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Tandem Wittig/Diels-Alder diversification of genetically encoded peptide libraries

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Abstract

In this paper, we developed a tandem of two carbon-carbon bond-forming reactions to chemically diversify libraries of peptides displayed on bacteriophage. Wittig reaction of a biotin-ester from a stabilized phosphorane ylide with model peptides containing N-terminal glyoxal exhibit reaction rates of 0.07 to 5 $M^{-1}s^{-1}$ in water at pH 6.5-8. The log(k) scaled linearly with pH from pH 6 to 8; above pH 9 the reaction is accompanied by hydrolysis of the ester functionality. Capture of the phage displaying the biotinylated product by streptavidin beads confirmed the rate of this reaction in a library of 10^8 peptides (k=0.23 $M^{-1}s^{-1}$ at pH=6.5) and also confirmed the regioselectivity of this modification. The olefins introduced in the Wittig reaction can act as Michael acceptors: addition of glutathione, cysteamine, and DYKDDDDKC ("FLAG-Cys") peptide occur with k=0.12-4.1 $M^{-1}s^{-1}$ at pH 7.8. Analogous reactions with DYKDDDDKC peptide take place on phage-displayed peptides modified *via* Wittig reaction. This reaction is manifested as a progressive emergence of FLAG-epitope on phage and detected by capture of this phage using anti-FLAG antibody. Olefins introduced in Wittig reaction also act as dienophiles in Diels-Alder reaction with cyclopentadiene. The conversion of the dienophile to norbornene-like adducts on phage was observed by monitoring the disappearance of the thiol-reactive olefin on phage. This report broadens the reaction scope for genetically-encoded peptide libraries displayed on phage, expanding the structural diversity of these platforms and increasing their potential to be used in screening against important protein targets. The possibility of monitoring tandem reactions by the use of different labels illustrates the feasibility of obtaining highly functionalized peptides with chemical motifs impossible to achieve by conventional translational machinery.

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Introduction

Chemically-modified peptide libraries are a valuable source for discovery of ligands for fundamental research, development of diagnostics and biomaterials as well as discovery of therapeutic leads.[1] Chemical post-translational modification of peptides made of 20 "natural" amino acids is the simplest strategy for production of such libraries because it bypasses the need of advanced constructs for incorporation of unnatural amino acids. Introduction of unnatural fragments into genetically-encoded peptide libraries makes it possible to equip these libraries with value-added functionality not encodable by conventional translational machinery.[1–4] Although there exists a rich palette of chemical transformations for site-specific modification of proteins made of natural amino acids, only a limited scope of chemical transformations have been adopted for diversification of genetically-encoded peptide libraries. These reactions include S_N2,[5–8] and S_NAr substitution,[9] Michael[10] and allenamide[11] addition reactions as well as tandem elimination-addition reaction[12] starting from sulfhydryl group of Cys; nucleophilic substitutions of selenocysteine,[13,14] addition of alpha-nucleophiles to aldehydes,[15–18] and cycloadditions to unnatural size chains containing azide or propargyl group[19].

Several carbon-carbon bond forming strategies—based on cycloadditions and transition metal catalyzed reactions—have been reported for modification of amino acid side chains of individual proteins or peptides in water[18,20–22]. However, the only example to date of a C-C bond formation reaction performed on the phage display context has been reported by Lin and coworkers, who described an elegant optimization of the Sonogashira cross coupling in a subset of peptides on phage libraries. This coupling, however, hinged on expression of an unnatural homopropargylglycine amino acid residue *via* amber suppression.[23]

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Wittig olefination is one of the attractive reactions to explore for diversification of peptide libraries on phage because it has already been employed to modify individual proteins in water as well as amino acids ligated to DNA strands.[24–26] Wittig chemistry has also been employed by Liu and coworkers for macrocyclization of DNA-templated libraries.[27] In proteins, aldehyde handles suitable for Wittig reaction are readily introduced by mild oxidative cleavage of natural residues that contain 1,2-aminoalcohols (N-Ser/Thr)[15,32,33] or 1,2-diols (glycans);[34] it can also be incorporated via UAA-mutagenesis,[35] or encoded as a L(C/A)T(P/A)S(A/R) peptide sequence recognized by the formylglycine generating enzyme.[18,36] Specifically, in peptide libraries displayed on phage, the orthogonal aldehyde handle can be quantitatively introduced by selective oxidation of N-terminal serine.[15] Conveniently, the Wittig reaction between N-terminal glyoxy-aldehyde and ester-stabilized ylide produces an olefin within an extended conjugation framework (Fig. 1) that enhances its performance both as Michael acceptor and dienophile. The introduced conjugated alkene functionality could enable survey of up to 10^6-10^9 Michael electrophiles to identify suitable covalent or reversible covalent inhibitors.[29–31] Introduction of norbornene-like moieties *via* tandem Wittig-Diels Alder reaction also opens the door for robust labeling *via* bioorthogonal aziridination[45] or inverse electron demand Diels-Alder cycloaddition with tetrazine[46] in a phage display context. While individual aforementioned reactions are well-established, the tandem use of such modifications in 10^6-10^9 scale genetically encoded libraries has not been investigated or reported to date.

