Baldwin and Whitehead's Manzamine Alkaloids Biosynthesis Hypothesis Involves a Finely Tuned Reactivity of C_3 Unit: a High-Throughput Experimentation Approach

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ABSTRACT: A rapid analysis of mass spectrometry data generated from 96 multicomponent reactions using a herein-provided chemoinformatic workflow, have pinpointed relevant conditions tuning the reactivity of acrolein to fulfill Baldwin and Whitehead's manzamine alkaloids biosynthetic hypothesis. This strategy can become part of a general method for the analysis of information-rich high-throughput experiments of multicomponent reactions applied to natural product biosynthetic scenario.

Tools at the interface of chemical synthesis and data science that have considerably affected the daily workflow of organic chemists have emerged in recent years.[1] As such, computer-assisted synthetic planning, prediction of organic reaction outcomes, assisted drug discovery, and optimization of reaction conditions with high-throughput experimentations (HTE), just to name a few, all provide examples in which leveraging contemporary developments in robotic and algorithmic techniques enhances the field of application of chemical synthesis.[2] HTE enables synthetic chemists – through a reiterative process of design, execution, data analysis, and hit identification – to efficiently explore conditions for reaction optimization and reactivity pattern discovery.[3] Although much of the recent effort has been invested in broadening the menu of reactions and synthetic targets (especially C–C and C–N bond-forming transformations), the use of HTE to tackle biochemical questions is still missing. More precisely, the *chemical feasibility* assessment of a biosynthetic proposal through HTE of multicomponent reactions (MCRs) has not been reported so far. Furthermore, deconvolution algorithms and automated workflows that process analytical read-out, generated from HTE-induced reaction space to highlight minor peaks as potentially novel reaction products, are still in their infancy.[4] Inspired by landmark advances provided by computational metabolomics tools[5] and recently applied to organic chemistry,[6] we developed a chemoinformatic workflow that processes HTE-based LC-MS/MS outputs to address Baldwin and Whitehead's hypothesis of manzamine alkaloids (retro)biosynthesis. Indeed, in 1992, in a milestone paper in the field of marine natural product chemistry entitled "On the Biosynthesis of Manzamines",[7] Baldwin and Whitehead disclosed their biosynthetic hypothesis for manzamine A (**1**, Scheme 1), one of the most intriguing alkaloids discovered in the 80ies,[8] and related manzamine-type alkaloids such as keramaphidin B (**2**) and ircinal A (**3**), the precursor of **1**. Notably, compounds **2** and **3** were discovered after the 1992 publication,[9] illustrating the sharp intuition of the authors. A MCR-type scenario involving fatty dialdehydes, a nitrogen source, and acrolein (**4**, Scheme 1) as a key C_3 unit was put forward by Baldwin and Whitehead to explain the formation of 3-alkyl substituted dihydropyridinium salts yielding manzamine-type alkaloids through an intramolecular Diels-Alder reaction (Scheme 1). This key C_3 unit can be traced in the structure of most manzamine-type alkaloids,[10] mainly isolated from Haplosclerida and Dictyoceratida marine sponges. While the *in vivo* existence of **4** is known,[11] its implication as a key intermediate in biosynthetic pathways remains totally elusive even 30 years after Baldwin and Whitehead's proposal despite the continuous discovery of new manzamine-type alkaloids.[12]

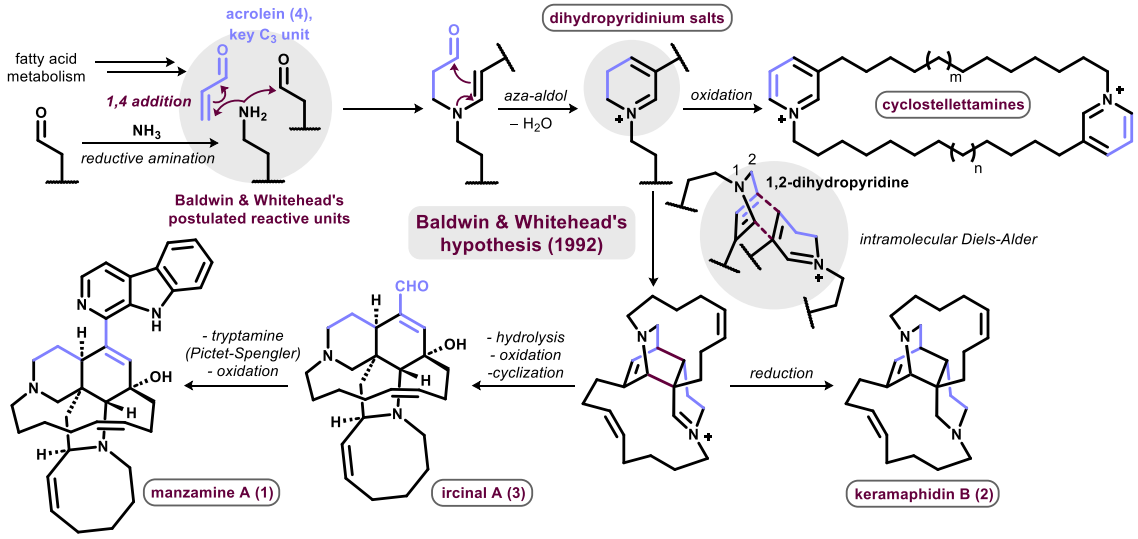
Based on this biosynthetic proposal, several bioinspired approaches were conducted, beautifully demonstrating the relevance of models starting from 3-alkyldihydropyridiniums. This culminated in the total synthesis of pyridinium salts cyclostellettamines and related compounds[13] as well as keramaphidin B.[14] However, an MCR-type scenario starting from free precursors, i.e., a primary amine, an aldehyde, and acrolein (**4**), the key C_3 unit, was never investigated probably due to their inherent high reactivity. Therefore, this work aims to investigate Baldwin and Whitehead's biosynthetic model in terms of "chemical feasibility" starting from the three above-mentioned free precursors following a HTE of MCRs.

