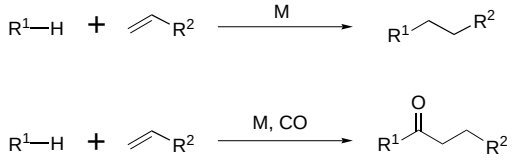
[11] See the Supporting Information for the variable-temperature ^{1}H, ^{31}P, and ^{29}Si NMR spectra of **5b**.
[12] ^{1}H and ^{31}P NMR spectra of **5a** in C_6D_6 were also complex at room temperature.
[13] Preparation of compound **2** was based on Tamao's method: K. Tamao, H. Yao, Y. Tsutsumi, H. Abe, T. Hayashi, Y. Ito, *Tetrahedron Lett.* **1990**, *31*, 2925 – 2928.
[14] It is known that M–SiH_3 bonds are shorter than M–$SiMe_3$ bonds (M = $(CO)_5Mn$ or $(CO)_5Re$): L. Manojlovic-Muir, K. W. Muir, J. A. Ibers, *Inorg. Chem.* **1970**, *9*, 447 – 452; D. W. H. Rankin, A. Robertson, *J. Organomet. Chem.* **1975**, *85*, 225 – 235; D. W. H. Rankin, A. Robertson, *J. Organomet. Chem.* **1976**, *105*, 331 – 340; M. C. Couldwell, J. Simpson, W. T. Robinson, *J. Organomet. Chem.* **1976**, *107*, 323 – 339.
[15] A preliminary study showed that nickel complexes catalyze the dehydrogenative dimerization of **2**.

A Mass Spectrometric Labeling Strategy for High-Throughput Reaction Evaluation and Optimization: Exploring C–H Activation**

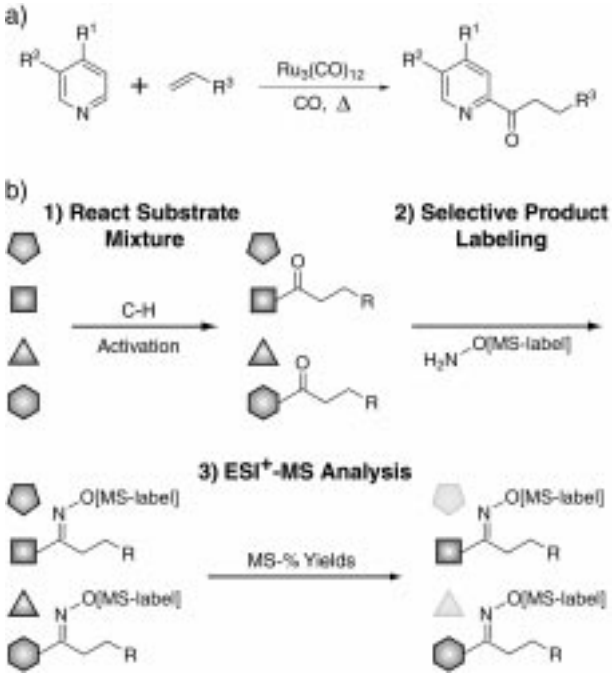
Jason W. Szewczyk, Rebecca L. Zuckerman, Robert G. Bergman,* and Jonathan A. Ellman*

Chemical transformations that combine C–H activation[1] and olefin insertion[2] present attractive opportunities for the rapid assembly of complex structures from unexploited classes of building blocks through carbon – carbon bond formation (Scheme 1).[3] Controlling the site of activation is



Scheme 1. The generality of C–H activation allows, in principle, the combination of aromatic heterocycles with alkenes to rapidly assemble diverse structures using unexploited classes of building blocks.

one of the key challenges of this chemistry. Moore et al. first demonstrated that [$Ru_3(CO)_{12}$] catalytically activates the *ortho* positions of pyridine toward acylation with CO and olefins (Scheme 2a).[4] They proposed that the aromatic nitrogen atom coordinates the ruthenium catalyst, and directs activation specifically to the *ortho* sp^2 C–H bonds. More recently, Murai and co-workers have extended this reaction to several different aromatic heterocycles and olefin substrates.[5] A better understanding of the rules for functional group compatibility and substrate generality demands that a large number of heterocyclic classes be investigated, yet current approaches cannot efficiently provide this information because of the large number of compounds that require testing.



Scheme 2. a) A mixture of diverse pyridines is C–H activated to provide a mixture of products each bearing a ketone group. b) A schematic representation summarizing the high-throughput strategy for the optimization of the reaction and discovery of new products.

