Tetrahedron 143 (2023) 133529

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

On matching and mismatching in protein chemistry☆

Dimitrios Mamalis [a,b], John R.J. Maynard [a], Sebastien R.G. Galan [b], Benjamin Bower [b], Amber L. Thompson [b], Benjamin G. Davis [a,b,c,*]

[a] The Rosalind Franklin Institute, Oxfordshire, OX11 0FA, UK
[b] Department of Chemistry, University of Oxford, OX1 3TA, UK
[c] Department of Pharmacology, University of Oxford, OX1 3QT, UK

ARTICLE INFO

Keywords:
Protein chemistry
Regioselectivity
Stereochemical cooperativity
Asymmetric reagents
Cysteine modification

ABSTRACT

Post-translational protein editing methods that rely upon precise chemical alteration of sequence and hence function are of increasing utility. Whilst the role of proteins as chiral environments is functionally ubiquitous in their interaction with substrates or ligands, the influence of stereochemical 'matching/mismatching' effects upon the reactivity of chemical reagents designed for such editing is essentially unexplored. The reagent dibromohexanediamide (DBHDA) is a useful reagent for the chemical transformation of cysteine to dehydroalanine (Dha) in proteins and peptides. Differences in reactivities of the stereoisomers of DBHDA, if any, in different protein substrates are unknown. Here, we report synthetic access to these isomers and reveal apparent stereochemically 'matched *vs* mismatched' differences in reactivity, that, in principle, in the future might be exploited to expand the methods available for residue- and site-selective editing.

1. Introduction

In the field of protein functionalization and synthetic post-translational modification [1] (protein 'editing') emerging methods have been explored to address chemo- [2] and, more rarely, regio- [3,4] selectivity (Fig. 1a). Control of selectivity in protein reactions has essentially focused on the nature of the functional groups used in reagents and their substituents (Fig. 1b) [4], while little focus has been placed on how the stereochemistry of such reagents may modulate reactivity and selectivity (and/or diminish side reactions) (Fig. 2). This is despite the fact that proteins provide powerful archetypes for reactive chiral environments [5].

The incorporation of dehydroalanine (Dha) into proteins and peptides has emerged as a method for generating structural and functional diversity in peptides and proteins. It cannot be directly incorporated using standard peptide synthesis strategies and thus a masked amino acid is commonly incorporated and subsequently converted to Dha. In several applications the Dha residue can act essentially as a Cα=Cβ-double bond sidechain 'stump' that may then be 'grafted onto' within proteins using various methods that make Cβ–Xγ bonds in proteins (where X can now = B, C, N, O, P, S, Se etc) [7]. This has enabled the post-translational alteration of side-chain identity and hence the primary sequence of proteins (to insert either canonical or non-canonical residues) without recourse to genetic intervention – a form of protein 'editing'.

Dha itself is an integral constituent of modified ribosomal peptides (so-called RiPPs [8]), such as the lanthipeptides and thiopeptides, being used to generate, for example, lanthionine and pyridine cross-links [9] as well as remaining unmodified in several lantibiotic structures. It has also been used as an elegant tool in chemical biology, for example, as an electrophilic probe of deubiquitinase activity and selectivity [10], and its enzymatic generation in mammalian-signalling kinases (MAPKs) by pathogen virulence factors has recently been suggested to have a potentially important effect upon mammalian physiology, against which humans have developed protective pathways [4].

Multiple complementary methods now exist for site-specific Dha incorporation into proteins (and are reviewed elsewhere [11]). One operationally simplistic formation process for Dha in proteins and peptides is that of Cys-selective bis-alkylation/elimination; it utilizes mild reaction conditions with reagents such as 2,5-dibromohexanediamide (DBHDA; Sigma-Aldrich, cat no 900607) and avoids the need for incorporation of non-canonical amino acids as precursors [11,12]. This can be combined with a Cys introduced via conventional mutagenesis to control the position of the desired 'tag/stump' site of interest; Dha acts

☆ Dedicated to Professor Chi-Huey Wong on the occasion of him receiving the 2022 Tetrahedron Prize.

* Corresponding author.
E-mail address: Ben.Davis@rfi.ac.uk (B.G. Davis).

https://doi.org/10.1016/j.tet.2023.133529
Received 4 June 2023; Accepted 26 June 2023
Available online 8 July 2023

