

Label-assisted mass spectrometry for the acceleration of reaction discovery and optimization

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The identification of new reactions expands our knowledge of chemical reactivity and enables new synthetic applications. Accelerating the pace of this discovery process remains challenging. We describe a highly effective and simple platform for screening a large number of potential chemical reactions in order to discover and optimize previously unknown catalytic transformations, thereby revealing new chemical reactivity. Our strategy is based on labelling one of the reactants with a polyaromatic chemical tag, which selectively undergoes a photoionization/desorption process upon laser irradiation, without the assistance of an external matrix, and enables rapid mass spectrometric detection of any products originating from such labelled reactants in complex reaction mixtures without any chromatographic separation. This method was successfully used for high-throughput discovery and subsequent optimization of two previously unknown benzannulation reactions.

High-throughput reaction-screening approaches that enable rapid and accurate detection of new products with unanticipated structures can substantially expand our knowledge of chemical reactivity. Although several innovative strategies to address this general problem have been reported^{1–11}, the development of a highly efficient, broadly useful and preparatively simple reaction-discovery platform remains challenging. We have recently used matrix-assisted laser desorption/ionization and time-of-flight mass spectrometry (MALDI–TOF–MS) to analyse chemical transformations on the surface of self-assembled monolayers of alkane-thiolates on gold¹². Despite the high throughput of the primary reaction screen and its ability to detect products with unanticipated structures, subsequent translation of the initially identified interfacial reactions to preparative, solution-phase processes have often required substantial effort.

We now describe the development of a new reaction-discovery strategy that features not only excellent screening throughput, but also a highly efficient translation of the initial ‘hits’ into catalytic, synthetically useful transformations. The reactions are rapidly analysed in solution using label-assisted laser desorption/ionization and time-of-flight mass spectrometry (LA–LDI–TOF–MS). This simple and highly effective approach is based on the incorporation of a readily available polyaromatic tag into the structure of a reactant, thereby greatly facilitating the desorption/ionization process and enabling rapid and selective MS analysis of hundreds of chemical reactions in solution under matrix-free conditions with excellent efficiency. After validation of the concept by monitoring the course of several known transformations, the technology was used to evaluate the outcome of 696 different reactant combinations, and led to the discovery of two previously unknown benzannulations.

Results and discussion

Rapid screening of chemical reactions by MALDI–TOF–MS is attractive for two main reasons. First, the efficiency and throughput of this approach compares favourably to the commonly used liquid chromatography–mass spectrometry (LC–MS) and gas chromatography–mass spectrometry (GC–MS) methods, because the reaction mixtures are analysed directly without any chromatographic fractionation. Second, the high sensitivity of this technique enables

MS analysis of reactions performed on an exceedingly small scale, enabling highly efficient miniaturization of experimental design. Indeed, accurate analytical data can be readily obtained using only picomoles of analyte. Despite such desirable features, development of the solution-based MALDI–TOF–MS reaction-discovery platform presents a substantial challenge because ionization of the matrix commonly used for the desorption/ionization process substantially complicates accurate detection of analytes with low molecular weights. A notable exception has been reported by Senkan, who used resonance-enhanced multiphoton ionization to selectively detect benzene in the presence of a cyclohexane¹³. Although this method was used to screen a relatively small library of heterogeneous catalysts for their ability to promote dehydrogenation, the approach is based on the detection of a specific reaction product and is not easily applicable to monitoring the efficiency of many other reactions.

In contrast, our main objective was to develop a broadly useful, practical reaction-discovery platform that can be readily used to identify and optimize a range of new chemical transformations. We envisioned that the introduction of an appropriate MS label into the structure of one of the reactants could promote a selective desorption/ionization process and enable accurate detection of products originating from such labelled analytes, completely eliminating the need for a matrix and greatly simplifying spectral analysis (Fig. 1a). The use of this MS labelling approach to facilitate the ionization process has been recognized^{14–18} and used to optimize at least two established reactions^{16,18}, but this powerful concept has not been used for the high-throughput discovery of new chemical transformations.

