Approaches to high throughput physical organic chemistry

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High throughput (HT) techniques are now extensively used for the synthesis of libraries of several thousands of compounds. More recently, HT methods began to be applied to other areas, such as physical organic chemistry. This has allowed for instance the development of tools for HT reaction assessment, HT kinetic and thermodynamic measurements, and physicochemical property profiling, using a broad set of analytical tools, ranging from mass spectrometry to image analysis based techniques. This article provides an overview of recent HT physical organic chemistry techniques. Special attention is given to the application of quantitative analytical constructs for HT monomer reactivity profiling and HT evaluation of Hammett parameters.

Introduction

Physical organic chemistry has had a fundamental impact on the way in which synthetic and mechanistic organic chemistry has developed. Take for example the detailed studies carried out on simple substitution chemistries in the mid 1930s, and it becomes clear that physical organic chemistry has allowed direct correlations to be established between the structure of organic molecules and their reactivity.[1] It has provided a platform from which fundamental rules of chemistry have developed, supplying explanations concerning molecular reactivity, and allowed detailed mechanistic understanding to be gained. The establishment of linear free energy relationships, based on the work of Hammett for instance, has provided an unequalled understanding of the reactivity of organic compounds with the development of parameters such as σ and ρ, while analysis and understanding of isotope effects has provided a range of subtle tools to probe reaction mechanisms. However, physical organic chemistry has often been tarnished (unfairly) with a "reputation" of tedium and repetition, with the vision of days if not weeks spent hunched in front of a high performance liquid chromatography (HPLC) system, analysing single reactions. This can be contrasted with high-throughput (HT) organic chemistry which has developed at a tremendous rate over the past decade, allowing chemists to rapidly and efficiently generate libraries of hundreds to thousands of compounds. As a direct consequence of these advances in HT synthetic chemistry, analytical chemistry tools have been developed to meet the challenges of being able to monitor the progress of large numbers of reactions *in situ*, to determine the purity of these library members and to enable automated purification and mass spectrometry (MS)

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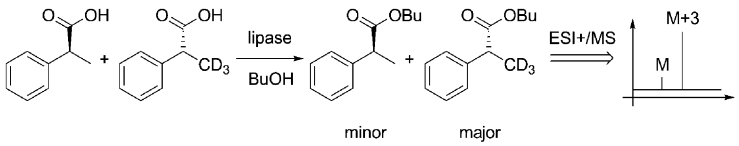


Scheme 1 Use of a pseudo-racemate for HT stereoselectivity evaluation.

based characterisation. As a result of these factors, unparalleled amounts of data are routinely being generated and larger monomer sets than ever before are being used in an increasingly large repertoire of chemistries. Clearly, these approaches open up a raft of opportunities to the physical organic chemist, with the accessibility of huge data collections, the ability to run much larger reaction sets and the ready availability of automation. It is also worth noting here the subtle boon provided by microwave heating, which allows not only unprecedented and highly accurate controlled heating of reactions, but, and importantly from a physical organic chemistry view-point, reproducible and known reaction times, temperatures and pressures in a manner normally impossible with traditional heating methods in organic synthesis.[2] This review will provide an overview of a number of approaches that have been used to enable increased throughput in physical organic chemistry, predominantly by the application of a number of HT tools that allow rapid reaction assessment and analysis, invigorating this whole process.

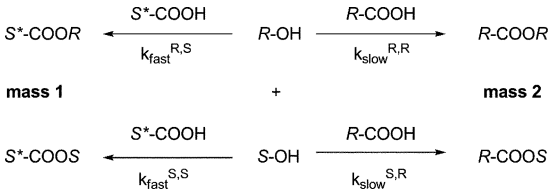
1 MS-based methods for HT reaction assessment

Since MS offers unrivalled speed of analysis and great sensitivity, it is often the tool of choice for the HT analysis of complex mixtures, and has seen application in areas ranging from the analysis of peptides and proteins (for example proteomics and serum profiling) to libraries of small organic compounds. In this review we shall concentrate on methods that focus predominantly on small molecules and that utilise, on the whole, soft ionisation techniques, although other MS based methods are also applicable.