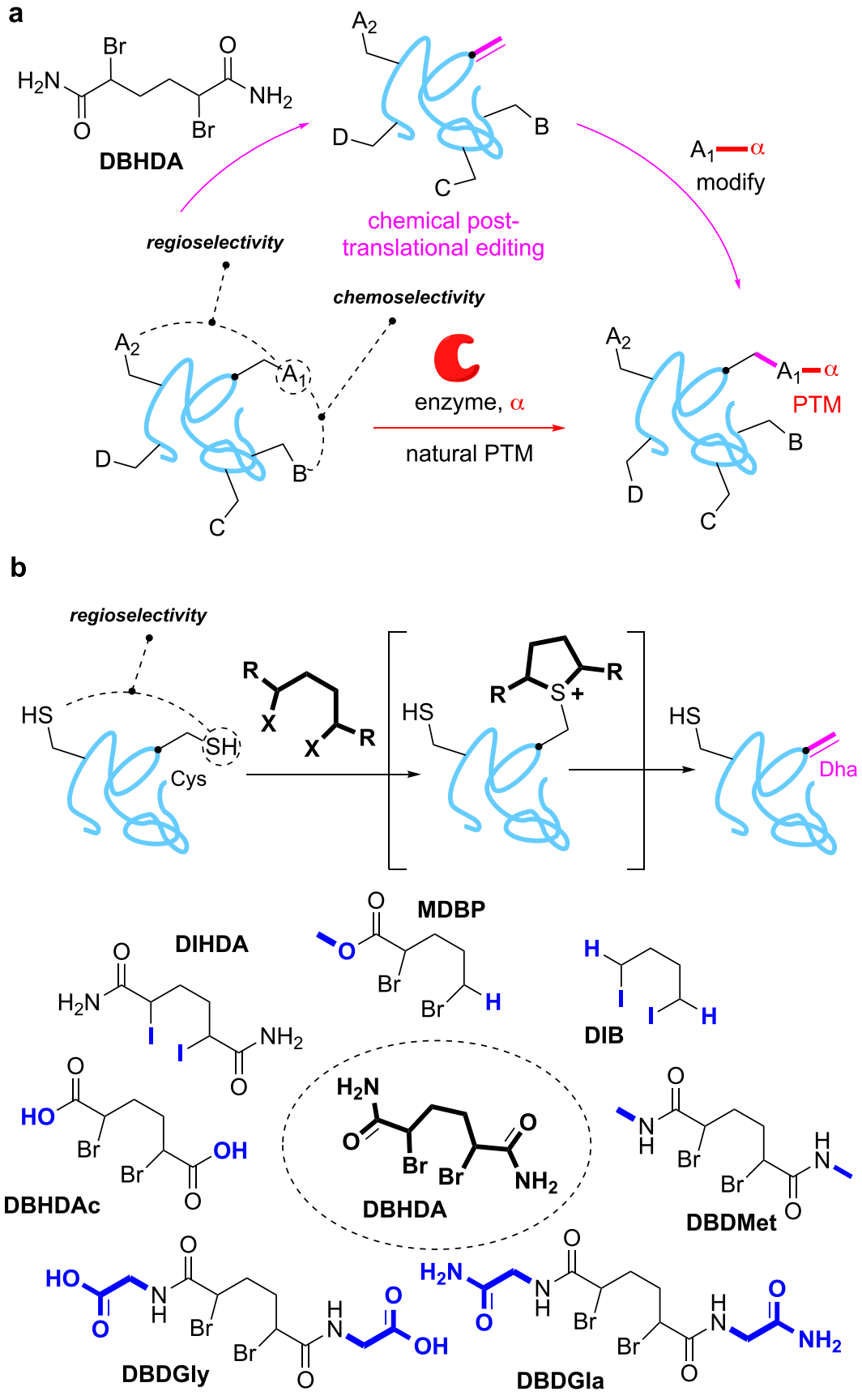


Fig. 1. **a**) Schematic representation of the concepts of regio- and chemo-selectivity in the chemical modification of proteins via Dha intermediates. **b**) Selected range of 2,5-dihalogenated alkylating agents previously explored in the chemoselective conversion of Cys to Dha via cyclic sulfonium intermediates [4].

as an intermediate, divergently-reactive 'tag' [13]. Variation of the functionality of the *bis*-alkylation/elimination reagents that are used has been shown to allow useful control of reactivity and selectivity (Fig. 1b) [4]. Yet, the role of stereochemical control upon reactivity has not been explicitly considered until now. Indeed, these reagents have been used to date essentially universally as a mixture of stereoisomers.

In reactions between chiral substrates and chiral reagents, asymmetric (resulting diastereomeric) effects can cooperate (matched pairs) or compete (mismatched pair) [14,15]. While matched and mismatched pairs are commonly considered in the field of asymmetric synthesis and catalysis (denoting if a pair of chiral reagents augment or reduce the resulting diastereomeric ratio of the reaction products compared to the use of an achiral reagent/catalyst/ligand) [14,15], similar principles can of course be applied to explain differential reactivity of stereoisomeric reagents. As Kagan has presciently highlighted [16], underlying regioselectivity can be either implicit or revealed by such effects. In the context of proteins as diversely chiral substrates for chemistry, this, in principle, could greatly affect the chemo- and site-selectivity of functional group-specific reagents, yet has not been thoroughly studied.

The three-step, *bis*-alkylation/elimination pathway of Dha formation (Fig. 2) provides an intriguing model system with which to explore the question of such matching/mis-matching. Whilst at first inspection it may seem that the stereochemical course is set following step 1 alkylation, we have previously determined [6] that the pathway involves the critical intermediacy of sulfonium ylids that therefore may allow for a stereoconvergent pathway (and then indeed loss of the key stereogenic elements) via the most efficient E_1cb-like elimination. This then allows for a step 3 that (via dynamic preequilibrium in the formation of the needed internal ylid general base) leads to optimal rate-limiting step 3 elimination to Dha regardless of the intermediates formed in steps 1 and 2. This then usefully confines potential matching or mismatching effects essentially to step 1 (and potentially step 2 in side-product formation). Here through the synthesis and kinetic characterization of enantioenriched DBHDA, as an archetypal *bis*-alkylation/elimination reagent, we have now established that such effects are indeed present.

2. Results

In all uses in protein chemistry, to our knowledge, DBHDA has been sold [17] and used as a mixture of D-/L-/*meso* diastereomers. However, until now no focus has been given to study potential differences in the reactivity of the different stereoisomers. Initial attempts at separation focused on selective crystallization. An updated [12,18–21], multigram-scale approach has been developed that not only bypasses the need for toxic solvents and reagents, but allows ready access to significant quantities of DBHDA, without the need for column chromatography (Fig. 3a). Recrystallization of a diastereomeric mixture (1:1 meso:D-/L-) of DBHDA from a minimum of hot methanol afforded small crystals of *meso*-DBHDA and allowed unambiguous identification via single crystal X-ray diffraction (Fig. 3a). However, separation of D-/L-(*R*,*R*- and *S*,*S*-) DBHDA with this method was not possible, prompting us to develop a brief stereoselective synthesis.

To enable stereoselective α-bromination, we applied Evans' auxiliary oxazolidinones [22], as a proven and facile method allowing us to access both *R*,*R* and *S*,*S* isomers with a single strategy (Fig. 3b) in two parallel routes. Starting from adipic acid, carbodiimide (DCC) mediated

