

Quantitative Analysis of Metabolic Mixtures by 2D ^{13}C -Constant-Time TOCSY NMR Spectroscopy

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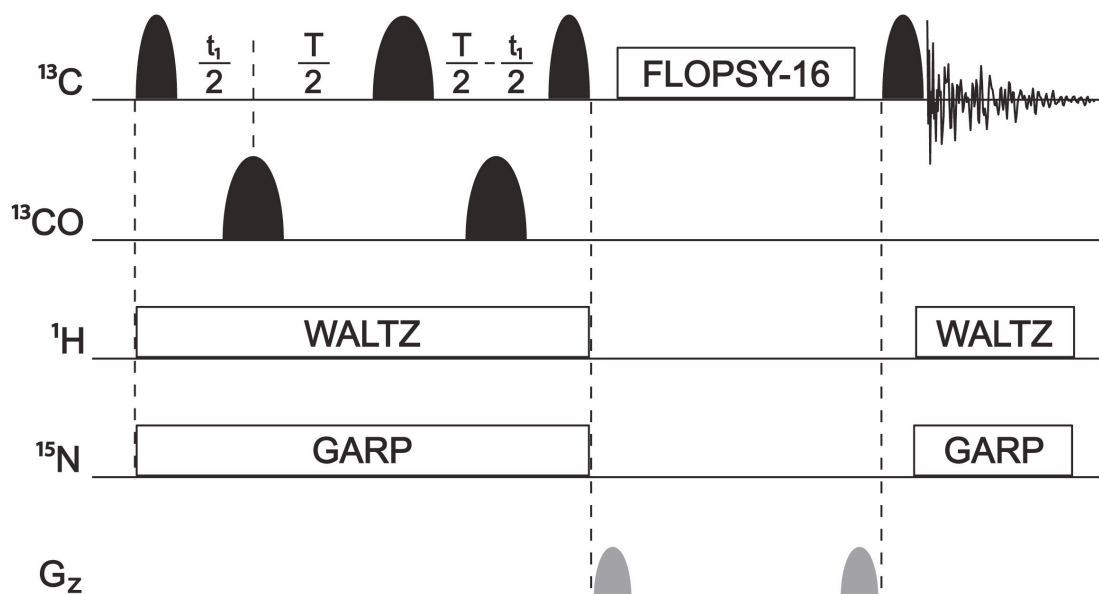


Figure S-1. Pulse sequence of 2D ^{13}C - ^{13}C constant-time (CT) TOCSY experiment. Narrow and wide black semi-elliptical shapes on the carbon channel (^{13}C) represent $\pi/2$ and π shaped radio-frequency pulses, respectively. The constant-time period T is chosen so that $T = 1/{}^1J_{CC} \cong 1/37.6 \text{ Hz} = 26.6 \text{ ms}$. Pulse phases are cycled as follows: three $\pi/2$ pulses from left to right = (x, -x), (x, x, x, x, -x, -x, -x, -x), (x, x, -x, -x); all three π pulses = (x); and receiver phase = (x, -x, -x, x, -x, x, x, -x). The pulse lengths are $248 \mu\text{s}$ for $\pi/2$ pulses and $192 \mu\text{s}$ for π pulses. The $\pi/2$ pulses are Gaussian cascade 4 ($G4$)¹ pulses and the π pulses are Quaternion cascade 3 ($Q3$)² pulses. The pulsed-field gradient pulses have a duration of 1 ms with a gradient strength of 38.5 G/cm and 33.7 G/cm, respectively. Quadrature detection in the indirect dimension is achieved by the States-TPPI method. ^1H and ^{15}N (in the case of ^{15}N enriched samples) are decoupled during t_1 and t_2 periods by WALTZ and GARP schemes, respectively. TOCSY mixing is performed by FLOPSY-16. The mixing times are set to 4.7 ms for short and 47 ms for long TOCSY mixing.

