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Simultaneous structure–activity studies and arming of natural products by C–H amination reveal cellular targets of eupalmerin acetate

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Natural products have a venerable history of, and enduring potential for the discovery of useful biological activity. To fully exploit this, the development of chemical methodology that can functionalize unique sites within these complex structures is highly desirable. Here, we describe the use of rhodium(II)-catalysed C–H amination reactions developed by Du Bois to carry out simultaneous structure–activity relationship studies and arming (alkynylation) of natural products at 'unfunctionalized' positions. Allylic and benzylic C–H bonds in the natural products undergo amination while olefins undergo aziridination, and tertiary amine-containing natural products are converted to amidines by a C–H amination–oxidation sequence or to hydrazine sulfamate zwitterions by an unusual N-amination. The alkynylated derivatives are ready for conversion into cellular probes that can be used for mechanism-of-action studies. Chemo- and site-selectivity was studied with a diverse library of natural products. For one of these—the marine-derived anticancer diterpene, eupalmerin acetate—quantitative proteome profiling led to the identification of several protein targets in HL-60 cells, suggesting a polypharmacological mode of action.

A renewed realization of the intrinsic value of bioactive small molecules isolated from natural sources (that is, natural products) for drug discovery[1] has spurred a renaissance in their study, including research on novel concepts for their efficient synthesis[2], the synthesis of natural product-like libraries (for a recent special issue on this topic, see ref. 3), the manipulation of biosynthetic pathways for synthetic biology, and their incorporation into high-throughput screens[4]. Natural products commonly demonstrate high structural diversity, cell permeability and high affinity for their cellular targets, arguably explaining why half the drugs in current clinical use are natural products or owe their intellectual origin to these bioactive small molecules[5]. Furthermore, the enduring value of natural products for advancing the knowledge of basic cell biology and their utility in the discovery of novel 'druggable' targets for human disease intervention cannot be overstated. In particular, natural products continue to facilitate the identification of both activators and inhibitors of proteins encoded in the human genome in addition to a number of natural product-inspired molecules that are providing advances in this post-genomic era[6,7]. Given that current pharmaceuticals are thought to access fewer than 500 of the estimated 3000–10,000 potential therapeutic targets for human disease intervention[8], natural products hold great potential for the discovery of novel cellular targets and drug discovery. One approach for fully exploiting this potential requires the synthesis of natural product conjugates that contain a covalently attached reporter tag, for example biotin or a fluorophore, appended by a flexible linker at a position in the natural product that does not abrogate binding to putative cellular receptor(s). However, natural products are often challenging to functionalize in a chemo- and site-selective manner because of their structural complexity, dense functionality and often limited availability. One approach to accessing natural products with strategically placed linkers is through total synthesis, which enables access to novel and unique attachment sites, but this approach may not be applied rapidly to access varied positions in complex natural products. Highly random derivatization techniques involving photo-crosslinking of natural products to affinity matrices have been reported. However, these methods provide limited structure–activity relationship (SAR) data for subsequent drug development and no information regarding the site of attachment in the event that cellular targets are not captured[9]. To fully exploit the inherent information content of complex, bioactive natural products[10,11], mild and generally applicable microscale strategies for site-selective derivatization of native natural products are required to enable SAR studies and the synthesis of natural product-based cellular probes. Such probes have proven useful for identifying the cellular targets of natural products and for providing a molecular-level understanding of how these small molecules exert their observed ameliorative or curative effects[7].

We have recently described several mild, microscale methods for simultaneous arming and SAR studies of natural products to address these issues, including a Rh(II)-catalysed OH and NH insertion of natural products bearing alcohols or amines[12,13], a mild In(III)-catalysed iodination of arene-containing natural products[14], and cyclopropanations of natural products bearing both electron-rich and electron-deficient alkenes[15]. These functionalization methods are dependent on the presence of native functional groups and are thus limited in terms of positional diversity. In addition, existing functional groups in natural products are often essential for maintaining biological activity. Accordingly, methods that enable the functionalization of C–H bonds would dramatically

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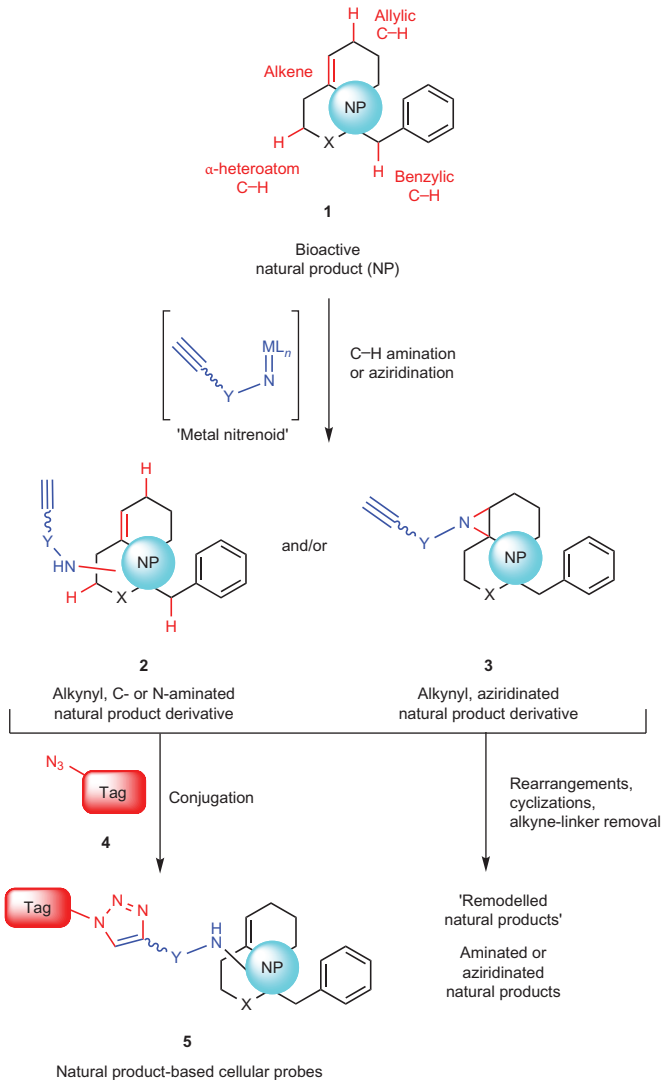


Figure 1 | A two-step C–H amination or aziridination–conjugation sequence for simultaneous arming and SAR studies of natural products.

increase the range of available sites on natural products for functionalization and improve the possibility of maintaining the bioactivity of derivatives.