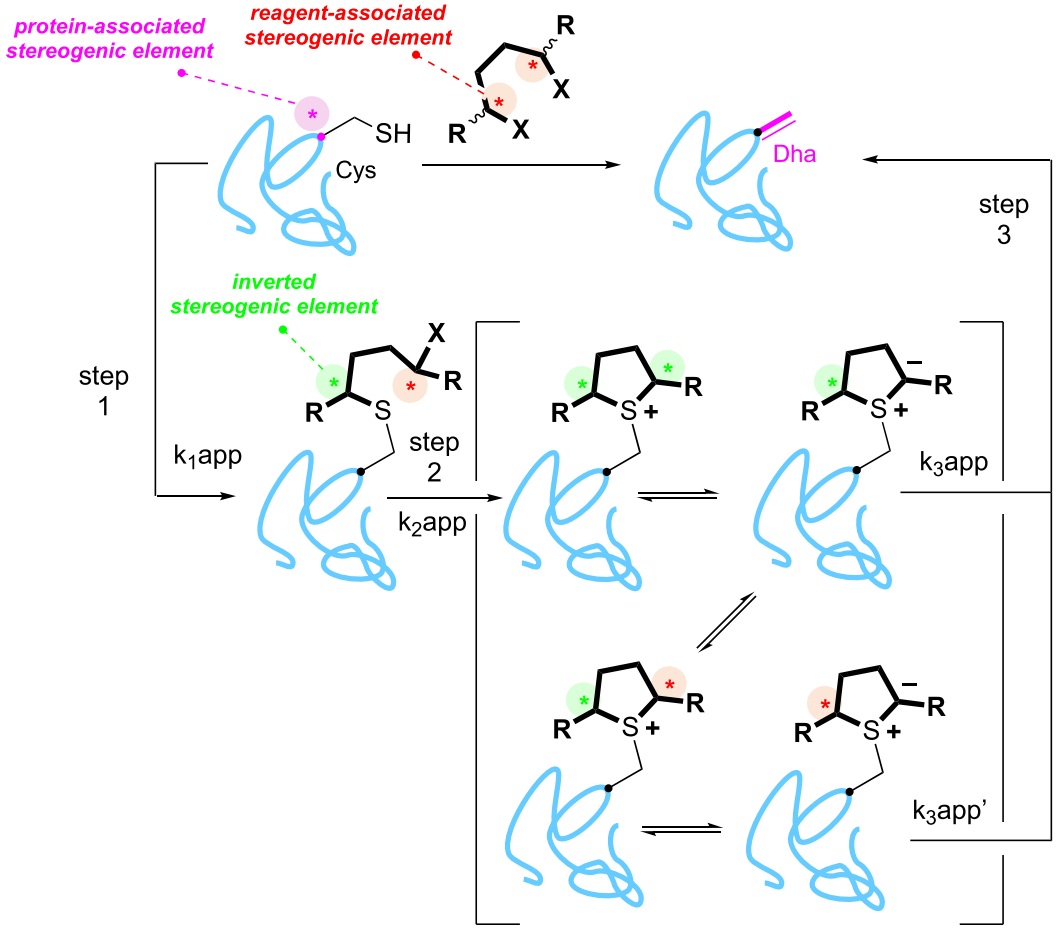


Fig. 2. Proposed mechanism for the DBHDA-mediated conversion of Cys to Dha [6], highlighting the potential role of sulfonium ylids as general bases in step 3 E_1cB elimination, including the potential epimerization of α-stereocentres via protonation of ylids.

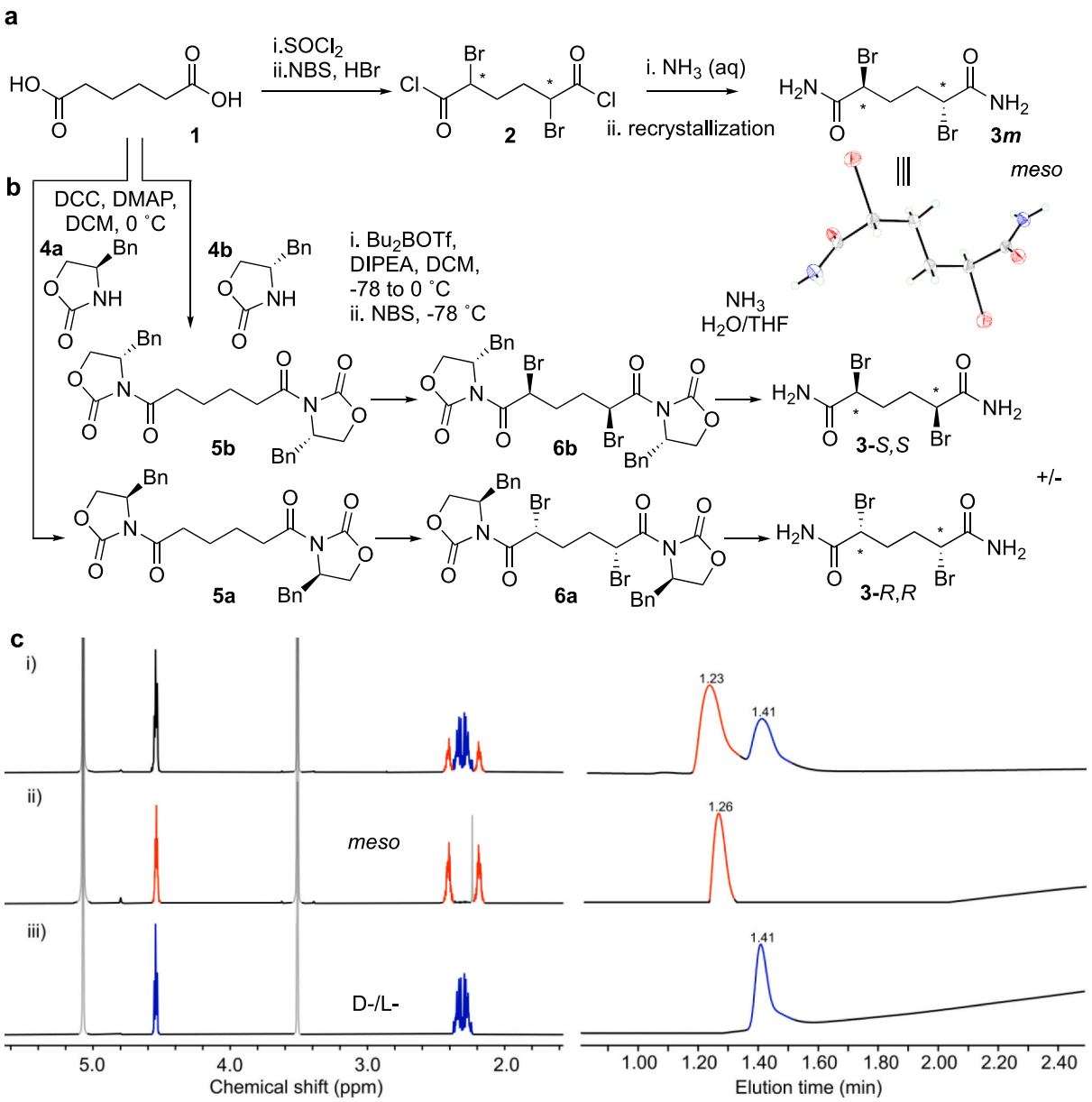


Fig. 3. **a**) Synthesis of meso-DBHDA by recrystallization of a stereoisomeric mix. Displacement ellipsoid plot for meso-DBHDA (inset); see ESI for more information. **b**) Stereoselective synthesis of DBHDA developed in this work. **c**) i) ^{1}H NMR spectrum and RP-HPLC trace of a mixture of *meso* and ± DBHDA. ii) ^{1}H NMR spectrum and RP-HPLC trace of recrystallised *meso*-DBHDA. iii) ^{1}H NMR spectrum and RP-HPLC trace of racemic-DBHDA.

coupling [23] with appropriate homochiral benzyl oxazolidinone, led to the formation of the desired imides. The *bis*-imides were treated with dibutylboryl triflate under basic conditions, followed by the addition of NBS at -78C. The reaction afforded product with 38%–46% yields and d.r.s 7:1:1 *R,R*: *S,R*: *R,S* or 7:1:1 *S,S*: *S,R*: *R,S* via HPLC analysis of the crude. Aminolysis afforded enantioenriched DBHDA that were assigned as *S,S*- or *R,R*-in both enantiomeric series (Fig. 3c and d) based on precedent [22].