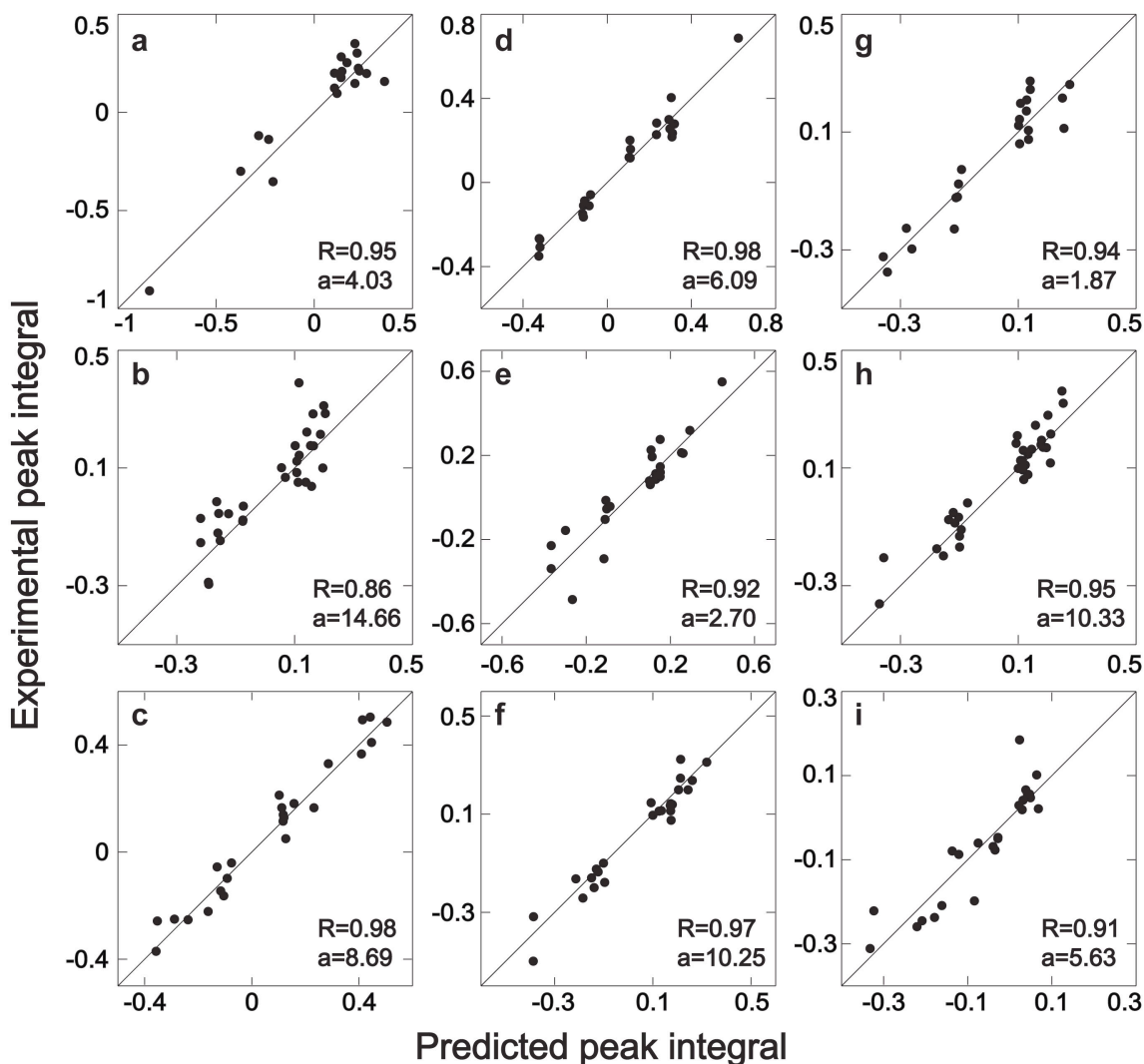


Figure S-2. Quantitative comparison of experimental and simulated (predicted) cross-peak integrals of 2D ^{13}C - ^{13}C constant-time (CT) TOCSY with a long mixing time ($\tau_m = 47$ ms) of 9 carbohydrate isomers: fructose β -furanose (a), fructose β -pyranose (b), glucose β -pyranose (c), glucose α -pyranose (d), ribose β -furanose (e), ribose β -pyranose (f), ribose α -furanose (g), galactose β -pyranose (h) and galactose α -pyranose (i). Fructose α -furanose is not shown in this figure. The predicted peak integrals are based on the quantum-mechanical treatment of Eq. (1) (Method A). Parameters R and a are defined as in Figure 2 and 3 of the main text.