Combinatorial methods have made a major impact on new catalyst discovery, but the development of rapid and efficient analyses remains a challenge.[7] Several recent high-throughput methods monitor reactions through the catalyst turnover and/or reaction conversion by IR thermography,[8] the formation of UV-active products,[9] or the production of acid[10] or carbon dioxide.[11] Although, these techniques are powerful, they cannot distinguish between products, by-products, and decomposition. Alternatively, serial methods (TLC, GC, and HPLC) have been employed to identify products and/or quantify yields,[12] but these methods severely limit the number of reactions that can be analyzed in a short period of time. In addition, fluorescent-labeled substrates have been used in the rapid monitoring of reactions.[13] Unfortunately, each substrate under investigation must be individually synthesized as a specifically labeled compound prior to reaction and analysis; thus experimental effort increases linearly with the number of different substrates to be evaluated. We report here the first optimization strategy that enables efficient quantitation of product yields at multiple time points for large numbers of substrates, thereby establishing structure – reactivity relationships and reaction compatibility of functional groups (Scheme 2b).

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[**] This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division, U.S. Department of Energy, under contract No. DE-AC03-76SF00098. We are grateful to the National Institutes of Health for support (GM-50353) and for a Postdoctoral Fellowship to J.W.S.

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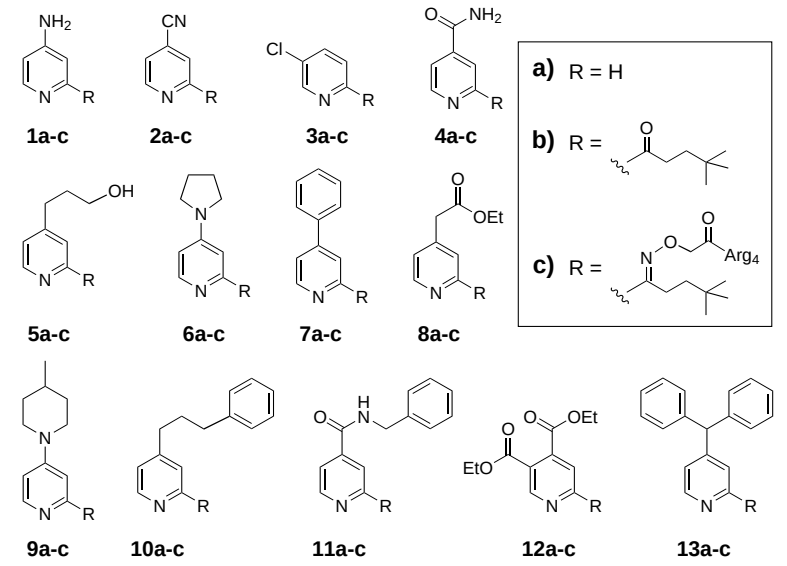
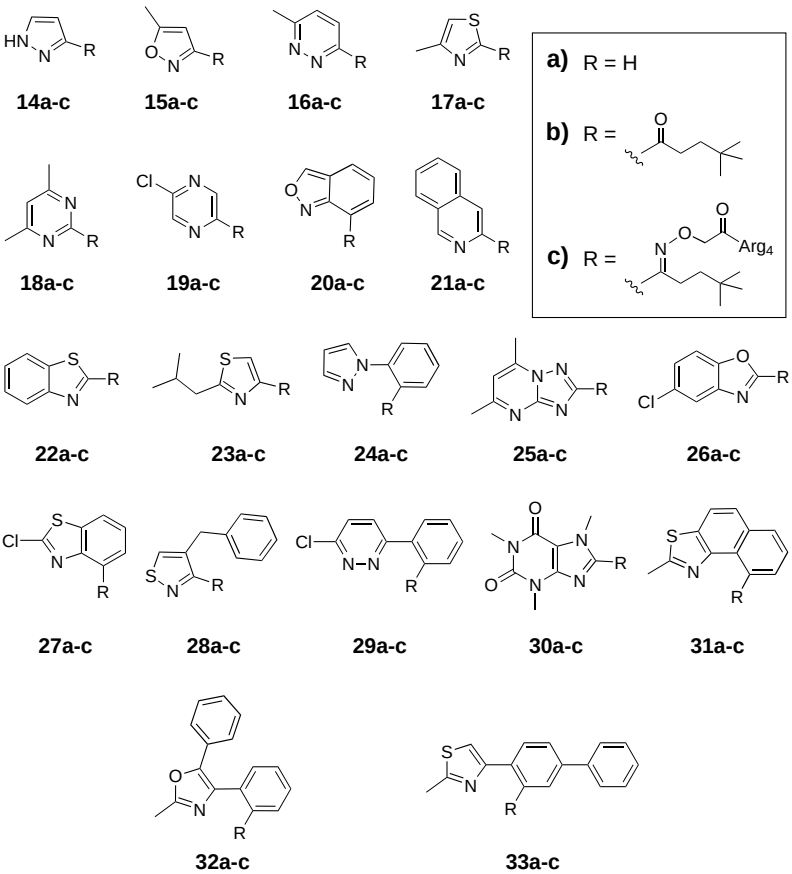
  0044-8249/01/11301-0222 $ 17.50+.50/0