Several mild and chemoselective functionalizations of C–H bonds adjacent to aryl groups, alkenes and heteroatoms (such as O and N) have recently been described that make use of carbenoid or nitrenoid reagents in both an intramolecular and intermolecular fashion[16]. Of particular interest to our efforts were intermolecular, Rh-catalysed C–H amination processes via metallo nitrenoids[16], as described by the groups of Du Bois[17,18], Lebel[19] and others, which can also deliver aziridines from electron-rich olefins with certain catalysts[20,21]. We envisioned that the application of C–H aminations using a metal nitrenoid precursor bearing an alkyne to derivatize native, bioactive natural products would enable simultaneous arming and SAR studies of natural products at 'unfunctionalized' positions. The attached alkyne enables subsequent conjugation to various tags (for example, biotin or fluorophores), providing natural product-based cellular probes useful for mode-of-action studies (Fig. 1). In addition, rearrangements or cyclizations of C–H amination products could lead to 'remodelled' natural products[22–25], while deprotection would lead to aminated or aziridinated natural products for SAR studies. Here, we describe a two-step strategy for the functionalization of natural products at unfunctionalized positions by Rh(II)-catalysed amination or aziridination processes with an alkynyl sulfamate reagent, which greatly expands the methods available for direct functionalization of native natural products. The described strategy provides a systematic approach for further exploiting natural products for chemical genetics and addressing the 'small molecule target identification problem'. The utility of this strategy is demonstrated by identifying the cellular targets of the anticancer marine natural product eupalmerin acetate (EuPA), by quantitative proteome profiling with an alkyne-substituted derivative obtained by C–H amination.

In considering natural product derivatization via C–H amination, several issues were considered: (i) intermolecular C–H amination is significantly more challenging than intramolecular processes[16]; (ii) high chemo- and site (chemosite) selectivity[12] is required for natural products, which often bear multiple functional groups; and (iii) microscale reaction conditions ($\leq$1 mg) may be required, because natural products are often available only in limited quantities. Mechanistic studies suggest that Rh-catalysed C–H amination processes proceed via a concerted asynchronous transition state involving a rhodium nitrene reactant and follow the reactivity trend $3^\circ > \alpha$-amino $> \alpha$-ethereal $\geq$ benzylic $> 2^\circ \gg 1^\circ$ C–H bonds[26].

Results and discussion

In our initial studies we compared Du Bois and Lebel C–H amination conditions and were drawn to the former because of the higher conversions observed with only 1.0 equiv. of nitrene precursor, which greatly simplifies purification despite the need for a stoichiometric oxidant. Building on the sulfamate design of Du Bois[17], we targeted a trichloroethyl substituted sulfamate with a terminal alkyne side chain to enable subsequent conjugation to reporter tags. Du Bois previously described the use of trichloromethyl substitution on sulfamate nitrene precursors to prevent intramolecular C–H amination and facilitate mild, reductive deprotection to the primary amine. We therefore targeted sulfamate **9**, which was readily prepared by a two-step sequence from commercially available trichloromethyl-β-lactone (Fig. 2). In control experiments, we verified the stability of the nitrene reagent sulfamate **9**, which could be recovered with good mass recovery. As an added benefit, trichloromethyl substitution provides a unique isotopic pattern to facilitate mass spectrometric identification of derivatized natural products.

For optimization studies, we chose carvone as a substrate, given its potential for both allylic C–H amination and alkene aziridination, and briefly studied various Rh(II) catalysts (Table 1). $Rh_2(esp)_2$ gave both allylic C–H amination and aziridination in a combined yield of 26% with 1.0 equiv. of nitrene precursor **9**, whereas $Rh_2(OAc)_4$, $Rh_2(OCOC_8H_{15})_4$ and $Rh_2(TPA)_4$ gave aziridination products exclusively, in modest yields (23–26%) but with high mass recovery. Although conversions were generally modest, the mass recovery of these derivatizations was excellent. In general, we view lower conversion with good mass recovery as a tolerable balance when applying this reaction to sample-limited natural products. Furthermore, in comparison with the overall

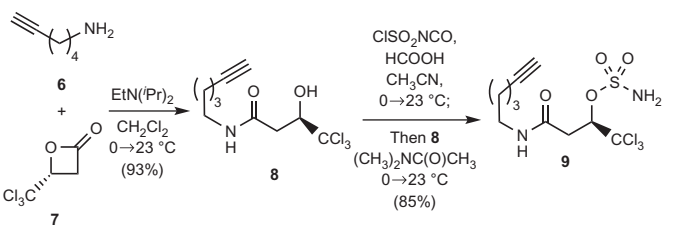
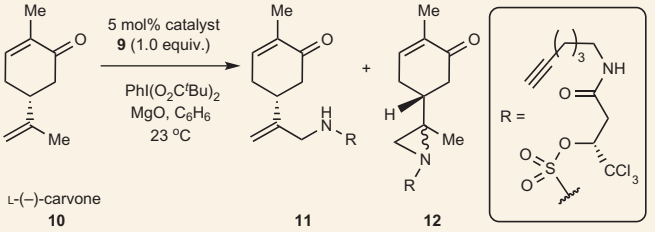


Figure 2 | Synthesis of alkynyl sulfamate 9. Ring opening of β-lactone **7** with alkynyl amine **6** and subsequent sulfamation of alcohol **8** provides **9**.

