

Reaction Discovery by Using a Sandwich Immunoassay**

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Despite the breadth of the synthetic chemist's arsenal, there is still a need to develop new organic reactions, to allow for unprecedented connection of reactive functions. A recent and still underdeveloped approach to reaction discovery involves the use of efficient screening techniques that allow test reactions to be systematized in a format that maximizes the opportunity for discovering new reactions. This "forced serendipity" approach is based on the assumption that the probability of serendipitous findings increases when a large number of chemical reactions are performed. Robust and general high-throughput screening techniques allowing the quick identification of new reactions are the critical point of such an approach.

Improvements in the automation of mass spectroscopy (MS) coupled to gas (GC) or liquid (LC) chromatography were the initial driving force of this serendipity-based strategy. Weber et al. reported in 1999 the discovery of a new Ugi-type reaction after screening thousands of reaction mixtures.^[1] Since this pioneering work, a few examples of new reactions discovered by MS-based screening have been reported. Porco, Jr. and co-workers developed a so-called multidimensional screening, wherein reactions are run in an array format and analyzed by LC-MS techniques.^[2] Several new interesting reactions were successfully identified, but because of moderate throughput of the screening, the approach was rather focused on one particular type of designed substrate or reaction process, which may leave many areas of chemical reactivity unexplored. Recently, MacMillan and co-workers discovered a useful photoredox-catalyzed C–H arylation reaction using robotic GC-MS.^[3] A copper-catalyzed alkyne hydroamination and two nickel-catalyzed hydroarylation reactions were also recently discovered by Robbins and Hartwig using a simpler MS instrument.^[4]

An alternative strategy for discovery of new reactions, initially used to facilitate MS analysis,^[5] relies on the use of tagged reactants. Liu and co-workers developed a powerful method based on DNA-tagged reactants allowing the quick identification of new chemical transformations.^[6] However, DNA tags require particular chemistry and might interact with the catalysts.

Herein, we discuss an approach to discover chemical reactions using an immunoassay screening technique that uses tags that are easier to synthesize and more compatible with the chemical reactions studied. As we reported,^[7] sandwich immunoassays can be adapted to monitor cross-coupling reactions by connecting small-molecule tags to chemically reactive groups. Products of coupling reactions can then be specifically detected by two anti-tag monoclonal antibodies: one antibody capturing the double-tagged coupling product onto a solid phase and a second acting as a detector. The required tags are respectively a *tert*-butoxycarbonyl (Boc)-protected imidazole and a *tert*-butyldimethylsilyl (TBDMS)-protected guaiacol derivative, both are linked to functional groups **A** and **B** by standard peptide coupling (Figure 1). We recently showed that any kind of cross-coupling products can be detected by this technique if the tags are separated by at least eight atoms.^[8]

Typically our sandwich immunoassay is run in microtiter plates and allows the measurement of at least 1000 reaction yields in a single day (for one person, without robotization). We anticipated that this throughput should be high enough to apply the technique to reaction discovery projects.

Our strategy (Figure 1) is therefore based on a three-step procedure: 1) parallel reactions of combinations of tagged functional groups **A** and **B** and catalysts are run in 96-well plates, 2) quenching, dilution, and transfer of crude reaction mixtures to determine cross-coupling yields by sandwich immunoassay, and 3) validation and evaluation of hits by reproducing the active combination with nontagged functional groups.

A particularly critical design element of this strategy relies on the choice of reactive functional groups that, in theory, should not be based on preconceived ideas of what will react. Herein, we conducted the core experiment with 21 tagged **A** functional groups and 16 tagged **B** functional groups (see Supporting Information for synthesis), most of them containing common functional groups (such as alcohol, nitro, amine, alkene, alkyne, azide, aldehyde, and nitrile). Assuming that the probability of identifying new reactions should be higher with functionalities whose chemistry has been less explored, we also selected some less common functional groups (such as skipped alkyne, *N*-hydroxy thiourea, alkynyl oxime, and amidoxime). Alkane groups were also added to the library as

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[**] This work was supported by the European Union, BioChemLig project and by ANR, ClickScreen project.

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.201201451>.