Next, we tested possible differences in reactivity on two different Cys-containing proteins. The first, representative of a well-folded region, used a cAbVCAM scaffold, a single-chain antibody (nanobody) that is against human and murine VCAM1, into which a Glu residue at site 118 was mutated to Cys, placing a cysteine target residue in an exposed yet structured area between two glycines on a β-sheet of the protein [24]. The second, to probe a more disordered region, used the Histone H3 scaffold, where an N-terminal histone tail site 2 introduces Cys into a site further from influence of proximal folds and residues. Both proteins cAbVCAM–Cys118 and $H3_{TEV}$–Cys2 were incubated (at 100 μM) with 100 eq of the stereoisomeric DBHDA reagents (from a 0.2 M DMSO stock) in sodium phosphate buffer pH 8, at 36 °C and the reaction progress monitored directly via intact protein mass spectroscopy (Fig. 4). This allowed not only monitoring of relative conversion but also the observation, notably, of individual alkylation/*bis*-alkylation/elimination intermediates. Each reaction was performed in triplicate.

In the case of cAbVCAM-Cys118 (Fig. 4a), we observed that while the consumption of Cys-containing protein was almost identical in the examples of *S,S*- and *meso*-DBHDA, *R,R*-DBHDA had a faster kinetic profile, showing an initial conversion rate characterized by $k_1$app approximately two-fold faster. The monoalkylated species were relatively short-lived in all cases and the more rapid consumption of cAbVCAM-Cys118 therefore resulted in a concomitant higher concentration of sulfonium formation ($k_2$app > $k_3$app). Notably, final elimination conversions proved convergent, potentially consistent with a pathway via a common epimerized intermediate (Fig. 3).

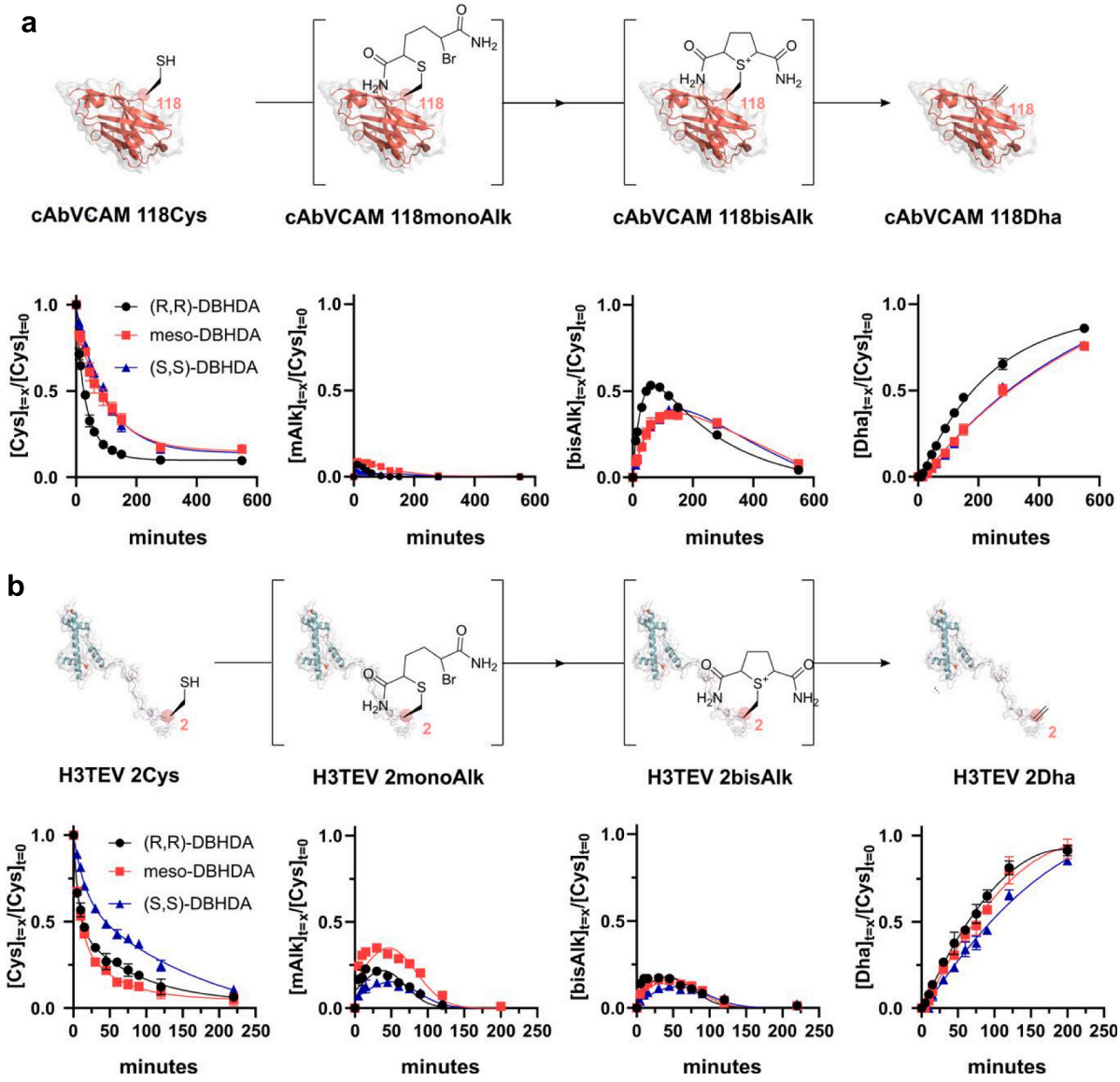


Fig. 4. Reaction timecourses for the formation of Dha, using different DBHDA stereoisomers on two distinct protein scaffolds: (**a**) cAbVCAM-Cys118 and (**b**) $H3_{TEV}$-Cys2.

Differences were also observed in the case of histone $H3_{TEV}$–Cys2, where *R,R*- and *meso*-DBHDA were now found to be the most reactive for the initial alkylation, with *S,S*-DBHDA again found to be slower (Fig. 4b). Here accumulation of the mono-/bis-alkylated intermediates was overall lower and more balanced, leading to an overall more rapid conversion to eliminated product. Nonetheless, interesting differential accumulation was observed this time of the mono-alkylated intermediate: *meso*-DBHDA samples were found to be more greatly enriched with monoalkylation product (ratio 2:1) suggesting potentially rate limiting sulfonium ring formation ($k_3$app > $k_2$app). Again, final elimination conversions proved convergent.