Table 1 | Exploratory C–H amination with L-(−)-carvone.



Entry	Catalyst	C–H amination product (11)*	Aziridination product (12)*	Recovered starting material
1	$Rh_2(esp)_2$	10%	16%	56%
2	$Rh_2(OAc)_4$	Trace†	26%	68%
3	$Rh_2(OCOCF_3)_4$	0	0	85%
4	$Rh_2(OCOC_8H_{15})_4$	Trace†	25%	67%
5	Rh_2 (TPA)$_4$	0	23%	75%

*Isolated yield. †Only trace amounts of product were detected by LC/MS.

yields likely to be obtained by *de novo* synthesis, the yield of these derivatives is quite acceptable for the goal of initial SAR and cellular probe synthesis, as only milligram to submilligram quantities of material are required.

Although higher turnover numbers were reported by Du Bois when $PhI(O_2CCMe_2Ph)_2$ was used as the oxidant[18], we found that $PhI(O_2C^tBu)_2$ gave less complex reactions and both higher product yields and mass recovery. The addition of inorganic bases (for example, K_2CO_3), Brønsted acids (for example, HOAc) or Lewis acids (for example, $In(OTf)_3$) did not lead to significant increases in conversion, but did have a major impact on the chemoselectivity of the reactions. For example, Brønsted and Lewis acid additives (for example, HOAc and $In(OTf)_3$) favoured C–H amination, whereas the use of K_2CO_3 favoured alkene aziridination, an observation important for altering chemoselectivity with complex natural products. Slow dropwise addition of a dilute solution of the oxidant did not improve conversion, and the use of MgO had no impact on conversion or yields. Thus, the direct addition of the solid oxidant in one portion enabled reaction at higher concentrations (~0.13 M), an important consideration for microscale derivatizations, and this procedure dramatically improved efficiency. Although benzene was the optimal solvent, dichloromethane and α,α,α-trifluorotoluene were acceptable alternatives. Ultimately, the conditions shown in the scheme in Table 1 (entry 1) were found to be optimal, so these conditions were subsequently applied in more complex settings.

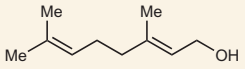
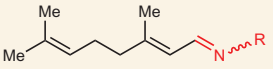
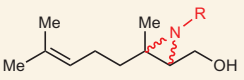
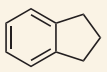
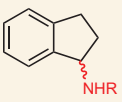
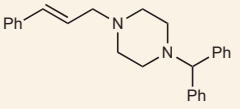
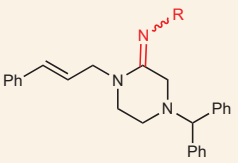
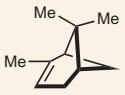
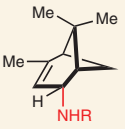
Scope of the $Rh_2(esp)_2$-catalysed C–H amination/alkene aziridination with a diverse set of natural products and drugs. The optimized C–H amination/aziridination conditions were applied to several commercially available natural products and drugs. Although conversions were modest in most cases, as with carvone, mass recovery was again generally excellent. As expected, chemosite selectivity is highly substrate-dependent, and the conformation of the natural products in benzene probably dictates the accessible positions for C–H amination or aziridination in some cases, overriding the site selectivities anticipated based on electronic considerations[17]. The structure and stereochemistry of derivatives was determined by extensive one- and two-dimensional NMR spectroscopy (Supplementary pages S32–64).

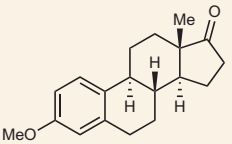
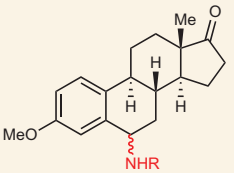
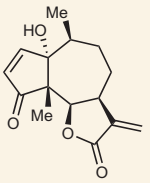
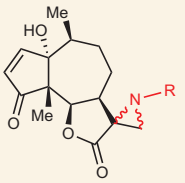
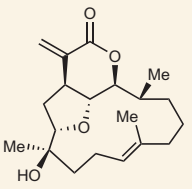
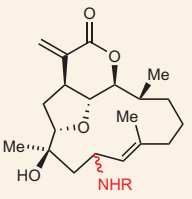
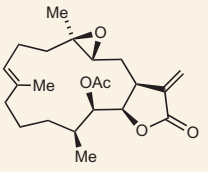
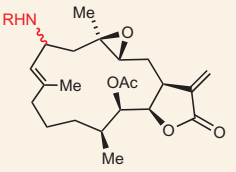
Application of the optimized conditions to several natural products and drugs led to either C–H amination/oxidation, amination/dehydration or aziridination (Table 2). Functionalization of geraniol (**13**) gave an ~3:2 mixture of imine **13a** (55%) and aziridine **13b** (37%). To ensure that imine **13a** (Table 2, entry 1) was not derived from simple allylic oxidation by $PhI(O_2C^tBu)_2$ and subsequent condensation of the resulting aldehyde with sulfamate **9**, a control experiment without sulfamate was performed. This gave primarily epoxidation of geraniol rather than allylic oxidation. Thus, imine **13a** may be derived from allylic C–H amination and subsequent dehydrative elimination. Aziridination of geraniol was site-selective for the less electron-rich alkene adjacent to the inductively electron-withdrawing alcohol, suggestive of a possible directing effect by the allylic alcohol. Benzylic C–H amination of indane (**14**) occurred to give sulfamate **14a**, as previously observed with a more simple sulfamate[17]. Cinnarizine (**15**) gave site-selective C–H amination on the piperazine ring adjacent to the less hindered tertiary nitrogen, providing amidine **15a**, presumably derived from subsequent oxidation of the initial C–H amination product (Table 2, entry 3). α-Pinene (**16**) underwent highly facially selective allylic C–H amination, leading to sulfamate **16a** as a single diastereomer in 21% yield (Table 2, entry 4). As with indane, benzylic C–H amination occurred with β-estrone 3-methyl ether (**17**), providing a 4:1 mixture of diastereomeric sulfamates **17a** in 40% yield (Table 2, entry 5). In the case of parthenin (**18**, Table 2, entry 6), aziridination was observed exclusively at the electron-deficient alkene, providing aziridines **18a** as a 1:1 mixture of diastereomers with low conversion. Site-selective allylic C–H amination occurred with both eupalmerolide (**19**) and EuPA (**20**), leading to the allylic sulfamates **19a** and **20a** as mixtures of diastereomers (d.r. ≈ 1:1), respectively (Table 2, entries 7 and 8).