To investigate Baldwin and Whitehead's hypothesis, Keramaphidin B (**2**) was selected as a case study and simplified to model compound **5** (Scheme 2), named herein "keramaphidin B scaffold". This latter was retrosynthetically disconnected to three reactive units consistent with the biosynthetic proposal: hexanal (**6**), butylamine (**7**), and acrolein (**4**). After a first « naive » MCR conducted in MeOH with NEt_3, an unexpected compound was isolated and characterized named therein "*iso*-scaffold" (**8**, Scheme 2) which is notably isomeric to **5** (Experimental procedure S2, Supporting Information). This type of scaffold was described earlier from various 1,4-tetrahydropyridines including biologically relevant nicotinamides and its importance in our study will be discussed below.[15]

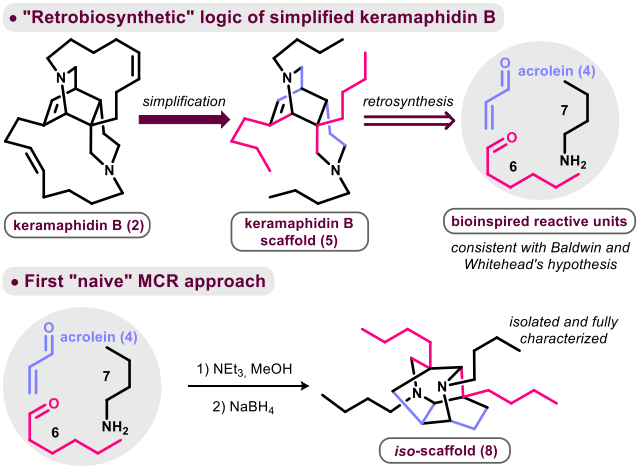
Our mechanism proposal (Scheme 3) for the formation of **8** would imply, in this context, the emergence of a new reactivity pattern from acrolein underlying formally an "upside-down" reactivity. Indeed, two different pathways are conceivable from the three reactive units **4**, **6**, and **7**. For the first one, the formal 1,4-addition of butylamine **7** onto acrolein **4** provides the needed 1,2-dihydropyridine **9** yielding keramaphidin B scaffold **5** through an intermolecular Diels-Alder reaction followed by a reduction. For the second one, the formal 1,2-addition provides a central 1,4-dihydropyridine **10** yielding *iso*-scaffold **8**.

Importantly, this second pathway clearly constitutes a dead-end scenario to fulfil Baldwin and Whitehead's model.

Scheme 1. Baldwin and Whitehead's Biosynthetic Hypothesis for Manzamine (1) and Manzamine-Type Alkaloids



Scheme 2. "Retrobiosynthetic" Logic from Keramaphidin B (2) and First MCR Approach



