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Published on Web 05/17/2003

Fluorescence Resonance Energy Transfer (FRET) as a High-Throughput Assay for Coupling Reactions. Arylation of Amines as a Case Study

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Abstract: A solution-phase assay based on fluorescence resonance energy transfer (FRET) was developed for high-throughput screening of palladium catalyzed aminations of aryl halides. Dansylpiperazine was used as the fluorescent component and a chloro- or bromoarene tagged with an azodye as the quenching partner. Fluorescence intensities of reaction aliquots correlated linearly with reaction yield after dilution to appropriate concentrations. A library of 119 phosphine and heterocyclic carbene ligands was evaluated in duplicate reactions of two combinations. In general, the FRET assay displayed excellent reproducibility, with less than 5% of the duplicate experiments showing significant variability in yields. Among reactions producing greater than 50% yield, the average percent uncertainty was just 5%. For a small subset of sterically hindered ligands, differences in yields between 10 and 20% were observed between the substrates bearing dyes for the FRET assay and substrates that are unfunctionalized. However, the remaining catalyst combinations gave yields similar to those expected from literature precedent. In addition to an evaluation of the accuracy of the FRET assay, this work includes the use of the FRET assay to investigate relative activities of various catalysts for the amination of aryl bromides and chlorides and to find conditions for aminations in more polar solvents. Reactions with K_3PO_4 base in aqueous mixtures of polar and nonpolar organic solvents were shown to be appropriate for the amination chemistry.

Introduction

The challenge of pinpointing the optimal catalysts for a particular reaction or generating an initial catalyst structure for studies of new processes has created the desire for methods to screen catalyst activity more rapidly. Several approaches to address this issue have emerged, and they provide the potential to accelerate the discovery of new reactions. For example, HPLC,[1,2] mass spectrometry,[3−6] colorimetric assays,[7,8] IR thermography,[9−11] capillary electrophoresis,[12,13] and fluorescence[14−16] have all been used in a high-throughput fashion to analyze catalyst activity. Each method has its attributes, but none of them applies to all problems. Each is slower than desired,[1,2] equipment intensive,[3−6,12,13] or narrow in the scope of reaction that can be analyzed.[7,8] Some analyze overall activity and do not probe directly the formation of a desired material.[9−11] We envisioned a rapid and general assay to screen for product formation that would use inexpensive, currently available equipment and would be highly sensitive, noninvasive, and time resolved.

To address this goal, we have developed a method to monitor product formation by the phenomenon of fluorescence resonance energy transfer (FRET).[17,18] We have applied this method to the analysis of three cross-coupling processes. The applications of this method to two of these processes have recently been communicated.[19,20] In one case, we uncovered conditions for the first general arylation of cyanoacetates. In the second, we uncovered conditions for the first Heck coupling of unactivated bromoarenes at room temperature.[21] We now describe the scope

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10.1021/ja034161p CCC: $25.00

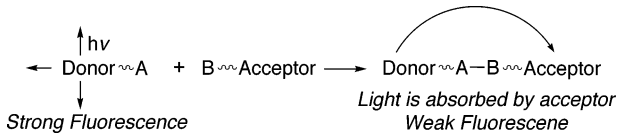

Figure 1. General approach to using FRET for analyzing the formation of covalent bonds.

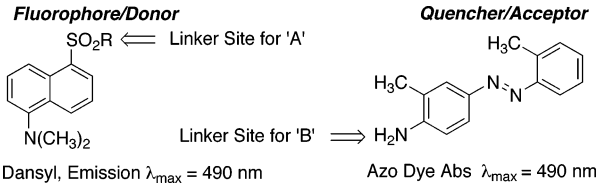

Figure 2. FRET pair chosen for derivatization with substrates for the amination chemistry.

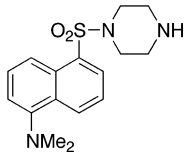

Figure 3. Amine substrate **3** used in the amination studies.

and limitations of this FRET-based assay for the palladium-catalyzed amination of aryl halides. A large number of ligands have already been screened for this reaction, and these previous results provide a framework for evaluating the accuracy of reaction yields obtained by the FRET method.

In addition to evaluating the accuracy of the FRET assay by studying the amination of aryl halides, we hoped to reveal whether the order of reactivity of catalysts containing different ligands depended on the identity of the halogen in the aryl halide and whether high yields could be obtained with bases weaker than NaO*t*-Bu in media more polar than arenes and ethers that are most commonly used for the amination process. The turnover-limiting step and reactions that deactivate the catalyst can depend on the halide and create different trends in catalyst reactivity for aryl chlorides, bromides, and iodides.[22,23] The use of weaker bases in more polar media would increase the scope of the amination, particulary with pharmacuetical intermediates that are often insoluble in aromatic and ether solvents.

General Strategy. To develop an assay that met the various criteria outlined in the previous section, we built upon a qualitative fluorescent method we published previously.[14] The previous method involved reactions of one reagent supported on polystyrene beads with a second reagent that was conveniently tagged with a fluorophore. This method provided a qualitative, rather than quantitative, assessment of catalytic activity and required isolation and washing of the resin-bound product. The modification of this method to generate a quantitative assay could permit the determination of kinetic parameters for multiple substrates in a parallel fashion. To develop such a quantitative assay, we changed the reaction partner from a reagent on a solid support to a reagent that would quench fluorescence by FRET.

FRET has been used over the past four decades as a versatile method for measuring noncovalent binding events in biological and macromolecular systems. FRET takes place when the fluorescence emission band of one molecule (donor) overlaps with an excitation band of a second (acceptor) that is within 20−80 Å of the donor.[17,18] At an appropriate constant total concentration of free and associated FRET pairs, the emission of the FRET donor is inversely proportional to the mole fraction of associated molecules.

