

Supporting Information

“Alkaline Phosphatase Mono- and Diesterase Reactions: Comparative Transition State Analysis.”

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Linear Free Energy Relationships with 4-Nitro and 4-Cyano Phenyl Leaving Groups

Introduction

The data compiled here from literature sources demonstrates that compounds with 4-nitro and 4-cyano phenyl substituents can deviate from LFERs when a strictly homologous series of phenyl leaving groups is used to define the correlation. Models to account for this behavior are discussed.

Deviations from LFERs can arise from trivial factors including experimental error, reactive impurities, and competing side reactions; non-trivial factors include changes in mechanism, steric effects, and resonance effects (S1, S2). While it is difficult to rule out trivial factors from a single observed deviation, repeated observation of an outlier substituent in different systems is good evidence for a true physical effect. These physical effects reflect the nature of the transition state, and comparisons of the magnitudes of the deviations for different reactions can therefore assist in characterizing transition state structure.

It is well established that substituent constants for the 4-nitro and 4-cyano groups derived from benzoic acid ionization constants are not applicable to many reactions of aryl groups, presumably due to resonance effects (S1, S2). Subsequent work demonstrated that the

magnitude of the deviations observed varied from reaction to reaction, and that these variations could often be rationalized based on whether resonance effects were expected to be significant for the reaction mechanism in question (S3, S4). 4-Nitro and 4-cyano phenyl substituents can also deviate from LFERs when the phenol pK_a is used as the substituent constant, presumably for the same reasons. However, these deviations are often obscured by the inclusion of several substituents capable of resonance interactions as well as ortho-substituted compounds that may deviate from LFERs due to steric effects. To demonstrate this phenomenon, data from several references are re-plotted below (Figures S1-S8) and summarized in Table S1. The linear least squares fits shown in Figures S1-S8 were obtained with data for compounds with phenyl leaving groups that lack ortho substituents and do not participate in significant resonance interactions (filled circles). 4-Nitro ($pK_a = 7.14$) and 4-cyano ($pK_a = 7.95$) leaving groups were plotted (open circles) but omitted from the linear fits.

Data from Literature Sources

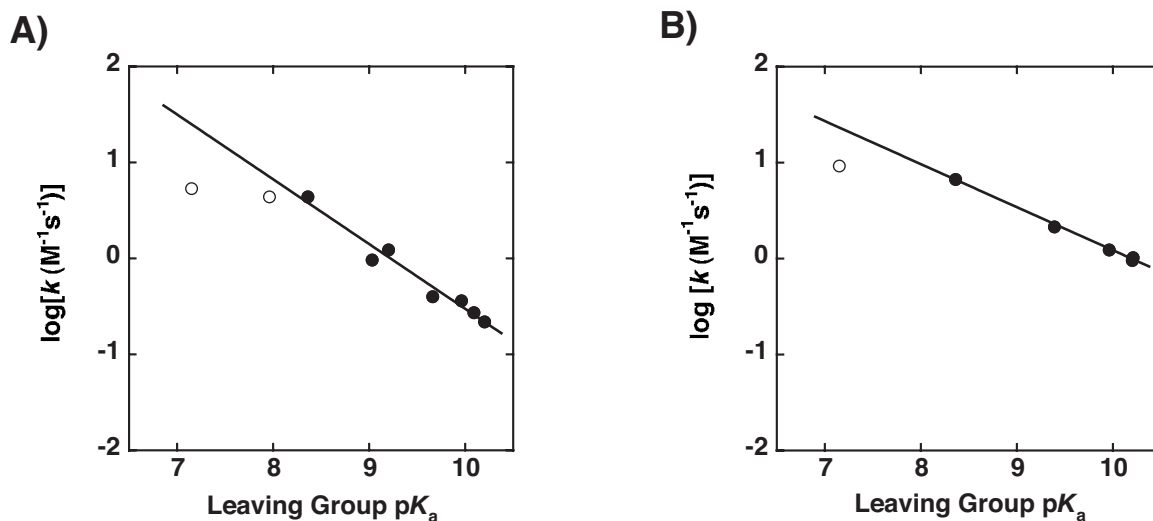


Figure S1. Alkaline hydrolysis of phenyl acetates. A) Data obtained at 20 °C and ionic strength <math><0.08</math> in 56% w/v aqueous acetone (S5). B) Data obtained at 25 °C and ionic strength 1.0 (S6).