Application of the amination conditions to amine-containing natural products led to either C–H amination/oxidation or a rare *N*-amination leading to a zwitterionic betaine ('inner salt') (for an intramolecular example of *N*-amination with a sulfamate nitrene see ref. 27, and for an example of *N*-amination via substitution using hydroxylamine derivatives see ref. 28), structurally verified following extensive ^{1}H NMR (Supplementary Table S3) and X-ray analysis of an *N*-aminated adduct (Supplementary Fig. S1). The derivatization of (*S*,*R*)-noscapine led to C–H amination of the *N*-methyl group of the isoquinoline ring and subsequent oxidation to give amidine **21a** ($E/Z \approx 1{:}1$, Table 3, entry 1). In addition, amination of the isoquinoline nitrogen occurred to give betaine **21b**. In a similar manner, dextromethorphan·HBr·H_2O salt, in the presence of K_2CO_3 to provide the free base *in situ*, gave amidine **22a** ($E/Z \approx 2{:}1$, Table 3, entry 2) and betaine **22b**. In the case of cinchonidine, *N*-amination was observed at the tertiary quinuclidine nitrogen, the quinoline nitrogen and both positions leading to betaines **23a** (verified by X-ray analysis, Supplementary Fig. S1, CCDC 930772), **23b** and **23c**, respectively (Table 3, entry 3). Brucine also led to amination of the tertiary amine to give betaine **24a**, and an eliminative oxidation with ring scission gave lactam **24b** (diagnostic ^{13}C signal at $\delta = 175.0$; for further characterization data see Supplementary Table S2) in 21% yield (Table 3, entry 4).

There is growing interest in structurally modifying or 'remodelling' natural products to identify new chemical entities for screening[29,30] by controlling chemosite selectivity of derivatizations using minimal peptides[31] or organometallic catalysts[12]. In the course of these studies, we observed instances of unexpected cyclizations, rearrangements and insertions that led to novel natural product derivatives. As described above, brucine provided lactam **24b** (Table 3, entry 4) derived from a ring scission event. Carvone led to both allylic C–H amination and aziridination (Table 1), as described above. However, after prolonged storage or in the presence of base, the allylic sulfamate **11** underwent an aza-Michael reaction to provide the interesting bicyclic carvone derivative **25** as an equilibrium 1:1 mixture with the starting allylic sulfamate (Fig. 3a). Under standard conditions, gibberellic acid methyl ester (GAME, **28**) did not give the expected aziridine (Fig. 3b, entry 1); instead, ketone **31** was isolated in 55% yield (44% recovered starting material). We propose that initial aziridination occurred to give the

Table 2 | Derivatives of natural products and drugs produced by C–H amination and aziridination.

Entry	Natural product/drug	Derivaties*,†,⊥
1	 Geraniol (**13**)	 **13a** (*E*/*Z* = 8:1, 55%)  **13b** (d.r. 1.2:1, 37%) (0% rec. SM)
2‡	 Indane (**14**)	 **14a** (d.r. 1.5:1, 48%) (+41% rec. SM)
3	 Cinnarizine (**15**)	 **15a** (*E*/*Z* = 2:1, 21%) (+74% rec. SM)
4	 α-(–)-pinene (**16**)	 **16a** (d.r. >19:1, 21%) (+70% rec. SM)

Entry	Natural product/drug	Derivaties
5	 β-estrone 3-methyl ether (**17**)	 **17a** (d.r. 4:1, 40%) (+51% rec. SM)
6	 Parthenin (**18**)	 **18a** (d.r. 1:1, 8%) (+90% rec. SM)
7	 Eupalmerolide (**19**)	 **19a** (d.r. 1:1, 52%) (+15% rec. SM)
8	 Eupalmerin acetate (**20**) (**EuPA**)	 **20a** (d.r. 1:1, 28%, **EuPAyne**) (+62% rec. SM)

*Reaction conditions: 2.0 equiv. **9**, 5 mol% $Rh_2(esp)_2$, 4 equiv. $PhI(O_2C^tBu)_2$, benzene, 23 °C, 30 min. †R = $SO_3CH(CCl_3)CH_2C(O)NH(CH_2)_4CCH$. ‡Diastereomers can be generated with achiral substrates, as in this case with indane, because the sulfamate reagent bears a stereogenic centre. ⊥Rec. SM, recovered starting material.