We used FRET as a quantitative probe for the formation of a desired covalent bond in a reaction product by the scenario in Figure 1. The coupling of a reactant A that is tethered to a donor fluorophore and a reactant B that is tethered to an acceptor dye or quencher could alter the fluorescence properties of the solution. If there is sufficient spectral overlap between the fluorophore and acceptor and a short enough distance between them, then energy transfer will take place. In this case, the fluorescence from A is quenched by the acceptor B. In addition, fluorescence from B is generated by this energy transfer if the acceptor attached to B is emissive.

The relative emission intensity can then be converted to reaction yield by the negative linear relationship between emission intensity and mole fraction of coupled product within an appropriate range of concentrations. An inexpensive, commercial fluorescence plate reader would require only 1 s per sample to measure the fluorescence intensities. The FRET assay could be miniaturized to evaluate sub-micromolar quantities of substrate per well and even less catalyst.

Design of Chromophoric Substrates. The two chromophores chosen for the FRET assay are shown in Figure 2. The dansyl group was selected as the fluorophore. It is inexpensive and possesses functional groups that are inert toward most types of coupling reactions. This chromophore can be attached to different reactants through the sulfonyl group and has a large Stokes' shift (~130 nm). An azodye was chosen as the acceptor molecule because it has an absorption band that overlaps with the fluorescence emission maximum of the dansyl group. The particular azodye selected for this study contains an ortho substituent to discourage coordination of the azo-nitrogens and an amino functionality to tether reactant B. A large number of nucleophiles and electrophiles could be attached to this FRET pair for screening of a variety of reactions.

Results and Discussion

Selection and Synthesis of the Chromophores. A method to analyze palladium-catalyzed arylation of amines with FRET requires a dye and quencher that are stable to the basic conditions of the amination process. The dansyl sulfonyl functionality was attached to a piperazine to form dansyl derivative **3** in Figure 3. Piperazines participate in aminations of aryl halides,[24−27] and the sulfonamide should be stable to the *tert*-butoxide base in organic solvents.[28]

Several methods for tethering aryl halides to azodye quenchers were explored (Scheme 1, **4**−**7**). Scheme 1 shows the four modified dyes examined for the arylation of **3** at 80 °C with

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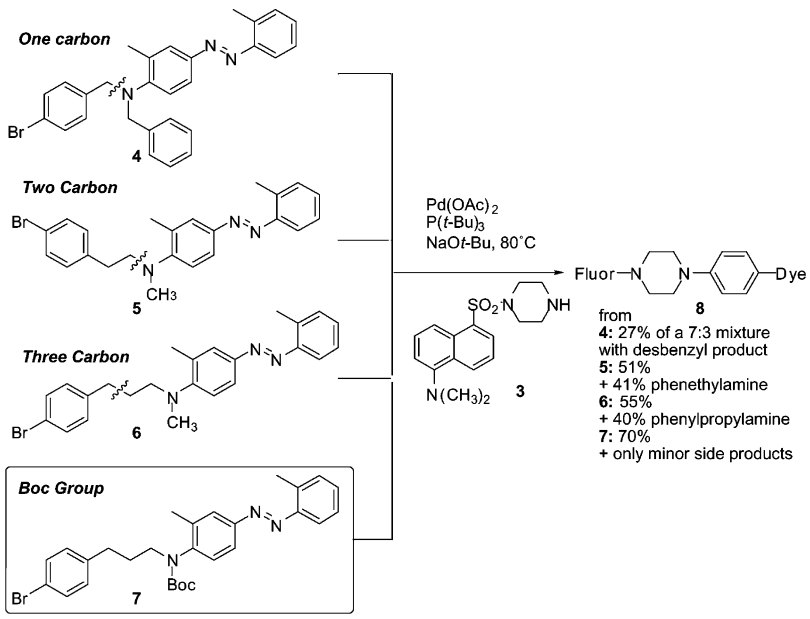
Scheme 1



NaO*t*-Bu as base and the combination of 3 mol % $Pd(OAc)_2$ and 2.8 mol % $(t\text{-Bu})_3P$ as catalyst.[29]

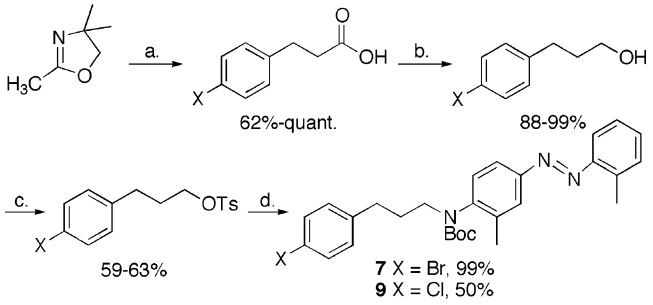
Modified dyes **4**−**6** underwent partial to complete cleavage of a carbon−carbon bond of the tether in competition with the formation of coupled product. Substrate **4** underwent debenzylation, and substrates **5** and **6** underwent C−C cleavage to generate an *N*-methyl arylamine and an *N*-ethyl, *N*-methyl arylamine, respectively. Isolated yields for the coupling process were roughly 50%, and isolated yields of the fragmented dye were in the range of 40%. No products from side reactions of the dansylamine were observed.

The mechanism for these fragmentations is unclear. The products from amination of **5** and **6** were subjected to the reaction conditions after they were isolated in pure form, and no cleavage products were detected. Thus, the cleavage occurs with the starting material or a reaction intermediate and not with coupled product.