The Wittig reaction of stabilized ylides can yield both E and Z isomers, and the rate and selectivity of this chemistry strongly depends on the reaction conditions.[36] Similarly to reactions in methanol, most “on-water” and “in-water” Wittig reactions of stabilized ylides with substituted benzaldehydes, vinyl aldehydes, heterocyclic derived aldehydes or alkyl aldehydes

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exhibit an increase in rates compared to analogous reactions in aprotic solvents at the expense of lower E selectivity.[37–41] In this report, we observed a strong influence of the aldehyde nature on the stereoselectivity of the Wittig reaction with a stabilized ylide *in water*. Products of N-terminal glyoxy-aldehyde handles and 2-nitrobenzaldehyde showed significantly lower E/Z selectivity than products for benzaldehyde at the same conditions. We invoke the dipolar moment effect reported by Harvey and Aggarwal to explain these observations[36,42] and when possible, we determine difference in chemical reactivity towards Michael addition and Diels-Alder of the two isomeric dienophiles.

Results

Nucleophilic addition of biotin-PEG-alcohol to Bestmann ylide yielded a carbonyl stabilized phosphonium salt (YEB) in 65% yield. The same positively charged product was obtained in two steps by reaction of the alcohol with bromoacetyl bromide and triphenylphosphine (Scheme 1), although scaling up of this second strategy generated bisacetylated adducts, considerably reducing the yield and making it less practical. To optimize the Wittig reaction in the environment akin to that of the glyoxal on phage-displayed peptides, we employed a model sequence o-VEKY produced by $NaIO_4$ oxidation of the peptide SVEKY.[15] The Wittig product (YEB-VEKY) was then used for evaluation of rates for Michael addition, retro- Michael and Diels Alder reactions.

The YEB phosphonium salt, with expected pKa of ~10-11 in water,[43] was sufficiently deprotonated in pH 6-8 buffered solution to undergo Wittig reaction. Mixtures of E/Z isomers were observed in reactions with benzaldehyde, 2-nitrobenzaldehyde, and the N-terminal glyoxal in o-VEKY (Fig. 1A and S1-5). Measured rate constants (Fig. S6-S8) and E/Z isomers ratio depended on the nature of the aldehyde. Benzaldehyde reacted with modest rate and produced

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high E/Z isomers selectivity (93:7 or 93% E) whereas 2-nitrobenzaldehyde reacted significantly faster but yielded 60-70% E isomer (Fig. S3-S5). The loss in E-selectivity in *o*-nitrobenzaldehyde, when compared to benzaldehyde were in accord with previous of Bergdahl and coworkers.[44] The rate of reaction and E/Z isomers selectivity (50-70 % E) of oxoacetamide were similar to that of 2-nitrobenzaldehyde and significantly different from that of benzaldehyde. Similar E/Z isomers ratios were observed *via* HPLC (Fig. S1-S2) and ^{1}H NMR analysis of either the crude reaction mixtures, or the purified Wittig products (Fig. S3-S5).

The rate of the Wittig reaction increased exponentially with pH: the graph for the log(k) vs. pH for all types of aldehydes had identical slope of ~1 (Fig. 1C and Fig. S6-S8) in the pH 6-8 range. The slope suggested that the rate-determining step involves an anion. Also, lack of any leveling of rate dependence at pH 8 suggested that these anionic species have pKa>9. This observation is in line with previously measured pKa of an ester stabilized phosphonium salt (10.5 in water)[43] and it differs from the reported value of 8.5 in DMSO.[45] While rate scaled dramatically with pH, the E/Z selectivity changed only subtly (Fig. S3). Rate of the Wittig reaction also modestly varied with changes in buffer concentration (Fig. S9B) and buffer compositions (Fig. S9A).

Z-isomer produced in Wittig reaction hydrolyzed significantly faster than E-isomer (Fig. S10). At pH 8, the half-life of hydrolysis of the E-ester group was $t_{1/2}$ = 18 hours, while Z-ester hydrolyzed completely in 7 hours. At pH 5, half-life of hydrolysis was >75 hours for both isomers. At these pH values, the rate of hydrolysis can be readily decoupled from the rate of Wittig reaction (Fig. S11) and at pH 5, the product can be stored for three days without any hydrolysis. Although we have not shown it explicitly, selective hydrolysis of Z-isomer can be used as one of the strategies to mitigate poor E/Z-selectivity in the product and yield

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predominantly E-product. Subsequent studies of Wittig reaction on peptides and phage displayed peptide libraries were performed in conditions that yield E-Z-mixture. Where possible, we focused on differential reactivity between the E and Z products.

To confirm the yield and specificity of Wittig reaction on phage displayed peptides, we employed a technology based on a streptavidin pull-down assay extensively used by our group.[15] Specifically, we use the phosphorane ylide containing a biotin tag (YEB) to modify a mixture of two phage types: (i) one displaying a SVEK sequence or SX_7 library of peptides displayed on pIII protein and *Lac*Z reporter; this phage produces blue plaques when plated on agar supplemented with X-Gal/IPTG and (ii) phage that displays N-terminal alanine and contains no *Lac*Z, forming white plaques on the same plates. In regioselective functionalization only "blue" phages should acquire biotin and this biotinylation can be detected by pull-down with streptavidin beads. The biotin capture technique[15] confirmed that the reaction of YEB with clonal phage displaying SVEK peptide at pH 7.8 exhibited nearly 90% conversion after one hour, whereas wild type phage in the same reaction, which do not possess the N-terminal serine, exhibited insignificant reactivity (Fig. 2B). The modification of 100,000,000-member SX_7 library of peptides displayed on phage reached 40-60% conversion in the same conditions (Fig. 2B). Incomplete conversion of Wittig reaction in phage libraries was not due to low reaction rates, as shown by measurement of kinetics of modification of the library (Fig. 2C). The half-life of library modification was ~20 min even at lower pH (6.5); the convergence exhibited a first order kinetic profile and reached saturation at ~50% after one hour. The same incomplete conversion has been observed in N-terminal modification of phage libraries.[5,8] To rule out sequence dependence of the oxidation step before Wittig reaction, SX_7 library was subjected to $NaIO_4$ treatment for different time intervals and then reacted with YEB. Increase in oxidation