Therefore, to drive acrolein reactivity towards **9** and to demonstrate the "chemical feasibility" of this long-lasting hypothesis, a set of MCRs was rationalized in terms of biomimetic logic aiming at creating abiotic but bioinspired conditions in a protecting group-free fashion. Briefly, different *chemical* environments of the enzymatic active site were tentatively mimicked with solvent of decreasing polarity (i.e., aqueous TRIS/HCl buffer, EtOH and, CH_2Cl_2), combined to diverse catalytic conditions such as general acido-basic catalysis (i.e., silica gel and Ketjen-Bekkum reagent[16]), Lewis acid catalysis (i.e., $ZnCl_2$ and $Bi(OTf)_3$) or covalent enamine catalysis (i.e., morpholine, pyrrolidine and, triethylamine) (Figure S49, Supporting Information). Moreover, the reagent addition order was also explored. In total, 96 individual MCRs were performed applying an HTE-based approach including a treatment with sodium borohydride as a way to mimic a needed hydrogenase-type reduction at a final step of the biosynthetic pathway. Each reaction was labelled as the following reaction code: #reaction number (1.x *for solvent* / 2.x *for catalyst* / 3.x *for the reagent addition order*). The exploration of those complex reaction mixtures, considered herein as "bioinspired synthetic metabolomes", required the design of a targeted mass spectrometry-based metabolomics profiling workflow (Figure 1) inspired from our previous "chemistry-first strategy" in natural product discovery.[17] According to this strategy, model compounds **5** (keramaphidin B scaffold) and **8** (*iso*-scaffold), both acting as synthetic probes, were upstream synthesized (Experimental procedure S1, Supporting Information) in order to easily access to their analytical read-out (i.e., HRMS, MS/MS, RT). Therefore, the formation of both probes in the 96 MCRs could be tracked with a high level of confidence (level 1 according to Metabolomics MSI guidelines).[18] To proceed, the 96 reaction mixtures were profiled by targeted UPLC-HRMS/MS (*m/z* = 389.389 $[M+H]^+$ corresponding to isomeric probes **5** and **8**) with a retention time constraint (Experimental procedure S4, Supporting Information). In addition, strict quality control was imposed to the study. In this way, synthetic probe vials were analyzed every ten injections to monitor the retention time drift (Table S2–S3 and Figure S52, Supporting Information) and to ensure the exactness of our results.[19] The obtained spectral space was processed using an MZmine3[20] batch mode in a sample centric manner and annotated with our standard MS/MS spectra (Experimental procedure S5 and Data availability, Supporting Information).

Satisfyingly, application of this strategy combining HTE and chemoinformatics, enhanced by an in-house Python script converting the annotation and quantification table files generated by MZmine 3 into cosine score and relative abundance-based heatmaps, has allowed an efficient and reliable analysis of the 96 MCRs resulting in the identification of keramaphidin B scaffold (**5**) and *iso*-scaffold (**8**) in 23 (#33, #34, #35, #36, #39, #40, #41, #42, #43, #44, #47, #48, #56, #57, #58, #59, #60, #61, #64, #72, #80, #88, #96) and 11 (#49, #50, #51, #52, #53, #54, #56, #71, #79, #87, #95) reaction mixtures, respectively (Figure S54 and Table S4, Supporting Information).

Scheme 3. Proposed Mechanism for the Formation of Keramaphidin B Scaffold (5) and *Iso*-Scaffold (8)