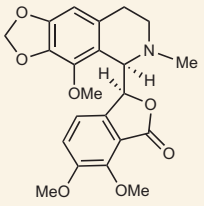
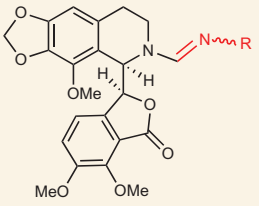
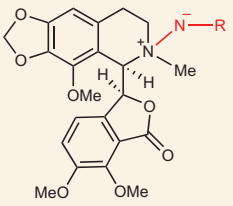
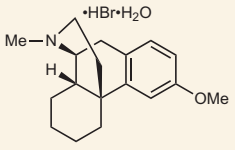
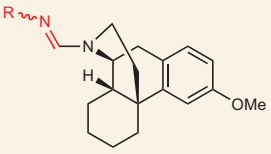
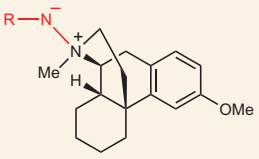
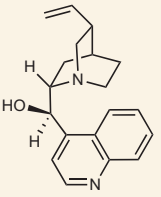
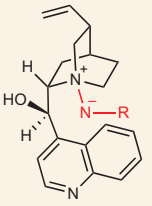
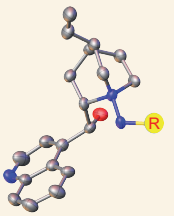
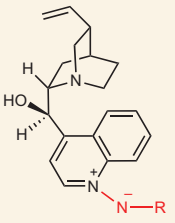
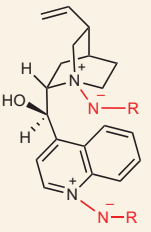
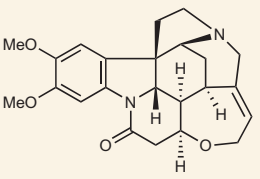
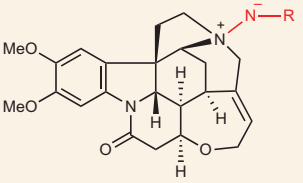
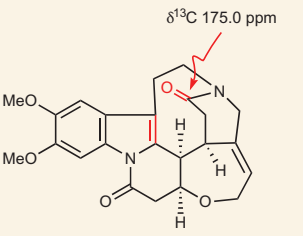
expected aziridine **29**, which underwent ring cleavage to afford a tertiary carbocation **30**, then a subsequent 1,2-pinacol rearrangement delivered ketone **31** (Fig. 3b). (For a related rearrangement of an epoxide derived from GAME (**28**) see ref. 32.) In efforts to isolate the intermediate aziridine, a brief solvent study was undertaken and it was found that use of CH_2Cl_2 did deliver unprotected aziridine **33** in 40% yield, accompanied by the rearranged ketone **31** (29%, Fig. 3b, entry 2). Use of α,α,α-trifluorotoluene significantly suppressed the rearrangement (6%) and gave lower conversion to the unprotected aziridine **33** (23%, Fig. 3b, entry 3). Further optimization and exploration of the CH amination/aziridination conditions were performed with GAME (Supplementary Table S1).

We studied removal of the alkynyl sulfamate by N–S cleavage, leading to the net addition of 'NH' (aziridination) or 'NH_2' (C–H amination) to natural products, which could serve to improve the water solubility of these products. Although several reductive methods for cleavage of trichloroethyl sulfamates could be applied[17], we chose the very mild conditions developed by Ciufolini, using a 10% Cd/Pb couple[33]. Deprotection of bicyclic ketone **25** under these conditions gave a 3:1 mixture of the expected deprotected azabicycle **26** and the ring-opened allylic amine **27** in 75% combined yield. Similarly, deprotection of the rearranged ketone **31** derived from GAME (**28**) gave the primary amine **32** in 85% yield, and the deprotection of betaine **24a** proceeded smoothly to give the fairly stable hydrazine inner salt **34** in 76% yield.

Quantitative proteomic profiling of EuPA targets in living HL-60 cells. EuPA has previously been shown to inhibit the proliferation of several cancer cell lines including leukaemia and non-small-cell lung, ovarian, breast and colon cancer cell lines at sub- to low micromolar concentrations (GI_{50} = 0.34–1.7 μM, where GI_{50} is the concentration for 50% of maximal inhibition of cell proliferation) in the NCI 60 cancer cell line panel[34]. EuPA also showed significant growth suppression in a mouse model of malignant glioma xenografts[35]. Despite the potential of EuPA as an anticancer therapeutic, the exact cellular target(s) of this natural product are unknown.

To demonstrate the utility of the described natural product derivatization strategy and illustrate the concept of simultaneous SAR studies and arming of natural products, we considered the application of quantitative proteomic profiling with the EuPA–alkyne derivative **20a** (Table 2, entry 8) to identify cellular targets of EuPA in the HL-60 human acute myeloid leukaemia cell line. Importantly, the described C–H amination of EuPA modified the

Table 3 | Derivatives of amine-containing natural products and drugs produced by C–H amination and *N*-amination.

Entry	Natural product/drug	Derivatives*,†
1	 (*S*,*R*)-noscapine (**21**)	 **21a** (*E*/*Z* = 1.3:1, 21%)  **21b** (37%) (0% rec. SM)
2‡	 Dextromethorphan•HBr•H_2O (**22**)	 **22a** (*E*/*Z* = 2:1, 27%)  **22b** (42%) (+10% rec. SM)
3	 Cinchonidine (**23**)	  **23a** (19%)  **23b** (37%)  **23c** (6%) (+37% rec. SM)
4	 Brucine (**24**)	 **24a** (28%)  **24b** (21%) (0% rec. SM)

*Reaction conditions: 2.0 equiv. **9**, 5 mol% $Rh_2(esp)_2$, 4 equiv. $PhI(O_2C^tBu)_2$, benzene, 23 °C, 30 min. † R = $SO_3CH(CCl_3)CH_2C(O)NH(CH_2)_4CCH$. ‡C-H amination of dextromethorphan•HBr monohydrate was performed in the presence of K_2CO_3 (6.0 equiv). ORTEP representation of the X-ray crystallographic structure of **23a**; thermal ellipsoids are shown at 50% probability.

allylic position of the macrocycle far removed from the electrophilic exocyclic alkene, the presumed pharmacophore, which probably serves as a Michael acceptor for nucleophilic residues of target proteins. We first compared the antiproliferative properties of EuPA and EuPAyne (**20a**) in HL-60 cells, and found a decrease by only a factor of approximately five compared to the natural product (concentration required to inhibit 50% of cell population $IC_{50} = 3.0$ μM versus 15 μM), suggesting that EuPAyne could serve as a chemical probe to profile the cellular targets of EuPA using quantitative, activity-based proteomic methods (Fig. 4a).

