Supplementary Material for Organic & Biomolecular Chemistry

Supplementary data

Acylation is rate-limiting in glycosylasparaginase-catalyzed hydrolysis of N^4-(4′-substituted phenyl)-L-asparagines

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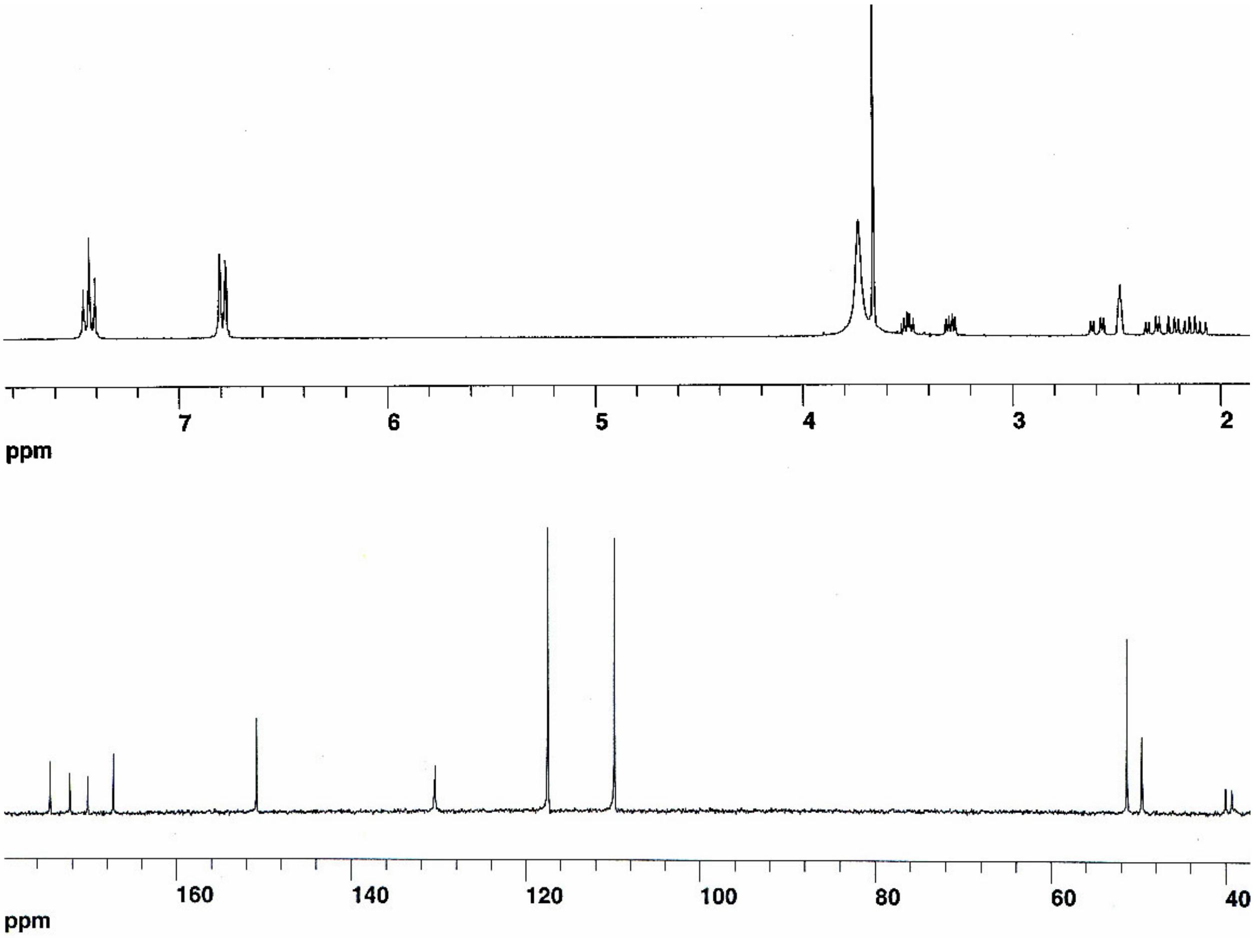
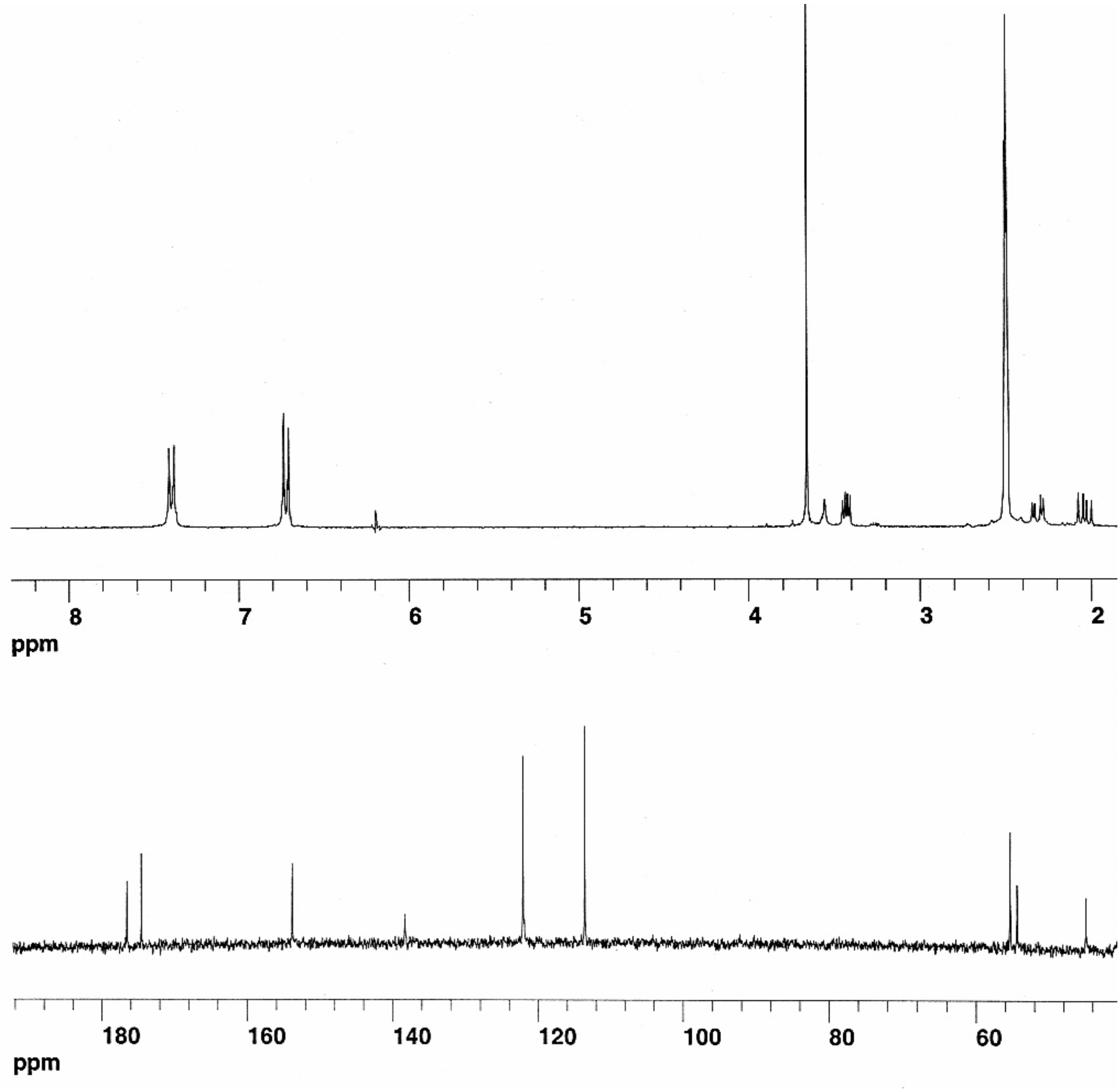
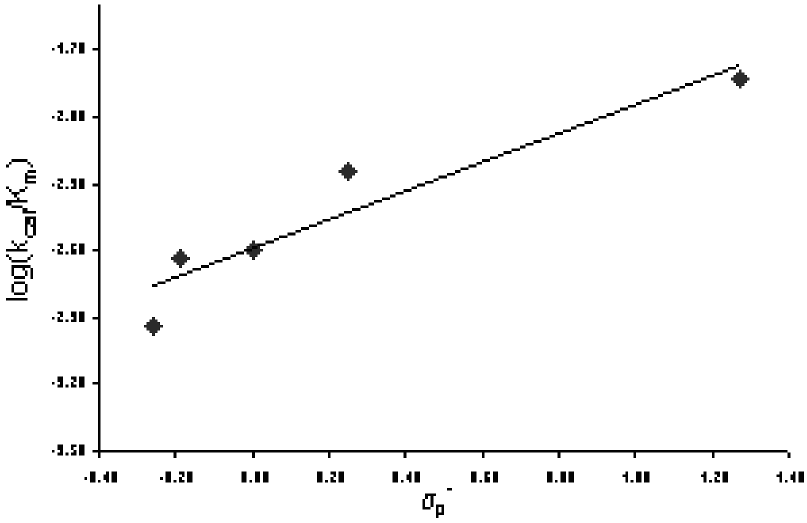