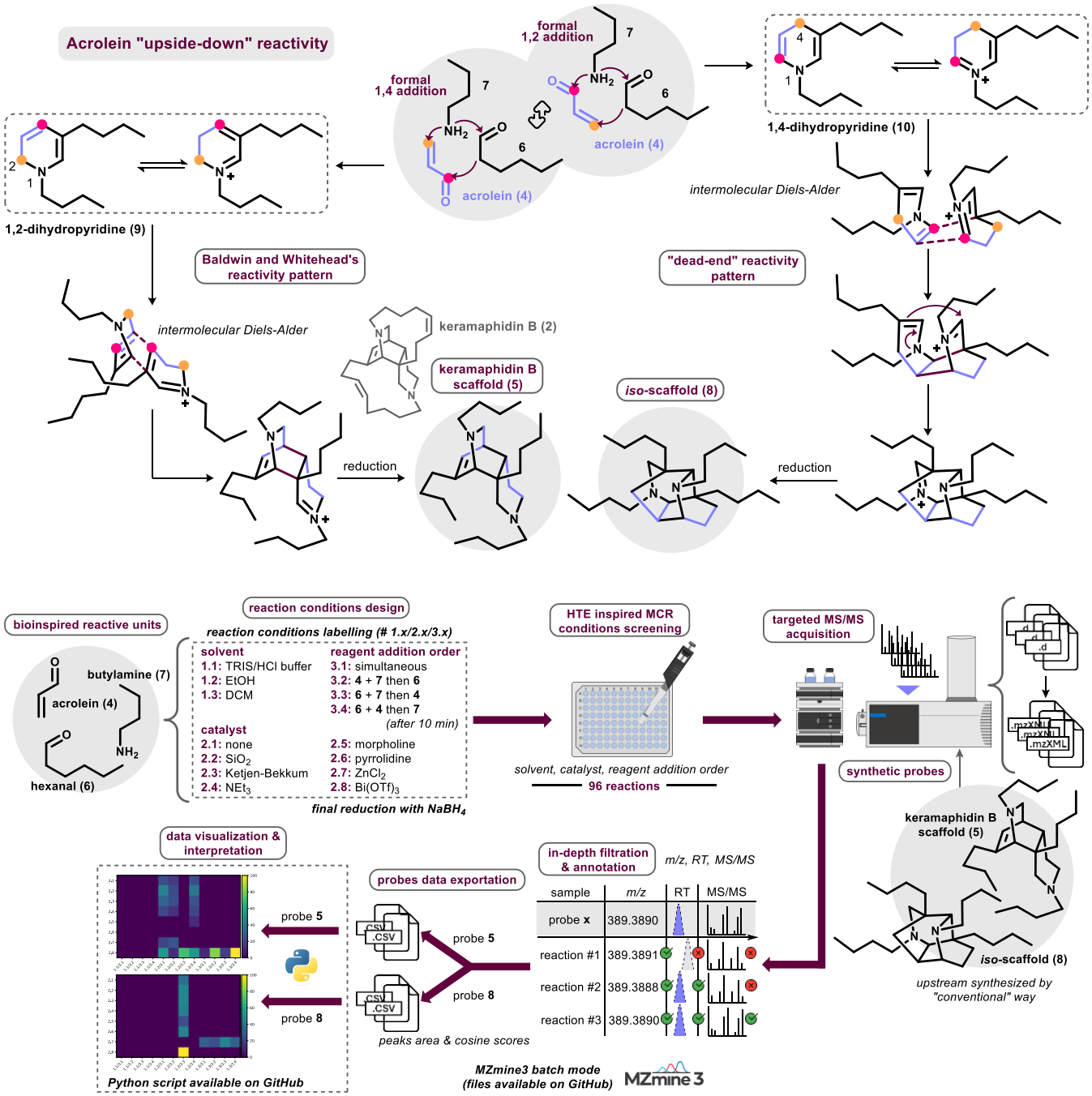


Figure 1. Overview of HTE of MCRs workflow coupled to targeted mass spectrometry analysis and chemoinformatic processing.

Notably, formation of both **5** and **8** pinpoints the "volatile" reactivity of acrolein (**4**) and demonstrates the emergence of Baldwin and Whitehead's chemical space by producing an impressive MCR cascade from this key starting material. Moreover, a cursory examination of the generated heatmaps (Figure 2) reveals salient reactivity patterns for keramaphidin B scaffold (**5**) and *iso*-scaffold (**8**).

Among the remarkable insights provided by the overall analysis of the heatmaps, it appears that both probes are largely promoted in EtOH (solvent(1.2)), which is probably due to its ability to perform hydrogen bonds with carbonyl compounds (i.e., hexanal and acrolein) for enhancing their electrophilic properties,[21] as well as with the use of Lewis acids $ZnCl_2$ (catalyst(2.7)) and $Bi(OTf)_3$ (catalyst(2.8)), which are also known to activate such chemical functions and catalyze Michael addition reactions.[22] Interestingly, **5** can be obtained in DCM (solvent(1.3)) if $Bi(OTf)_3$ (catalyst(2.8)) is used as a catalyst (Figure 2, top). A similar reactivity has been reported elsewhere.[22a] In the same fashion, $ZnCl_2$ (catalyst(2.7)) enables the formation of *iso*-scaffold (**8**) in DCM (solvent(1.3)) (Figure 2, bottom). In addition, these both Lewis acids appear to be insensitive towards reagent addition order. However, when EtOH (solvent(1.2)) was used as a solvent, the reagent addition order seems to have a noteworthy influence on the MCR outcomes.

For instance, Figure 2 (bottom) shows that, except when $Bi(OTf)_3$ (catalyst(2.8)) was used, the addition order (3.3) involving hexanal (**6**) with butylamine (**7**) followed by acrolein (**4**), *iso*-scaffold (**8**) was selectively obtained. The importance of the reagent addition order on MCR outcomes has been already observed in various scenarios.[23] At last, since **5** and **8** were not formed in TRIS/HCl aqueous buffer (solvent(1.1)), it appears that such solvent is not adapted to our study.

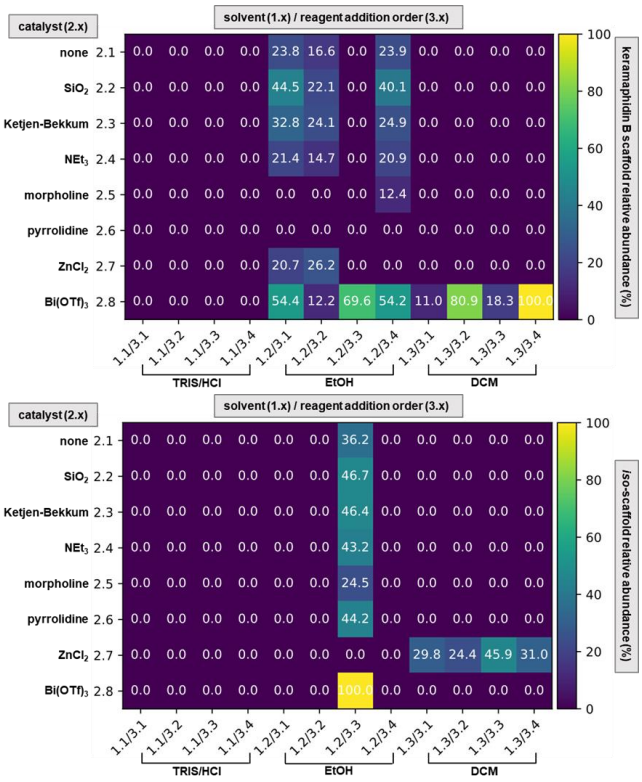


Figure 2. Heatmaps depicting keramaphidin B scaffold (**5**) relative abundance (top) and *iso*-scaffold (**8**) relative abundance (bottom) depending on reaction conditions.

