Chemoselective Enrichment as a Tool to Increase Access to Bioactive Natural Products: Case
Study Borrelidin

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ABSTRACT: Chemoselective purification technologies have seen great success in biomolecule isolation, with a classic example being the genetically-encoded His tag utilized to enrich desired proteins from a crude lysate. We sought to translate this purification tactic into a powerful tool for the isolation of natural products and demonstrate that chemoselective enrichment can reduce the number of purification steps required and increase the yield obtained for important natural products, as compared to the use of traditional chromatography methods alone. To date, we have reported reversible enrichment tags for three functional groups, carboxylic acids and aliphatic or aryl hydroxyls. To illustrate the power of chemoselectivity-mediated purification of natural products, we present here an improved isolation of borrelidin. Application of our carboxylic acid tag yielded pure borrelidin in only two steps and with double the yield acquired with traditional chromatography methods. These results highlight the utility of this orthogonal strategy to facilitate the isolation of natural products, which are often present in minute quantities in their source materials.

Natural products, derivatives thereof and compounds inspired by these often complex molecular scaffolds represent a significant proportion, ~60%, of the therapeutic agents currently on the market.¹ As the drug discovery pipeline continues to shrink, a resurgence of natural product discovery is needed as these molecules have consistently played pivotal roles in antibiotic, anticancer, and antifungal therapy.² However, the identification of bioactive natural products still remains a daunting task despite recent advances in purification and detection techniques. Even after a bioactive lead has been uncovered, synthesis and evaluation of a number of derivatives is often required to minimize undesirable properties such as off-target effects and cytotoxicity. Structure activity relationship (SAR) studies both enable assignment of the

molecular features required for the desired activity and reveal the moieties that can be removed or replaced to improve pharmacokinetic properties. An example of a natural product whose scaffold possesses a wide range of biological activities and for which SAR studies have, and should continue to be pursued, is borrelidin (**1**; **Figure 1b**). This macrolide was discovered from the extract of *Streptomyces rochei* in 1949 and subsequently detected in other *Streptomyces* strains.³ Numerous synthetic,⁴⁻⁶ isolation,⁷⁻⁹ and bioactivity¹⁰⁻¹³ studies have been performed highlighting borrelidin's significance. Of note, this compound has activity against the bacterium *Borrelia*, the causative agent of Lyme disease, for which borrelidin was named.³

Figure 1. Chemoselective enrichment scheme and the target molecule for these studies. (a) Reversible enrichment resins for isolation of the carboxylic acid or hydroxyl moiety. Chemoselective capture dramatically simplifies the compound pool that must subsequently be separated. Natural products are captured onto resin in the presence of base (Et_3N) and when desired, released using a fluoride source ($\text{HF}\cdot\text{pyridine/pyridine}$, $\text{pH} \sim 3.5$). (b) Borrelidin, a natural product with myriad biological activities.

Recently, there has been a resurgence of studies of borrelidin derivatives as it has been identified as a potentially potent means for treatment of malaria, a parasitic protozoan transmitted by mosquitoes.^{14, 15} Chloroquine and artemisinin have been critical treatments for this infection, unfortunately resistance to both drugs has arisen.¹⁶ Borrelidin inhibits threonine-tRNA synthetase (ThrRS), preventing the amino acid activation step and halting protein synthesis¹⁷ and is effective against malaria, including drug-resistant strains.^{12, 18} Unfortunately, borrelidin is not selective for the malarial ThrRS over the human homolog. Last year, Novoa *et al.* reported several borrelidin analogs that are highly selective for malaria ThrRS and retain potency similar

to chloroquine.¹⁴ This work highlights the value of derivatization of known natural products to identify compounds with desired properties. Such studies often require a significant amount of the natural product, which can be obtained through isolation or total synthesis.

Traditional isolation techniques utilize a molecule's inherent physiochemical properties, such as size, solubility, polarity, and charge to dictate separation. Often, multiple rounds of separation, concentration, and analysis are required to obtain a purified natural product. This process can result in significant loss of product, as well as sample degradation, making the isolation of compounds that often represent <1% by weight of the extract difficult.¹⁹ Alternatively, a functional group-enriched pool is likely to possess compounds with diverse physicochemical properties, enabling ready separation in an ensuing chromatography step. With this in mind, we recently reported reagents to enable chemoselective isolation of natural products from a crude extract.²⁰⁻²³ Briefly, a resin bead is affixed with a reactive group that targets a specific functional group (e.g., carboxylic acid; **Figure 1a**). This resin is exposed to a crude natural product extract and subsequently washed to remove non-covalently associated molecules. Captured compounds are tracelessly released from the resin under mild conditions. These resins can be recycled without a decrease in enrichment yield making them a viable separation matrix.²¹ Additionally, chemoselective isolation is not biased towards the most abundant molecules in a sample. In fact, when we exposed the previously reported carboxylic acid-selective resin **2** to two model compounds that were added to a crude extract from *Streptomyces coelicolor*, the recovered yield (>95%) of these molecules was not affected even when they were extremely minor components (800:1 extract:model compound, 0.125% of sample; **Figure 2**).