Because commercial MALDI–TOF–MS instruments are typically equipped with lasers that irradiate in the ultraviolet region of the electromagnetic spectrum, the effective MS label must readily undergo the photoionization/desorption process upon laser irradiation, without the assistance of an external matrix. In addition, such MS labels should be chemically inert under a range of commonly used reaction conditions in organic and organometallic chemistry. It has been established that many polyaromatic compounds efficiently undergo the photoionization/desorption process upon laser-induced irradiation in the ultraviolet region, presumably due to their high molar absorptivity and ability to form

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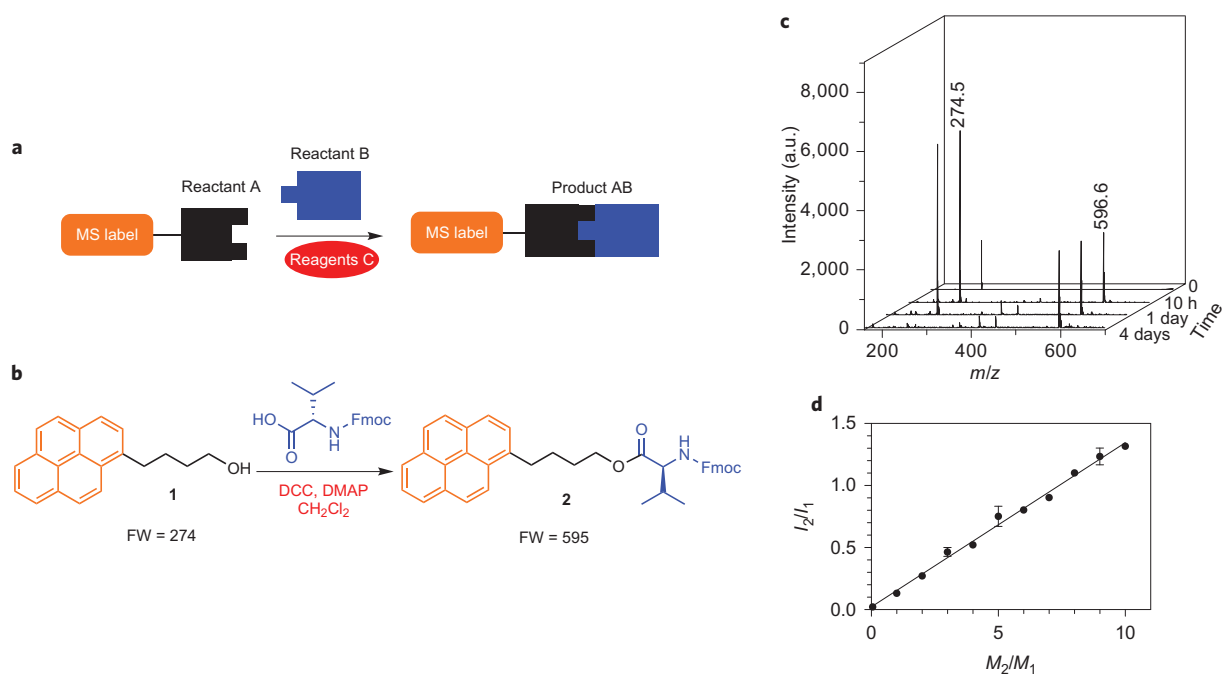


Figure 1 | Use of LA-LDI-TOF-MS to monitor the progress of a representative known reaction. **a**, General strategy for monitoring the progress of chemical reactions using LA-LDI-TOF-MS, which entails labelling one of the reactants with a tag that permits matrix-free laser-induced desorption/ionization and rapid detection of any products originating from the labelled analyte. **b**, Reaction scheme of a representative chemical transformation of **1** to **2**, which was studied using LA-LDI-TOF-MS. **c**, MS spectra for conversion of alcohol **1** to ester **2**. **d**, Plot of relative ion intensity ratio (I_2/I_1) versus mole ratio (M_2/M_1) ($y = 0.1324x + 0.0216$, $R^2 = 0.99365$). Error bars represent standard deviations. Fmoc, fluorenylmethyloxycarbonyl; DCC, dicyclohexylcarbodiimide; DMAP, 4-dimethylaminopyridine.