The lines give values of β_{lg} of -0.68 ± 0.06 and -0.45 ± 0.01 , respectively.

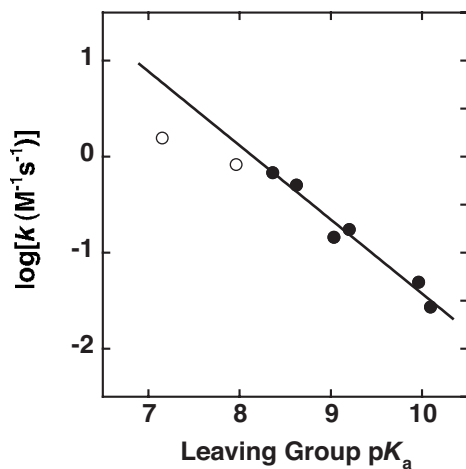


Figure S2. Alkaline hydrolysis of phenyl benzoates at 25 °C and ionic strength 0.005 in 56% w/v aqueous acetone (S7). The line gives a value of β_{lg} of -0.77 ± 0.07 .

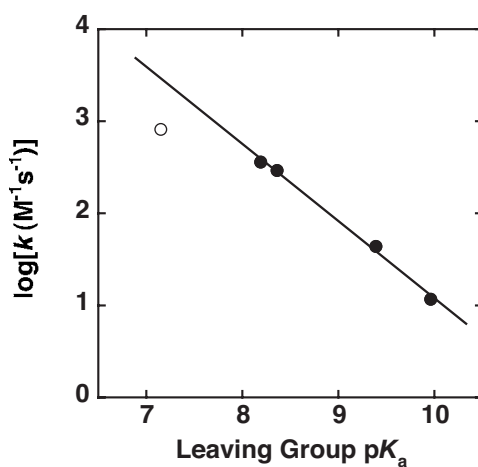


Figure S3. Alkaline hydrolysis of uridine 3'-phenyl phosphates at 25 °C and ionic strength 0.25 (S8). The line gives a value of β_{lg} of -0.84 ± 0.04 .

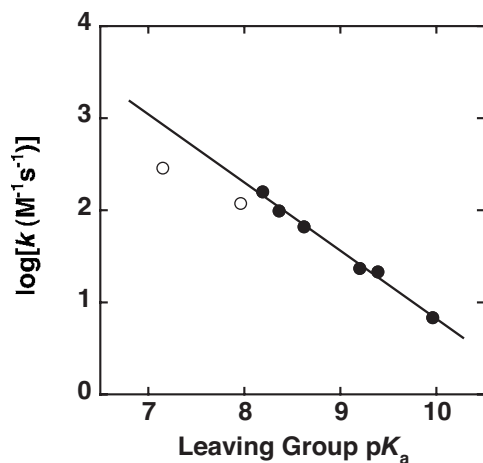


Figure S4. Alkaline hydrolysis of uridine 3'-phenyl phosphorothioates at 25 °C and ionic strength 0.25 (S9). The line gives a value of β_{lg} of -0.74 ± 0.03 .

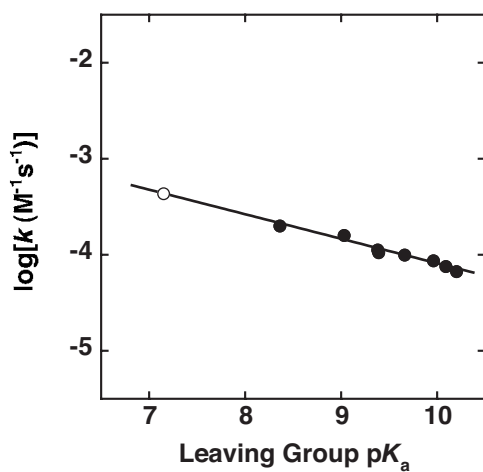


Figure S5. Hydrolysis of neutral phenyl sulfates at 49 °C and ionic strength 0.13 (S10). The line gives a value of β_{lg} of -0.26 ± 0.02 .