(a) Pseudo-enantiomers and pseudo-diastereomers

For compounds that show good MS ionisation, HT qualitative reaction assessments can be made by the direct injection of reaction mixtures. However, the technique has obvious limitations for the analysis of compounds that have the same molecular weight, such as enantiomers or diastereomers. This has led to the introduction of the concept of pseudo-enantiomers which solves this dilemma and allows MS methods to be used for HT determination of enantiomeric excesses. The approach involves "isotopic tagging" whereby one of the enantiomers of a compound is synthesised or chemically modified to allow the incorporation of an isotopic label (for example a CD_3 replacing a CH_3 group). This "tagged" enantionomer is then mixed in a 1 : 1 manner with the unlabelled enantiomer. The two compounds are said to be "pseudo-enantiomers", since one of the two isomers is "heavier" than the other thanks to the presence of the CD_3 group: they form a racemic mixture whose analysis by electrospray ionisation MS (ESI/MS) is now possible. The stereoselectivity of a chemical transformation can thus be assessed by MS, provided this transformation is not affected by the modified group. Pseudo-diastereomers can be considered in the same way.[3] Using this approach, Reetz *et al.* developed a HT screening method to examine the enantioselectivity of a number of catalysts.[4] This allowed, for example, the evaluation of 1000 enantiomeric excesses (*ee*'s) a day for the lipase catalysed stereoselective esterification of 2-phenylpropionic acid, as shown in Scheme 1. This work also demonstrated that the results obtained *via* the use of pseudo-enantiomers compared very well to those obtained with other techniques, proving that the labelling of one of the enantiomers did not induce changes in reaction selectivity.

Guo *et al.* developed a related approach to allow the HT *ee* determination of alcohols and amines.[5] Thus, the alcohols (or amines) under investigation, *R*-OH and *S*-OH were coupled to a pseudo-racemate of a carboxylic acid (*R*–COOH and *S**-COOH). The esterification of the various pairs of chiral reagents proceeded with different speeds (each one of the pseudo-enantiomers reacted preferentially with a given enantiomer of the alcohol, as shown in Scheme 2), with ESI/MS analysis of the final mixture making it possible to evaluate the *ee* of the starting mixture of alcohols following Horeau and Nouaille's work.[3]



Scheme 2 Use of a pseudo-racemate for HT *ee* evaluation.

(b) MS "Tagging"

To allow the most to be made of MS techniques, and apply them to as broad a range of chemistries and compounds as possible, especially those with poor ionisation abilities, a variety of MS tagging strategies have been successfully applied. One representative example of this is the work reported by Szewczyk *et al.* and the development of a solution phase MS labelling method for HT reaction evaluation and optimisation.[6] The method, as described in Scheme 3, consists of a one pot acylation of a library of pyridine based substrates **1a–d** with 3,3-dimethylbut-1-ene and carbon monoxide, in the presence of $[Ru_3(CO)_{12}]$. The "tag", composed of four arginine residues and an *N*-terminal alkoxyamine (H_2N–O–$GlyArg_4$) was used to selectively label the products of the reaction mixture (**2a–c**) by oxime formation with any ketone functionality generated in the reaction. The "tag" not only guaranteed the ionisation of the products **3a–c** for MS detection, but also dominated it, allowing quantitative conclusions to be drawn from the integration of the MS peak areas, by comparison to an internal reference (2-pyridinecarboxaldehyde labelled with the (H_2N–O–$GlyArg_4$) tag). Furthermore, thanks to

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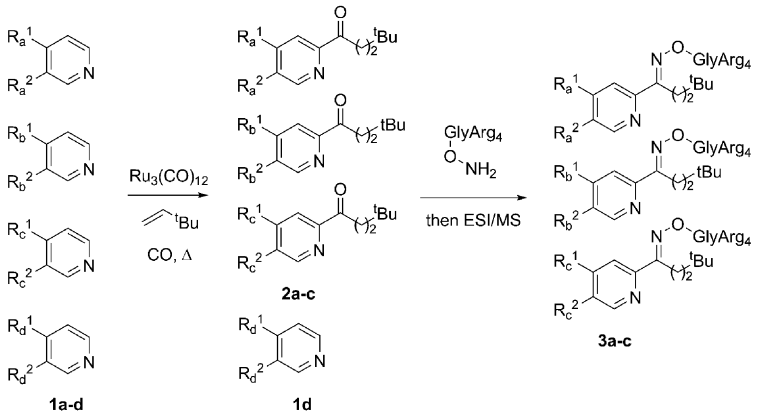


Scheme 3 MS tagging approach for HT reaction evaluation and optimisation (products **2a–c** are prepared with varying levels of success and their relative levels can be determined *via* ESI/MS following derivatisation).

the tag, only peaks corresponding to the products of the reaction will be identified whereas unreacted reagents *etc.*, are not ionised or detected.