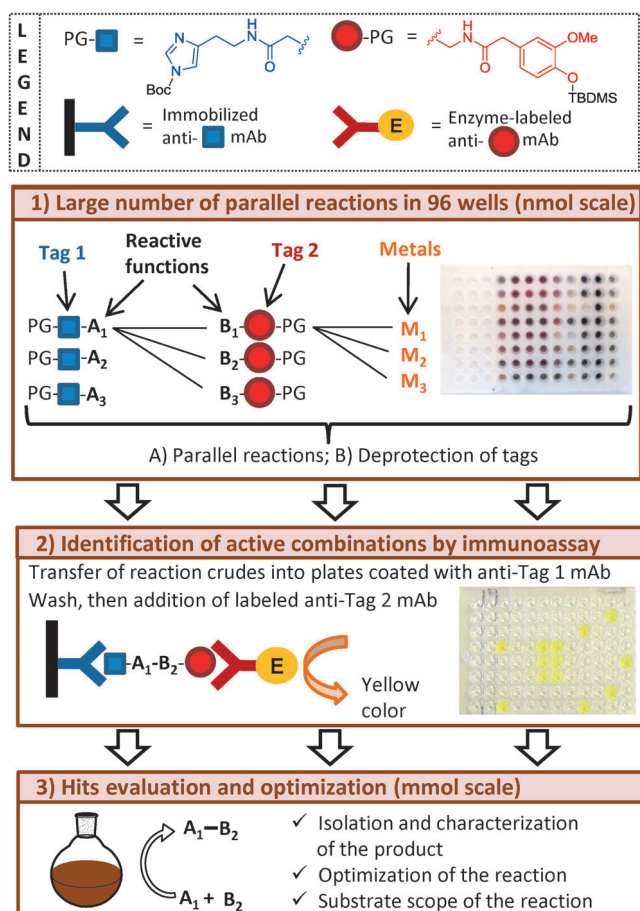


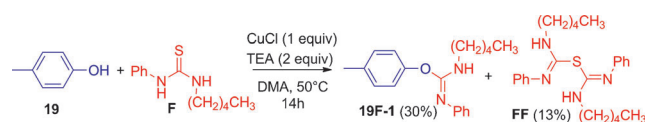
Figure 1. Outline of the immunoassay-based reaction discovery process. PG: Tag protecting groups.

negative controls. Reactants were combined in a parallel manner and exposed to one set of reaction conditions with or without transition-metals (Cu, Pd, Au, and Ru). To maximize the chances of discovering new reactions, a stoichiometric amount of metal was used. Reactions were run in dimethylacetamide (DMA) at 50 °C in the presence or absence of triethylamine (TEA) leading to a total of 3360 parallel reactions performed in 96-well plates. It should be noted that, because of the high sensitivity of the screening method, reactions were carried out with only 20 μ L total volume and 100 nmol of reactants. Following treatment by a solution of tetrabutylammonium fluoride (TBAF) and HCl (quenching and tag deprotection), the crude reaction mixtures were transferred into microtiter plates coated with anti-Tag 1 monoclonal antibody (mAb). After washing, the enzyme-labeled anti-Tag 2 mAb was added and the enzymatic activity quantitated (Figure 1). Yields were calculated using a calibration curve obtained with a double-tagged model compound (see Supporting Information). All combinations were screened by sandwich immunoassay in 2 days to reveal 57 coupling reactions. Representative results obtained with Cu, Pd, and Au catalysts in the presence of TEA are indicated in Figure 2.

Yields were globally poor and did not exceed 30%, probably because of the reaction conditions used, but clear

hits were identified. Among them we focused our interest on the 22 hits which generated more than 5% of the cross-coupled products. Of these hits, 13 combinations, confirmed by LC/MS analysis, corresponded to well-known reactions: Henry, Knoevenagel, Michael reactions (**11H**, **13H**, **11L**, and **19L**), Cu-catalyzed alkyne-azide cycloaddition (**2D**, **5D**, and **6D**), alkyne-alkyne dimerization (**2E**, **5E**, and **6E**), and Pd-catalyzed Sonogashira and Heck reactions (**2G** and **3G**). The remaining nine combinations were thus carefully investigated by reproducing the reactions on the mmole scale using non-tagged reactants. These experiments highlighted only two interesting reactions resulting from copper-mediated coupling of phenols and thioureas (combination **19F**) and coupling of alkynes with *N*-hydroxy-thioureas (combination **2O**). The other seven combinations gave complex mixtures containing several coupling products and by-products and were therefore considered less promising.

Examination of the combination **19F** was first carried out using *para*-cresol **19** and 1-pentyl-3-phenylthiourea **F** under the reaction conditions used in the screening. Two products were isolated: the isourea **19F-1** and the byproduct **FF** (Scheme 1).



Scheme 1. Target reaction 1 (yields of isolated product).