In contrast, Boc-substituted, propyl-linked **7** produced the coupled product **8** without side reactions. This substrate formed the product of aryl halide amination in 70% isolated yield under the previous conditions. Thus, we used this substrate and its chloride analogue for all further studies. Substrate **7** was prepared by a straightforward sequence starting from 2,4,4-trimethyl-2-oxazoline and an appropriate benzyl halide, as shown in Scheme 2. By this reaction sequence, we produced multiple grams of the bromo- and chloroarene substrates **7** and **9** in five steps.

Evaluation of Changes in Emission Intensity upon Coupling. To generate a calibration curve for determining reaction yields, we obtained the data summarized in Figure 4. Solutions containing various ratios of coupled product **8** and starting materials **3** and **7** (1:1 ratio) were prepared, and the fluorescence intensities of the solutions were obtained with a fluorescence plate reader. Correlation coefficients for such plots were typically 0.99. These plots were used to determine percent yield from the fluorescence intensity of aliquots removed from the amination reactions (see Supporting Information for further details).

Scheme 2 [a]



[a] Reagents and conditions: (a) (i) *n*-BuLi, −75 °C; (ii) $4\text{-}ClC_6H_4CH_2Cl$ or $4\text{-}BrC_6H_4CH_2Br$; (iii) HCl; (b) $BH_3{-}SMe_2$; (c) TsCl, Et_3N; (d) (i) LiNHAr (Ar = azodye); (ii) NaHMDS, Boc_2O.

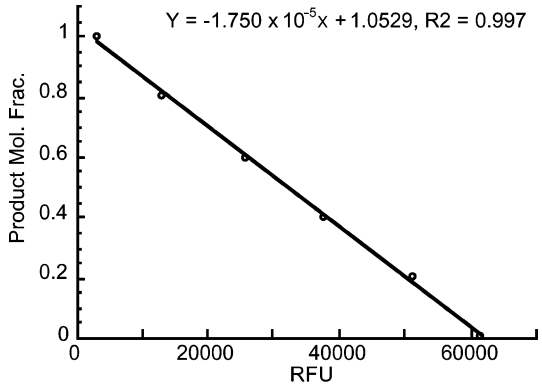


Figure 4. Mole fraction of product versus fluorescence intensity ($\lambda_{excitation}/\lambda_{emmision}$ = 360 nm/460 nm) for solutions of product **8**, fluorophore **3**, and quencher **7** at a total concentration of 10^{-5} M containing 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 mol fraction of **8**.

Library of Dative Ligands. Concurrent with the development of the FRET assay, we expanded our library of ancillary ligands. We assembled an array of pure ligands that contained representatives from families that spanned a broad spectrum of both steric and electronic properties. New structures were based on

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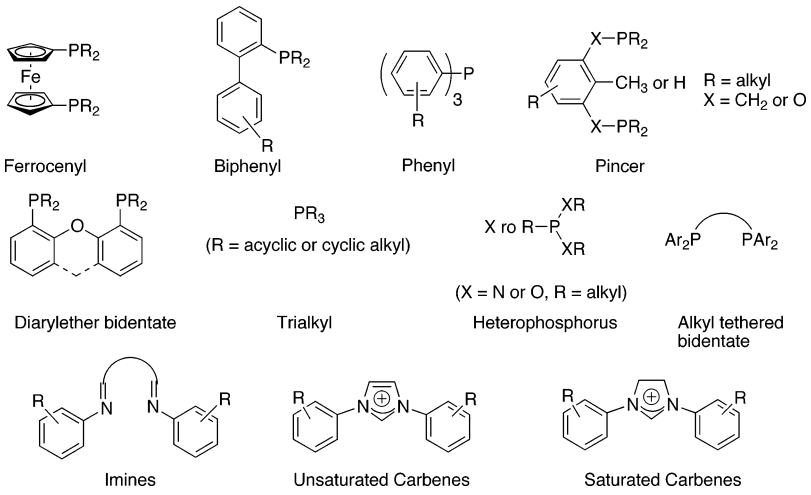

Figure 5. General structural classes contained in the ligand library.

recent advances in ligand design.[29−34] Some of the more prevalent families of ligands in the library are sumarized in Figure 5. The structures of each member of the 119-member library are provided as Supporting Information. One-third of the ligands were obtained from commercial sources, one-third were prepared according to literature procedures, and the remaining 39 were new structures.[19,20] Of the 39 new ligands, classes that could be prepared by a divergent synthesis were explored most widely. These classes included ferrocenyl, biphenyl, and alkyl phosphines.

Conditions and Reproducibility of Screening the Reactions of Dansylpiperazine with Bromo- and Chloroarene Dyes 7 and 9. Yields of reactions of the tagged substrates determined by FRET were compared to yields of reactions of related untagged substrates determined by conventional methods. Reactions of the tagged substrates were conducted initially with 12 different ligands in 0.5 dram screw-top vials. The reaction vessels contained 15 μmol of amine **3** and bromide **7**, 3 mol % ligand and 3 mol % $Pd(OAc)_2$, and 1.5 equiv of NaO*t*-Bu in approximately 100 μL (0.14 M) of solvent. Reactions were assembled in a drybox, and all reagents were added by pipet from stock solutions. The vials were placed in an aluminum block, heated at 60 °C, and agitated with a STEM shaker.