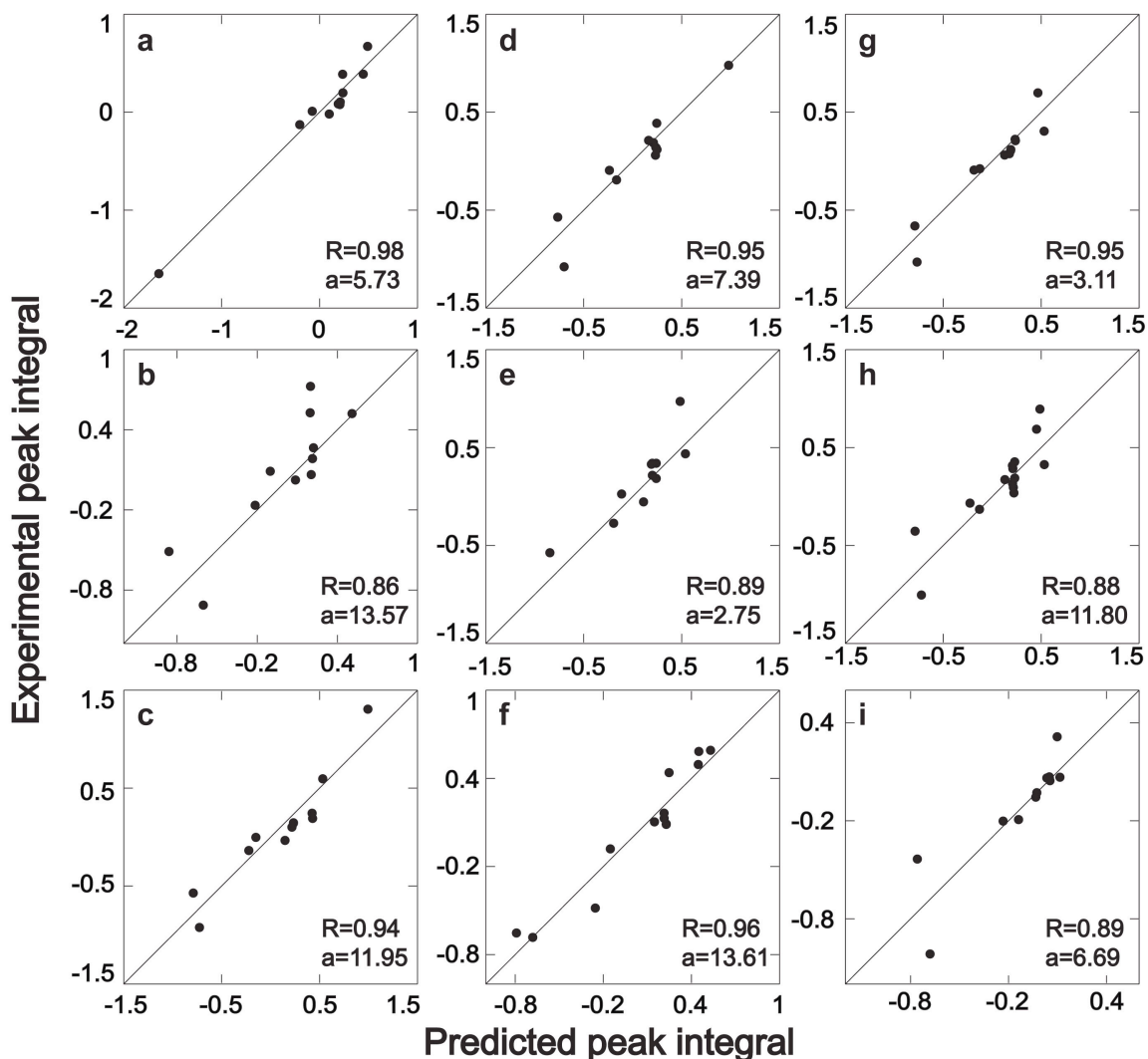


Figure S-3. Quantitative comparison of experimental and simulated (predicted) cross-peak integrals of 2D ^{13}C - ^{13}C constant-time (CT) TOCSY with a short mixing time ($\tau_m = 4.7$ ms) of the same 9 carbohydrate isomers as in Figure S-2: fructose β -furanose (a), fructose β -pyranose (b), glucose β -pyranose (c), glucose α -pyranose (d), ribose β -furanose (e), ribose β -pyranose (f), ribose α -furanose (g), galactose β -pyranose (h) and galactose α -pyranose (i). The predicted peak integrals are based on the quantum-mechanical treatment of Eq. (1) (Method B). Parameters R and a are defined as in Figure 2 and 3 of the main text.