Electrospray ionization mass spectrometry (ESI-MS) has drawn increasing attention for the analysis of combinatorial libraries as a consequence of its mild ionization conditions, rapid data acquisition, and the recent availability of affordable equipment.[14] However, all previous combinatorial approaches employ only a single substrate or a few substrates to optimize reaction parameters.[15] Since a typical mass spectrum surveys a window of thousands of Daltons simultaneously, mass spectrometry can theoretically be used to evaluate hundreds of substrates in a single experiment. In our strategy a mixture consisting of aromatic heterocycles and a single alkene was subjected to catalytic C–H activation with $[Ru_3(CO)_{12}]$ and CO (Scheme 2). Next, products bearing a ketone group were selectively labeled.[16] This step silences remaining starting materials and/or decomposition products and allows the yields of the desired products to be determined directly by positive-ion ESI-MS (ESI^+-MS). As proof of principle, a small library of functionalized pyridines was treated with $[Ru_3(CO)_{12}]$, alkene, and CO to determine the compatibility of functional groups and substrate generality (Scheme 3). In addition, a second array of diverse heterocycles was investigated using this combinatorial method to further expand the number of heterocyclic classes that were compatible with this chemistry (Scheme 4).



Scheme 3. The library of pyridine substrates used to investigate the functional group compatibility of directed C–H activation.



Scheme 4. The library of diverse heterocycles used to investigate the substrate generality of directed C–H activation.

A quantitative mass spectrometric assay requires uniform ionization and negligible fragmentation of all analytes so that the yields can be determined by peak integration. As a control, a simple peptide label composed of four arginine residues and an N-terminal alkoxylamine ($H_2NOGlyArg_4$) was treated with an equimolar mixture of 13 purified pyridylketone products prepared individually in the C–H activation reaction. The resulting oximes were then analyzed by ESI^+-MS. Complete conversion into the oxime products was always observed as a result of the high efficiency of this labeling reaction. Notably, this labeling strategy allows a wide range of functional groups to be present in the starting heterocycle. The MS label ($H_2NOGlyArg_4$) was sufficient to dominate the ionization of all 13 oximes and provided a uniform response for all the substrates within experimental error ($< \pm 10\,\%$, Figure 1), which permits for the accurate determination of yields by the direct integration of peak areas relative to that of an external standard. Also, the high molecular weight of the labeled products (> 700 Da) ensures that ions of the product oximes are not obscured by ions from residual reactants and decomposed material.

In a typical timed run the pyridine mixture (1 mmol/substrate) was placed in a stainless steel bomb and allowed to react with $[Ru_3(CO)_{12}]$ (40 mol %) and neohexene (5 equiv) in toluene under CO (300 psi, 160 °C). A 40 mol % catalyst loading was chosen to ensure that the yield and relative rate data obtained were accurate and not biased by catalyst availability. The product mixture was then cooled, concentrated, dissolved in acetonitrile, and an external standard (2-pyridinecarboxalde-

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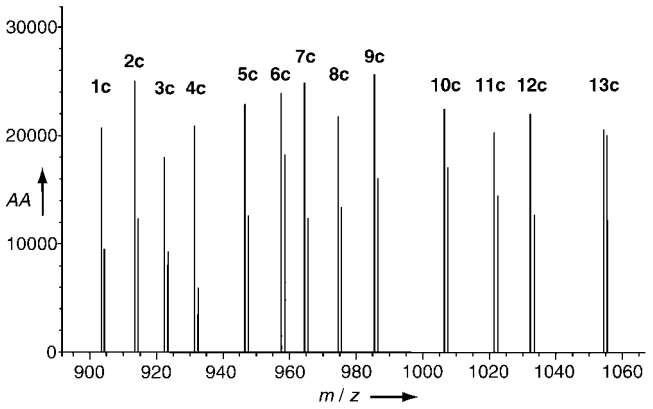

Figure 1. A control positive-ion ESI mass spectrum demonstrating that the mass spectrometric label (-GlyArg$_4$) ionizes the product oximes uniformly regardless of the functional group present (AA = absolute abundance of the ion).