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time did not change the percentage of functionalization in the SX_7 library context (Fig. S12). Wittig reaction, thus, effectively diversified phage displayed library of 10^8 peptides with only a modest impact on integrity of the library: phage "viability" decreased only to ~50% after three hours reaction, which is similar to viabilities observed in other reactions.[5,9]

The Wittig product contains a Michael acceptor that could potentially form Michael addition products by attack of thiols. We tested glutathione, cysteamine and the peptide sequence DYKDDDDKC, aka "FLAG-Cys" peptide, with this Michael acceptor and rates were ranging from 0.12 to 4.1 $M^{-1}s^{-1}$ at pH 7.8 (Fig. S13). We noticed a minor difference in reactivity in Michael addition of FLAG-Cys thiol to E and Z alkenes; (Fig. S13C) however, both isomers were quantitatively converted to the Michael addition adducts. The Michael acceptor can form either irreversible or reversible covalent interactions with cysteine-like nucleophiles. Therefore, Wittig-diversified libraries can be potentially used for discovery of either irreversible or reversible covalent inhibitors. We evaluated the efficiency of Michael warhead using the approach of Tauton and co-workers.[29] Monitoring the retro-Michael reaction in isolated addition products of thiols with different pKa values (GSH and cysteamine) at pH $\geq$ 6.5[29,46,47] uncovered little to no retro-Michael reaction (Fig. S14), indicating that these Michael acceptors favor irreversible additions. Lack of reversibility of analogous Michael adducts has also been reported by Bernardes and coworkers.[48]

We applied Michael addition on phage-displayed alkene-peptide obtained by Wittig derivatization of SVEK monoclonal phage to confirm the integrity of the Wittig product on phage. Removal of the excess reagent was critical for this reaction and we lowered the excess YEB from initial 4 mM to a sub-nanomolar concentration *via* dialysis of the modified phage against pH 5 buffer, which minimized possible hydrolysis. After 5-6 buffer exchanges were

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performed in 48 hours, we observed < 20% loss of modification (Fig. S16); the concentration of small organic molecule reference (fluorescein) present in this condition was reduced to single digit nM concentrations (Fig. S15C). Purified Wittig product displayed on phage (YEB-VEK phage) reacted effectively with FLAG-Cys and were captured by anti-FLAG (Fig. 3). Neither the WT phages nor purified YEB-VEK phage not exposed to FLAG-Cys peptide were captured in this condition (Fig. 3). To rule out non-specific reactivity with thiol, we confirmed that the phage that was SVEK phage not exposed to YEB did not exhibit significant reactivity in Michael addition (Fig. 4C).

The Wittig product can be "quenched" with cyclopentadiene *via* the Diels-Alder reaction to suppress any Michael addition to the olefin functionality. Aqueous Diels-Alder reaction in the model peptide exhibited a differential reactivity of E and Z isomers: after 48 hours E-isomer exhibited 100% conversion but only minimal conversion for Z isomer was observed (Fig. S16). Identity of the residual Z olefin was confirmed by NMR (Fig. S16D). Addition of co-solvents (acetonitrile or DMF) or Lewis acids (LiCl or $Cu(NO_3)_2$) known to catalyze the Diels-Alder reaction,[32,49] did not improve the reactivity of the Z isomer (Fig. S17). Changing pH had no effect on the reaction and, therefore, Diels Alder can be effectively performed at pH 5 in conditions that suppress hydrolysis of ester. In these conditions, the E isomer is consumed after 20 hours, while conversion of the Z isomer is negligible (Fig. S16B). Most importantly, the olefin that did not react in Diels-Alder preserved its ability to act as Michael acceptor in reaction with FLAG-Cys thiol (Fig. S16C). This observation was used for subsequent "pulse-chase" quantification of the Diels-Alder reaction on phage-displayed peptides.

The Wittig modified phage can also be diversified *via* Diels-Alder reaction with cyclopentadiene. To evaluate the efficiency of the Diels Alder reaction on phage, we employed

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the pulse-chase technique developed in our group.[5,15] Specifically, exposure of purified YEB-modified SVEK phage (Fig. 4B) to FLAG-Cys (pH 7.8, 2 h, RT) renders phage tagged if and only if it has accessible Michael acceptor. As phage was "pulsed" with cyclopentadiene at pH 5 for 48 hours, the alkene was consumed in the Diels-Alder reaction and the labeling in the "chase" reaction with FLAG-Cys peptide decreased. The pulse-chase approach suggested that 70 % of the population modified *via* Wittig reaction was converted to a norborene product (Fig. 4D left bar). We recognized that the loss of Michael reactivity in "pulse chase" approach can be due to: i) blocking of the Michael acceptor due to Diels-Alder reaction, ii) hydrolysis of the ester in α-position to the double bond, iii) reaction with other nucleophiles present in the reaction buffer. To rule out possibilities ii) and iii), we demonstrated that Wittig-modified phage preserved its ability to react with FLAG-Cys peptide even after incubation with pH 5 buffer for 48 hours (Fig. 4D middle bar). We note that incomplete (70%) conversion in Diels-Alder reaction mirrors the lack of reactivity of the Z Wittig adduct observed in reaction on synthetic peptide. While direct structural analysis of E/Z isomers on phage-displayed peptides is presently impossible, the results of pulse-chase study strongly suggest that: i) Wittig product is formed on phage in a similar E/Z ratio as was observed in model synthetic peptide (~70% E), ii) Diels-Alder reaction selectively converts phage-population displaying the E-isomer to *trans*-norborene products; iii) subset of library remaining after Diels-Alder reaction is significantly enriched in Z-isomer.

