## **SUPPLEMENTARY MATERIAL**

Identification, localization, and relative quantitation of pseudouridine in RNA by tandem mass spectrometry of hydrolysis products

Monika Taucher, Barbara Ganisl, and Kathrin Breuker\*

Institute of Organic Chemistry and Center for Molecular Biosciences (CMBI), University of Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria

\* Address reprint requests to Kathrin Breuker, Institute of Organic Chemistry and Center for Molecular Biosciences (CMBI), University of Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria, phone: ++43 512 507 5240, fax ++43 512 507 2892



**Figure S1**: (a) CAD-MS spectrum of  $(M - 5H)^{5-}$  ions of 22 nt RNA (U-sequence, 1  $\mu$ M in 1:1 H<sub>2</sub>O/CH<sub>3</sub>OH, 25 mM piperidine/25 mM imidazole), laboratory frame collision energy 85 eV; (b) fragment ion map illustrating sequence coverage. A<sub>b</sub> and G<sub>b</sub> stand for nucleobases of adenosine and guanosine, respectively.



**Figure S2**: Ratio of *c*, *y*, and *w* ion yields from CAD of  $(M - 5H)^{5-}$  ions of U- and  $\Psi$ -sequences versus cleavage site, higher collision energy is 85 eV, lower collision energy is 70 eV.



Figure S3: ESI-MS spectra of 22 nt RNA (U-sequence, 1  $\mu$ M in 1:1 H<sub>2</sub>O/CH<sub>3</sub>OH) with (a) 1% Vol. TEA, (b) 0.1% Vol. DBU, and (c) 20 mM CHP.