Isoureas are versatile esterification and alkylation reagents^[9] usually prepared from reaction of alcohols or phenols with carbodiimides.^[10] To the best of our knowledge intermolecular coupling between a thiourea and phenols has only been described once using activated *N,N'*-bis-(Boc)thiourea by way of the formation of the corresponding carbodiimide upon addition of stoichiometric amounts of Hg^{II} salts.^[11] Anticipating that our reaction should give rise to an easier and more general synthesis of isoureas, optimization of the reaction conditions was investigated (Table 1). The reaction was found to proceed efficiently at room temper-

Table 1: Optimization of reaction 1 conditions.^[a]

Entry	Copper	Base ^[b]	Conditions	F19 ^[c]	FF ^[c]
1	CuCl (1 equiv)	-	N ₂ , 50 °C	trace	45%
2	CuCl (1 equiv)	TEA	N ₂ , 50 °C	30%	13%
3	CuCl (1 equiv)	<i>t</i> BuOK	N ₂ , 50 °C	32%	4%
4	CuCl (1 equiv)	NaH	N ₂ , 50 °C	38%	19%
5	CuCl (1 equiv)	K ₂ CO ₃	N ₂ , 50 °C	48%	13%
6	CuCl (1 equiv)	K ₂ CO ₃	N ₂ , 25 °C	47%	20%
7	CuCl (0.1 equiv)	K ₂ CO ₃	N ₂ , 25 °C	48%	20%
8	CuI (0.1 equiv)	K ₂ CO ₃	N ₂ , 25 °C	39%	11%
9	CuOTf (0.1 equiv)	K ₂ CO ₃	N ₂ , 25 °C	15%	5%
10	Cu(OAc) ₂ (0.1 equiv)	K ₂ CO ₃	N ₂ , 25 °C	46%	14%
11	CuCl ₂ (0.1 equiv)	K ₂ CO ₃	N ₂ , 25 °C	52%	22%
12	CuCl ₂ (0.1 equiv)	K ₂ CO ₃	air, 25 °C	78%	16%

[a] Reactions were conducted with 0.1 M substrates in DMF. [b] 3 equiv. [c] Yields as determined by NMR spectroscopy.