To conclude, by applying a chemoinformatic workflow that processes HTE-based LC-MS/MS outputs, we were able to demonstrate the chemical feasibility of Baldwin and Whitehead's hypothesis for manzamine-type alkaloids biosynthesis. The entire data processing was automated by applying a MZmine3 batch mode associated to an in-house Python script accessible at https://github.com/AxelLebld/Peaklist-to-heatmap. We foresee that this strategy will be part of a general method to efficiently analyze the outputs generated by HTE-based MCRs. In this study, 96 individual MCRs were designed according to biosynthetic considerations. Three starting reactive units were used as surrogates of postulated biosynthetic precursors including acrolein (**4**) as the key C_3 unit. An "upside-down" reactivity pattern of acrolein was revealed and highlighted leading whether to the correct arrangement to fulfill Baldwin and Whitehead's proposal with the formation of keramaphidin B scaffold (**5**) or to a dead-end outcome exemplified by *iso*-scaffold (**8**). This work is obviously not a proof of the involvement of such a metabolic pathway *in vivo* but it allows not to refute the hypothesis outright. Manzamine alkaloids are clearly one of the still unlocked main challenges in the field of natural product biosynthesis. In that context, the C_3-unit hypothesis put forward by Baldwin and Whitehead which was slightly modified by Marazano and coll. in the 1990s and 2000s (with the intervention of malondialdehyde and leading in 2008 to a mixed scenario)[13a, 24] may be considered as a guiding principle for genome mining of putative biosynthetic gene clusters and enzymes.

ASSOCIATED CONTENT

Data Availability Statement

The Python script and data sets (MZmine output files) used to produce the reported results can be found at https://github.com/Axel-Lebld/Peaklist-to-heatmap.

Supporting Information

Full description of the experimental procedures including probes **5** and **8** synthesis, 1D and 2D NMR spectra, experimental design for HTE, analytical details about targeted mass spectrometry analysis, data processing workflow, and data availability.

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Author Contributions

The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Shen, Y.; Borowski, J. E.; Hardy, M. A.; Sarpong, R.; Doyle, A. G.; Cernak, T. Automation and computer-assisted planning for chemical synthesis. *Nat. Rev. Methods Primers* **2021**, *1* (1), 1–23.

(2) (a) Wołos, A.; Koszelewski, D.; Roszak, R.; Szymkuć, S.; Moskal, M.; Ostaszewski, R.; Herrera, B. T.; Maier, J. M.; Brezicki, G.; Samuel, J.; et al. Computer-designed repurposing of chemical wastes into drugs. *Nature* **2022**, *604* (7907), 668–676. (b) Mahjour, B.; Shen, Y.; Cernak, T. Ultrahigh-throughput experimentation for information-rich chemical synthesis. *Acc. Chem. Res.* **2021**, *54* (10), 2337–2346. (c) Mahjour, B.; Hoffstadt, J.; Cernak, T. Designing Chemical Reaction Arrays Using Phactor and ChatGPT. *Org. Process Res. Dev.* **2023**, *27* (8), 1510–1516. (d) Shim, E.; Tewari, A.; Cernak, T.; Zimmerman, P. M. Machine Learning Strategies for Reaction Development: Toward the Low-Data Limit. *J. Chem. Inf. Model.* **2023**, *63* (12), 3659–3668. (e) Mullowney, M. W.; Duncan, K. R.; Elsayed, S. S.; Garg, N.; van der Hooft, J. J. J.; Martin, N. I.; Meijer, D.; Terlouw, B. R.; Biermann, F.; Blin, K.; et al. Artificial intelligence for natural product drug discovery. *Nat. Rev. Drug Discov.* **2023**. (f) Molga, K.; Szymkuc, S.; Grzybowski, B. A. Chemist ex machina: advanced synthesis planning

by computers. *Acc. Chem. Res.* **2021**, *54* (5), 1094–1106. (g) Zahrt, A. F.; Henle, J. J.; Rose, B. T.; Wang, Y.; Darrow, W. T.; Denmark, S. E. Prediction of higher-selectivity catalysts by computer-driven workflow and machine learning. *Science* **2019**, *363* (6424), eaau5631. (h) Schwaller, P.; Laino, T.; Gaudin, T.; Bolgar, P.; Hunter, C. A.; Bekas, C.; Lee, A. A. Molecular transformer: a model for uncertainty-calibrated chemical reaction prediction. *ACS Cent. Sci.* **2019**, *5* (9), 1572–1583. (i) Taylor, C. J.; Pomberger, A.; Felton, K. C.; Grainger, R.; Barecka, M.; Chamberlain, T. W.; Bourne, R. A.; Johnson, C. N.; Lapkin, A. A. A Brief Introduction to Chemical Reaction Optimization. *Chem. Rev*. **2023**, *123* (6), 3089–3126.