This approach allowed the rapid evaluation of large numbers of substrates to define structure–reactivity relationships and reaction compatibility of functional groups. One big asset of this method is that it is applicable to virtually any reaction that generates a carbonyl group, thus allowing tag attachment.

(c) Analytical constructs

In the area of solid phase synthesis, a number of tools have been developed that have enhanced reaction analysis and those termed "analytical constructs" are perhaps the best known (Fig. 1). These "analytical constructs" incorporate features that allow the rapid and reliable qualitative analysis of reactions by the incorporation of a "MS sensitizer" tag, which guarantees uniform MS ionisation and sometimes also a "mass splitter" for the rapid identification of relevant peaks from the mass spectrum. These analytical constructs have been used mainly to identify products and monitor solid phase reactions,[7] but they have also proven useful in other applications, such as functional group compatibility studies,[8] and linker development.[9–11] Quantitative conclusions have been achieved, either by the incorporation of an ultraviolet (UV) chromophore into the construct, or using MS based quantitation, which clearly enhances the power of the concept. Such quantitative analytical constructs were developed on the basis of a quaternary ammonium species as an MS sensitizer and ionisation leveller and an aryl bromide as a peak splitter (see Fig. 1). Cleavage of mixtures of compounds linked to the construct and their direct positive ESI/MS (ESI+/MS) analysis afforded a set of peaks whose intensities were proportional to the amount of product in the mixture (Fig. 2).[12]

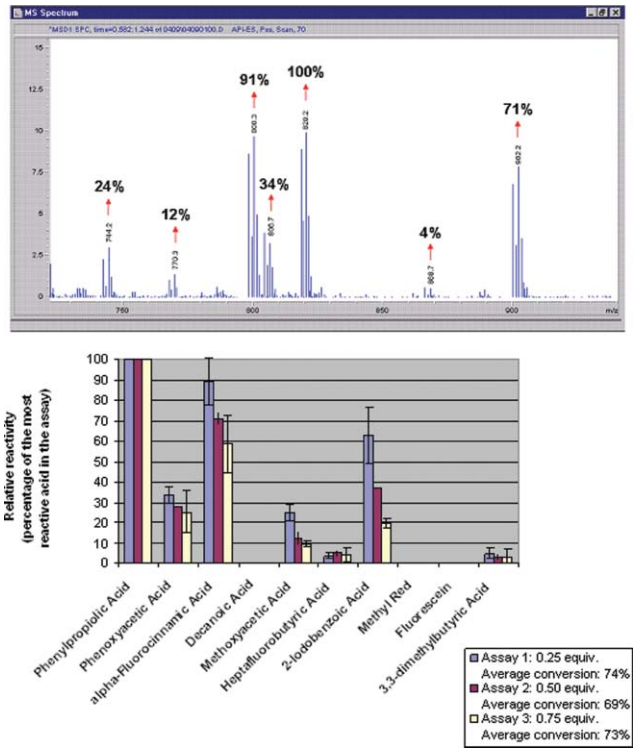


Fig. 2 Top: MS trace (of a crude reaction mixture, single injection) and bottom: monomer reactivity data obtained for the reaction described in Scheme 4.

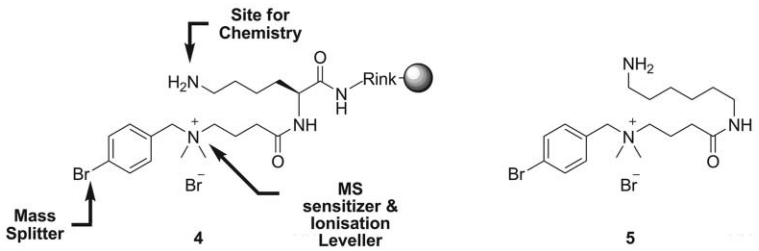


Fig. 1 Solid phase MS analytical construct (**4**) and its solution phase variant (**5**).