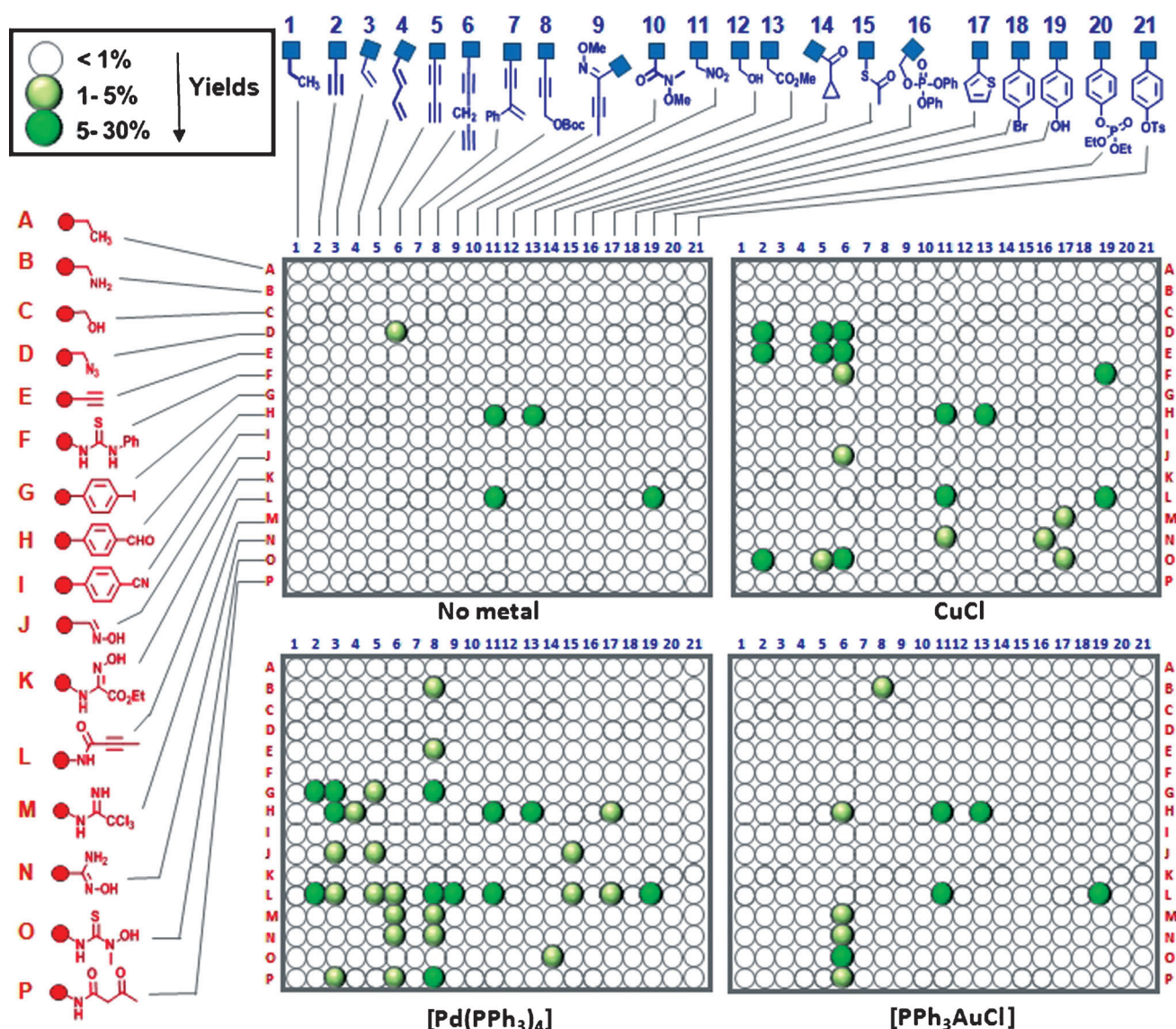
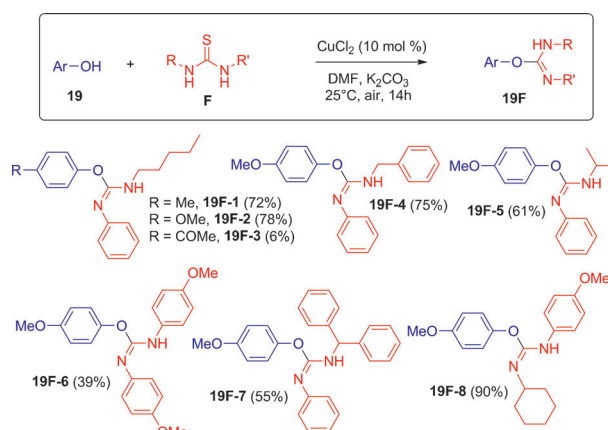


Figure 2. Screening results. Reactions were carried out with 5 mm reactants, 10 mm TEA, and with or without 5 mm metal in 40 μ L of DMA.

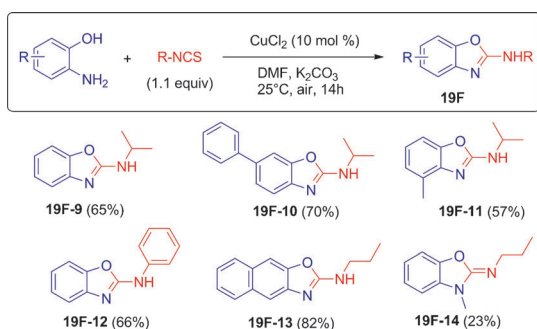
ature using K_2CO_3 in the presence of catalytic amounts of Cu^{II} salts under air.

To look at the scope of the reaction, a series of reactions were conducted with the optimized reaction conditions. As shown in Scheme 2, the reaction efficiency appeared to be strongly influenced by the phenol nucleophilicity but seemed less sensitive to the thiourea structure.

The reaction was then successfully extended to the intramolecular version to form *N*-substituted 2-aminobenzoxazoles from 2-aminophenols. Upon treatment with alkyl- or arylisothiocyanates in the presence of catalytic amounts of $CuCl_2$, aminophenols underwent rapid formation of the corresponding thiourea intermediates followed by copper-catalyzed cyclodesulfurization to form the desired aminobenzoxazoles in good yields (Scheme 3). Cyclodesulfurization methods were previously reported using stoichiometric amounts of toxic heavy-metal oxides,^[12] or strong oxidants.^[13] Therefore, this reaction represents an interesting milder



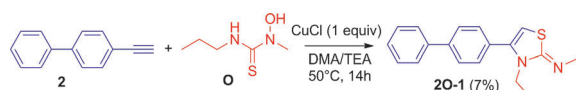
Scheme 2. Scope of target reaction 1 (yields of isolated product).