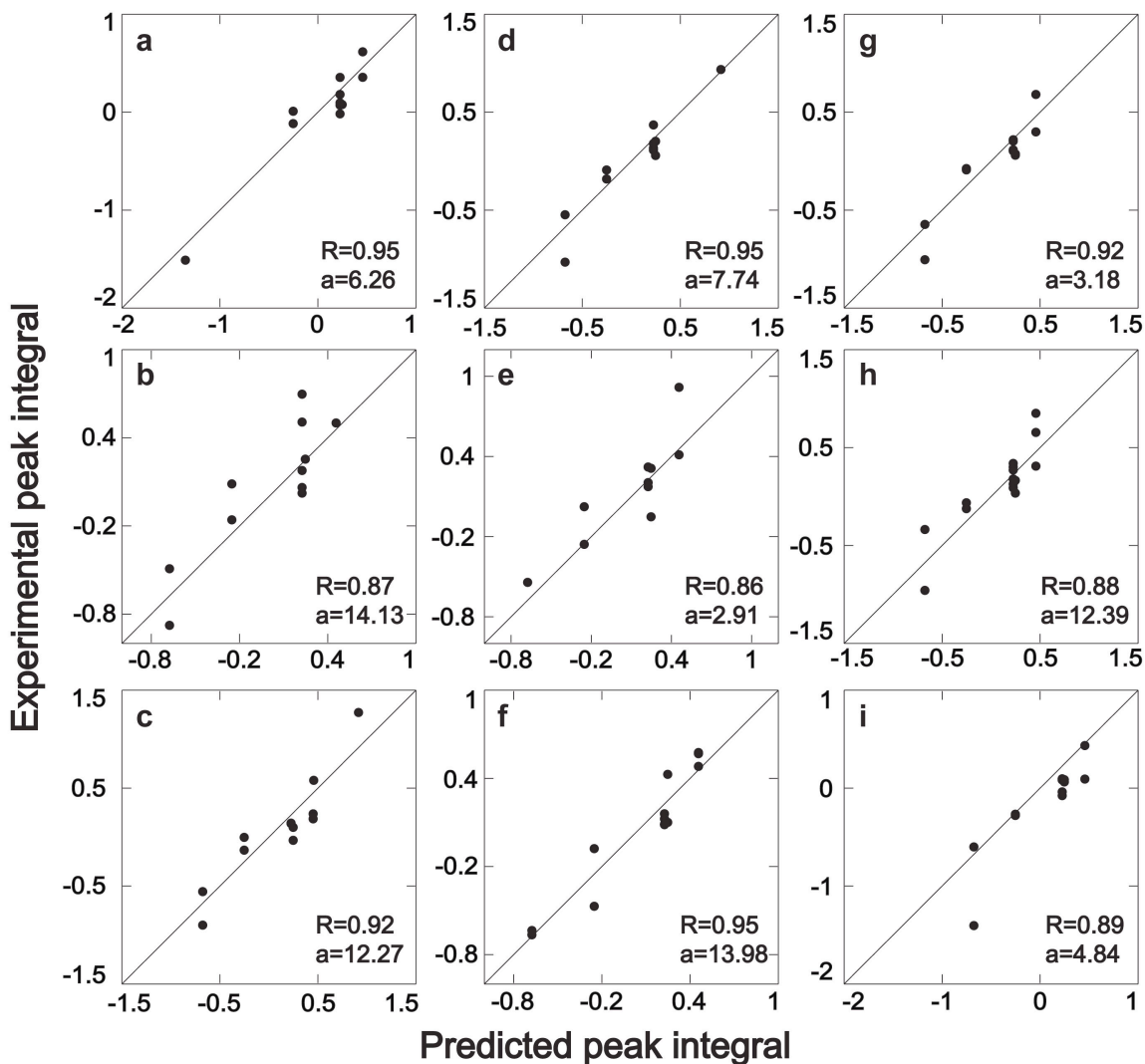


Figure S-4. Quantitative comparison of experimental and simulated (predicted) cross-peak integrals of 2D ^{13}C - ^{13}C constant-time (CT) TOCSY with a short mixing time ($\tau_m = 4.7$ ms) of the same 9 carbohydrate isomers as in Figure S-2: fructose β -furanose (a), fructose β -pyranose (b), glucose β -pyranose (c), glucose α -pyranose (d), ribose β -furanose (e), ribose β -pyranose (f), ribose α -furanose (g), galactose β -pyranose (h) and galactose α -pyranose (i). The predicted peak integrals are based on the analytical approximations (Method C).

References

- (1) Emsley, L.; Bodenhausen, G. *Chem. Phys. Lett.* **1990**, *165*, 469-476.
- (2) Emsley, L.; Bodenhausen, G. *J. Magn. Reson.* **1992**, *97*, 135-148.