(i) HT monomer reactivity profiling. Currently, one of the bottlenecks of HT synthesis is the time and money wasted in the elaboration of libraries where combinations of reactants do not give the desired product in satisfactory yield and/or purity. One way to prevent this would be to "scan" rapidly all monomer combinations to determine if the desired compounds will be generated efficiently before embarking on the production of the complete library. However, it would be incredibly time consuming to test the monomers one by one, and therefore HT methods have been elaborated to achieve this. Quantitative analytical constructs turned out to be an extremely efficient means of evaluating the relative reactivity of a range of ten carboxylic acids in the Ugi-4 component condensation (4CC), with ESI+/MS as the sole analytical tool. To do so, the carboxylic acids **6a–j** were mixed and then reacted with the analytical construct **4**, hydrocinnamaldehyde **7** and cyclohexyl isonitrile **8**, to afford a mixture of α-acylamino amides **9a–j** as shown in Scheme 4.[12]

Identification and quantification of the cleaved products **9a–j** was rapidly achieved thanks to the properties of the analytical construct: quantification was made possible by the ionisation levelling property of the construct, while the relevant peaks were located in a "clean" region of the spectrum thanks to the added mass of the construct and were easily identified due to the bromine isotope pattern. Subsequent correlation of this data to the corresponding monomers **6a–j** allowed an assessment of their relative reactivities (Fig. 2) with the most reactive carboxylic acid being defined as 100% with the reactivity of the other building blocks expressed in relation to this.

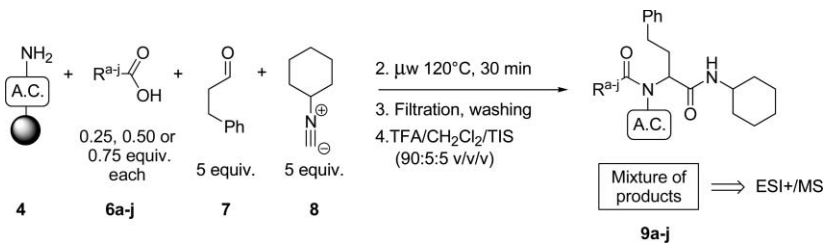
The mixtures of monomers **6a–j** could be studied at various concentrations to study the effect of relative building block concentration on reactivity (Fig. 2). The approach was also extremely efficient in terms of material, since the amount of each monomer used was typically around 100 μg (a few mg for the other components), while less than 2 mL of solvent were used per experiment. Several building blocks turned out to be unreactive in the Ugi-4CC, and would sensibly have to be taken out of the pool of starting materials if the Ugi-4CC were to be used to generate a library. Similar studies were undertaken with mixtures of ten isonitriles and ten aldehydes, where variations in concentration showed no remarkable change in reactivity profiles. The method was also carried out using the solution phase analytical construct **5**, allowing monomer reactivity profiling with just 0.1 eq. of each carboxylic acid.

The relative reactivity profiling of building blocks for a given reaction allows them to be gathered into groups of similar reactivity to ensure good yields and purities when it comes to prepare the final library of α-acylamino amides. Moreover, the fact that only the reactivity of the carboxylic acids happened to be concentration dependent has mechanistic implications. Analysis of these results from a broader perspective, suggests that the versatility of the ESI+/MS analytical construct, makes them applicable to a broad range of reactions, monomer rehearsal and reaction optimisation (Fig. 1).

(ii) HT Hammett parameter assessment. As shown previously, ESI+/MS quantitative analytical constructs allow the rapid investigation of relative reaction rates. In the case of families of substrates ranking enables an evaluation of the effect of substituents across the family. This principle was applied to the competitive displacement of a pentafluorophenyl ester functionality placed on the reactive site of the construct **4** (Fig. 1), by reaction of an equimolar mixture of aniline and various substituted anilines (*meta* or *para*). In such a case, the Hammett equation applies: $\log\left(\frac{k_X}{k_H}\right) = \rho\sigma_x$ and the relative reaction rates, translated by the ratio of the amides generated, depend on the intrinsic electronic effect of the substituent (σ parameter) and how these electronic effects are transmitted to the reaction centre (ρ parameter). One "pot" combinatorial Hammett plots were generated to allow assessment of the value of ρ for the reaction.[13] Having determined this value a HT method was designed to successfully measure σ_X's on more than 30 *para* and *meta* anilines (see Table 1), allowing rapid yet accurate assessment of σ's for any substituent. This is particularly useful for values not reported in the literature (custom-made groups and complicated substituents incompatible with previous methods of evaluation, *etc.*).