Importantly, after incubation of all three DBHDA stereoisomers under representative conditions (sodium phosphate buffer pH 8, 37 °C for 3 h) minimal background epimerization was observed (<10%, D-/L- to *meso*), further confirming that the observed differences arose from individual matching/mismatching effects of each stereoisomeric reagent.

3. Discussion

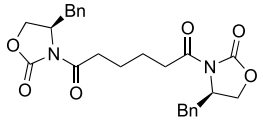
Our results, in the specific case of conversion to Dha from Cys using DBHDA as a *bis*-alkylation/elimination reagent revealed that differential reactivity is observed for stereoisomeric reagents. This differential reactivity is seemingly altered by the nature of the cysteine residue's location in an alternative protein site, implying that context (and not simply the local stereogenic Cα centre of the reacting protein L-Cys residue) is a determining factor in this difference.

In addition, in the particular case of DBHDA it appears that such differential reactivity is primarily observed in the initial alkylation steps (with apparently different rate-limiting steps in the two examples observed here). However, in both cases the reaction progress converges for all stereoisomeric reagents in the final elimination step to Dha, leading to effectively complete conversion. Such an observation is consistent with the observed [6] C–H exchange, via ylid, in sulfonium moieties formed from Cys on proteins; this would in principle lead to stereoconvergence following any stereospecific alkylation events, thereby mitigating any reactivity differences in this final step (Fig. 3).

Together these observations suggest two key things in the context of selective protein chemistries. First, that regardless of the commercial supply and general use of DBHDA as a stereoisomeric mixture (and given its typical use in excess), such differential activities do not affect the final outcome in the chemoselective formation of Dha from Cys. DBHDA can be used as a mixture as an efficient reagent for creating Dha in proteins. Second, such differential reactivity implies [16] potential in regioselective protein chemistries that may, according to context in a given protein framework, allow differentiation between one Cys residue and another. Therefore, whilst not demonstrated in this work, the potential to generate regioselectivity simply through the use of stereoisomeric reagents is now excitingly suggested by these initial results. This strategy would complement prior regioselective strategies for regioselectivity in protein modification [3,4] and is currently under investigation. Moreover, these results suggest that similar effects may be observed for not only other *bis*-alkylation/elimination reagents but for other chemistries and at other residues. As such they may provide insight into site selectivity in other methods in protein science, including cross-linking reagents [25], and covalent protein inhibitors [26].

4. Methods

4.1. 1,6-bis((R)-4-benzoyl-2-oxooxazolidin-3-yl)hexane-1,6-dione **5a**



Based on the method of Andrade et al., [23] adipic acid (2.0 g, 1.0 eq., 13.7 mmol), (*R*)-4-benzyloxazolidin-2-one (7.4 g, 3.0 eq., 41.8 mmol) and DMAP (320 mg, 0.2 eq., 2.7 mmol) were mixed in 40 mL of DCM and cooled down to 0 °C. DCC (11.2, 4.0 eq., 54 mmol) were added, and the suspension was left to reach room temperature. After 16 h, the suspension was filtered over Celite, and the precipitate was washed with DCM (10 mL). The filtrate was washed with sat. $NaHCO_3$ (10 mL), 1 M $HCl_{(aq)}$ (10 mL), water 2 × 10 mL and brine, dried with $MgSO_4$ and concentrated to afford the crude product. The crude material was dissolved in 60 mL of ethyl acetate at reflux temperature, and then petrol ether was added dropwise until minimal cloudiness was observed. The solution was left to cool at room temperature, affording white needle-like crystals of the product (2.73 g, 44% yield).

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.33–7.21 (m, 10H, 10 × Ph-**H**), 4.71–4.63 (ddt, *J* = 10.6, 7.4, 3.1 Hz, 2H, 2 × N–C**H**–CH_2), 4.24–4.13 (m, 4H, 2 × Ph-C**H$_2$**), 3.31 (dd, *J* = 13.4, 3.4 Hz, 2H, CH–C**H$_2$**–O), 3.06–2.92 (m, 4H, C**H$_2$**–CO), 2.77 (dd, *J* = 13.4, 9.7 Hz, 2H, CH–C**H$_2$**–O), 1.84–1.78 (m, 4H, 2 × C**H$_2$**–CH_2–CO).

^{13}C NMR (150 MHz, $CDCl_3$): δ 173.0 (CH_2–**C**O–N), 153.6 (O–**C**O–N), 135.5 (Ph), 129.6 (Ph), 129.1 (Ph), 127.5 (Ph), 66.4 (CH–**C**H_2–O), 55.3 (N–**C**H–CH_2), 38.1 (Ph-**C**H_2), 35.3 (**C**H_2–CO), 23.7 (**C**H_2–CH_2–CO).

HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{26}H_{29}N_2O_6$: 465.2026; Found: 465.1958.

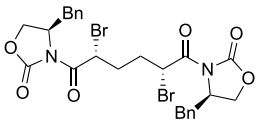
R_f (2:3 EtOAc:pet. ether 40-60): 0.6

m.p. (EtOAc:pet. ether 40-60): 136.4 °C.

$[\alpha]_D^{25}$ (*c* 0.6, CH_2Cl_2): −82.7

IR (cm^{-1}): 2969, 1776, 1698, 1387, 1211, 704.

4.2. (2R,5R)-1,6-bis((R)-4-benzyl-2-oxooxazolidin-3-yl)-2,5-dibromohexane-1,6-dione **6a**



Based on the method of Evans et al., [22] bis-oxazolidinone (500 mg, 1eq., 1.08 mmol) was dissolved in 10 mL of dry DCM and cooled to −78 °C. Dry DIPEA (500 μL, 2.7 eq. 2.9 mmol) was added to the stirred solution followed by dropwise addition of dibutylboron trifluoromethanesulfonate (3 mL, 1 M in DCM, 2.8 eq., 3 mmol). The yellowish solution, was stirred at −78 °C for 20 min and then at 0 °C for 1.5 h. In a separate flask, *N*-bromosuccinimide (540 mg, 2.8 eq., 3.0 mmol) was mixed with 5 mL of dry DCM and the slurry was precooled to −78 °C. The boron enolate solution was transferred rapidly to the precooled NBS flask and the reaction was stirred at −78 °C for 2 h. The reaction was quenched with 1 M sodium thiosulfate, washed with water 2 × 10 mL, brine 1 × 10 mL, dried over anhydrous $MgSO_4$, filtered and the filtrate concentrated under vacuum. The d.r. was found to be 7:1:1 b y HPLC analysis of the crude mix. Diastereomerically pure *bis*--bromo-*bis*-imide was isolated by flash column chromatography (ethyl acetate: pet. ether 40–60, 0–40% gradient) as a white solid (260 mg, 39% yield).