Discussion

Successful implementation of chemical transformations to diversify a library of substrates requires understanding of the mechanistic aspects of this transformation. The active species in the Wittig reaction is the phosphorane ylide, which results after deprotonation of the phosphonium salt. When the reaction is conducted at pH << pKa, the log(k) of such reaction

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should scale with pH and level off at pH near this pKa. This model is overly simplistic as the rates could also be affected by variations in $[H^+]$ due to the associated changes in hydrogen bonding, solvation effects and dipole-dipole interactions with the transition state (Fig. S9). In peptide-aldehyde substrates, additional attenuation of the transition state can stem from neighboring amino acid side chains. The most important conclusion emanating from the pH profile is the invariance of E/Z selectivity at all tested pH values. Increase in proton concentration, thus, does not significantly change the location of the transition state nor the mechanism of the reaction; the location of the TS is only substrate dependent (Fig. S3).

Kinetically controlled formation of an O-apical oxaphosphetane *via* [2+2] cycloaddition through an asynchronous transition state is also accepted for the Wittig reaction *in* and *on water* and standard, electron rich aldehydes (reference[50,51] and references within). A comparison of transition states between the Wittig reaction of oxoacetamides and benzaldehyde can be visualized *via* More O'Ferrall Jencks (MOJ) diagram (Scheme 2). Lowering of the betaine corner due to the presence of the α-carbonyl that stabilizes the negative charge developed on the aldehyde oxygen results in an *anti*-Hammond movement towards the betaine. As a result, the TS2 for oxoacetamide should have more betaine character (or higher asynchronous character, where C-C bond is formed while P-O bond has significant charge character)[42] when compared to the TS1 for benzaldehyde-like aldehydes. We anticipated that such a shift in the pKa of the transition state could be manifested as a different pH-dependence of rate for different aldehydes at the pH near the pKa of the betaine-like transition state (Scheme 2). Unfortunately, hydrolysis of the substrate and products above pH 10 complicated such studies. We note that the location of the transition state on MOJ plot alone does not reflect the 3D conformation of TS.[50,52,36] 1,2-interactions, 1,3-interations and dipole-dipole interactions in *cis* and *trans* TSs towards

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oxaphosphetanes (OPA-TS) can influence the 3D conformation and determine the final E/Z selectivity. The latter statement assumes irreversibility of the OPA formation, which has been observed experimentally[52,53] and suggested theoretically.[36]

While many mechanistic investigations of Wittig reaction were based on benzaldehyde-like substrates, there exists a critical difference in dipole-dipole interactions between OPA-TA of benzaldehyde and peptide-oxoacetamide substrates. The conformations of OPA-TS were hypothesized to minimize the overall dipole moment while minimizing 1,2 and 1,3-interactions. The TS of reaction originating from the benzaldehyde substrate towards the *trans* OPA was proposed to be semi-puckered due to the generation of favorable dipole-dipole interactions.[50,51,36,54] As the additional α-amide of oxoacetamide introduces an extra dipole, we hypothesize that the TS originating from oxoacetamide towards *trans* OPA cannot adopt the same geometry as benzaldehyde TS due to incomplete cancellation of dipole moment of the carbonyl group. In a similar analysis to the one done by Harvey and Aggarwal on α-alcoxy aldehydes,[42] we propose that the additional dipole destabilizes the puckered *trans* TS, making it similar in energy to a nearly planar *cis* TS and thus, decreasing the expected steroeselectivity of the Wittig reaction with stabilized ylides towards the E isomer (Scheme 3). Similar dipole-induced de-stabilization of the *trans*-puckered TS could potentially explain the lower E/Z selectivity of 2-nitrobenzaldehyde.

The mechanistic hypothesis underpinning the E/Z selectivity in peptide aldehydes can be further attacked by profiling of reactions in various peptide substrates that contain amino-acid side chains capable of screening or enhancing dipole-dipole interactions. Implementation of the Wittig reaction in a population of 100 million diverse peptides open doors to numerous mechanistic studies that involve selection of substrates of higher reactivity and even selection of

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subsets of library that yield exclusively E or Z products (e.g., using a differential reactivity of these isomers in a Diels-Alder or ester hydrolysis reactions). We believe that fundamental mechanistic investigations of Wittig reaction and a downstream Michael addition and Diels-Alder reactions will serve as a robust foundation for future substrate profiling studies within a library of genetically-encoded substrates.