After 9 h, an aliquot was removed from each vial and diluted to 10^{-5} M with *m*-xylene. The fluorescence intensities of the samples were measured and converted to percent yield with a curve, such as that shown in Figure 4. The standard deviation in yield determined by fluoresence ranged from 1 to 8% ($n \geq 3$) for the 12 model reactions conducted three or more times. In general, reactions with catalysts that produced coupled product in less than 20% yield displayed the greatest uncertainty, while reactions with catalysts that produced coupled product in high yields displayed good reproducibility.[35]

The trends in reaction yields were consistent with the relative activity of these catalysts for reactions of secondary amines with aryl bromides observed during previous studies. For example, reactions with catalysts bearing Tol-BINAP or bis(di-*tert*-butylphosphino)ferrocene were found to generate coupled product after 9 h at 60 °C in greater than 70% yield, as determined by FRET, with a standard deviation less than 5%. These data are consistent with literature data obtained by conventional methods for reactions with these pairs of ligands and substrates.[29,36] Moreover, reactions catalyzed by complexes of ligands, such as tri(*o*-tolyl)phosphine and dppe that are known to generate catalysts with low activity at room temperature,[37,38] gave yields less than 20%, as determined by FRET.

The reactions were then conducted in a Zinsser 96-well glass plate. A plate with 45° corners and 300 μL wells sandwiched between two aluminum plates (see Experimental Section for details) was used to conduct reactions with 50 μL volumes. With the sensitive fluorescence detection method and appropriate reaction blocks, the reactions could be further reduced in volume, and reagents could be added with standard robotic liquid dispensors.

With this methodology, the 119-member library of ligands was evaluated with the bromide **7** and chloride **9** under a variety of reaction conditions. For the broader screening in 96-well plates, CpPd(allyl) was used instead of $Pd(OAc)_2$ as catalyst precursor because of its enhanced solubility. Depending on the batch, $Pd(OAc)_2$ precipitated from dioxane within 15−30 min at room temperature; however, $Pd(OAc)_2$ was utilized with certain ligands (vide infra). Palladium(0) precursors, such as $Pd_n(dba)_m$ ($n = 1, 2$; $m = 2, 3$), were not soluble enough to generate stock solutions that would allow for a final substrate concentration above 0.1 M. To minimize variability in catalyst incubation time, the ligand stocks were added first, and the palladium source was added just before addition of the base. Stock solutions of CpPd(allyl), fluorophore, and NaO*t*-Bu were stored no longer than 1 day at −30 °C in a drybox. Stock solutions of the ligand library and reactants **7** and **9** were stored for several weeks at −30 °C in a drybox.

CpPd(allyl) is a soluble catalyst precursor that is readily reduced to common Pd(0) complexes.[39,40] Free phosphine

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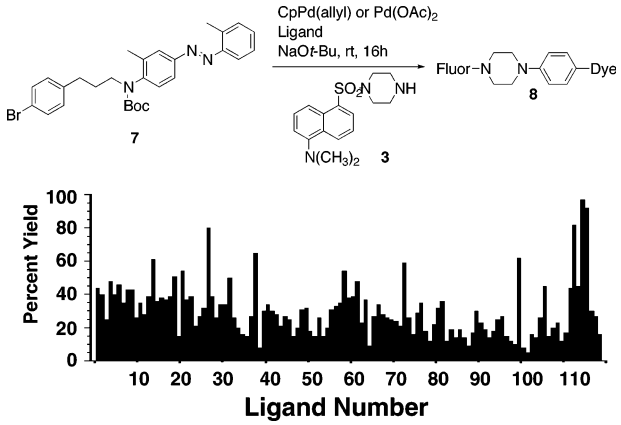

Figure 6. Yields determined by fluorescence measurements for the coupling of **3** with **7** in the presence of palladium catalysts containing 119 different ligands.

ligands induce reduction of CpPd(allyl) to Pd(0) phosphine complexes.[40] However, reactions of imidazolium and dihydroimidazolium salts with CpPd(allyl) may form the Pd(0) complexes slowly or in low yield because the ligand was added in its protonated form and base was added after the palladium. Indeed, reactions catalyzed by imidazolium and dihydroimidazolium salts in combination with (allyl)PdCp occurred in yields below 30%, but reactions catalyzed by these ligand precursors and $Pd(OAc)_2$ occurred in yields over 80%. Thus, we tested in parallel both CpPd(allyl) and $Pd(OAc)_2$ as precursors for reactions with the imidazolium and dihydroimidazolium salts.[41,42]

Figure 6 depicts the average percent yields deduced from fluorescence intensity measurements for duplicate reactions with each member of the ligand library. All reactions were conducted for 16 h at room temperature with fluorophore **3** and bromoarene **7** (see Supporting Information for complete details, including statistical variation). Results from duplicate runs indicated excellent reproducibility of the yields obtained by the fluorescence method from reactions that occurred in moderate to high yields. In the set of 119 duplicate reactions, only 16 reactions showed a percent uncertainty[43] greater than 30, and of these 16 reactions, only three (***L15***, ***L28***, and ***L66***) occurred in greater than 40% yield in either run. Thus, only 3 of the 119 reactions showed significant variation in cases that could have synthetic value. Moreover, analysis of a second aliquot removed from the second run of these three reactions indicated the formation of coupled product in yields that were within 30% of those obtained from the first run of these three reactions. Thus, an error in dilution of the first aliquot taken from the second run with these three ligands accounts for the larger variation in yield. This type of error could be recognized in all cases, as long as the experiment is conducted in duplicate.

Trends from Reaction of 3 with 7. Figure 7 shows the structures of the ligands that consistently generated catalysts that formed coupled product in yields greater than 50% from the test substrates. With the exception of ***L100***, the phosphine ligands in catalysts that produced a higher than 50% yield contained one or two *tert*-butyl groups. These results are in accord with studies conducted over the past few years that have generated highly active catalysts for cross-coupling processes.[21,23,25,33,44−49] These studies have shown that reaction rates for aryl halide aminations catalyzed by complexes of sterically hindered alkyl phosphines are much faster than those catalyzed by complexes of arylphosphines. The most effective phosphines for reactions of the two dyes at room temperature contained a ferrocenyl (***L4***-***L21***), a biphenyl (***L27***-***L38***), or a third alkyl (***L69***-***L76***) group in addition to one or two *tert*-butyl groups.

