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, 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-thiols on gold. 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 cyclohexane. 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 and used to optimize at least two established reactions, 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...
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 regent(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, which was studied using LA–LDI–TOF–MS. MS spectra for conversion of alcohol 1 to ester 2. d. Plot of relative ion intensity ratio ($I_1/I_2$) versus mole ratio ($M_2/M_1$) ($y = 0.132x + 0.0216$, $R^2 = 0.99365$). Error bars represent standard deviations. Fmoc, 1-fluorenylethoxycarbonyl; DCC, dicyclohexylcarbodiimide; DMAP, 4-dimethylaminopyridine.

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 ($I_1/I_2$) versus mole ratio ($M_2/M_1$) ($y = 0.132x + 0.0216$, $R^2 = 0.99365$). Error bars represent standard deviations. Fmoc, 1-fluorenylethoxycarbonyl; DCC, dicyclohexylcarbodiimide; DMAP, 4-dimethylaminopyridine.
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 CO₂ from the initially produced cycloadducts 21. However, the tandem cycloaddition/fragmentation pathway described in Fig. 3 has not been reported. This process successfully tolerated various substitution patterns of siloxy alkynes (R₁) and 2-pyrones (R₂ and R₃). Reactions of unsubstituted 2-pyrone (R₂=¼R₃=¼H) with two alkyl-substituted siloxy alkynes resulted in efficient formation of the corresponding salicylic acids 10a and 10b. Introduction of electron-withdrawing groups (R₃=¼CO₂Me) into the 2-pyrone structure gave the expected benzannulation products 10c and 10d. Presence of the aromatic substituent (R₂=¼Ph) was also well-tolerated and afforded biaryl product 10e. Finally, the use of 5-chloro-2-pyrone (R₂=¼Cl) 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...
Mechanism and scope of gold-catalysed benzannulation of siloxy alkyne with 2-pyrones. Siloxy alkyne 6 undergoes a [4+2] cycloaddition with 2-pyrene 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. R is a generic alkyl or aryl substituent.

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 presumptively 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 substitutents of the siloxy alkyne (R³=alkyl), as exemplified by the efficient formation of benzannulation products 10f–l. The structures of 10a and 10c were verified by X-ray crystallography.

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,
followed by ring-opening and aromatization. Such reactions represent previously unknown modes of benzannulation reactivity of alkynes,28,29 and provide a simple and efficient synthetic entry into substituted salicylic acids and highly functionalized naphthols. We envision that this broadly useful reaction-discovery platform will find a range of future applications for identification and optimization of chemical transformations.

**Methods**

LA–LDI–TOF–MS reaction screen. Solutions of alkyne 3 (reactant A, 0.3 M in 30 ml of 1,2-dichloroethane), each of the 23 reactants B (0.3 M in 8 ml of 1,2-dichloroethane) and each of the 28 reagents C (0.006–0.3 M in 3 ml of 1,2-dichloroethane) were prepared manually. Using a Perkin Elmer Multiprep liquid handler, 30 µl of the solution of alkyne 3 was dispensed into 696 wells in eight 96-deep-well plates (Axygen Scientific P-DW-500-C). Using the same liquid handler, each well was treated with solutions of the 23 reactant B (30 µl per well) and 28 reagents C (30 µl per well), as well as the negative controls for both B and C containing only solvent, so that each well received a unique pairwise combination of B and C to give 969 reaction mixtures. The plates were sealed with aluminum foil and left for 1 h at room temperature. The seals were removed. Using a Perkin Elmer Janus automated workstation equipped with a 96-channel pipetting head, a 0.8 µl aliquot of each reaction mixture was transferred onto standard stainless-steel plates as used by MALDI–TOF mass spectrometers. The plates were allowed to air dry, and were analysed in automatic mode on a Bruker UltrafleXtra MALDI–TOF/TOF mass spectrometer equipped with a 355 nm Bruker smartbeam-II laser, using the positive ion reflector mode. The reaction plates were resealed and MS analysis was repeated after 24 h and 4 days as described.

**Benzannulation of siloxy alkenes with 2-pyrones.** A mixture of 2-pyrone (0.15 mmol) and (Johnphos)AuNCMe-SbF6 (0.25 mol%) was dissolved in dichloromethane (0.1 ml) and treated dropwise with siloxyalkyne (1.5 equiv.) dissolved in dichloromethane (0.3 ml) over 2 h. The reaction mixture was warmed to room temperature and stirred until the reaction was complete (typically 2–12 h). The solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (2.5 ml), placed in an ice bath, and treated dropwise with a solution of HF-pyridine (0.05 ml, 70% aqueous HF and 30% pyridine). Following warming to room temperature, the reaction mixture was treated with water (1 ml) and extracted with ethyl acetate. The combined organic layers were dried over anhydrous MgSO4, concentrated under reduced pressure and subjected to column chromatography on silica gel to deliver benzannulation product.

**Benzannulation of siloxy alkenes with isoquinoline analogues.** A mixture of isoquinoline N-oxide 11 (0.15 mmol) and (Johnphos)AuNCMe-SbF6 (0.3, 3 mol%) in dichloromethane (0.3 ml) was treated with siloxyalkyne 6 (1.5 equiv.) dissolved in dichloromethane (0.3 ml). The reaction mixture was stirred for 12 h and concentrated under reduced pressure. The residue was dissolved in dichloromethane (2.5 ml), placed in an ice bath, and treated dropwise with TBAF (1.1 equiv. 1 M in dichloromethane) was treated with siloxyalkyne in tetrahydrofuran. Following warming to room temperature, the reaction mixture was warmed to room temperature, the reaction mixture was treated with water (1 ml) and extracted with ethyl acetate. The combined organic layers were dried over anhydrous MgSO4, concentrated under reduced pressure and subjected to column chromatography on silica gel to deliver benzannulation product.

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**Author contributions**

J.R.C.-P. developed the reaction-screening platform, and performed and analysed all reactions using MS. D.I.C and S.L. carried out the reaction optimization and scope studies. Additional information

Supplementary information and chemical compound information are available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to S.A.K.

**Competing financial interests**

The authors declare no competing financial interests.