In conclusion, we have successfully implemented the Wittig reaction to diversify a library of peptides displayed on phage. The modification is highly regio- and chemo-selective and it exhibits only a minimal effect on infectivity of phage. We further diversified the Wittig-products displayed on phage *via* Michael addition and Diels-Alder reactions. The efficiency of both transformations can be monitored in phage-displayed context; neither of these reactions have a significantly impact on the infectivity of the virions. This report broadens the scope of reactions compatible with genetically-encoded libraries of phage-displayed peptides and increases the potential of this platform for identification of diverse ligands for important protein targets

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†**Electronic Supplementary Information** (ESI) available: Fig. S1-S16, synthesis and characterization of YEB and its peptide derivatives, experimental procedures for reactions on model peptide and phage libraries. MatLab scripts for kinetic fit in Figures 1-2 and S1 See DOI: 10.1039/x0xx00000x

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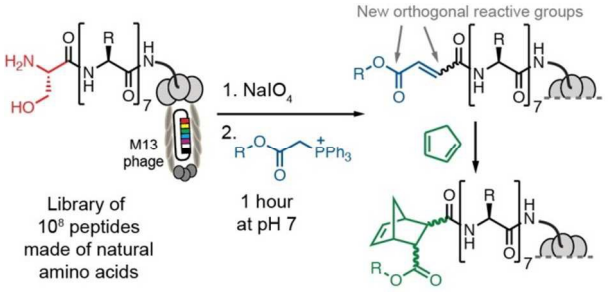

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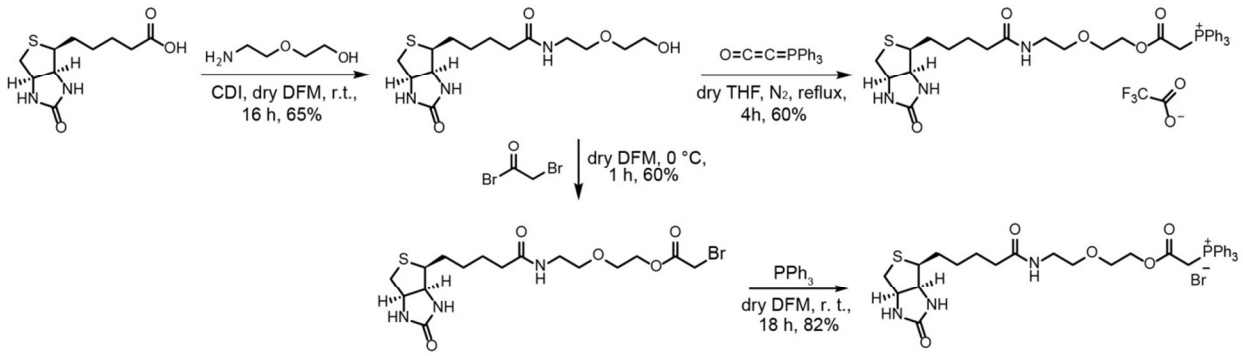

Scheme 1. Synthetic pathways towards ylide ester biotin phosphonium salt precursor (YEB).

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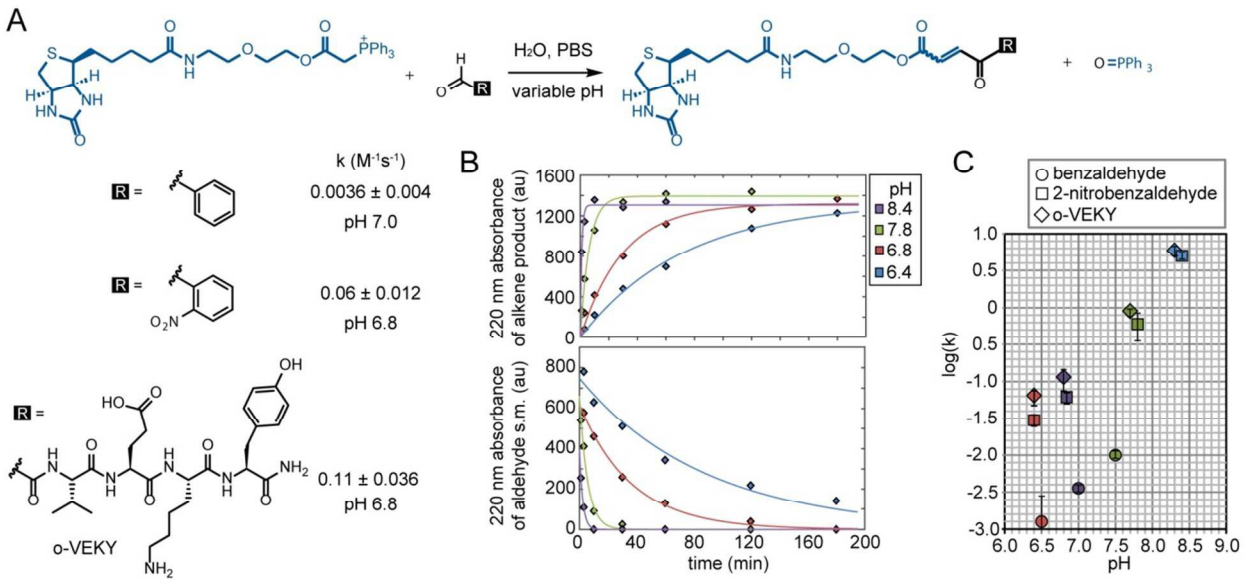