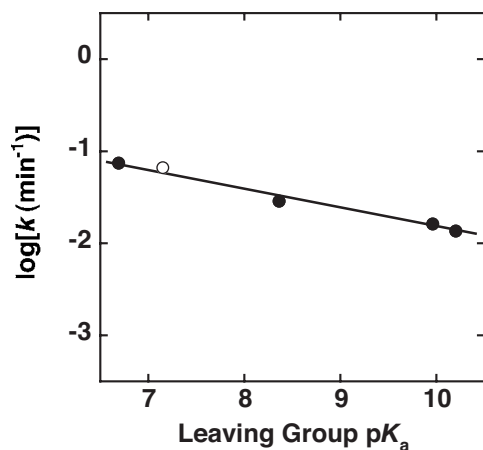


Figure S6. Hydrolysis of phosphate monoester monoanions at 100 °C and ionic strength 1.0 (S11). The line gives a value of β_{lg} of -0.20 ± 0.03 .

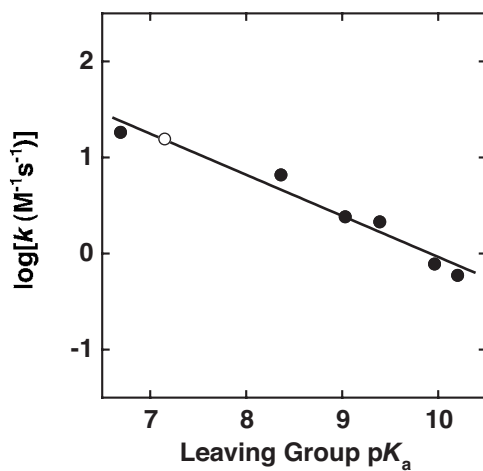


Figure S7. Hydrolysis of aryl dimethylphosphinates at 25 °C and ionic strength 0.1 (S12). The line gives a value of β_{lg} of -0.43 ± 0.05 .

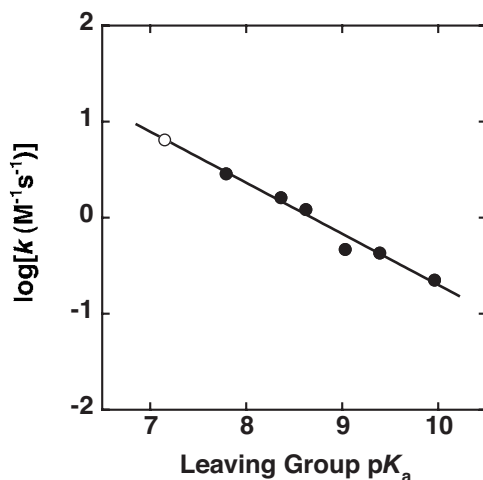


Figure S8. Hydrolysis of aryl dimethylphosphinothioates at 25 °C and ionic strength 1.0 (S13).

The line gives a value of β_{lg} of -0.53 ± 0.05 .

Table S1: Values of β_{lg} and R^2 for LFERs with phenyl substituents.^a

Substrate	Omitting 4-nitro/4-cyano		Including 4-nitro/4-cyano	
	β_{lg}	R^2	β_{lg}	R^2
Methyl phenyl phosphate diesters	-0.94	0.98	-0.81	0.96
Phenyl Acetates (Figure S1 A)	-0.68	0.97	-0.51	0.92
Phenyl Acetates (Figure S1 B)	-0.45	>0.99	-0.34	0.95
Phenyl Benzoates	-0.77	0.97	-0.61	0.94
Uridine 3'-phosphates	-0.84	>0.99	-0.67	0.95
Uridine 3'-phosphorothioates	-0.74	0.99	-0.59	0.95
Neutral phenyl sulfates	-0.26	0.97	-0.26	0.99
Phosphate monoester monoanions	-0.20	0.98	-0.21	0.98
Aryl dimethylphosphinates	-0.43	0.96	-0.43	0.97
Aryl dimethylphosphinothioates	-0.53	0.97	-0.53	0.98

^a Data from Figures S1-S8 and Figure 3 of the main text.

Table S2: Statistical significance of deviations of 4-nitrophenyl substituents.^a

Substrate	$\text{Log}(k_{\text{predicted}}^{4\text{-nitro}}) - \text{log}(k_{\text{observed}}^{4\text{-nitro}})^{\text{b}}$	Standard Deviation ^c	P-value ^d
Methyl phenyl phosphate diesters	0.60	0.10	5×10^{-4}
Phenyl acetates [Figure S1 (A)]	0.67	0.09	3×10^{-4}
Phenyl acetates [Figure S1 (B)]	0.39	0.02	1×10^{-4}
Phenyl benzoates	0.57	0.10	2×10^{-3}
Uridine 3'-phosphates	0.55	0.05	4×10^{-3}
Uridine 3'-phosphorothioates	0.47	0.04	2×10^{-4}
Neutral phenyl sulfates	-0.01	0.03	0.38
Phosphate monoester monoanions	-0.07	0.04	0.11
Aryl dimethylphosphinates	-0.02	0.12	0.44
Aryl dimethylphosphinothioates	0.00	0.07	0.49

^a Data from Figures S1-S8 and Figure 3 of the main text. ^b $\text{log}(k_{\text{predicted}}^{4\text{-nitro}})$ is the expected value of $\text{log}(k)$ for a compound with a 4-nitrophenyl leaving group based on the LFER for each reaction.