radical cations that can be detected by MS^{19,20}. We initially examined a range of polyaromatic compounds and identified pyrene as an effective label for selective ionization. The progress of each reaction can be readily analysed by monitoring the conversion of MS-labelled reactant A into the expected MS-labelled product AB in the presence of reagent(s) C (Fig. 1a), enabling selective detection of only two species in crude reaction mixtures under matrix-free conditions. To validate the utility and generality of this method, we analysed the progress of several known transformations. Treatment of pyrene-containing alcohol **1** with Fmoc-protected valine under standard esterification conditions produced the expected ester **2** (Fig. 1b). The course of this reaction was readily analysed by the disappearance of the peak of the reactant at m/z 274.5 (Fig. 1c) and formation of the product peak at m/z 596.6 ($M+1$). In addition to the qualitative assessment of the reaction progress, LA-LDI-TOF-MS could be used readily to quantify the conversion of **1** to **2** by measuring the intensities of the MS peaks corresponding to the two compounds (Fig. 1d). Having examined several other known reactions (Supplementary Fig. S1), we clearly established that LA-LDI-TOF-MS allows the efficient and accurate detection of pyrene-labelled products, validating the generality of this analytical method and setting the stage for its further implementation to search for new chemical reactivity.

Electron-rich alkynes, especially siloxy alkynes, represent a fertile ground for developing new carbon-carbon and carbon-heteroatom bond-forming reactions^{12,21–29}. Such reactions can be catalysed by a range of transition metals, as well as Brønsted acids or bases, and typically proceed under mild conditions. To further explore the reactivity of this important functional group, we used the LA-LDI-TOF-MS reaction screening platform with a pyrene-containing siloxy alkyne **3** (Fig. 2a). This labelled substrate A was treated with 23 different reactants B as well as a negative control. Each of the reactions was evaluated in the absence and presence of 29 individual reagents C (including a negative solvent control),

corresponding to 696 discrete experiments, which were set up in 1,2-dichloroethane at ambient temperature in a 96-well format using a conventional robotic liquid handler. The progress of each experiment was analysed after 1 h, 1 day and 4 days by spotting a 0.8 μ l aliquot of each reaction mixture onto a standard stainless-steel plate, as used by MALDI-TOF instruments. Following solvent evaporation, each spot was analysed directly using LDI-TOF-MS in positive-ion reflector mode. The high throughput of this screening platform is noteworthy, as 696 spectra were typically collected within 2 h by a conventional MALDI-TOF spectrometer working in an automated data acquisition mode.

We then analysed all the MS spectra, and several reactions that had produced unanticipated products were repeated on a larger scale to enable their complete structural characterization by other commonly used analytical methods. This effort identified two benzannulation reactions that have not previously been described. Treatment of alkyne **3** with 2-pyrone in the presence of 5 mol% AuCl₃ afforded carboxylic acid **4** with m/z 537.8, which was isolated in 75% yield (Fig. 2b, Supplementary Fig. S2). In addition, reaction of alkyne **3** with isoquinoline *N*-oxide in the presence of 10 mol% AgNTf₂ gave oxime **5** with m/z 585 in 52% yield (Fig. 2c, Supplementary Fig. S3). Although the initial structural assignments of **4** and **5** relied on NMR spectroscopy, the structures of both products were ultimately secured by X-ray crystallography. It is also noteworthy that neither of the two transformations occurred in the absence of a catalyst, even at elevated temperatures.

Our next efforts centred on optimizing the efficiency of the transformation shown in Fig. 2b. To this end, we examined several known gold and silver complexes and monitored each experiment using either LDI-TOF-MS or more conventional NMR spectrometry (Supplementary Table S1). This study demonstrated that LA-LDI-TOF-MS could be used for rapid reaction optimization and that gold(I) complex **8**, containing highly sterically congested Johnphos ligand, proved excellent in catalysing the reaction