Fig. 1 (A) Wittig reaction on model aldehydes. (B) Absorbance of reactant and product (extracted as absolute area of the peaks in HPLC trace) of reaction between YEB and o-VEKY at different times and pH values. The data was fit to a equation $A_t = A(1–e^{-k[YEB]t})$, where k is the presuto-first order rate constant, [YEB] – initial concentration of the ylide, and A is the maximum absorbance. (C) Rate constants of reactions of three aldehydes at different pH values

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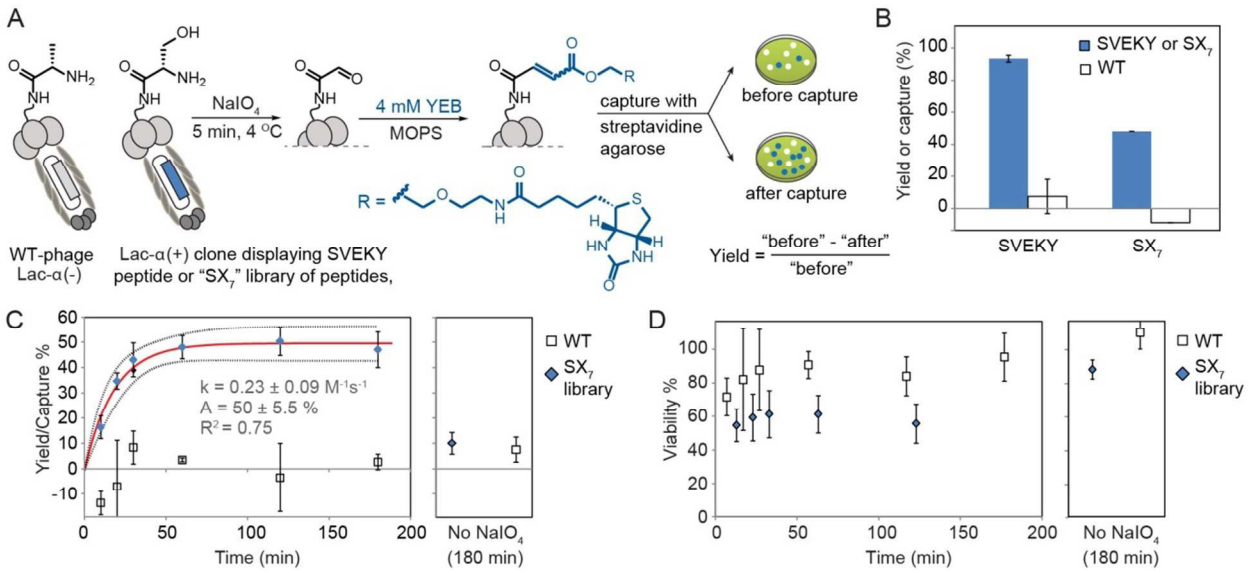

Fig. 2 (A) Quantification of yield and selectivity of Wittig reaction on phage using biotin-capture technique. (B) Capture % of phage clones displaying a SVEK-sequence and phage displaying the SX_7 library of peptides in presence of WT phage as control at pH 7.8 after one hour reaction with 1 mM YEB. (C) Kinetics of reaction between SX_7 library and YEB (4 mM) at pH 6.5. (D) Monitoring of viability of SX_7 library in the reaction described in (C).

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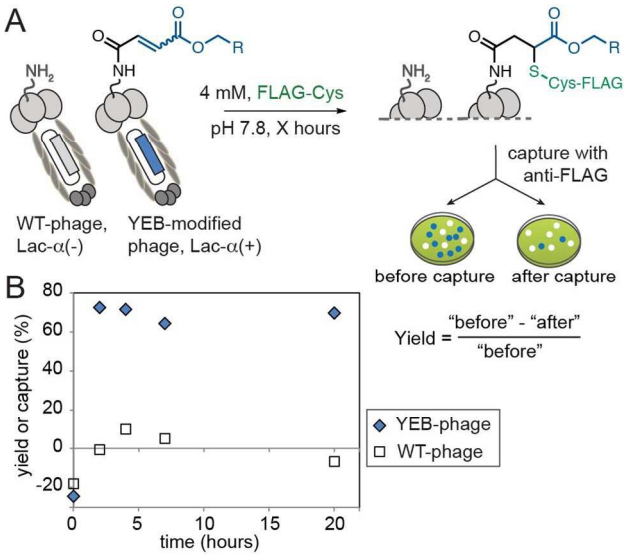

Fig. 3 Reaction of Wittig functionalized phage and control WT phage with DYKDDDDKC peptide (FLAG-Cys peptide). Pull down assay with anti-FLAG immobilized on protein G magnetic beads quantified the yield of incorporation of FLAG and confirmed that FLAG was not incorporated into WT-phage