^c The standard deviation from the linear fit is defined as $\sqrt{\frac{1}{N-1} \sum_i^N (\text{log}(k_{\text{predicted}}^i) - \text{log}(k_{\text{observed}}^i))^2}$

and includes data for all compounds except those with 4-nitro and 4-cyano phenyl substituents.

^d The P-value is defined as $(1 - \text{CI})$, where CI is a one-sided confidence interval that extends to the data point for the 4-nitrophenyl leaving group, assuming a Student's t -distribution with $N - 2$ degrees of freedom. N is the number of data points included in the linear fit.

Table S3: Statistical significance of deviations of 4-cyanophenyl substituents.^a

Substrate	$\log(k_{\text{predicted}}^{4\text{-cyano}}) - \log(k_{\text{observed}}^{4\text{-cyano}})^{\text{b}}$	Standard Deviation ^c	P-value ^d
Methyl phenyl phosphate diesters	0.14	0.10	0.11
Phenyl acetates [Figure S1 (A)]	0.21	0.09	0.03
Phenyl benzoates	0.23	0.10	0.04
Uridine 3'-phosphorothioates	0.25	0.04	2×10^{-3}

^a Data from Figures S1-S8 and Figure 3 of the main text. ^b $\log(k_{\text{predicted}}^{4\text{-cyano}})$ is the expected value of $\log(k)$ for a compound with a 4-nitrophenyl leaving group based on the LFER for each reaction.

^c The standard deviation from the linear fit is defined as $\sqrt{\frac{1}{N-1} \sum_i^N (\log(k_{\text{predicted}}^i) - \log(k_{\text{observed}}^i))^2}$ and includes data for all compounds except those with 4-nitro and 4-cyano phenyl substituents.

^d The P-value is defined as $(1 - \text{CI})$, where CI is a one-sided confidence interval that extends to the data point for the 4-nitrophenyl leaving group, assuming a Student's t -distribution with $N - 2$ degrees of freedom. N is the number of data points included in the linear fit.

Discussion

The rate constants for reactions of 4-nitro and 4-cyano phenyl substituents appear to deviate from several of the LFERs shown above. The P-values reported in Tables S2 and S3 represent the fraction of data points that would be expected to be farther away from the line than those for compounds with 4-nitro or 4-cyano phenyl substituents, assuming a Student's *t*-distribution of points around the best-fit line. For the hydrolysis reactions of methyl phenyl phosphate diesters (main text), phenyl acetates (Figure S1), phenyl benzoates (Figure S2), uridine 3'-phosphates (Figure S3), and uridine 3'-phosphorothioates (Figure S4), the 4-nitrophenyl group is a significant outlier, with P-values less than 5×10^{-3} (Table S2). The 4-cyanophenyl group is a significant outlier from the uridine 3'-phosphorothioate hydrolysis reaction, with a P-value of 2×10^{-3} (Figure S4 and Table S3). In other reactions for which data are available with the 4-cyanophenyl leaving group, the P-values are greater than 0.01 (Table S3) and the significance of the deviations is not convincing.

In general, outliers from linear least squares fits are difficult to detect, particularly when the outlier is at an extreme of the data, and it is not surprising that 4-nitro and 4-cyano phenyl substituents are typically included in LFERs. The effects of inclusion of these compounds on the values of β_{lg} are not large (Table S1) and are unlikely to lead to qualitative changes in interpretations or conclusions for most purposes, although for the comparisons described in the main text of this work the changes are significant and should not be neglected.

Two simple models predict that compounds with 4-nitro and 4-cyano phenyl substituents can deviate from LFERs for solution reactions. First, resonance delocalization might lag behind charge buildup (i.e. nonperfect synchronization) such that the leaving group pK_a is an overestimate of the ability of 4-nitro substituents to stabilize negative charge buildup in the

transition state (S14, S15). Second, the Hammond postulate predicts that increased stabilization of charge buildup in the transition state could shift the transition state structure towards less charge buildup at the leaving group, leading to downward curvature in the LFER (S6, S16). This model is applicable to leaving groups that stabilize charge buildup through resonance or inductive and field effects.