The most effective imidazolium or dihydroimidazolium ligand precursors contained hindered 2,6-diisopropylphenyl groups on nitrogen. These results were also consistent with the high activity of sterically hindered ligands. These results led us to test the dihydroimidazolium salts for a variety of aryl halide amination reactions. The scope of room-temperature aminations catalyzed by palladium and this ligand precursor was published recently.[41] Nolan has also investigated the activity of this ligand for aminations at elevated temperatures,[50] for related arylations of carbonyl compounds,[51] and for more classic couplings.[52−54]

Although the differences in activities were small between derivatives of the pentaphenylferrocenyl phosphines (Q-phos)[55] bearing methoxy, hydrogen, and trifluoromethyl groups at the *para*-position of the aryl rings, some trends could be assigned. The Q-phos derivative bearing electron-withdrawing trifluoromethyl groups (***L19*** in Figure 7) appeared to generate catalysts that were more active toward bromoarenes than those lacking this group or bearing electron-donating groups. Consistent with this result from the FRET experiments, reactions of 0.5 mmol bromotoluene with morpholine at room temperature catalyzed by 3% $Pd(dba)_2$ and 3% ***L19*** occurred 2−3 times faster and to higher conversions after 48 h than the same reaction catalyzed by 3% $Pd(dba)_2$ and 3% parent Q-phos ligand ***L17***.

The catalyst generated from Xantphos ***L100***, first reported by van Leeuwen,[56] was the only one that lacked alkyl groups and formed over 50% yield of coupled product after 16 h at room temperature. In contrast, ligand ***L64***, the bis(di-*tert*-butyl) analogue of ***L100***, was only moderately effective for the test reaction (37 ± 9% yield). The more commonly used catalysts containing BINAP[36] and DPPF[57] showed little or no activity for reactions of aryl bromides at room temperature.[58]

Two false positives emerged from this screen. Catalysts derived from ligands ***L59*** and ***L106*** gave yields that were

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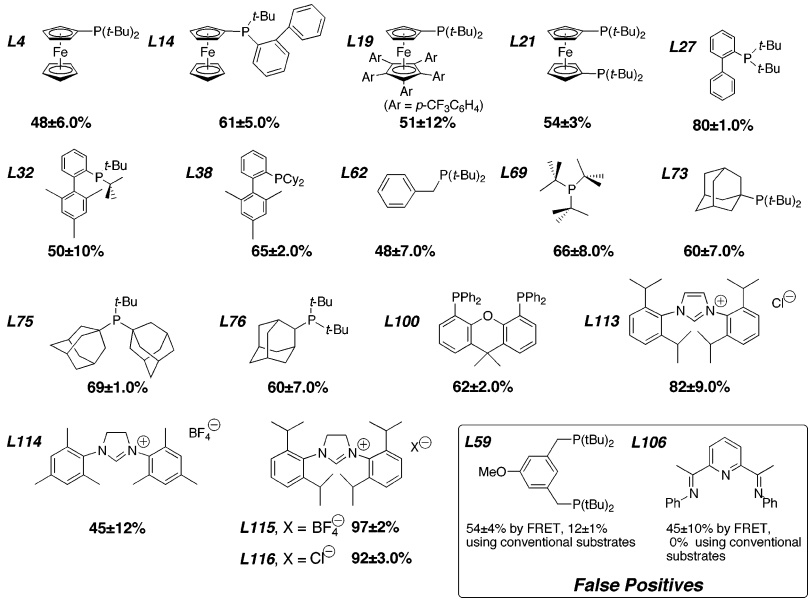

Figure 7. Ligands that gave >50% yield for the coupling of **3** with **7**.

reproducibly higher than 50%, as determined by the fluorescence measurement. The structures of these two ligands were much different from others shown to be active by a combination of this assay and more conventional methods.[46,59−64] Thus, we tested the activity of ***L59*** and ***L106*** for the related reaction of morpholine with bromotoluene. The reaction conducted with ***L59*** gave 12% yield, and the reaction conducted with ***L106*** gave no product after 16 h at room temperature. We do not have a firm explanation for the high yields determined by fluorescence with these ligands, but reactions conducted with bisimine ligand ***L106*** were black in color. This ligand is poised for aldol chemistry, which could generate unsaturated polymers that would either absorb the incident light or quench the free amine fluorescence.

Reaction of Dansylpiperazine 3 with Choroarene Dye 9. We also tested the coupling of amine **3** with chloroarene **9** at 70 °C with catalysts derived from the library of ligands. Again, a 96-well plate was loaded sequentially with ligand (3.0 mol % for monodentate and 1.5 mol % for bidentate ligands), fluorophore (**3**, 7.5 μmol), chlorodye **9** (7.5 μmol), NaO*t*-Bu, and CpPd(allyl) or $Pd(OAc)_2$ (3 mol %). The reactions were conducted in duplicate at 70 °C for 12 h with ligands ***L1***−***L119***. An aliquot was removed from each well after 12 h of reaction, and the aliquot was diluted to 10^{-5} M. The percent yield of product was determined as described previously. Figure 8 shows the average percent yields for the aminations of chloroarene **9** with catalysts bearing the 119 ligands.