Table 1 Values of Hammett σ parameters determined for *meta* and *para* substituents on anilines using the HT MS approach

Substituent	σ_p^- lit.[14]	σ_p^- exp.[15]	σ_m lit.[14]	σ_m exp.[15]
Me	−0.17	−0.17 ±0.01	−0.07	−0.04 ± 0.01
^{t}Bu	−0.13	−0.13 ±0.02	−0.10	−0.12 ± 0.02
Cl	0.19	0.19 ±0.01	0.37	0.36 ± 0.01
I	0.27	0.27 ±0.01	0.35	0.31 ± 0.01
CF_3	0.65	0.64 ±0.01	0.43	0.40 ± 0.01
OH	−0.37	−0.37 ±0.01	0.12	0.05 ± 0.01
OMe	−0.26	−0.27 ±0.01	0.12	0.07 ± 0.02
SMe	0.15	0.12 ±0.01	0.15	0.12 ± 0.01
NMe_2	−0.16	−0.13 ±0.01	−0.16	−0.13 ± 0.01



Scheme 4 Solid phase Ugi-4CC using the "analytical construct" resin **4** as the amine component.

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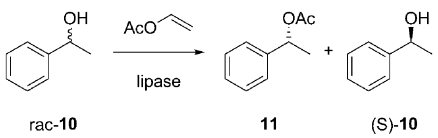
2 Image based methods for HT reaction kinetics and thermodynamic assessment

(a) Thermographic imaging

Davies *et al.* reported a general method to allow HT kinetic measurements of chemical reactions to be carried out by monitoring the change in temperature of the reaction mixture by thermographic imaging of microtitre plates.[16] Monitoring the temperature changes in the reaction mixtures permitted thermodynamic data to be obtained, in an effort to evaluate reaction enthalpies. The success of the study was relative since the development of the method required tedious modelling steps and the enthalpies obtained were much less than values obtained *via* reaction calorimetry. In the case of a calorimeter, heat losses to the environment are minimised whereas performing the experiment in an open microtitre plate leads to large and uncontrolled heat losses; however, this work made it possible to afford a correct ranking of the enthalpies in a HT manner and represents an important preliminary piece of work towards HT reaction enthalpy determination.

In the same way infrared (IR) thermography was successfully used as a HT screening method for looking at enantioselective reactions involving biocatalysts or chiral transition metal catalysts.[17] The work was based on the use of a modified microtitre plate where reactions, such as the lipase catalysed enantioselective acylation of 1-phenylethanol (Scheme 5) were carried out and temperature changes in the reaction wells monitored periodically thanks to the presence of an IR camera. Enantioselectivity was screened by performing the reaction in three separate wells respectively containing *rac*-**10**, (*S*)-**10** and (*R*)-**10** and comparing the temperature changes in the three wells, affording qualitative selectivity data. Again, this work is a very important step towards HT assessment of *ee*'s using IR thermography.



Scheme 5 Enzymatic resolution of *rac*-**10**.

On bead IR thermographic imaging was used by Taylor and Morken for the discovery of new polymer bound multifunctional catalysts for acyl transfer reactions from acetic anhydride to ethanol.[18] After mixing the reagents and the catalysts, the hot beads were picked and decoded (Fig. 3).