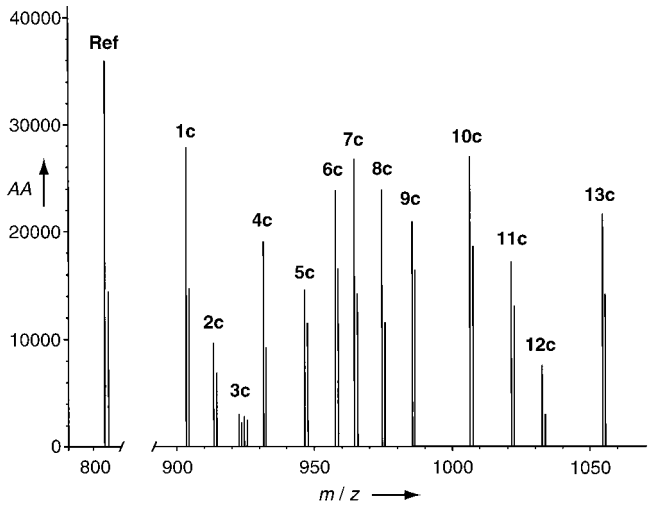

Figure 2. A typical positive-ion ESI mass spectrum obtained for the timed run (over 5 h) from the unpurified pyridine reaction mixture using 2-pyridinecarboxaldehyde as the reference (Ref).

hyde, 1 mmol) added that reacted efficiently with the MS label. With no purification required, a sample of the product mixture was treated with a fourfold excess of the MS label (H_2NOGlyArg$_4$) overnight to ensure complete conversion. This solution was diluted with 5% trifluoroacetic acid (TFA) in MeCN (30 μM/substrate) and analyzed by ESI$^+$-MS. The yields of the products relative to the standard were found to be independent of concentration.

Data from the library of 13 pyridine derivatives shows the scope of the functional group compatibility of [Ru$_3$(CO)$_{12}$]. Directed C–H bond activation occurs in good yield for pyridine substrates bearing nitriles, amides, alcohols, esters, biaryls, benzhydryls, and amines (Table 1). Surprisingly, aromatic chlorides **3a** react in good yield, which suggests that the acylation reaction may be combined with palladium-mediated chemistry[17] to enable more highly functionalized structures to be built rapidly. Furthermore, by executing a series of timed runs, the relative rates of product formation from all 13 pyridine substrates could be monitored simultaneously to provide optimized reaction conditions for each substrate (Figure 2). Trends in the rate data demonstrate that electron-withdrawing substituents slow the rate of reaction, while electron-donating groups increase the rate. The success of this high-throughput strategy for the challenging case of substrate mixtures elegantly demonstrates the deconvolution power of an analytical strategy based on mass spectrometry.

Table 1. Yields obtained by mass spectrometry for the C–H activation of the pyridine library.

Compound	Calcd m/z	Found m/z	Yields [%][a]			
			3 h[b]	5 h[b]	10 h[b]	20 h[b]
1c	903.59	903.50	65(±5)	**80(±6)**	54(±14)	32(±2)
2c	913.57	913.45	25(±3)	30(±5)	48(±3)	**63(±4)**
3c	922.54	922.43	26(±3)	27(±10)	54(±13)	**57(±7)**
4c	931.58	931.48	43(±1)	48(±6)	**56(±7)**	31(±3)
5c	946.62	946.56	48(±12)	**48(±5)**	46(±2)	9(±1)
6c	957.63	957.55	50(±6)	**68(±5)**	38(±3)	18(±2)
7c	964.61	964.50	71(±4)	83(±10)	**86(±11)**	66(±3)
8c	974.61	974.54	71(±6)	66(±8)	**84(±10)**	64(±5)
9c	985.66	985.61	45(±6)	**71(±12)**	54(±8)	26(±2)
10c	1006.65	1006.58	63(±5)	**79(±6)**	71(±5)	56(±1)
11c	1021.63	1021.55	42(±1)	56(±6)	**63(±6)**	55(±4)
12c	1032.62	1032.48	15(±1)	20(±3)	37(±8)	**43(±1)**
13c	1054.65	1054.58	57(±4)	**69(±5)**	66(±2)	50(±2)

[a] The reported yields are the mean values measured from at least three experiments. [b] Standard deviations are shown in parentheses.

A series of control experiments were performed to test the accuracy of the yields determined by MS and the effect of catalyst loading. Two products (**4b** and **7b**) were synthesized individually under conditions identical to those employed in the mixture and the yields determined by mass balance (57 and 95%, respectively). The yields for the individual runs are in excellent agreement with those measured by ESI$^+$-MS for the same compounds when run in the library format. Additionally, the yields determined by MS compare favorably with previous experiments in which lower catalyst loadings and extended reactions times were employed to prepare the 13 authentic pyridine standards.