(3) (a) Mahjour, B.; Zhang, R.; Shen, Y.; McGrath, A.; Zhao, R.; Mohamed, O. G.; Lin, Y.; Zhang, Z.; Douthwaite, J. L.; Tripathi, A.; et al. Rapid planning and analysis of high-throughput experiment arrays for reaction discovery. *Nat. Commun.* **2023**, *14* (1). (b) Biyani, S. A.; Moriuchi, Y. W.; Thompson, D. H. Advancement in organic synthesis through high throughput experimentation. *Chem. Methods* **2021**, *1* (7), 323–339.

(4) (a) Le, M. T.; Morato, N. M.; Kaerner, A.; Welch, C. J.; Cooks, R. G. Fragmentation of polyfunctional compounds recorded using automated high-throughput desorption electrospray ionization. *J. Am. Soc. Mass. Spectrom.* **2021**, *32* (8), 2261–2273. (b) Troshin, K.; Hartwig, J. F. Snap deconvolution: An informatics approach to high-throughput discovery of catalytic reactions. *Science* **2017**, *357* (6347), 175–181. (c) McNally, A.; Prier, C. K.; MacMillan, D. W. Discovery of an α-amino C–H arylation reaction using the strategy of accelerated serendipity. *Science* **2011**, *334* (6059), 1114–1117.

(5) (a) Beniddir, M. A.; Kang, K. B.; Genta-Jouve, G.; Huber, F.; Rogers, S.; Van Der Hooft, J. J. Advances in decomposing complex metabolite mixtures using substructure-and network-based computational metabolomics approaches. *Natural product reports* **2021**. (b) Fox Ramos, A. E.; Evanno, L.; Poupon, E.; Champy, P.; Beniddir, M. A. Natural products targeting strategies involving molecular networking: different manners, one goal. *Natural Product Reports* **2019**, *36* (7), 960–980.

(6) (a) Chung, H.-H.; Kao, C.-Y.; Wang, T.-S. A.; Chu, J.; Pei, J.; Hsu, C.-C. Reaction Tracking and High-Throughput Screening of Active Compounds in Combinatorial Chemistry by Tandem Mass Spectrometry Molecular Networking. *Anal. Chem.* **2021**, *93* (4), 2456–2463. (b) Turpin, V.; Beniddir, M. A.; Genta-Jouve, G.; Skiredj, A.; Gallard, J. F.; Leblanc, K.; Le Pogam, P.; Poupon, E. In silico anticipation of metabolic pathways extended to organic chemistry reactions: a case study with caffeine alkaline hydrolysis and the origin of camellimidazoles. *Chem. Eur. J.* **2020**, *26* (57), 12936–12940. (c) Gentry, E. C.; Collins, S. L.; Panitchpakdi, M.; Belda-Ferre, P.; Stewart, A. K.; Carrillo Terrazas, M.; Lu, H. H.; Zuffa, S.; Yan, T.; Avila-Pacheco, J.; et al. Reverse metabolomics for the discovery of chemical structures from humans. *Nature* **2023**. (d) Leblond, A.; Houari, I.; Beauxis, Y.; Leblanc, K.; Poupon, E.; Beniddir, M. A. Chemoinformatic Exploration of "Bioinspired Metabolomes" Illuminates Diacetyl Assembly Pathways Toward Nesteretal A-Like Cage Molecules. *Org. Lett.* **2022**, *24* (5), 1247–1252.

(7) Baldwin, J. E.; Whitehead, R. C. On the Biosynthesis of Manzamines. *Tetrahedron Lett.* **1992**, *33* (15), 2059–2062.

(8) Sakai, R.; Higa, T.; Jefford, C. W.; Bernardinelli, G. Manzamine A, a novel antitumor alkaloid from a sponge. *J. Am. Chem. Soc.* **1986**, *108* (20), 6404–6405.