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.29–7.17 (m, 10H, 10 × Ph-**H**), 5.57 (dd, *J* = 7.5, 5.7 Hz, 2H, 2 × C**H**Br), 4.67 (ddt, *J* = 9.5, 7.8, 3.4 Hz, 2H, 2 × N–C**H**–CH_2), 4.25–4.11 (m, 4H, 2 × Ph-C**H$_2$**), 3.24 (dd, *J* = 13.6, 3.4 Hz, 2H, 2 × CH–C**H$_2$**–O), 2.74 (dd, *J* = 13.5, 9.5 Hz, 2H, 2 × CH–C**H$_2$**–O), 2.32–2.14 (m, 4H, 2 × C**H$_2$**–CHBr).

^{13}C NMR (150 MHz, $CDCl_3$): δ 168.8 (CHBr–CO–N), 152.6 (O–CO–N), 134.9 (Ph), 129.6 (Ph), 129.2 (Ph), 127.6 (Ph), 66.4 (CH–CH_2–O), 55.3 (N–CH–CH_2), 42.9 (CHBr–CO), 37.1 (Ph- CH_2), 31.7 (CH_2–CHBr).

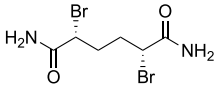
HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{26}H_{27}Br_2N_2O_6$: 621.0236, 623.0216, 625.0195; Found: 621.0269, 623.0278, 625.0267.

R_f (2:3 EtOAc: pet. ether 40–60): 0.2

m.p.: 108.1 °C.

$[\alpha]_D^{25}$ (*c* 0.27, CH_2Cl_2): −60.7

4.3. (2R,5R)-2,5-dibromohexanediamide **3-R,R**



Bis-bromo-*bis*-imide (150 mg, 0.24 mmol) was dissolved in 5 mL THF: 33% NH_3 (aq.) 4:1 and stirred at 0 °C for 15 min. The solution was concentrated under vacuum, and the crude residue was solubilized in 0.5 mL of DMSO and purified via reverse phase C18 prep-HPLC (5%–95% MeCN in H_2O, 0.1% formic acid) to yield (22 mg, 30%) as a white solid.

^{1}H NMR (600 MHz, CD_3OD) δ 4.37–4.31 (m, 2H, CBr**H**–CO), 2.18–2.03 (m, 4H, C**H$_2$**– CBrH–CO).

^{13}C NMR (150 MHz, CD_3OD) δ 173.8 (CBrH–**C**O–N), 48.0 (**C**HBr–CO), 34.1 (**C**H_2– CBrH–CO).

HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_6H_{11}Br_2N_2O_2$: 300.9187, 302.9167, 304.9147; Found: 300.9225, 302.9210, 304.9190.

R_f (EtOAc): 0.4

m.p.: 101.0 °C.

$[\alpha]_D^{25}$ (c = 0.10, DMSO): +42

IR (cm^{-1}): 3436, 3303, 3199, 1681, 1606, 1219, 565, 533.

4.4. meso-2,5-dibromohexanediamide [12] **3m**

^{1}H NMR (600 MHz, CD_3OD) δ 4.34 (ddt, *J* = 7.0, 4.7, 2.4 Hz, 2H,

CBrH–CO), 2.26–2.16 (m, 2H, CH2– CBrH–CO), 2.03–1.94 (m, 2H, CH2–CBrH–CO).

^{13}C NMR (150 MHz, CD_3OD) δ 173.7 (CBrH–CO–N), 47.8 (CHBr–CO), 34.2 (CH2– CBrH–CO).

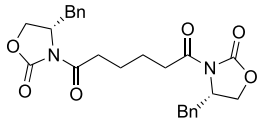
R_f (EtOAc): 0.4

m.p.: 109 °C.

IR (cm^{-1}): 3435, 3302, 3198, 1677, 1606, 1220, 567, 533.

Crystal structure: Monoclinic, C 2/c (a = 18.6110 (8) Å, b = 12.3548 (4) Å, c = 18.9052 (7) Å, β = 109.697(4)°, V = 4092.6(3) $Å^3$). Full solution and refinement details in ESI/CIF [27–29]; Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (CCDC 2281273) and can be obtained via www.ccdc.cam.ac.uk/data_request/cif.

4.5. 1,6-bis((S)-4-benzoyl-2-oxooxazolidin-3-yl)hexane-1,6-dione **5b**



To a suspension of adipic acid (0.50 g, 3.42 mmol, 1.0 eq.) in CH_2Cl_2 (6.8 mL) at 0 °C was added DCC (1.41 g, 6.84 mmol, 2.0 eq.), followed by DMAP (83 mg, 0.68 mmol, 0.2 eq.), and the mixture was allowed to stir for 5 min. (*S*)-oxazolidinone (1.27 g, 7.18 mmol, 2.1 eq.) was added in a single portion, and the reaction was allowed to warm to ambient temperatures. After 16 h the reaction mixture was filtered, washing the precipitate with minimal CH_2Cl_2. The isolated solution was washed with sat. $NaHCO_3$, then brine, before drying over Na_2SO_4. Filtration and concentration under reduced pressure gave crude bis-imide as a yellowish solid, which was subsequently recrystallised from hot EtOAc-pet. ether 40–60 to yield the *bis*-oxazolidinone as a white solid (680 mg, 1.46 mmol, 43%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.40–7.33 (m, 4H), 7.32–7.30 (m, 2H), 7.26–7.22 (m, 4H), 4.79–4.65 (m, 2H), 4.29–4.16 (m, 4H), 3.34 (dd, *J* = 13.4, 3.3 Hz, 2H), 3.12–2.93 (m, 4H), 2.79 (dd, *J* = 13.4, 9.6 Hz, 2H), 1.97–1.77 (m, 3H).

^{13}C NMR (150 MHz, $CDCl_3$): δ 168.8, 152.6, 134.9, 129.6, 129.2, 127.6, 66.5, 55.3, 42.9, 37.1, 31.7.

HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{26}H_{29}N_2O_6$: 465.2026; Found: 465.2046.

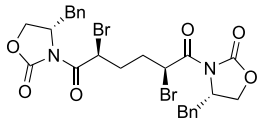
R_f (2:3 EtOAc:pet. ether 40–60): 0.6.