The variations in yields by fluorescence for the two runs with chloroarene **9** were similar to the variations with bromoarene **7**. Only a few reactions showed significant variability in yields for the two experiments, and these reactions occurred in less

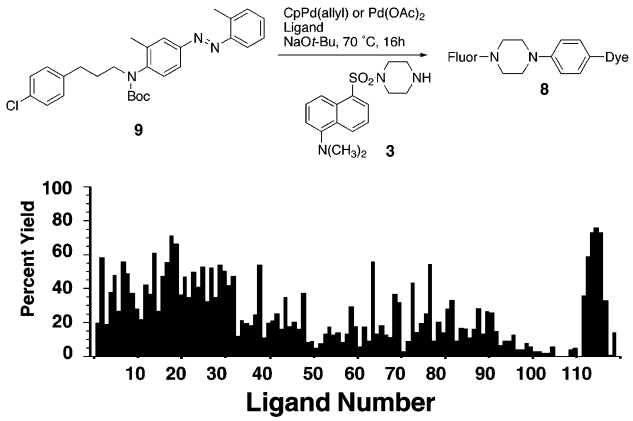

Figure 8. Yields determined by fluorescence measurements for the coupling of **3** with **9** in the presence of palladium catalysts containing 119 different ligands.

than 20% yield. All reactions that occurred in yields by fluorescence greater than 30% showed a percent uncertainty less than 30. The 23 reactions that occurred in yields greater than 50% in one or two of the runs showed a percent uncertainty between 1 and 12%. The average uncertainty of the yields from these reactions was just 5%.

Trends from Reactions of 3 with 9. The structures of the 23 ligands that generated product in greater than 50% yield are shown in Figure 9. The carbene ligands provided the most active systems for the coupling of aryl chlorides under the conditions of the screen. The pentaphenylferrocenyl ligands (Q-phos) ***L17***−***L19*** were the next most active. Some trends could again be assigned for the relative activities of the heterocyclic carbene ligands. Catalysts generated from the dihydroimidazolium salts ***L115*** and ***L116*** were more active for reactions of aryl chlorides than were those generated from the imidazolium salts. This trend was confirmed by subsequent studies in our labs[41] and has been published recently by Nolan.[50] In contrast to reactions of bromoarenes at room temperature catalyzed by complexes of the three Q-phos derivatives, FRET results indicated that the three Q-phos derivatives ***L17***, ***L18***, and ***L19*** generated catalysts with nearly equal activity toward chloroarene **9**.

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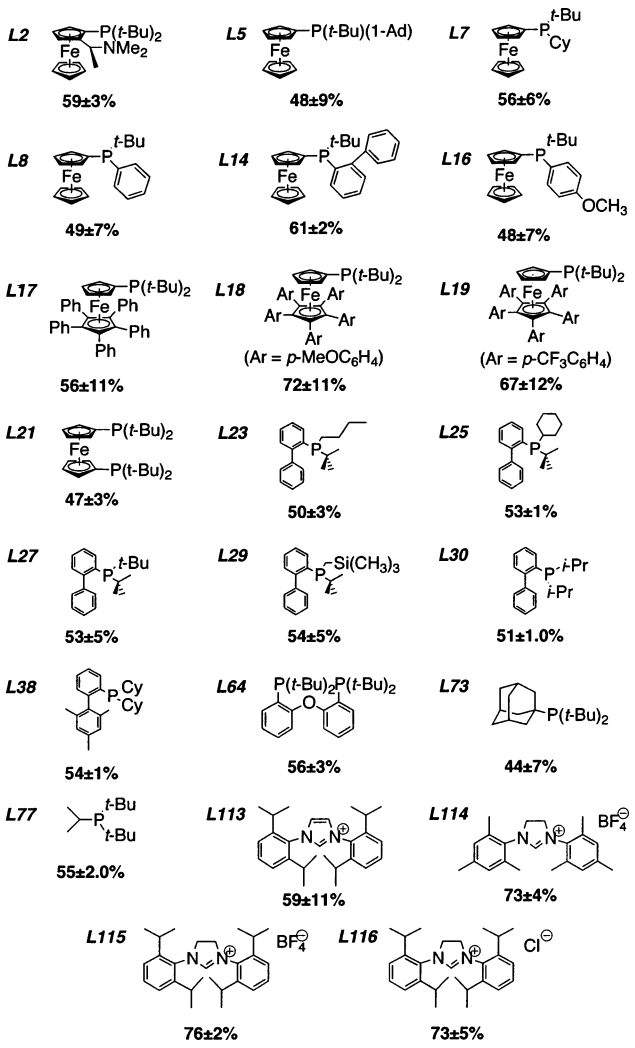


Figure 9. Ligands that gave >50% yield for reaction of **3** with **9**.

Comparison of Reactions of 3 and 7 with Reaction of 3 with 9. Catalysts derived from ligands in the library coupled bromo- and chloroarenes in different yields. The results in Table 1 indicate that some ligands created catalysts that formed coupled product from the chloroarene in *higher* yields than from the bromoarene. For example, carbene ligand precursor ***L114***, which generated catalysts with only modest activity toward the reaction of bromoarene **7** at room temperature (45%), showed good activity toward the reaction of chlorodye **9** at 70 °C. In addition, reactions conducted with ligands ***L29*** and ***L64*** both occurred in slightly higher yields with chloroarene **9** than with bromoarene **7**. These higher yields for the reaction of the chloride **9** in the presence of these catalysts is likely a result of the higher temperatures at which the reactions of chloroarenes were conducted. If catalysts formed from ***L29*** and ***L64*** react cleanly with both aryl bromides and chlorides, but reactions with these substrates occur at similar rates and require elevated temperatures, then yields from the reaction of chloride **9** would be higher than yields from the reaction of bromide **7**.