(9) (a) Kobayashi, J. i.; Tsuda, M.; Kawasaki, N.; Matsumoto, K.; Adachi, T. Keramaphidin B, a novel pentacyclic alkaloid from a marine sponge Amphimedon sp.: a plausible biogenetic precursor of manzamine alkaloids. *Tetrahedron Lett.* **1994**, *35* (25), 4383–4386. (b) Kondo, K.; Shigemori, H.; Kikuchi, Y.; Ishibashi, M.; Sasaki, T.; Kobayashi, J. Ircinals A and B from the Okinawan marine sponge Ircinia sp.: plausible biogenetic precursors of manzamine alkaloids. *J. Org. Chem.* **1992**, *57* (8), 2480–2483.

(10) Piwko, A. T.; Miller, B. G.; Smith, J. M. Revisiting the manzamine biosynthetic hypothesis. *Nat. Prod. Rep.* **2023**, *40* (5), 964–971.

(11) (a) Stevens, J. F.; Maier, C. S. Acrolein: sources, metabolism, and biomolecular interactions relevant to human health and disease. *Mol. Nutr. Food Res.* **2008**, *52* (1), 7–25. (b) Zhang, J.; Sturla, S.; Lacroix, C.; Schwab, C. Gut Microbial Glycerol Metabolism as an Endogenous Acrolein Source. *mBio* **2018**, *9* (1), 10.1128/mbio.01947–01917.

(12) (a) Kim, C. K.; Riswanto, R.; Won, T. H.; Kim, H.; Elya, B.; Sim, C. J.; Oh, D. C.; Oh, K. B.; Shin, J. Manzamine Alkaloids from an Acanthostrongylophora sp. Sponge. *J. Nat. Prod.* **2017**, *80* (5), 1575–1583. (b) Kubota, T.; Nakamura, K.; Kurimoto, S. I.; Sakai, K.; Fromont, J.; Gonoi, T.; Kobayashi, J. Zamamidine D, a Manzamine Alkaloid from an Okinawan Amphimedon sp. Marine Sponge. *J. Nat. Prod.* **2017**, *80* (4), 1196–1199. (c) Kurimoto, S. I.; Suzuki, S.; Ueno, M.; Fromont, J.; Kobayashi, J.; Kubota, T. Zamamiphidins B and C, Manzamine-Related Alkaloids from an Amphimedon sp. Marine Sponge Collected in Okinawa. *J. Nat. Prod.* **2022**, *85* (9), 2226–2231.

(13) (a) Kaiser, A.; Billot, X.; Gateau-Olesker, A.; Marazano, C.; Das, B. C. Selective entry to the dimeric or oligomeric pyridinium sponge macrocycles via aminopentadienal derivatives. Possible biogenetic relevance with manzamine alkaloids. *J. Am. Chem. Soc.* **1998**, *120* (32), 8026–8034. (b) Wanner, M. J.; Koomen, G. J. Synthesis of the Cyclostellettamines A− F and Related Bis (3-alkylpyridinium) Macrocycles. *Eur. J. Org. Chem.* **1998**, *1998* (5), 889–895. (c) Timm, C.; Volk, C.; Sasse, F.; Köck, M. The first cyclic monomeric 3-alkylpyridinium alkaloid from natural sources: identification, synthesis, and biological activity. *Organic & Biomolecular Chemistry* **2008**, *6* (21), 4036–4040. (d) Morimoto, Y.; Yokoe, C. Total synthesis of haliclamine A, a macrocyclic marine alkaloid related to the key biogenetic intermediate of manzamines. *Tetrahedron Lett.* **1997**, *38* (52), 8981–8984. (e) Shorey, B. J.; Lee, V.; Baldwin, J. E. Synthesis of the Arctic sponge alkaloid viscosaline and the marine sponge alkaloid theonelladin C. *Tetrahedron* **2007**, *63* (25), 5587–5592. (f) Baldwin, J.; Melman, A.; Lee, V.; Firkin, C.; Whitehead, R. Biomimetic synthesis of (-)-Xestospongin A,(+)-Xestospongin C,(+)-Araguspongine B and the correction of their absolute configurations. *J. Am. Chem. Soc*. **1998**, *120* (33). (g) Kaiser, A.; Marazano, C.; Maier, M. First Synthesis of Marine Sponge Alkaloid Niphatoxin B. *J. Org. Chem.* **1999**, *64* (10), 3778–3782.

(14) (a) Baldwin, J. E.; Claridge, T. D.; Culshaw, A. J.; Heupel, F. A.; Lee, V.; Spring, D. R.; Whitehead, R. C. Studies on the biomimetic synthesis of the manzamine alkaloids. *Chem. Eur. J.* **1999**, *5*, 3154–3161. (b) Baldwin, J. E.; Claridge, T. D.; Culshaw, A. J.; Heupel, F. A.; Lee, V.; Spring, D. R.; Whitehead, R. C.; Boughtflower, R. J.; Mutton, I. M.; Upton, R. J. Investigations into the manzamine alkaloid biosynthetic hypothesis. *Angew. Chem. Int. Ed.* **1998**, *37* (19), 2661–2663.