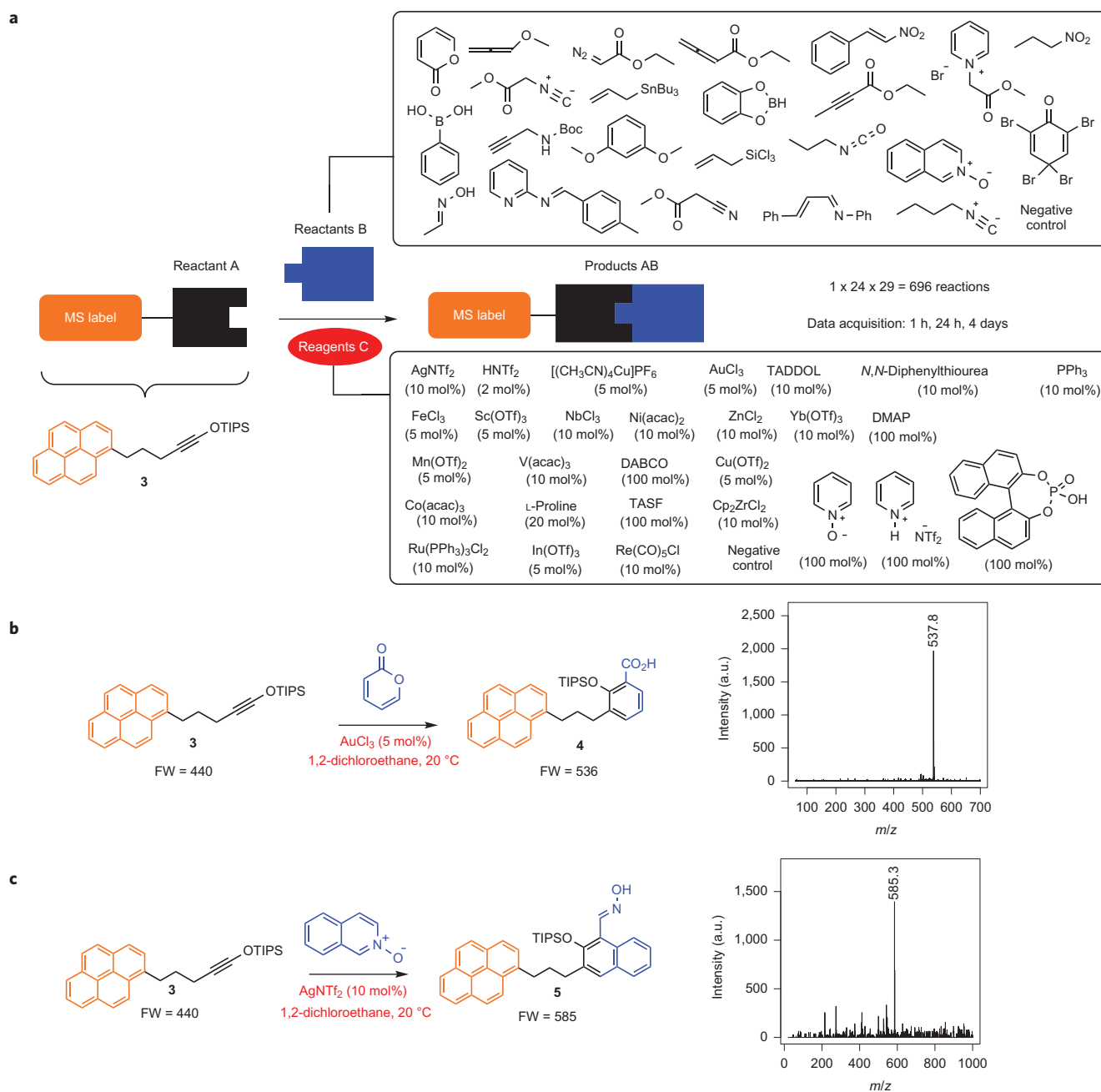


Figure 2 | Reaction screen using LA-LDI-TOF-MS and identification of two catalytic benzannulations. **a**, High-throughput screening of 696 potential reactions. Siloxy alkyne **3** contains a pyrene tag, which enables matrix-free detection of any products originating from this compound using the LA-LDI-TOF-MS platform. TIPS, triisopropylsilyl; Tf, trifluoromethanesulfonyl; TADDOL, ($\alpha,\alpha,\alpha,\alpha$ -tetraaryl-1,3-dioxolane-4,5-dimethanol); acac, acetylacetonate; DMAP, 4-dimethylaminopyridine; DABCO, 1,4-diazabicyclo[2.2.2]octane; TASF, tris(dimethylamino)sulfonium difluorotrimethylsilicate; Cp, cyclopentadienyl. **b**, Initial identification of the benzannulation of siloxy alkyne **3** with 2-pyrone in the presence of AuCl_3 to give carboxylic acid **4**. **c**, Initial identification of the benzannulation of siloxy alkyne **3** with *N*-isoquinoline oxide in the presence of AgNTf_2 to give oxime **5**.