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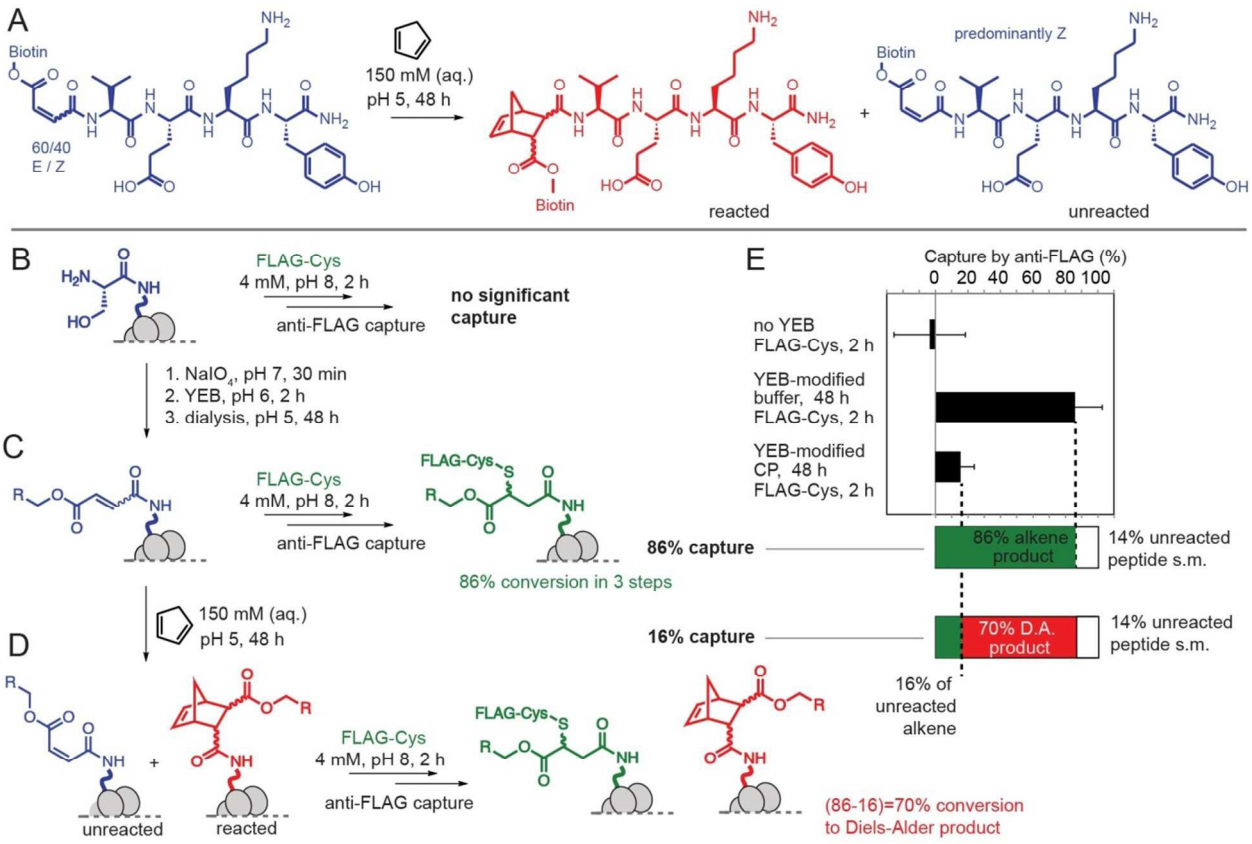

Fig. 4 (A) Diels-Alder reaction on Wittig product of model peptide. E selectivity was confirmed by analyzing the recovered unreacted starting material by NMR (Supporting Figure S16D). (B) Wittig reaction on phage clone displaying SVEK peptide. Phage not subjected to Wittig reaction did not exhibit any detectable capture whereas (C) alkene-functionalized phage, when reacted with FLAG-Cys peptide captured by anti-FLAG yields 86% capture. (D) Exposure of phage for 48 h with 250 mM cyclopentadiene after which an aliquot was taken and reacted with FLAG-Cys peptide quantified the efficiency of Diels-Alder modification. for two hours. (E) Bar graph summary: 86% of the phage population reacted with FLAG-Cys and was captured by anti-FLAG when no cyclopentadiene was added. After exposure to cyclopentadiene for 48 hours, only 16% of phage remained reactive towards FLAG-Cys. These observations extrapolate a 70% conversion for Diels-Alder reaction.

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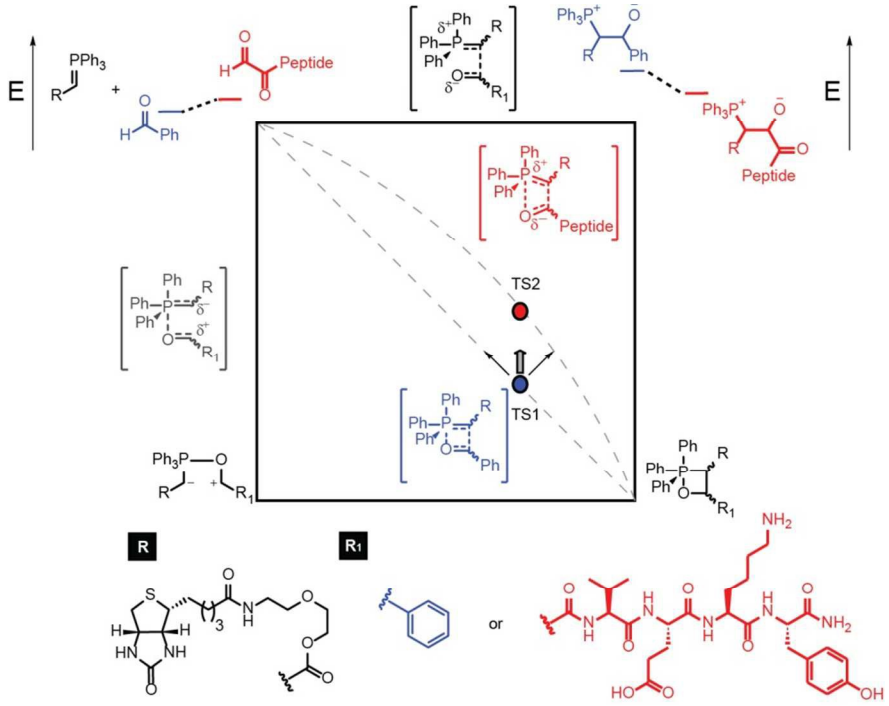

Scheme 2. More O'Ferrall Jencks plot showing transition states of Wittig reactions that proceed *via* concerted oxaphosphetane (TS1) and movement of TS1 towards asynchronous betaine-like transition state (TS2) in response to changes in electronic properties of the aldehyde.