Among the 3150 catalysts that were screened, 23 beads were selected and the activity of the catalysts was investigated. The results obtained showed that, to a rough approximation, the HT assay was representative of the catalytic efficiency, and that further development of the technique might allow catalytic reaction kinetics to be followed on bead. However there are important issues with these experiments such as rates of energy transfer and the need to have beads that float or reagents that are IR transparent. As an alternative to IR thermography for catalytic activity screening, Connolly and Sutherland used an array of thermistors that allowed the monitoring of temperature changes of chemical and biochemical reactions by direct immersion in the reaction mixture.[19] Thanks to the 96-well plate format, the technique is readily adaptable to problems requiring HT thermal analysis, allowing increased accuracy, as well as measurements to be carried out through non IR transparent materials. The method could also be further developed to monitor other physical parameters by replacing the array of thermistors by an array of miniaturized probes, such as for pH, *etc.*

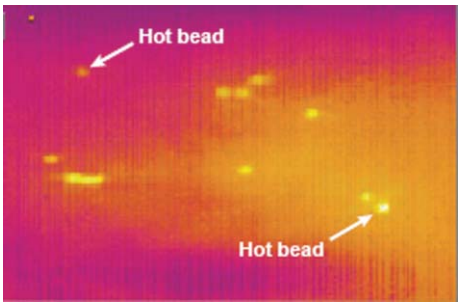


Fig. 3 IR thermographic image of 14 visible hot beads in the presence of several thousand noncatalyst beads (reproduced with permission).

(b) Thin layer chromatography (TLC) based analysis

In the key area of the discovery of new catalysts where the development of HT techniques is essential, colorimetric assays occupy a significant position. Quantitative TLC based assays have for example been developed by Garbacia *et al.* to perform HT screening of the composition of reaction mixtures.[20] By image analysis (with calibration), they were able to deduce the degree of reaction conversion from the intensity of the product spot, under 12 different reaction conditions (11 different metals or no metal). The technique not only enabled monitoring of conversion but also the qualitative assessment of the reaction mixture, making it possible to identify the existence of unexpected products, or reaction intermediates (see Fig. 4). The discovery of a new ruthenium-based catalyst for the Sonogashira coupling was made possible *via* this technique.

Conclusion

HT techniques have already demonstrated their power in enhancing synthetic processes, specifically in the development of new chemical entities. This naturally has led to the development of a wide portfolio of HT analytical tools and techniques many of which can be applied to studies in physical organic chemistry. HT reaction assessment has begun to be possible with the development of quantitative analytical constructs, which have been shown to be particularly powerful in allowing reaction conversion monitoring and these have now been applied in various applications, such as HT determination of enantiomeric excesses, HT monomer reactivity profiling and HT assessment of Hammett parameters. Those tools have shown much promise in terms of ease of use, versatility and throughput, and other applications of these constructs are expected in the near future. Other techniques also offer paths into the area, ranging from sophisticated thermal imaging to the use of simple, yet powerful quantitative parallel TLC analysis. However, whatever tools are applied the conclusion is clear, HT methods will fundamentally change the nature of physical organic chemistry and will offer routes to data generation,

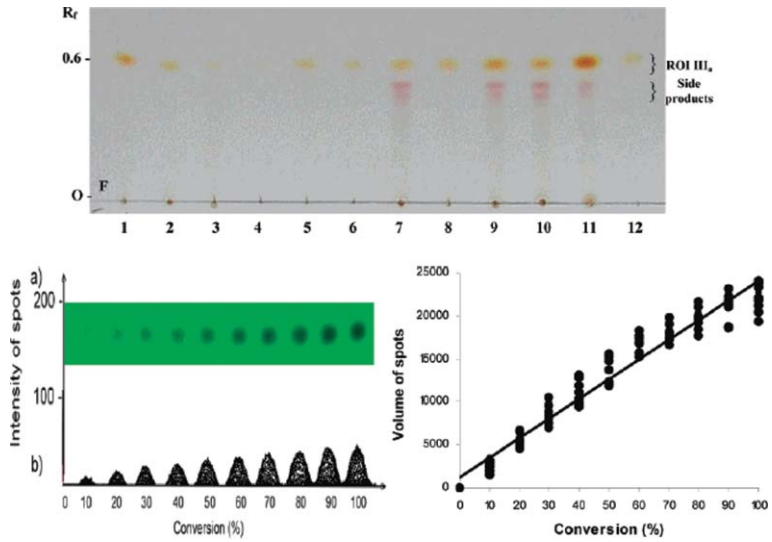


Fig. 4 Image of a TLC plate showing the composition of reaction mixtures (desired product is III_a) generated using different catalysts. Quantification was obtained by image analysis of the region of interest (ROI) and calibration (reproduced with permission).

and tools to understand reactions and mechanisms in a manner that will unshackle the field of physical organic chemistry.

Acknowledgements

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