Instructions for Selective MT imino experiment

You will need to copy TS/wavemaker/excitation/cw file to TS/wavemaker/inversion/cw_i

1. Select imino protons' peaks from 1D (Watergate or 11-echo spectrum) using peak picking – and save it
2. Create new Selective MT experiment (use parameter set, not prosol compatible)
3. Use command "had_pl"
4. Type the experiment number with 1D spectrum where peaks are saved
5. Based on spectral resolution (peak separation) and broadness of the peaks – choose maximum saturation B1 field in order to encode two peaks that are closest – If closest two imino resonances are 40 Hz apart, choose cnst23=5Hz at 600 MHz – this will be base B1 field strength.
6. Since 5 Hz saturation is not enough to perturb efficiently fast exchanging iminos, they can be saturated stronger – this can be done by creating .va list in lists/va directory. The list will be in the form of integers, multipliers of base frequency selected by cnst23. Integers should be ordered according to selected peaks in 1D experiment in decaying chemical shift order (15 ppm→10 ppm) The B1 field multipliers list should be stored with the same name as the shapefile used by wvm to create the encoding saturation pulses, with the extension .va (for example seq_cw.va). If the file is missing, constant amplitudes will be used.
7. Choose p28, duration of cw saturation – standard option is 600-800ms for imino-imino. This will only be attributed to reference pulse used for phase cycling
8. For 15N labeled samples, in order to allow long saturation in the presence of 15N decoupling, without damaging the probe and heating up the sample, very soft decoupling should be used (for example pcpd7=500 us)
9. Since Selective MT encoding targets one frequency at the time, very selective decoupling can be applied. This requires prior knowledge of 15N-1H HSQC/HMQC spectrum (low resolution spectrum is sufficient). FQ3LIST should be created with 15N offsets in ppm for every peak, again in the decreasing chemical shift order like in the VALIST. Alternatively, only two chemical shifts can be used, one in the middle of Gs, and another in the middle of Us, but still they need to be ordered in the list according to peaks from 1.
10. At the end set userA1: sp55(p34):wvm:seq_cw:f1 cw_i(800 ms; B1max=5 Hz) ss=5u

where duration should be set equal to p28, and B1max equal to cnst22. This includes parameters that will be used to make imino encoding pulses.

Also, since acquisition block is sofast-like, set cnst54 and cnst55, parameters of 90 and 180 deg pulses for selective echo. These pulses will be created together with Selective MT saturation pulses

11. Use au program seq_sl to create all the pulses – it will ask again for 1D experiment number and please input the right one.
12. Use “proc_seq” to process the spectrum – zero filling set up with SI will artificially increase F1 resolution

When setting up experiment for the first time, ased will complain that it doesn’t contain necessary pulses – after “had_pl”, do immediately “wvm -a” or “seq_sl” which will create pulses using default parameters and will allow you to go through pulse parameters.