Both of the models described above predict that no deviations will be observed for neutral phenyl sulfate (Figure S5) and phosphate monoester monoanion hydrolysis (Figure S6). These reactions share the common feature of little charge buildup on the leaving group oxygen in the transition state due to proton transfer from the transferred group to the leaving group oxygen (S11, S17, S18). Therefore, any effects due to nonperfect synchronization will be insignificant. Alternatively, because the slopes of the LFERs are shallow, any downward curvature due to a Hammond effect will be small. This prediction is confirmed by the P-values of 0.38 and 0.11, respectively, for the 4-nitrophenyl leaving group in these reactions (Table S2).

No deviations are observed for the hydrolysis reactions of aryl dimethylphosphinates (Figure S7) and phosphinothioates (Figure S8), which have P-values of 0.44 and 0.49 respectively for the 4-nitrophenyl leaving group. However, the values of β_{lg} of ~ -0.5 suggest that there is substantial charge buildup in the transition state and that resonance or Hammond effects could be important. It is unclear why deviations are not observed for these reactions. Differences in solvation may be responsible, but this suggestion is purely speculative.

Conclusions

The literature data presented above demonstrate that the 4-nitro and 4-cyano phenyl groups can deviate from LFERs for solution reactions. It is therefore reasonable to omit these substituents from fits to determine β_{lg} . Comparisons of the magnitudes of these deviations may

provide additional information to characterize transition state structure, as carried out in the main text.

Log(k) vs. Log(k) Plots for AP-Catalyzed Reactions

Compounds with 4-nitro and 4-cyano phenyl leaving groups are outliers from LFERs in several AP-catalyzed hydrolysis reactions (S19-21). In a plot of the log of the rate constant for the AP-catalyzed reactions of sulfate monoesters versus that for the corresponding phosphorothioate monoesters, the 4-nitro and 4-cyano outliers fall on the same correlation line as the other leaving groups in the series (Figure S9 A), demonstrating that the magnitudes of the deviations are the same for both reactions (S21). However, in a plot of the log of the rate constant for the AP-catalyzed reactions of phosphate diesters versus that for the corresponding phosphorothioate monoesters, the 4-nitro and 4-cyano outliers do not fall on the same correlation line as the other leaving groups in the series (Figure S9 B), demonstrating that the magnitudes of the deviations are different for the phosphate diester reaction than for the phosphorothioate or sulfate monoester reactions (see also Figure 5 of the main text).