$[\alpha]_D^{25}$ (*c* 0.37, CH_2Cl_2): +81

m.p.: (EtOAc/pet. ether 40–60): 132–134 °C.

IR (cm^{-1}): 2921, 1772, 1697, 1386, 1208, 703.

4.6. (2S,5S)-1,6-bis((S)-4-benzyl-2-oxooxazolidin-3-yl)-2,5-dibromohexane-1,6-dione **6b**



To a solution of bis-imide (500 mg, 1.05 mmol, 1.0 eq.) in CH_2Cl_2 at −78 °C was added ^{i}Pr$_2$NEt (459 μL, 2.59 mmol, 2.4 eq.), followed by dropwise addition of a 1.0 M solution of dibutylboryl triflate in CH_2Cl_2 (2.34 mL, 2.34 mmol, 2.1 eq.). The reaction mixture was stirred for 15 min at −78 °C before warming to 0 °C and stirring for a further 1 h. The solution of boron enolate was again cooled to −78 °C, whilst a slurry of *N*-bromosuccinimide (416 mg, 2.34 mmol, 2.2 eq.) was prepared in CH_2Cl_2 (2.34 mL) and cooled to −78 °C. The boron enolate solution was rapidly added to the flask containing the *N*-bromosuccinimide slurry and bromination was allowed to proceed for 90 min at −78 °C. The reaction mixture was quenched by pouring on to 1 M $Na_2S_2O_3$, and the aqueous layer was extracted with EtOAc (x 3). Combined organics were washed with 1 M $Na_2S_2O_3$ and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Flash chromatography (SiO_2, 0–30% EtOAc in pet. ether 40–60) gave bis-bromo-bis-imide (192 mg, 0.31 mmol, 29%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29–7.17 (m, 10H, 10 × Ph-**H**), 5.64–5.52 (m, 2H, 2 × C**H**Br), 4.66 (ddt, *J* = 9.6, 7.9, 3.4 Hz, 2H, 2 × N–C**H**–CH2), 4.24–4.11 (m, 4H, 2 × Ph-C**H$_2$**), 3.23 (dd, *J* = 13.6, 3.5 Hz, 2H, 2 × CH–C**H$_2$**–O), 2.74 (dd, *J* = 13.6, 9.5 Hz, 2H, 2 × CH–C**H$_2$**–O), 2.32–2.16 (m, 4H, 2 × C**H$_2$**–CHBr).

^{13}C NMR (150 MHz, $CDCl_3$): δ 168.8 (CHBr–**C**O–N), 152.6 (O–**C**O–N), 134.9 (Ph), 129.6 (Ph), 129.2 (Ph), 127.6 (Ph), 66.4 (CH–**C**H$_2$–O), 55.3 (N–**C**H–CH$_2$), 42.9 (**C**HBr–CO), 37.1 (Ph-**C**H$_2$), 31.7 (**C**H$_2$–CHBr).

HRMS (ESI-TOF) *m/z*: [M+H]+ Calcd for C26H27Br2N2O6: 621.0236, 623.0216, 625.0195.

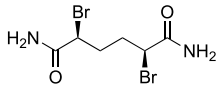
R_f (2:3 EtOAc:pet. ether 40–60): 0.2.

$[\alpha]_D^{25}$ (*c*, 0.91, CH_2Cl_2): +60.2

m.p.: 105 °C.

IR (cm^{-1}): 3029, 2924, 1773, 1696, 1387, 1207, 703.

4.7. (2S,5S)-2,5-dibromohexanediamide **3-S,S**



Bis-bromo-bis-imide (18 mg, 28.9 μmol, 1.0 eq.) was dissolved in 2:1 THF-33% aq. ammonia (289 μL) and rapidly stirred for 20 min. At this point, the formation of a white precipitate was noted and no starting material was detected by TLC (EtOAc), so methanol (2 mL) was added. The reaction was concentrated under reduced pressure, and purified directly by reverse phase prep-HPLC (5%–95% MeCN in H_2O, +0.1% formic acid), yielding *S,S*-DBHDA as a white solid (2.3 mg, 26%).

^{1}H NMR (600 MHz, CD_3OD): δ 4.42–4.28 (m, 2H, 2 × C**H**Br), 2.21–2.01 (m, 4H, 2 × C**H$_2$**).

^{13}C NMR (151 MHz, CD_3OD): δ 173.8 (**C**O), 48.0 (**C**Br), 34.1 (**C**H$_2$).

HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_6H_{11}Br_2N_2O_2$: 300.9187, 302.9167, 304.9147; Found: 300.9225, 302.9210, 304.9190.

R_f (EtOAc): 0.4.

$[\alpha]_D^{25}$ (*c* 0.23, DMSO): -37

m.p.: 104 °C.

IR (cm^{-1}): 3435, 3302, 3199, 1680, 1606, 1220, 565, 530.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We are grateful to the following funders: the BBSRC with AstraZeneca (studentship to S.R.G.G.), UCB (studentship to B.J.B.). The Chemistry theme at the Rosalind Franklin Institute is supported by the EPSRC (V011359/1 (P)).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tet.2023.133529.

References

[1]. B.G. Davis, Mimicking posttranslational modifications of proteins, Science 303 (5657) (2004) 480–482, https://doi.org/10.1126/science.1093449.

[2]. O. Boutureira, G.J.L. Bernardes, Advances in chemical protein modification, Chem. Rev. 115 (5) (2015) 2174–2195, https://doi.org/10.1021/cr500399p.

[3]. S.I. van Kasteren, H.B. Kramer, H.H. Jensen, S.J. Campbell, J. Kirkpatrick, N. J. Oldham, D.C. Anthony, B.G. Davis, Expanding the diversity of chemical protein modification allows post-translational mimicry, Nature 446 (7139) (2007) 1105–1109, https://doi.org/10.1038/nature05757.

[4]. K.-Y. Lai, S.R.G. Galan, Y. Zeng, T.H. Zhou, C. He, R. Raj, J. Riedl, S. Liu, K. P. Chooi, N. Garg, et al., LanCLs add glutathione to dehydroamino acids generated at phosphorylated sites in the proteome, Cell 184 (10) (2021) 2680–2695.e2626, https://doi.org/10.1016/j.cell.2021.04.001.

[5]. W.P. Jencks, Catalysis in Chemistry and Enzymology, Courier Corporation, 1987.

[6]. S.R.G. Galan, J.R. Wickens, J. Dadova, W.-L. Ng, X. Zhang, R.A. Simion, R. Quinlan, E. Pires, R.S. Paton, S. Caddick, et al., Post-translational site-selective protein backbone α-deuteration, Nat. Chem. Biol. 14 (10) (2018) 955–963, https://doi.org/10.1038/s41589-018-0128-y.