To profile specific EuPA targets, we treated HL-60 cells with EuPAyne (5 μM) and EuPA (0–15 μM). Following this treatment, the cells were homogenized and the probe adducts were conjugated to rhodamine-azide under copper-catalysed azide–alkyne cycloaddition conditions[36], separated by SDS–polyacrylamide gel electrophoresis, and visualized by in-gel fluorescent scanning. As shown in Fig. 4b, several EuPAyne bands compete in a concentration-dependent manner with EuPA, indicating that they are specific cellular targets of this natural product. To identify these proteins, we performed competitive ABPP-SILAC (activity-based protein profiling-stable isotope labelling by amino acids in cell culture), a quantitative, mass spectrometry (MS)-based chemoproteomic method that has been used to identify enzyme targets of activity-based probes[37,38] and small molecules in cells[39,40] and *in vivo*[37,39]. Using competitive ABPP-SILAC, we observed several EuPAyne targets that were competed by pretreatment with EuPA, suggesting that these proteins are selective targets of EuPA (Fig. 4d, Supplementary Table S4). Interestingly, the high-affinity target Derlin-1 (DERL1) is associated with cancer cell proliferation, and cytochrome b5 type B (CYB5B)[41] and thromboxane A synthase (TBXAS1)[42] are overexpressed in cancer[43–45]. These three high-affinity targets were confirmed by overexpression in 293T cells and were shown to interact specifically with EuPAyne **20a** as they were competed out with EuPA in a concentration-dependent manner (Fig. 4e, Supplementary Fig. S2A,B). Thus, EuPAyne may prove to be a useful cellular probe for gaining further understanding of the significance of these proteins in cancer proliferation. Another minor number of EuPA targets are either completely