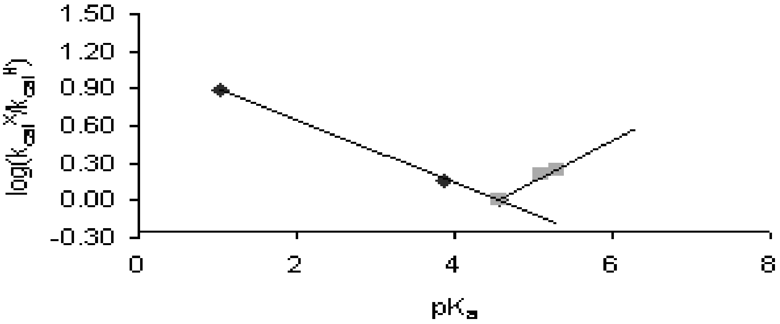
Supplementary Figure 1. The ^{1}H NMR spectrum (top) and ^{13}C NMR spectrum (bottom) of the product of the synthesis (Figure 1) using 4-methoxyaniline shows an equal mixture of *N*^{4}-(4′-methoxyphenyl)-L-asparagine (**6**) and *N*^{1}-(4′-methoxyphenyl)-L-asparagine (**7**). One set of signals for each isomer is present in the ^{1}H NMR spectrum; δ 2.50 is dimethyl sulfoxide as internal reference and δ 3.75 is HDO. Two signals each for C-1 and C-4 (δ ≈ 170) and C-3 (δ ≈ 40) in L-asparagine are present in the ^{13}C NMR spectrum.



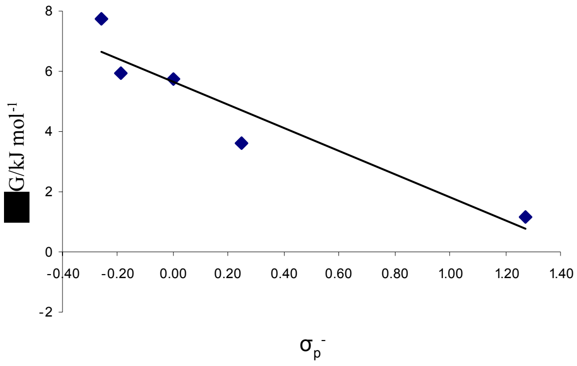
Supplementary Figure 2. After separation of the mixture in Supplementary Figure 1, the signals in the ^{1}H NMR spectrum (top) and ^{13}C NMR (bottom) were assigned to the pure isomer *N*^{4}-(4′-methoxyphenyl)-L-asparagine (**6**).



Supplementary Figure 3. A Hammett plot of $\log(k_{cat}/K_m)$ vs σ_p^- is linear with a slope $\rho = 0.65$ ($r = 0.95$). The positive slope is indicative of a favorable effect on the binding step with increasingly electron-withdrawing substituents. The substituent effect suggests that the electron distribution of the substrate is perturbed toward the transition state.



Supplementary Figure 4. A Brønsted plot of $\log(k_{cat}^{X}/k_{cat}^{H})$ vs pK_a for the conjugate acid of the leaving group is biphasic. The charge on the nitrogen atom in the anilines containing electron-withdrawing groups is (slope) $\beta_{lg} = -0.25$ ($r = 1.00$) and in anilines containing electron-donating groups is (slope) $\beta_{lg} = 0.43$ ($r = 0.98$).



Supplementary Figure 5. A plot of the free energy (incremental) change of binding ($\Delta\Delta G_b$) for the substituted anilides relative to the natural substrate vs σ_p^- is linear (slope $\rho = -3.84_5$, $r = 0.95$). The data indicate that, in the enzyme-substrate transition state complexes, the substitution of a substituted phenyl group for the pyranosyl group results in an overall loss of binding energy equivalent to a weak hydrogen bond, the magnitude of which is dependent on the electronic properties of the substituent group.