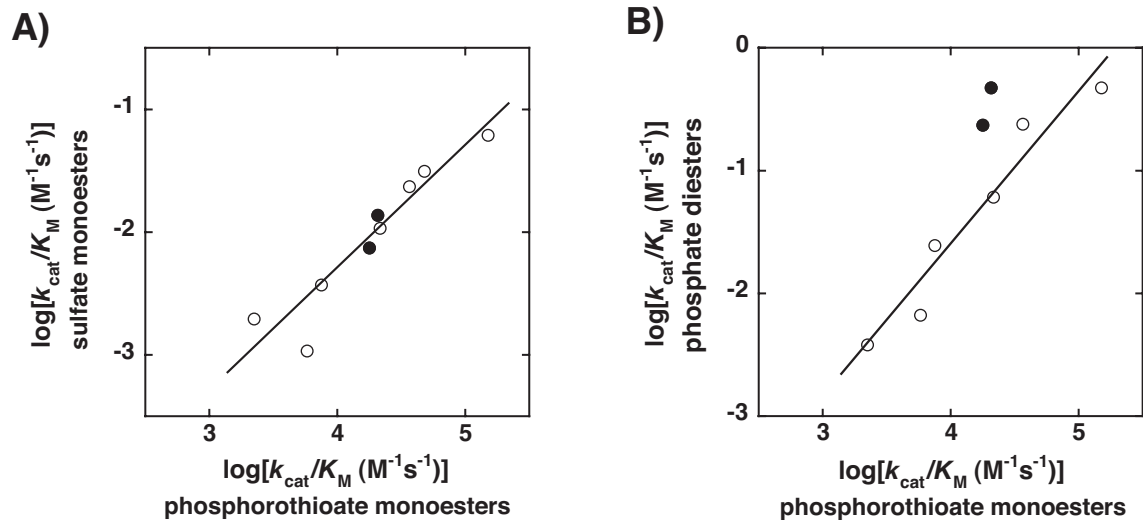


Figure S9. Log(k) vs. log(k) plots for AP-catalyzed reactions. A) Plot of the log of the rate constant for the AP-catalyzed sulfate monoester reaction (S21) versus that for the phosphorothioate monoester with the same phenyl leaving group (S19). B) Plot of the log of the rate constant for the AP-catalyzed phosphate diester reaction (data from the main text) versus that for the phosphorothioate monoester with the same phenyl leaving group (S19). Filled circles are rate constants for compounds with 4-nitro and 4-cyano phenyl leaving groups and were not included in either fit. The slopes of the correlations for A and B are 1.00 ± 0.15 and 1.24 ± 0.16 respectively.

NMR Chemical Shift Data for Methyl Phenyl Phosphate Diesters

Purified methyl phenyl phosphate diesters were characterized by ^1H and ^{31}P NMR on a Varian Mercury 400 MHz instrument in D_2O solvent at room temperature. ^1H chemical shifts are reported relative to the solvent residual HDO peak at δ 4.79 (S22) and ^{31}P shifts are reported relative to an external standard of 85% H_3PO_4 . The chemical shift data are compiled in Table S4. Single ^{31}P NMR signals were observed for each diester except methyl 4-chloro-3-nitro phenyl phosphate, for which a contaminant was observed at δ -9.95 (<5% of total ^{31}P signal). The contaminant peak is consistent with a symmetrical dimethyl pyrophosphate generated as a synthesis byproduct (S23). This contaminant had no effect on reaction kinetics, as the observed rate constant increased linearly over a 10-fold range of phosphate diester concentrations.

Table S4: ^1H and ^{31}P NMR chemical shift data for methyl phenyl phosphate diesters

Phenyl substituent	^{31}P	^1H
4-nitro	-3.00	8.27 (d, 2H, Ph)
		7.35 (d, 2H, Ph)
		3.70 (d, 3H, CH_3 , $J_{\text{CH}_3\text{-P}} = 11.2$ Hz)
4-chloro-3-nitro	-2.68	7.80 (d, 1H, Ph)
		7.62 (d, 1H, Ph)
		7.43 (dd, 1H, Ph)
		3.68 (d, 3H, CH_3 , $J_{\text{CH}_3\text{-P}} = 11.3$ Hz)
4-cyano	-2.89	7.77 (d, 2H, Ph)
		7.31 (d, 2H, Ph)
		3.68 (d, 3H, CH_3 , $J_{\text{CH}_3\text{-P}} = 11.2$ Hz)
3-nitro	-2.46	8.03 (m, 2H, Ph)
		7.58 (m, 2H, Ph)
		3.70 (d, 3H, CH_3 , $J_{\text{CH}_3\text{-P}} = 11.2$ Hz)
3,4-dichloro	-2.41	7.49 (d, 1H, Ph)
		7.36 (d, 1H, Ph)
		7.09 (dd, 1H, Ph)
		3.66 (d, 3H, CH_3 , $J_{\text{CH}_3\text{-P}} = 11.2$ Hz)
3-chloro	-2.31	7.35 (t, 1H, Ph)
		7.23 (m, 2H, Ph)
		7.11 (d, 1H, Ph)
		3.68 (d, 3H, CH_3 , $J_{\text{CH}_3\text{-P}} = 11.2$ Hz)
		7.36 (q, 1H, Ph)
3-fluoro	-2.34	6.98 (m, 3H, Ph)
		3.67 (d, 3H, CH_3 , $J_{\text{CH}_3\text{-P}} = 11.2$ Hz)
		7.37 (d, 2H, Ph)
4-chloro	-2.20	7.14 (d, 2H, Ph)
		3.67 (d, 3H, CH_3 , $J_{\text{CH}_3\text{-P}} = 11.2$ Hz)
		7.13 (m, 4H, Ph)
4-fluoro	-1.88	3.68 (d, 3H, CH_3 , $J_{\text{CH}_3\text{-P}} = 11.1$ Hz)
		7.40 (t, 2H, Ph)
		7.20 (t, 3H, Ph)
Parent	-2.03	3.68 (d, 3H, CH_3 , $J_{\text{CH}_3\text{-P}} = 11.2$ Hz)

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