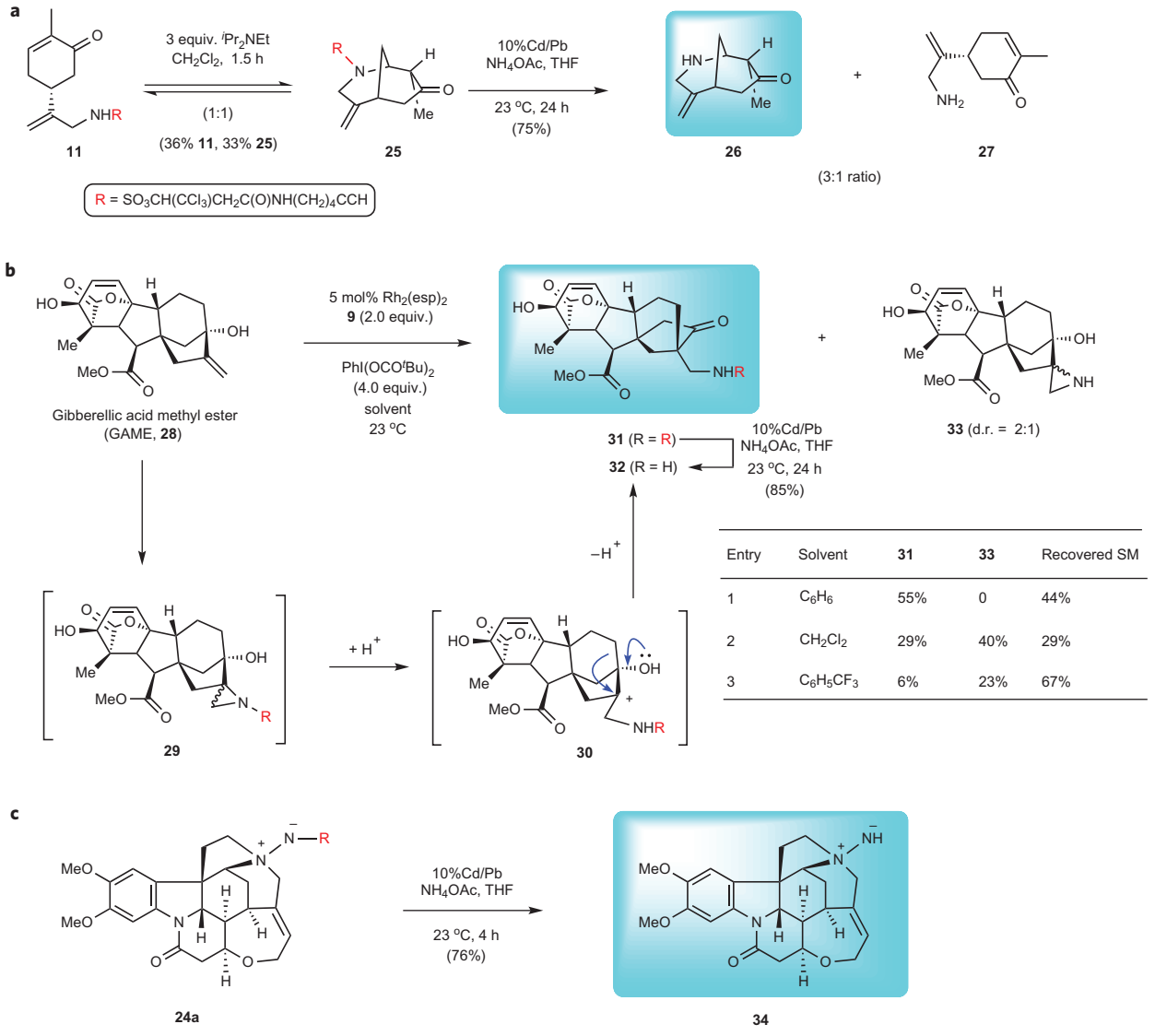
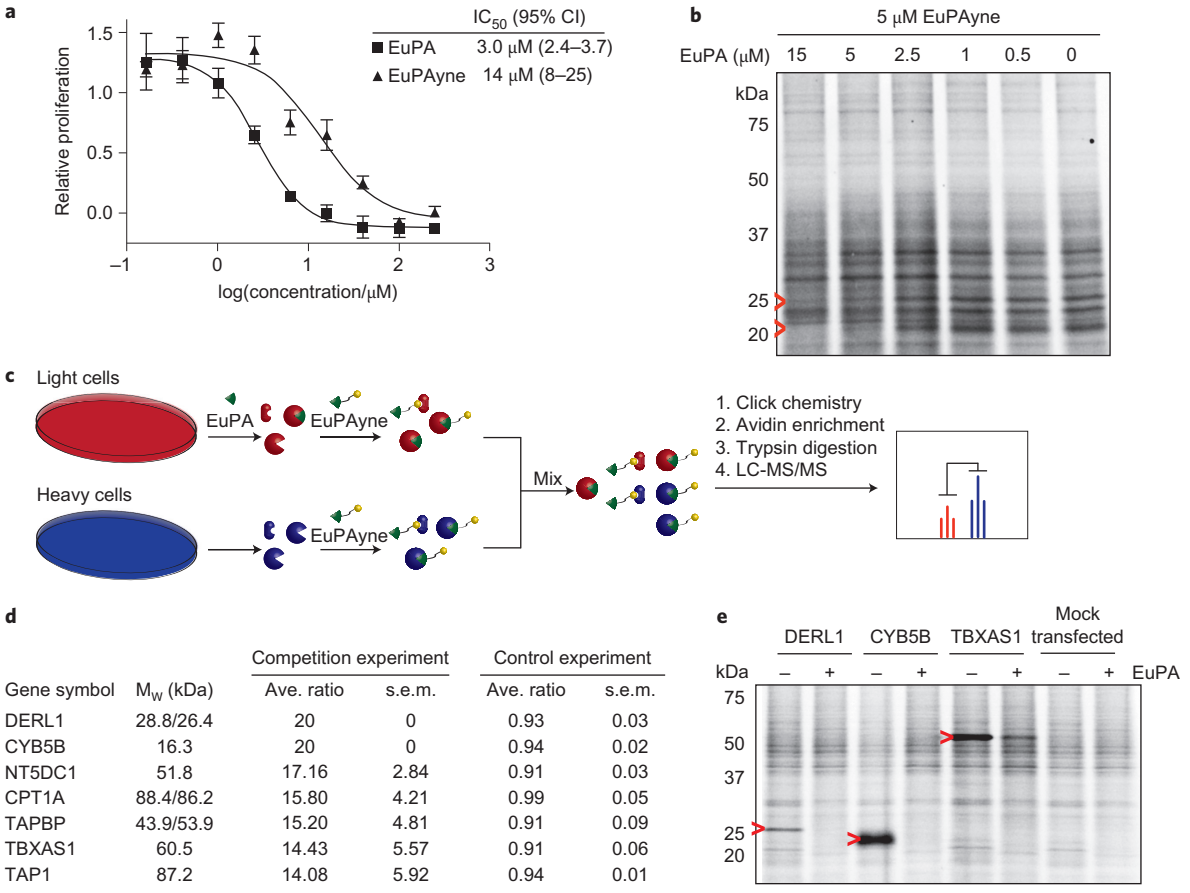


Figure 3 | Natural product remodelling. a, Cyclization of the carvone C–H amination product via aza-Michael addition, leading to the bridged bicyclic derivative **26**. **b**, Rearrangement of a GAME-derived aziridine leading to the remodelled cyclopentanone **31** via pinacol rearrangement, and solvent study leading to variations in product distribution. **c**, Reductive cleavage of the N–S bond in betaine **24a** with Cd/Pb couple.

uncharacterized (NUDT8, NT5DC1) or have unknown molecular functions (CDKAL1).

In summary, a method for performing simultaneous SAR studies and arming of bioactive natural products through Du Bois C–H amination and alkene aziridination is described using an alkynyl sulfamate nitrene precursor and $Rh_2(esp)_2$ as catalyst. These derivitizations are mild enough to tolerate a number of functional groups found in natural products, including alcohols, ethers, lactones, lactams, esters, quinolines and piperazines. In several cases, the described derivatization is found to be both chemo- and site-selective, enabling functionalization of allylic and benzylic positions via C–H amination or alkenes through aziridination. In addition, *N*-aminations of tertiary amines occur readily, giving inner salts, and this process provides another avenue for tagging amine-containing natural products. Future studies will explore the potential utility of these inner salts for mode-of-action studies including proteomic profiling. For the purpose of SAR studies, the ability to initially obtain low chemosite selectivity is ideal for sampling various positions of a given natural product in a tractable manner. In addition, attachment of an alkynyl substituent ('arming') enables direct conjugation of the derived natural product congeners to a variety of tags in a subsequent step. Mild conditions were identified for cleavage of the sulfamate group leading to net amination or aziridination of the parent natural product, which can improve water solubility when required. The utility of this method for natural product derivitization was demonstrated by application to EuPA, a marine-derived anticancer diterpene with unknown cellular targets. A unique, allylic C–H aminated derivative of EuPA ($IC_{50} = 3.0\ \mu M$), coined EuPAyne ($IC_{50} = 15\ \mu M$), enabled the use of gel proteomic profiling of HL-60 cells for the initial analysis of cellular protein targets in this cancer cell line. Competitive ABPP-SILAC enabled quantitative proteome analysis of HL-60 cells and the identification of several cellular protein targets of EuPA, including several associated with cancer proliferation. The described derivatization strategy greatly expands the methods available for direct functionalization of native natural products and provides a rapid and systematic approach for further exploiting natural products for chemical genetics and addressing the 'small molecule target identification problem'. Other emerging methods for direct C–H functionalization[46–48] are also expected to be applicable and will potentially enable a truly universal approach for exploiting natural products for chemical genetics[26].