Scheme 3. Extension of reaction 1 to aminobenzoxazoles synthesis (yields of isolated product).

alternative to known procedures. Although no clear mechanism of the reaction can be provided at this time, control experiments conducted with thiourea **F** in the presence of CuCl_2 indicated that no carbodiimide was formed during the reaction. The formation of compound **19F-14** from *N*-disubstituted thiourea also supports the absence of carbodiimide intermediate formation during the reaction process. No H_2S or elemental sulfur was detected in this desulfurization reaction, suggesting an oxidative mechanism from the Cu^{2+} /air system.

Examination of combination **2O** was then performed using 4-ethynylbiphenyl **2** and 1-hydroxy-1-methyl-3-propylthiourea **O** as model substrates under the conditions used during the screening procedure. LC/MS analysis of the crude material revealed a minor coupling product with a molecular weight equal to the sum of the starting reactants with loss of H_2O . Purification of the crude afforded two products: the 2-iminothiazolidine **2O-1** obtained in 7% yield together with the alkyne dimer product (53% yield) resulting from Glaser–Hay oxidative coupling (Scheme 4).^[14]



Scheme 4. Target reaction 2 (yields of isolated product).

Convinced by the usefulness of this new reaction, we performed a systematic optimization study by varying all the parameters that might influence the reaction efficiency. Part of this study is given in Table 2. Varying bases and copper salts was unsuccessful (Table 2, entries 1–6). The use of high temperatures or controlled atmosphere (to alleviate the formation of the alkyne dimer) also did not improve the reaction (Table 2, entries 4 and 8). Improvement of the reaction was finally possible by using excess $\text{Cu}(\text{OAc})_2$ in refluxing EtOH under air (Table 2, entries 13 and 14).

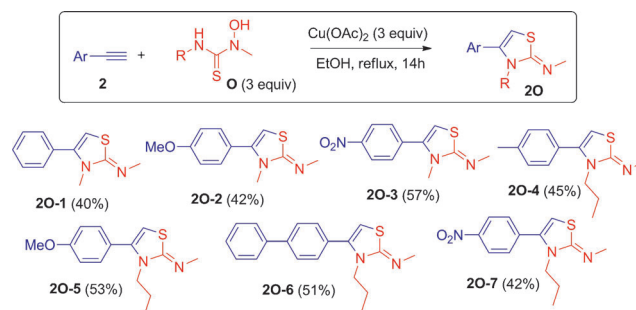
We then examined the scope of the reaction using 3 equivalents of $\text{Cu}(\text{OAc})_2$ in EtOH at 80°C under an atmosphere of air. The reaction was tolerant of electron-rich and electron-poor alkynes affording the desired 2-iminothiazolidines **2O** in moderate yields (Scheme 5). The structures of the products were determined by NMR spec-

Table 2: Optimization of reaction 2 conditions.^[a]

Entry	Copper	Base ^[b]	Conditions	2O ^[c]
1	CuCl (1 equiv)	TEA	DMF, air, 50°C	7%
2	CuCl_2 (1 equiv)	TEA	DMF, air, 50°C	5%
3	$\text{Cu}(\text{OAc})_2$ (1 equiv)	TEA	DMF, air, 50°C	12%
4	$\text{Cu}(\text{OAc})_2$ (1 equiv)	TEA	DMF, Ar, 50°C	n.d.
5	$\text{Cu}(\text{OAc})_2$ (1 equiv)	K_2CO_3	DMF, air, 50°C	8%
6	$\text{Cu}(\text{OAc})_2$ (1 equiv)	Cs_2CO_3	DMF, air, 50°C	11%
7	$\text{Cu}(\text{OAc})_2$ (1 equiv)	-	DMF, air, 50°C	15%
8	$\text{Cu}(\text{OAc})_2$ (1 equiv)	-	DMF, air, 100°C	7%
9	$\text{Cu}(\text{OAc})_2$ (1 equiv)	-	H_2O , air, 80°C	5%
10	$\text{Cu}(\text{OAc})_2$ (1 equiv)	-	CH_3CN , air, 80°C	n.d.
11	$\text{Cu}(\text{OAc})_2$ (1 equiv)	-	DMSO, air, 80°C	n.d.
12	$\text{Cu}(\text{OAc})_2$ (1 equiv)	-	EtOH, air, 80°C	24%
13	$\text{Cu}(\text{OAc})_2$ (3 equiv)	-	EtOH, air, 80°C	33%
14	$\text{Cu}(\text{OAc})_2$ (3 equiv) ^[d]	-	EtOH, air, 80°C	54%

[a] Experiments were carried out with 1 equiv of *N*-hydroxy thiourea **O**.