between siloxy alkynes **6** and 2-pyrones **7** to give siloxy acid **9** (Fig. 3). Subsequent one-flask desilylation with HF-pyridine afforded the corresponding salicylic acids **10**. The initial step presumably proceeded via a formal [4+2] cycloaddition to give bicyclic intermediate **A**, which underwent subsequent fragmentation of the C–O bond and aromatization. Although 2-pyrones are known to undergo [4+2] cycloadditions, such reactions generally require high temperatures and proceed typically with complete loss of CO_2 from the initially produced cycloadducts²¹. However, the tandem cycloaddition/fragmentation pathway described in Fig. 3 has not been reported. This process successfully tolerated various

substitution patterns of siloxy alkynes (R^1) and 2-pyrones (R^2 and R^3). Reactions of unsubstituted 2-pyrone ($\text{R}^2=\text{R}^3=\text{H}$) with two alkyl-substituted siloxy alkynes resulted in efficient formation of the corresponding salicylic acids **10a** and **10b**. Introduction of electron-withdrawing groups ($\text{R}^3=\text{CO}_2\text{Me}$) into the 2-pyrone structure gave the expected benzannulation products **10c** and **10d**. Presence of the aromatic substituent ($\text{R}^2=\text{Ph}$) was also well-tolerated and afforded biaryl product **10e**. Finally, the use of 5-chloro-2-pyrone ($\text{R}^2=\text{Cl}$) allowed us to test a wide range of substitution patterns on siloxy alkynes including various alkyl and aryl substituents. All reactions proceeded efficiently to give the corresponding

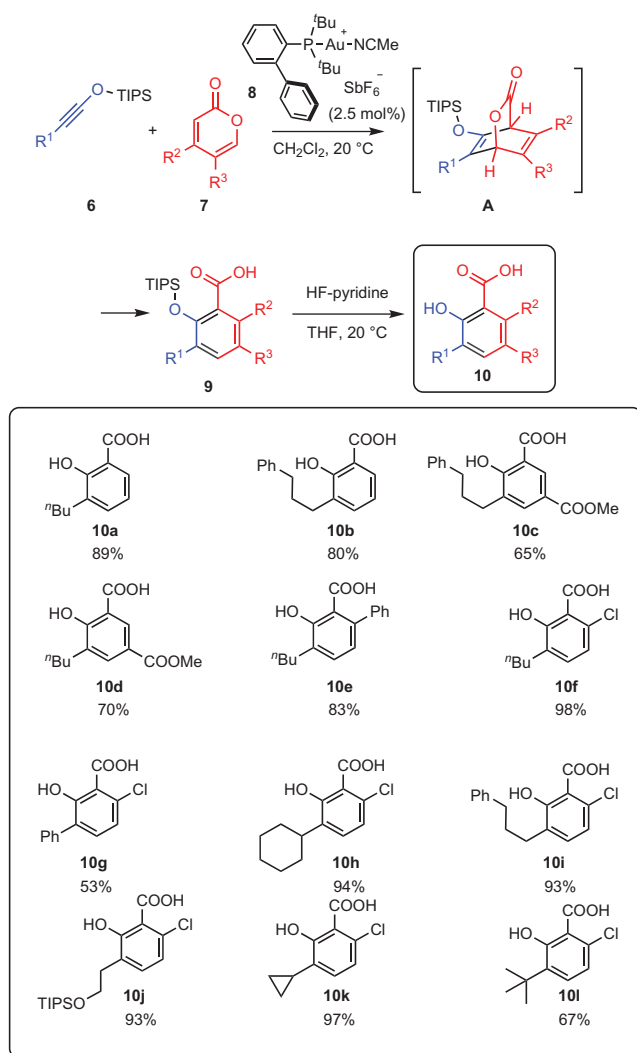


Figure 3 | Mechanism and scope of gold-catalysed benzannulation of siloxy alkyne with 2-pyrones. Siloxy alkyne **6** undergoes a [4+2] cycloaddition with 2-pyrone **7** to give a putative intermediate **A**, which undergoes subsequent fragmentation to deliver carboxylic acid **9**. Compound numbers are shown in bold. Isolated yields are shown below each compound number. R is a generic alkyl or aryl substituent.

benzannulation products **10f–l**. The structures of **10a** and **10e** were verified by X-ray crystallography.