Figure 2. Demonstration of the ability of resin **2** to recover model carboxylic acids spiked into a natural product extract. Each capture and release was performed in triplicate with the error bars corresponding to the standard deviation.

We sought to illustrate the utility of chemoselective enrichment for natural product purification by isolation of borrelidin (**1**). Amongst its functional group content is a carboxylic acid, a moiety found in ~15% of all known natural products,²⁴ suggesting that separation of an extract by way of this group could dramatically enhance our ability to access borrelidin, while quickly eliminating the vast majority of the extract material. Isolation of carboxylic acid-containing compounds such as borrelidin could be accomplished with resin **2**.²¹ To compare the use of chemoselective enrichment for the purification of borrelidin to previously reported strategies, we produced this compound by culture of *S. rochei* (2 L).⁸ The resulting extract material was split in half to enable direct comparison (**Figure 3**; total ion chromatogram of the crude material, Supporting Information Figure S1). Isolation of borrelidin by traditional methods was performed utilizing hydrophobic/hydrophilic and size exclusion chromatography as has been reported.^{3, 7, 25, 26} Silica gel separation is both amenable to large-scale purification and economical, making it a clear choice for the first purification step.²⁶ Following this separation, fractions containing a substantial amount of borrelidin, as assessed by liquid chromatography-mass spectrometry (LC-MS; Supporting Information Figure S2), were subjected to a LH-20

Sephadex column as had been reported (Supporting Information Figure S3).^{9, 25} Finally, separation with reversed phase chromatography (C18; HPLC)^{9, 26} provided pure material (Supporting Information Figure S4 and S5). Over three chromatography steps, 2-3 mg of borrelidin was obtained per liter of *S. rochei* culture (yield range obtained from three independent trials).

Figure 3. Comparison of our chemoselective isolation method with purification performed using traditional chromatography tools alone. The chemoselective method eliminated the need for a purification step and facilitated isolation of significantly more of the desired natural product. Full isolation was performed three times to yield the provided mass ranges; weight of initial crude extract was 200-300 mg per experiment.

We postulated that we could purify natural products in fewer steps and/or higher yield by incorporation of a chemoselective enrichment step and envisioned that use of this strategy at the start of the isolation protocol would be most advantageous as it provides a pool of molecules with disparate physicochemical properties. As such, the chemoselective purification scheme for isolation of borrelidin began with application of resin **2** to selectively enrich all carboxylic acid-containing molecules from the crude extract (**Figure 3**). Borrelidin was efficiently captured as judged by LC-MS analysis (Supporting Information Figure S6 and S7). In this step, we eliminated the vast majority of the compounds from the mixture that were of similar polarity to borrelidin as is evidenced in **Figure 4**. Examination of the total ion chromatogram obtained from the crude *S. rochei* extract (**Figure 4a** and **b**) indicates that borrelidin is flanked by several species with similar polarity, making these contaminants difficult to remove using standard methods. Alternatively, when resin **2** was applied to the crude *S. rochei* extract, the molecules that co-elute with borrelidin were eliminated (**Figure 4c**).

Figure 4. Borrelidin was detected by LC-MS in the crude extract (**a**), but co-elutes with other species (zoom in of chromatogram; **b**). Application of resin **2** to the crude extract separated borrelidin from co-eluting molecules and enabled the use of only one additional chromatography step to isolate the pure natural product (**c**).

Following carboxylic acid enrichment, this pool of molecules was subjected to HPLC purification using the same conditions that had been applied in the traditional purification scheme (Supporting Information Figure S8). Borrelidin was readily separated from the other components in the carboxylic acid fraction, yielding 5-7 mg of material after only two purification steps (yield range obtained from three independent trials; Supporting Information Figure S9). The increased yield and decrease in the overall number of steps required to obtain the pure natural product can be attributed to the ability of **2** to effectively capture borrelidin from the crude extract, and the generation of a pool of molecules that could subsequently be readily separated by their inherent physicochemical properties. Overall, this represents a ~350% gain in yield per separation step.

To confirm that isolation of borrelidin using chemoselective resin **2** did not cause degradation or epimerization of this compound, we performed co-elution studies and NMR analysis (^1H , ^{13}C , ^1H - ^1H COSY). The purified material was compared to a purchased borrelidin standard (Supporting Information Figures S10, S13-19 and Table S1).^{4-6, 25-27} These data confirm that all borrelidin samples (chemoselectively-isolated, traditionally-isolated, and purchased standard) are identical.