To assess the generality of the reaction a mixture of 20 diverse heterocyclic substrates was investigated using the same C–H activation conditions as those employed for the pyridine library (Scheme 4). Labeling and ESI$^+$-MS analysis revealed a number of new substrate classes for activation (pyridazines, thiazoles, isoquinolines, and benzothiazoles) which proceeded in good yield and with rates of reaction comparable to that of pyridine (Table 2). Further investigation of the unreactive substrates at an elevated temperature (180 °C) did not increase product yields. Intriguingly, the two thiazole substrates **17a** and **23a** both present C–H bonds for activation; however reactivity at C2 (**17a**) is accelerated nearly sixfold over that at C4 (**23a**). The observed reactivity trend was independently confirmed by the C–H activation of unsubstituted thiazole, where the C2 and C4 activated products were produced in a 9:1 ratio. This additional level of selectivity could allow for the efficient elaboration of multicomponent structures through a series of controlled C–H activations where different olefin building blocks are introduced regioselectively without the need for blocking groups. After the completion of these studies, Murai and co-workers published an independent report on a subset of the

  0044-8249/01/11301-0224 $ 17.50+.50/0

Table 2. Yields determined by mass spectrometry for the C–H activation of the heterocyclic library.

Compound	Calcd *m/z*	Found *m/z*	Yields [%][a]			
			3 h[b]	6 h[b]	20 h[b]	40 h[b]
14c	877.57	877.56	3(±1)	3(±2)	2(±1)	5(±1)
15c	892.57	892.43	2(±1)	3(±1)	2(±1)	2(±1)
16c	903.59	903.51	**38(±6)**	16(±4)	13(±9)	7(±3)
17c	908.55	908.49	36(±5)	46(±3)	65(±4)	**91(±2)**
18c	917.60	917.55	4(±1)	5(±1)	4(±2)	7(±3)
19c	923.53	923.50	3(±1)	3(±1)	5(±2)	4(±4)
20c	928.57	928.63	2(±1)	2(±1)	2(±1)	4(±2)
21c	938.59	938.52	35(±4)	32(±5)	**49(±10)**	22(±4)
22c	944.55	944.54	42(±4)	59(±6)	**95(±5)**	78(±6)
23c	950.59	950.56	6(±1)	8(±2)	16(±1)	**32(±5)**
24c	953.60	953.51	4(±2)	3(±2)	5(±1)	7(±3)
25c	957.61	957.51	2(±1)	2(±1)	2(±2)	3(±1)
26c	962.53	962.35	3(±3)	4(±2)	3(±2)	4(±1)
27c	978.51	978.39	1(±1)	1(±2)	1(±1)	4(±1)
28c	984.58	984.55	2(±1)	2(±1)	2(±1)	7(±1)
29c	999.56	999.55	1(±1)	1(±1)	1(±1)	3(±2)
30c	1003.61	1003.59	2(±1)	2(±1)	3(±2)	3(±2)
31c	1008.58	1008.56	2(±1)	2(±2)	4(±1)	3(±1)
32c	1044.63	1044.65	1(±1)	1(±1)	4(±3)	3(±2)
33c	1060.61	1060.54	1(±1)	1(±1)	2(±1)	3(±2)

[a] The reported yields are the mean values measured from at least three experiments. [b] Standard deviations are shown in parentheses.

heterocycles described here, which completely supports our results for the small number of compounds investigated in both studies.[18]

In summary, a rapid method to evaluate large numbers of substrates was developed which defines structure–reactivity relationships and the reaction compatibility of functional groups. Additionally, no purification and only a structurally simple mass spectrometric label are required to directly determine the desired products and their yields. In three simple experiments 33 different substrates were submitted to C–H activation conditions and analyzed over time to provide more than 170 determinations of yield. This information identifies acceptable and unacceptable substrates, relative rates of product formation, and optimal reaction times for achieving high yields. While substrate mixtures were appropriate for these investigations, the method is applicable to spatially separate reactions, which may simply be sampled, pooled, and assayed as described. Notably, this approach can be directly applied to any reaction where a carbonyl group is introduced into the product. Furthermore, simple modification of the attachment functionality and linking group allows the mass spectrometric labeling strategy to be applied, in principle, to a broad range of transformations.[19]

Received: July 17, 2000
Revised: October 26, 2000 [Z15466]

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[19] A variety of linking groups could readily be introduced into the mass spectrometric label since the label is prepared by solid-phase synthesis with the linking group attached as the final step before cleavage from the solid support.

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