Table 1 also reveals the expected finding that several ligands, such as ***L27***, ***L38***, and ***L100***, are more active for the amination of aryl bromides than for the amination of chlorides. The difference in activity of catalysts generated from Xantphos ***L100*** for reactions of aryl bromides and chlorides underscores the low activity of palladium complexes of aryl phosphines as catalysts for the reactions of aryl chlorides and the accuracy of the FRET method when screening a library of ligands.

Table 1. Comparison of FRET Data for the Amination of Bromo and Chloroarene Dyes with the 30 Ligands that Generated the Most Active Catalysts for Reactions of **3** with **7** and **9**[a]

Ligand	Yield with Br-dye **7**	Yield with Cl-dye **9**	Ligand	Yield with Br-dye **7**	Yield with Cl-dye **9**
L2	40±4	**59±3**	***L30***	34±4	**51±1**
L4	**48±6**	38±8	***L32***	**50±0**	48±1
L5	40±5	**48±9**	***L38***	**65±2**	**54±1**
L7	35±3	**56±6**	***L62***	**48±7**	18±4
L8	43±5	**49±7**	***L64***	37±9	**56±3**
L14	**61±5**	**61±2**	***L69***	**66±8**	37±5
L16	38±7	**48±7**	***L73***	**59±6**	**44±6**
L17	37±1	**56±1**	***L75***	**69±1**	19±2
L18	39±7	**72±1**	***L76***	**58±6**	26±14
L19	**51±2**	**67±2**	***L77***	35±8	**55±2**
L21	**54±3**	**47±3**	***L100***	**62±2**	6±7
L23	39±1	**50±3**	***L113***	**82±9**	**59±1**
L25	27±0	**53±1**	***L114***	**45±12**	**73±4**
L27	**80±1**	**53±5**	***L115***	**97±2**	**76±2**
L29	26±4	**54±5**	***L116***	**92±3**	**73±5**

[a] Ligands in bold indicate those generating above 50% yield. Ligands in red were used in the screen for reactions in polar solvents with K_3PO_4 as base (see text).

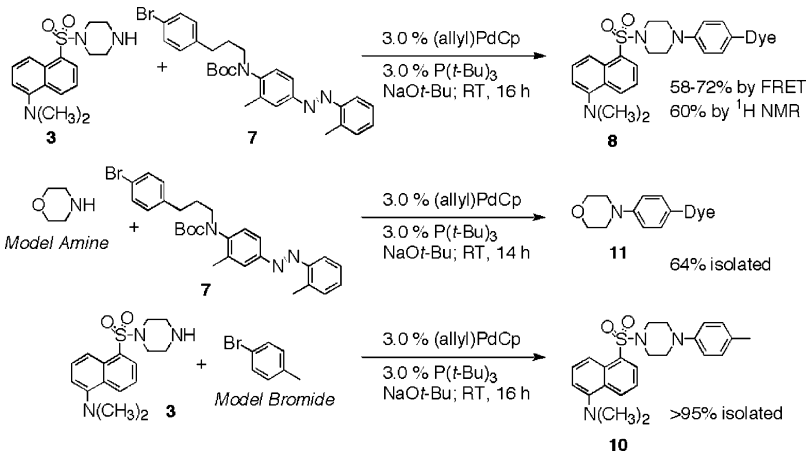
Investigation of Potential False Negatives. Reactions of the bromo- and chlorodyes with catalysts containing certain ligands, particularly ***L4***, ***L21***, ***L27***, and ***L69***, occurred in yields by the fluorescence analysis that were lower than those reported previously for reactions of related unlabeled substrates. Catalysts containing these four ligands are known to couple electron neutral aryl chlorides and bromides with cyclic secondary amines in yields greater than 80%.[29,65] To confirm that these ligands provide high yields for related substrates under the conditions of the FRET assay, we evaluated the reaction of bromotoluene with morpholine in the presence of (allyl)PdCp and (*t*-Bu)$_3$P (***L69***). As expected, *N*-tolylmorpholine was formed in 98% yield by GC yield after 16 h.

To probe the origin of these lower yields in the FRET assay, we evaluated whether the method for assembly of reactions, the method of analysis, or the type of substrate created the moderate yields. Initially, we tested whether an inaccuracy in the Pd/L ratio from assembly of small-scale reactions was contributing to the low yields;[29,66] however, experiments with 2.8 or 3.0 mol % ligand and 3.0 mol % (allyl)PdCp added carefully from stock solutions generated no significant difference in the yields of coupled product formed from dyes **3** and **7** in the presence of (allyl)PdCp and any of the four ligands. In addition, increasing the amount of catalyst to 6 mol % (allyl)-PdCp and P(*t*-Bu)$_3$ and allowing the reaction to occur over several days did not increase the yield, as determined by FRET.

Thus, we conducted the three reactions in Scheme 3. First, we determined by ^{1}H NMR spectroscopy the yield for the coupling of **3** with **7** in the presence of 3 mol % CpPd(allyl) and P(*t*-Bu)$_3$ in C_6D_6 solvent. This reaction produced the coupled product in 60% yield, and this yield is consistent with those determined by FRET. To evaluate whether halide **7** or amine **3** caused the yields to be lower than those observed with simpler

(65) Wolfe, J. P.; Buchwald, S. L. *Angew. Chem., Int. Ed.* **1999**, *38*, 2413−2416.
(66) The difficulty in controlling the metal-to-ligand ratio when handling small quantities of solids was noted previously in ref 1.