(15) Jakubowicz, K.; Wong, Y.-S.; Chiaroni, A.; Bénéchie, M.; Marazano, C. New polycyclic diamine scaffolds from dimerization of 3-alkyl-1, 4-dihydropyridines in acidic medium. *J. Org. Chem.* **2005**, *70* (19), 7780–7783.

(16) Roelofsen, D.; Van Bekkum, H. The synthesis of enamines and ketimines using molecular sieves. *Recl. Trav. Chim. Pays-Bas* **1972**, *91* (5), 605–610.

(17) Bonneau, N.; Chen, G.; Lachkar, D.; Boufridi, A.; Gallard, J.-F.; Retailleau, P.; Petek, S.; Debitus, C.; Evanno, L.; Beniddir, M. A.; et al. An Unprecedented Blue Chromophore Found in Nature using a "Chemistry First" and Molecular Networking Approach: Discovery of Dactylocyanines A–H. *Chem. Eur. J.* **2017**, *23* (58), 14454-14461.

(18) Schymanski, E. L.; Jeon, J.; Gulde, R.; Fenner, K.; Ruff, M.; Singer, H. P.; Hollender, J. Identifying small molecules via high resolution mass spectrometry: communicating confidence. *Environ. Sci. Technol.* **2014**, *48* (4), 2097–2098.

(19) Broadhurst, D.; Goodacre, R.; Reinke, S. N.; Kuligowski, J.; Wilson, I. D.; Lewis, M. R.; Dunn, W. B. Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies. *Metabolomics* **2018**, *14* (6), 72.

(20) Schmid, R.; Heuckeroth, S.; Korf, A.; Smirnov, A.; Myers, O.; Dyrlund, T. S.; Bushuiev, R.; Murray, K. J.; Hoffmann, N.; Lu, M.; et al. Integrative analysis of multimodal mass spectrometry data in MZmine 3. *Nat. Biotechnol.* **2023**, *41* (4), 447–449.

(21) Shirakawa, S.; Shimizu, S. Hydrogen-Bond-Promoted C-C Bond-Forming Reaction: Catalyst-Free Michael Addition Reactions in Ethanol. *Synlett* **2007**, *2007* (20), 3160–3164.

(22) (a) El-Remaily, M. A. E. A. A. Bismuth triflate: A highly efficient catalyst for the synthesis of bio-active coumarin compounds

via one-pot multi-component reaction. *Chinese J. Catal.* **2015**, *36* (7), 1124–1130. (b) Borthakur, U.; Saikia, A. K. Bismuth(III)-Triflate-Catalyzed Highly Diastereoselective Synthesis of Substituted Tetrahydrothiophene via Tandem Isomerization, Michael and Aldol Reactions. *ChemistrySelect* **2019**, *4* (37), 11136–11139. (c) Khan, M. M.; Saigal; Khan, S. One-Pot Knoevenagel–Michael–Cyclization Cascade Reaction for the Synthesis of Functionalized Novel 4H-pyrans by Using ZnCl2 as a Catalyst. *J. Heterocycl. Chem.* **2019**, *56* (3), 1020–1029.

(23) (a) Zhu, Q.; Jiang, H.; Li, J.; Liu, S.; Xia, C.; Zhang, M. Concise and Versatile Multicomponent Synthesis of Multisubstituted Polyfunctional Dihydropyrroles. *J. Comb. Chem.* **2009**, *11* (4), 685–696. (b) Wang, M.; Fu, Z.; Feng, H.; Dong, Y.; Liu, J.; Liu, Q. Tandem [4 + 1 + 1] annulation and metal-free aerobic oxidative aromatization: straightforward synthesis of highly substituted phenols from one aldehyde and two ketones. *Chem. Commun.* **2010**, *46* (47), 9061–9063. (c) Shao, J.; Houghten, R. A.; Dooley, C. T.; Cazares, M.; McLaughlin, J. P.; Eans, S. O.; Ganno, M. L.; Hoot, M. R.; Giulianotti, M. A.; Yu, Y. A one-pot multicomponent approach to a new series of morphine derivatives and their biological evaluation. *Org. Biomol. Chem.* **2017**, *15* (37), 7796–7801. (d) Belabbes, A.; Selva, V.; Foubelo, F.; De Gracia Retamosa, M.; Sansano, J. M. Synthesis of Spiro{pyrrolidine-3,1′-pyrrolo[3,4-c]pyrrole} Basic Framework by Multicomponent 1,3-Dipolar Cycloaddition. *Eur. J. Org. Chem.* **2021**, *2021* (29), 4229–4236.

(24) Wypych, J. C.; Nguyen, T. M.; Nuhant, P.; Bénéchie, M.; Marazano, C. Further insight from model experiments into a possible scenario concerning the origin of manzamine alkaloids. *Angew. Chem. Int. Ed.* **2008**, *47* (29), 5418–5421.