Here, we have demonstrated that chemoselective enrichment can enable the purification of a biologically active natural product, borrelidin, in two steps. Chemoselective enrichment produced a fraction that contains only carboxylic acid-functionalized molecules, instead of a pool with similar physicochemical properties, simplifying subsequent separation steps. Application of resin **2** resulted in a dramatic gain in yield per separation step (~350%) and the devised purification strategy will provide borrelidin in significant quantities for future derivatization studies and biological screening. More globally, this example demonstrates that use of chemoselective enrichment will be advantageous for the isolation of many important natural products.

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References

1. Newman, D.J., Cragg, G.M. *J. Nat. Prod.* 2012, 75, 311.
2. Butler, M.S. *Nat. Prod. Rep.* 2005, 22, 162.
3. Berger, J., Jampolsky, L.M., Goldberg, M.W. *Arch. Microbiol.* 1949, 22, 476.
4. Duffery, M.O., LeTiran, A., Morken, J.P. *J. Am. Chem. Soc.* 2003, 125, 1458.
5. Vong, B.G., Kim, S.H., Abraham, S., Theodorakis, E.A. *Angew. Chem. Int. Ed.* 2004, 43, 3947.
6. Nagamitsu, T., Takano, D., Fukuda, T., Otoguro, K., Kuwajima, I., Harigaya, Y., Omura, S. *Org. Lett.* 2004, 6, 1865.
7. Lumb, M., Macey, P.E., Spyvee, J., Whitmarsh, J.M., Wright, R.D. *Nature* 1965, 206, 263.
8. Singh, S.K., Gurusiddaiah, S., Whalen, J.W. *Antimicrob. Agents. Chemother.* 1985, 27, 239.
9. Gao, Y.M., Wang, X.J., Zhang, J., Li, M., Liu, C.X., An, J., Jiang, L., Xiang, W.S. *J. Agric. Food. Chem.* 2012, 60, 9874.
10. Habibi, D., Ogloff, N., Jalili, R.B., Yost, A., Weng, A.P., Ghahary, A., Ong, C.J. *Invest. New Drugs* 2012, 30, 1361.
11. Ruan, B., Bovee, M.L., Sacher, M., Stathopoulos, C., Poralla, K., Francklyn, C.S., Soll, D. *J. Biol. Chem* 2005, 280, 571.
12. Ishiyama, A., Iwatsuki, M., Namatame, M., Nishihara-Tsukashima, A., Sunazuka, T., Takahashi, Y., Omura, S., Otoguro, K. *J. Antibiot.* 2011, 64, 381.
13. Vino, S., Lokesh, K.R. *Advanced Biotech.* 2008, 6, 22.
14. Novoa, E.M., Camacho, N., Tor, A., Wilkinson, B., Moss, S., Marin-Garcia, P., Azcarate, I.G., Bautista, J.M., Mirando, A.C., Francklyn, C.S., Varon, S., Royo, M., Cortes, A., de Pouplana, L.R. *Proc. Natl. Acad. Sci. USA* 2014, 111, E5508.
15. Schulze, C.J., Bray, W.M., Loganzo, F., Lam, M.-H., Szal, T., Villalobos, A., Koehn, F.E., Linington, R.G. *J. Nat. Prod.* 2014, 77, 2570.
16. Goldberg, D., Siliciano, R., Jacobs, W. *Cell* 2012, 148, 1271.
17. Fang, P., Yu, X., Jeong, S.J., Mirando, A., Chen, K., Chen, X., Kim, S., Francklyn, C.S., Guo, M. *Nat. Communications* 2015, 6, 6402.
18. Otoguro, K., Ui, H., Ishiyama, A., Kobayashi, M., Togashi, H., Takahashi, Y. *J. Antibiot.* 2003, 56, 727.
19. Cannell, R.P. *How to approach the isolation of a natural product, Natural Products Isolation*, Humana Press, 1998, pp. 1.
20. Odendaal, A.Y., Trader, D.J., Carlson, E.E. *Chem. Sci.* 2011, 2, 760.
21. Trader, D.J., Carlson, E.E. *Org. Lett.* 2011, 13, 5652.
22. Trader, D.J., Carlson, E.E. *J. Org. Chem.* 2013, 78, 7349.
23. Trader, D.J., Carlson, E.E. *Tetrahedron* 2014, 70, 4191.
24. Henkel, T., Brunne, R.M., Muller, H., Reichel, F. *Angew. Chem. Int. Ed.* 1999, 38, 643.
25. Cao, Z., Khodakaramian, G., Arakawa, K., Kinashi, H. *Biosci. Biotechnol. Biochem.* 2012, 2, 353.
26. Saisivam, S., Bhikshapathi, D.V., Krishnaveni, J., Kishan, V. *Indian J. Biotechnol* 2008, 7, 349.
27. Kuo, M.S., Yurek, D.A., Kloosterman, D.A. *J. Antibiot.* 1989, 32, 1006.

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