Although the initial discovery of the reaction between siloxy alkyne **3** and isoquinoline *N*-oxide was made using silver-based catalyst, we found during subsequent optimization studies (Supplementary Table S2) that the same gold(i) complex **8** proved to be optimal for catalysing this benzannulation process (Fig. 4). In this case, the optimization study was performed using conventional NMR methods due to the high propensity for ion fragmentation of the major reaction product under LDI-TOF-MS conditions. This process represents another example of a formal [4+2] cycloaddition/fragmentation pathway, which begins presumably via the formation of tricyclic intermediate **B**, followed by C–N bond fragmentation and aromatization to give oxime **12**. Subsequent one-flask desilylation can be efficiently achieved using tetrabutyl ammonium fluoride (TBAF) to give 2-naphthols **13**. This reaction tolerated a wide range of substitutions of the siloxy alkyne (R^1 =alkyl), as exemplified by the efficient formation of benzannulation products **13a–d**. Furthermore, the use of 1-methyl isoquinoline *N*-oxide (R^2 =Me) afforded the corresponding naphthalene derivative **13e**. Finally, a variety of halogenated isoquinoline

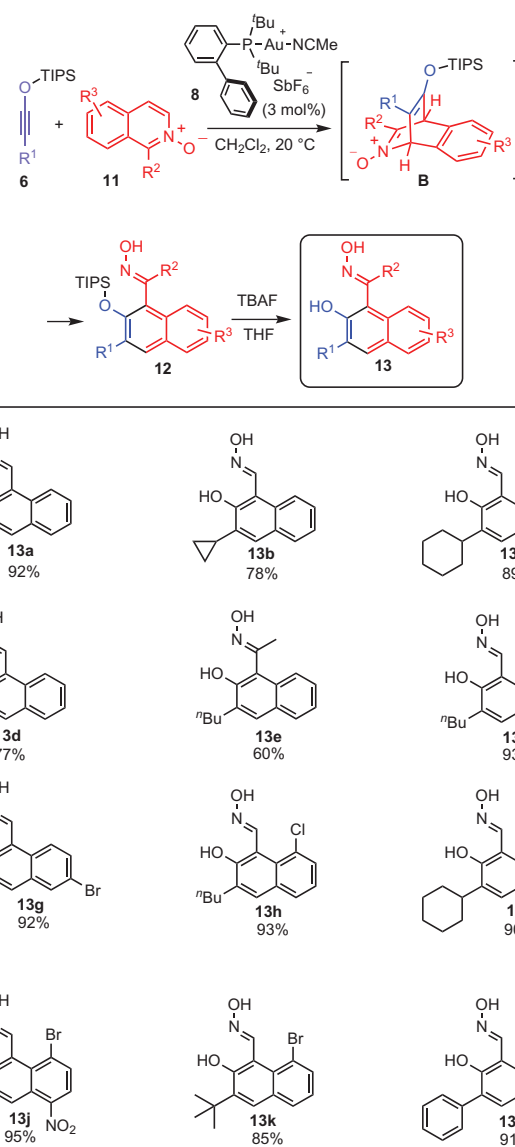


Figure 4 | Mechanism and scope of gold-catalysed benzannulation siloxy alkynes with isoquinoline *N*-oxides. Siloxy alkyne **6** undergoes a [4+2] cycloaddition with isoquinoline *N*-oxide **11** to give a putative intermediate **B**, which undergoes subsequent fragmentation to deliver oxime **12**. Compound numbers are shown in bold. Isolated yields are shown below each compound number. R is a generic alkyl or aryl substituent. Owing to instability, the yield of compound **13h** was determined by NMR using an internal standard. The product of this reaction was subsequently isolated as the corresponding imine in 82% yield following treatment of **13h** with MoCl_5 and Zn in acetonitrile, as described in the Supplementary Information.

N-oxides successfully afforded the expected products **13f–l**, representing a range of highly functionalized, synthetically useful naphthalene derivatives. The structures of **13a** and **13f** were established by X-ray crystallography.

In summary, we have described a broadly useful platform for rapid reaction discovery. Our general approach is based on the introduction of a polyaromatic label into the structure of one of the reactants. As a result, any conversion of such a compound into any other products can be monitored easily using matrix-free LDI-TOF-MS, even in complex reaction mixtures without any chromatographic fractionation. We demonstrated a direct application of this screening strategy to the discovery of two benzannulation reactions, which proceed via initial [4+2] cycloaddition,