Gene symbol	M_W (kDa)	Competition experiment Ave. ratio	Competition experiment s.e.m.	Control experiment Ave. ratio	Control experiment s.e.m.
DERL1	28.8/26.4	20	0	0.93	0.03
CYB5B	16.3	20	0	0.94	0.02
NT5DC1	51.8	17.16	2.84	0.91	0.03
CPT1A	88.4/86.2	15.80	4.21	0.99	0.05
TAPBP	43.9/53.9	15.20	4.81	0.91	0.09
TBXAS1	60.5	14.43	5.57	0.91	0.06
TAP1	87.2	14.08	5.92	0.94	0.01

Figure 4 | Cytoxicity and proteomic profiling of EuPA and EuPAyne. a, Inhibition of HL-60 cell proliferation by EuPA and EuPAyne as measured using a WST-1 assay. Error bars indicate the standard error from three replicates. **b**, *In situ* treatment of HL-60 cells with EuPAyne alone or in competition with EuPA followed by conjugation to an azide-rhodamine reporter tag, SDS-PAGE and fluorescent scanning. Red arrows highlight competed signals. **c**, Schematic of competitive ABPP-SILAC. Cells are enriched with heavy or light isotopic tags and treated with DMSO or EuPA, respectively, followed by the addition of EuPAyne. EuPA-labelled proteomes are mixed, conjugated to biotin using click chemistry, enriched, and analysed by Multi-dimensional Protein Identification Technology (MudPIT). **d**, List of putative EuPA targets in HL-60 cells. Average ratios are from duplicate runs and standard error results are reported. In the control experiment, heavy and light cells are treated with EuPAyne alone. **e**, Validation of three high-affinity targets DERL1, CYB5B and TBXAS1 by labelling of transiently transfected 293T cells with EuPAyne (5 μM) ± EuPA (15 μM). Red arrows indicate the target's expected molecular weight. These data illustrate how EuPAyne can be used to profile the covalent molecular targets of EuPA in living cells.

Methods

Representative procedure for C–H amination/aziridination of complex natural products. The natural product or drug (0.05 mmol, 1.0 equiv.), sulfamate **9** (0.1 mmol, 2.0 equiv.) and $Rh_2(esp)_2$ (0.0025 mmol, 0.05 equiv.) were mixed in a small vial and evacuated under high vacuum for 10 min before being purged with N_2. (Note that if the natural product/drug is volatile, it should be added after this evacuation process.) Benzene (0.4 ml) was added to give a green suspension (0.125 M) and this mixture was stirred at 23 °C under N_2 for 10 min. $PhI(O_2C^tBu)_2$ (0.2 mmol, 4.0 equiv.) was then added in one portion and the reaction mixture was vigorously stirred at 23 °C for an additional 30 min. The crude reaction mixture, following initial analysis by liquid chromatography-mass spectrometry (LC-MS), was loaded on a silica gel column directly without workup and purified by flash column chromatography (eluting with EtOAc/hexane) to isolate the desired product(s). For natural products only available in minute quantities, the reaction could be successfully performed on a 1 mg scale using 0.2 ml of solvent (concentration is ~20 mM in this case). The amount of all other reagents is reduced accordingly. If separation of sulfamate from the product is problematic, it can be removed by extraction with 0.01 N (aq.) NaOH assuming stability of the natural product to these conditions. The reaction mixture is dissolved in CH_2Cl_2 and washed twice with 0.01 N (aq.) NaOH solution (0.2 vol.).

Competitive ABPP-SILAC. Briefly, HL-60 cells were cultured under isotopically 'light' conditions (with medium containing $^{12}C_6^{14}N_2$-lysine and $^{12}C_6^{14}N_4$-arginine) or 'heavy' conditions (with $^{13}C_6^{15}N_2$-lysine and $^{13}C_6^{15}N_4$-arginine). For competition experiments, light and heavy cells were treated with EuPA (15 μM) or dimethylsulfoxide (DMSO), respectively, for 30 min and then subsequently treated with EuPAyne (5 μM) for an additional 30 min. Cell proteomes were harvested and mixed (1:1), reacted under click chemistry conditions with biotin-azide, enriched with avidin beads, digested on-bead with trypsin, and analysed by liquid chromatography–tandem MS (LC-MS/MS) using an LTQ-Orbitrap instrument. Light and heavy signals were quantified from parent ion peaks (MS1) (see Supplementary Information) and the corresponding proteins identified from product ion profiles (MS2) using the ProLuCID search algorithm[49]. Control experiments were carried out in the same manner with the exception that both heavy and light cells were treated with EuPAyne alone.

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Author contributions

D.R. and B.F.C. jointly conceived the study and supervised the work. D.R., B.F.C., J.L., J.S.C. and C.Z. were involved with the design of the studies, performance of the experiments, and analysis of the results. J.L., J.S.C. and D.R. wrote the manuscript. H.W. assisted with acquisition and analysis of 2D and cryoprobe NMR data. A.D.R. and B.V. isolated and purified samples of eupalmerolide and EuPA and assisted with the NMR analysis of the resultant alkynylated derivatives. All authors edited the manuscript.

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 D.R.

Competing financial interests

The authors declare no competing financial interests.