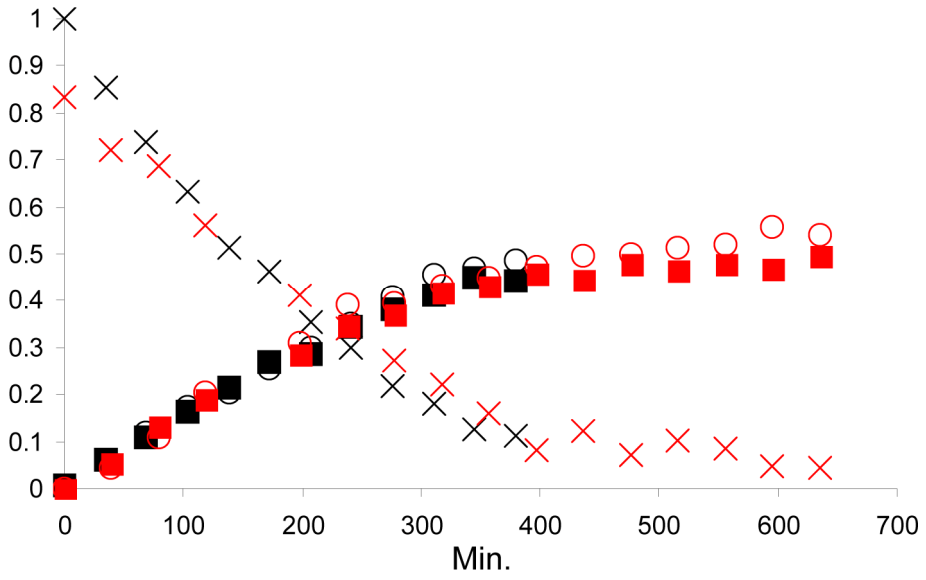

Figure S5: Details of [^{1}H-^{15}N] HSQC showing resonances of residues Cys52 (left spectra) or Ala101 (right spectra) of [^{15}N]-CypA in the CypA-CsA, CypA-Alisporivir or CypA-EthV4Cs complexes. Superimposed are the 1D-projections. Experiments were performed on a 900 MHz Bruker spectrometer equipped with a cryogenic probe head, with 240 µM [^{15}N]-CypA incubated for 15 hours with a mixture of 1:1 Alisporivir:CsA (upper spectra) or Alisporivir:EthV4Cs (lower spectra) dried in 5 mm NMR tubes.

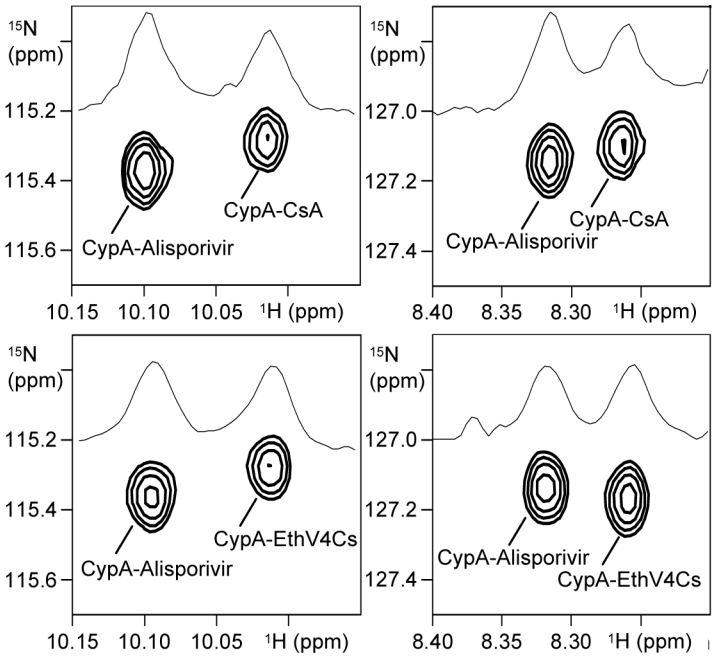

Figure S6: Reverse phase chromatography on a C18 phase of 100 µl of a saturated aqueous solution of CsA (green line), Alisporivir (red line), EthV4Cs (purple stars), MeV4Cs (light blue crosses). The pink line represents the gradient of acetonitrile in 0.05 % aqueous solution of TriFluoroacetic Acid (TFA). The left scale is in milli-absorption units at 215 nm and the right scale is the percentage of acetonitrile.