Scheme 3



substrates, the reaction of morpholine with bromodye **7** in C_6D_6 for 14 h was conducted. This reaction occurred in 64% isolated yield after chromatography (Scheme 3, middle). In contrast, the reaction of fluorescent amine **3** and bromotoluene (Scheme 3, bottom) formed the *N*-tolylamine in 95% isolated yield. Thus, the yield of coupled product from the reaction of **3** with **7** determined by fluorescence was accurate, and the reaction of the haloarene tethered to the azodye quencher catalyzed by some of the metal ligand combinations occurred in lower yields than reactions of less functionalized aryl halides.

During previous studies, yields from reactions of acrylates (Heck couplings)[20] and cyanoesters[19] with the haloarene azodyes catalyzed by palladium complexes of P(*t*-Bu)$_3$ were similar to those with less functionalized haloarene substrates. These previous results contrast with results in the current study with some of the catalysts. The difference in the strength of the base between the previous studies and the current work may account for these contrasting results. Phosphate and amine bases were used in the previous studies, while NaO*t*-Bu base was used in the current work.

Reactions of Amine 3 with Bromoarene 7 in Polar Solvents with K_3PO_4 as Base. A final set of reactions was performed at 80 °C with bromodye **7**, fluorophore **3**, and the milder K_3PO_4 base for three reasons: to evaluate if the strong base led to the lower yields with the azodye, to develop conditions for aminations in more polar solvents, and to demonstrate the utility of screening to find a new medium for a reaction. This base was delivered as an aqueous solution, and this aqueous solution

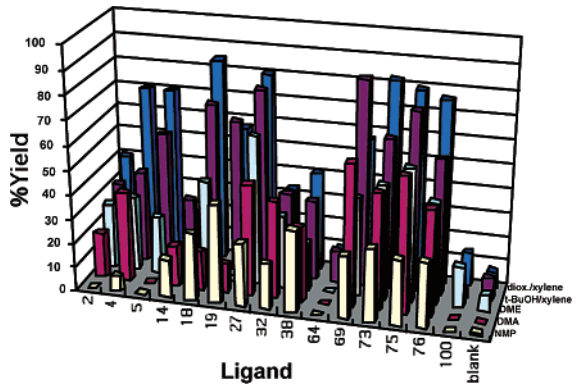


Figure 10. Yields determined by FRET for the reaction of **3** with **7** at 80 °C for 16 h in the presence of K_3PO_4 as base.

was approximately 17% of the total volume of the reactions. Six different polar solvents and solvent mixtures were tested, and CpPd(allyl) was used as the source of palladium. Fifteen ligands that generated the most active catalysts in the coupling of the bromo- and chlorodye were chosen to screen for activity in the presence of K_3PO_4 as base.

The results from this experiment are shown in Figure 10. A mixture of dioxane and *m*-xylene with K_3PO_4 as base was included to compare results from reactions with this base to those in a similar medium with NaO*t*-Bu as base described earlier. In addition, DME, NMP, DMSO, DMA, and a mixture of *t*-BuOH and *m*-xylene were tested as solvent. Reactions in DMSO formed an intractable tar, and the results from these reactions are not shown. Figure 10 shows that reactions catalyzed by palladium and certain ligands occurred in high yield in the presence of K_3PO_4 when conducted in the appropriate solvent mixtures. Three of these ligands, ***L4***, ***L27***, and ***L69***, generated complexes that catalyzed reactions of the azodye in mixtues of dioxane and xylene in much higher yield than when NaO-*t*-Bu was used as base in this medium. These higher yields from reactions conducted with K_3PO_4 agree with our conclusion that the yields for reactions with the azodye quencher and NaO-*t*-Bu as base differ from yields with other aryl bromides and from yields obtained with the FRET assay during previous studies with phosphate or amine as base[19,20] because of the difference in strength of the base.

None of the ligands gave high yields in the most polar solvents. However, several ligands gave high yields in mixtures of xylene and *tert*-butyl alcohol. Reactions catalyzed by complexes of 8 of the 15 ligands (***L4***, ***L5***, ***L18***, ***L27***, ***L69***, ***L73***, ***L75***, and ***L76***) gave yields over 70% in this medium, and ferrocenyl ligands ***L14*** and ***L19*** and trialkylphosphines ***L69*** and ***L73*** gave yields in the range of 80%. Thus, reactions in mixtures of polar and nonpolar organic solvents can be appropriate for the amination chemistry.[67]

Summary

Overall, this work shows that FRET can be used to screen homogeneous catalysts for the amination of aryl halides and,

(67) The ability to conduct reactions with a two-phase mixture of toluene and water with hydroxide base in the presence of a phase-transfer agent was demonstrated recently but not with polar substrates that are insoluble in the nonpolar toluene: Kuwano, R.; Utsunomiya, M.; Hartwig, J. F. *J. Org. Chem.* **2002**, *67*, 6479−6486.

by analogy, to screen catalysts for other processes. The study reported here evaluated catalysts derived from a library of phosphine and heterocyclic carbene ligands for two substrate combinations in several media. The reaction yields obtained by the FRET method were reproducible and agreed in most cases with yields obtained by conventional methods. Some differences in yields were observed between the dye substrates and unfunctionalized substrates, but only with a small subset of the ligands. As a test of the FRET method to evaluate new reaction media, conditions to conduct the amination chemistry with K_3PO_4 in polar solvent mixtures were developed.

Acknowledgment. We thank the NIH-NIGMS (GM55382) for support of this work, and S.R.S. thanks NIH (GM-20191-01) for postdoctoral fellowship support. We thank Kevin Shaughnessy for initial fluorescent studies and James Stambuli, Sunwoo Lee, and Quinetta Shelby for providing several of the phosphine ligands.

Supporting Information Available: Full Experimental Section including the structures and synthetic methods for preparation of ligands used in the screening assay and procedures for all catalyst screening (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA034161P