[7]. M. Zhang, P. He, Y. Li, Contemporary approaches to α,β-dehydroamino acid chemical modifications, Chem. Res. Chin. Univ. 37 (5) (2021) 1044–1054, https://doi.org/10.1007/s40242-021-1307-z.

[8]. P.G. Arnison, M.J. Bibb, G. Bierbaum, A.A. Bowers, T.S. Bugni, G. Bulaj, J. A. Camarero, D.J. Campopiano, G.L. Challis, J. Clardy, et al., Ribosomally synthesized and post-translationally modified peptide natural products: overview and recommendations for a universal nomenclature, Nat. Prod. Rep. 30 (1) (2013) 108–160, https://doi.org/10.1039/C2NP20085F.

[9]. Z. Zhang, G.A. Hudson, N. Mahanta, J.I. Tietz, W.A. van der Donk, D.A. Mitchell, Biosynthetic timing and substrate specificity for the thiopeptide thiomuracin, J. Am. Chem. Soc. 138 (48) (2016) 15511–15514, https://doi.org/10.1021/jacs.6b08987.

[10]. R. Meledin, S.M. Mali, O. Kleifeld, A. Brik, Activity-based probes developed by applying a sequential dehydroalanine formation strategy to expressed proteins reveal a potential α-globin-modulating deubiquitinase, Angew. Chem. Int. Ed. 57 (20) (2018) 5645–5649, https://doi.org/10.1002/anie.201800032.

[11]. J. Dadová, S.R.G. Galan, B.G. Davis, Synthesis of modified proteins via functionalization of dehydroalanine, Curr. Opin. Chem. Biol. 46 (2018) 71–81, https://doi.org/10.1016/j.cbpa.2018.05.022.

[12]. J.M. Chalker, S.B. Gunnoo, O. Boutureira, S.C. Gerstberger, M. Fernández-González, G.J.L. Bernardes, L. Griffin, H. Hailu, C.J. Schofield, B.G. Davis, Methods for converting cysteine to dehydroalanine on peptides and proteins, Chem. Sci. 2 (9) (2011) 1666–1676, https://doi.org/10.1039/C1SC00185J.

[13]. J.M. Chalker, G.J.L. Bernardes, B.G. Davis, A "tag-and-modify" approach to site-selective protein modification, Accounts Chem. Res. 44 (9) (2011) 730–741, https://doi.org/10.1021/ar200056q.

[14]. A. Horeau, H.-B. Kagan, J.-P. Vigneron, Syntheses asymétriques par double induction, Bull. Chim. Soc. Fr. (1968) 3795–3797.

[15]. S. Masamune, W. Choy, J.S. Petersen, L.R. Sita, Double asymmetric synthesis and a new strategy for stereochemical control in organic synthesis, Angew Chem. Int. Ed. Engl. 24 (1) (1985) 1–30, https://doi.org/10.1002/anie.198500013.

[16]. R.R. Kumar, H.B. Kagan, Regioselective reactions on a chiral substrate controlled by the configuration of a chiral catalyst, Adv. Synth. Catal. 352 (2–3) (2010) 231–242, https://doi.org/10.1002/adsc.200900822.

[17]. www.sigmaaldrich.com/FR/fr/product/aldrich/900607, www.tocris.com/products/dbhda_6175, www.bldpharm.com/products/99584-96-0.html. www.rndsystems.com/products/dbhda_6175.

[18]. S. Galan, Site-selective Chemical Phosphorylation and Activation of Kinases, 2016. D.Phil. Thesis, University of Oxford.

[19]. B. Bower, Novel Protein Modification Strategies, 2018, D.Phil. Thesis, University of Oxford..

[20]. B. Bower, S.R.G. Galan, B.G. Davis, Chromatography-Free and Chlorinated-solvent-free Preparation of 2,5-dibromohexanediamide (DBHDA), submitted.

[21]. For original preparations see Chalker et al, 2011, citation [11].

[22]. D.A. Evans, T.C. Britton, J.A. Ellman, R.L. Dorow, The asymmetric synthesis of . alpha.-amino acids. Electrophilic azidation of chiral imide enolates, a practical approach to the synthesis of (R)- and (S)-.alpha.-azido carboxylic acids, J. Am. Chem. Soc. 112 (10) (1990) 4011–4030, https://doi.org/10.1021/ja00166a045.

[23]. C.K.Z. Andrade, R.O. Rocha, O.E. Vercillo, W.A. Silva, R.A.F. Matos, DCC/DMAP-Mediated coupling of carboxylic acids with oxazolidinones and thiazolidinethiones, Synlett 2003 (15) (2003) 2351–2352, https://doi.org/10.1055/s-2003-42117.

[24]. A. Broisat, S. Hernot, J. Toczek, J. De Vos, L.M. Riou, S. Martin, M. Ahmadi, N. Thielens, U. Wernery, V. Caveliers, et al., Nanobodies targeting mouse/human VCAM1 for the nuclear imaging of atherosclerotic lesions, Circ. Res. 110 (7) (2012) 927–937, https://doi.org/10.1161/CIRCRESAHA.112.265140.

[25]. L. Piersimoni, P.L. Kastritis, C. Arlt, A. Sinz, Cross-linking mass spectrometry for investigating protein conformations and protein–protein Interactions—A method for all seasons, Chem. Rev. 122 (8) (2022) 7500–7531, https://doi.org/10.1021/acs.chemrev.1c00786.

[26]. L. Boike, N.J. Henning, D.K. Nomura, Advances in covalent drug discovery, Nat. Rev. Drug Discov. 21 (12) (2022) 881–898, https://doi.org/10.1038/s41573-022-00542-z.

[27]. L. Palatinus, G. Chapuis, Superflip - a computer program for the solution of crystal structures by charge flipping in arbitrary dimensions, J. Appl. Crystallogr. 40 (4) (2007) 786–790, https://doi.org/10.1107/S0021889807029238.

[28]. P.W. Betteridge, J.R. Carruthers, R.I. Cooper, K. Prout, D.J. Watkin, CRYSTALS version 12: software for guided crystal structure analysis, J. Appl. Crystallogr. 36 (2003), 1487.

[29]. R.I. Cooper, A.L. Thompson, D.J. Watkin, CRYSTALS enhancements: dealing with hydrogen atoms in refinement, J. Appl. Crystallogr. 43 (5 Part 1) (2010) 1100–1107, https://doi.org/10.1107/S0021889810025598.