[b] 3 equiv. [c] Yields as determined by NMR spectroscopy. [d] 3 equiv of *N*-hydroxy thiourea **O** were used. n.d. = not detected

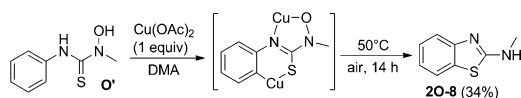


Scheme 5. Scope of target reaction 2 (yields of isolated product).

troscopy analysis and further confirmed by X-ray diffraction analysis of products **2O-3** and **2O-7** (see Supporting Information). Although the product yields could still be improved, an interesting feature of this reaction is its regioselectivity. In all cases, only one regioisomer (surprisingly the most sterically hindered one) was formed during the reaction. No other heterocycles were isolated; the only byproducts were the alkyne dimer and products from *N*-hydroxy thiourea degradation.

Whereas a detailed mechanism remains to be determined, thiolation of the C–H bond of the terminal alkyne by way of copper-oxidative coupling^[15] followed by subsequent cyclization seems to be one possible mechanism. In support of this hypothesis, heating $\text{Cu}(\text{OAc})_2$ with the *N*-phenyl-*N'*-hydroxy thiourea **O'** interestingly undergoes C–S bond formation through C–H activation at only 50°C to yield benzothiazole **2O-8** (structure determined by X-ray analysis; Scheme 6).

In conclusion, this work shows that sandwich immunoassays are a suitable and effective screening tool for “serendipitously” discovering new chemical transformations. Using this method, thousands of reactions were run and screened in a few days, revealing two new reactions: a copper-catalyzed desulfurization reaction of thioureas leading to isoureas and a copper-promoted cyclization reaction leading to thiazole derivatives from alkynes and *N*-hydroxy thioureas.



Scheme 6. Reaction of **O'** with Cu^{2+} (the structure of the copper complex is hypothetical).

The presented screening strategy has no particular limitations and could be used to explore any kind of bond formation reactions in a large panel of experimental conditions. Although the need to connect tags to reactive functions may be a drawback of the screening technique, because it requires some time for tagged reactant synthesis, it is also a strong advantage. The tags allow for easy isolation of cross-coupling products, ensuring a high level of selectivity of the screen. Control experiments showed that the method is able to detect the coupling products in very complex reaction mixtures, including biological media, allowing identification of reactions with yields as poor as 0.01 % if necessary. This represents a significant advantage over described screening methods that cannot determine reaction yields in a high throughput manner. We envision in the near future using the high selectivity, sensitivity, and throughput of this screening approach to search for new chemoselective, bioorthogonal reactions, which represents one of the fascinating next steps of the “forced serendipity” approach.

Experimental Section

General procedure for parallel reactions and screening by sandwich immunoassay: Reactant **1-21** (1 equiv; 5 μL of a 20 mM DMA solution), reactant **A-P** (1 equiv; 5 μL of a 20 mM DMA solution), metal salts (1 equiv; 5 μL of a 20 mM DMA solution), and TEA (2 equiv; 5 μL of a 40 mM DMA solution) were mixed in a 96-well microtiter plate. No precautions were taken to exclude oxygen. The reaction plates were incubated at 50°C with stirring for 24 h and then quenched by addition of 110 μL of a 0.1M HCl/TBAF aqueous solution.

The crude mixtures were then diluted 1:10⁵ in EIA buffer (containing 0.1M phosphate buffer and 1 mgmL⁻¹ BSA, pH 7.4). 100 μL of these diluted solutions was transferred to the wells of a microtiter plate previously coated with anti-Tag 1 mAb (directly adsorbed to the polystyrene support). After 3 h of incubation at room temperature, the plates were washed and 100 μL of anti-Tag 2 mAb-acetylcholinesterase (AChE) conjugate was added (mAb-AChE conjugate was prepared and stored as previously described).^[16] After 12 h of immunological incubation at 4°C, the plates were washed and Ellman's reagent was added. The absorbance related to the solid phase-bound AChE activity was measured at 414 nm. Immunological reagents (antibody for the capture step and enzyme conjugate antibody) were used in excess compared with the concentration of the samples. All measurements for standards or samples were made in duplicate.

Received: February 21, 2012

Revised: April 10, 2012

Published online: May 4, 2012

Keywords: copper · high-throughput screening · immunoassays · reaction discovery

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