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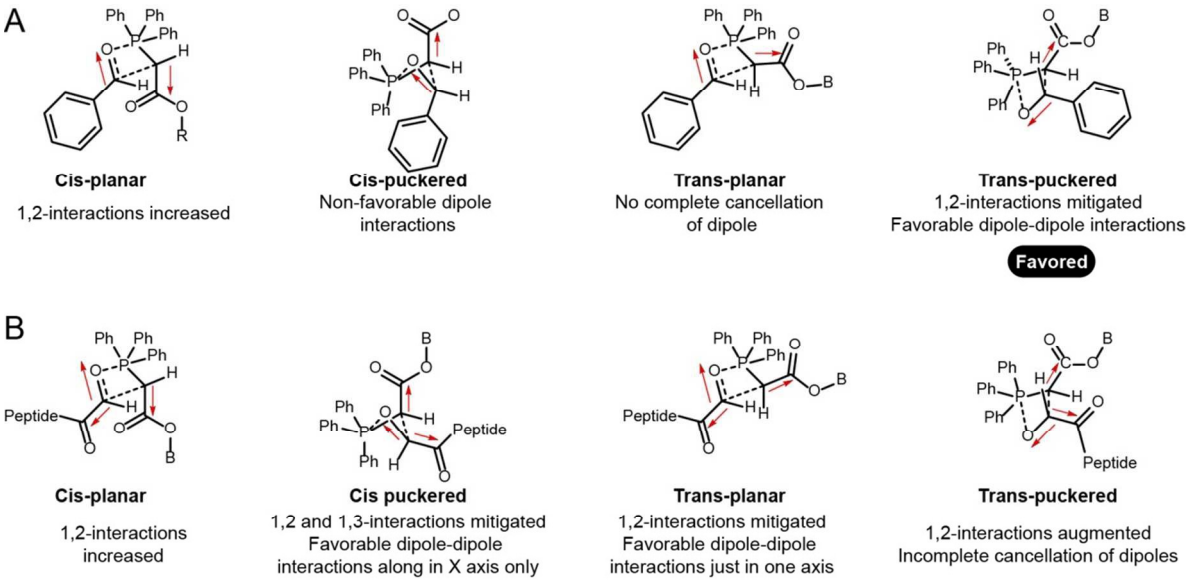

Scheme 3. (A) Transition states towards oxaphosphetane of Wittig reaction between benzaldehyde and triphenylphosphine ester stabilized type ylides showing a conformer that minimizes 1,2 and 1,3-interactions as well as dipole-dipole interactions. (B) Analogous transition states towards oxaphosphetane of Wittig reaction between oxoacetamide and triphenylphosphine ester-stabilized type ylides cannot completely cancel dipole moment in any conformation.

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References

1 C. Heinis and G. Winter, *Curr. Opin. Chem. Biol.*, 2015, **26**, 89–98.

2 S. Li, S. Millward and R. Roberts, *J. Am. Chem. Soc.*, 2002, **124**, 9972–9973.

3 T. T. Takahashi, R. J. Austin and R. W. Roberts, *Trends Biochem. Sci.*, 2003, **28**, 159–165.

4 S. Li and R. W. Roberts, *Chem. Biol.*, 2003, **10**, 233–239.

5 M. R. Jafari, L. Deng, P. I. Kitov, S. Ng, W. L. Matochko, K. F. Tjhung, A. Zeberoff, A. Elias, J. S. Klassen and R. Derda, *ACS Chem. Biol.*, 2014, **9**, 443–450.

6 C. Heinis, T. Rutherford, S. Freund and G. Winter, *Nat. Chem. Biol.*, 2009, **5**, 502–507.

7 K. Iwasaki, Y. Goto, T. Katoh and H. Suga, *Org. Biomol. Chem.*, 2012, **10**, 5783–5786.

8 S. Ng and R. Derda, *Org. Biomol. Chem.*, 2016, **14**, 5539–5545.

9 S. Kalhor-Monfared, M. R. Jafari, J. T. Patterson, P. I. Kitov, J. J. Dwyer, J. M. Nuss and R. Derda, *Chem. Sci.*, 2016, **7**, 3785–3790.

10 S. Chen, D. Bertoldo, A. Angelini, F. Pojer and C. Heinis, *Angew. Chemie Int. Ed.*, 2014, **53**, 1602–1606.

11 M. R. Jafari, J. Lakusta, R. J. Lundgren and R. Derda, *Bioconjug. Chem.*, 2016, **27**, 509–514.

12 S. A. K. Jongkees, S. Umemoto and H. Suga, *Chem. Sci.*, 2017, **8**, 1474–1481.

13 K. E. Sandman, *Nucleic Acids Res.*, 2003, **31**, 2234–2241.

14 J. Beech, L. Saleh, J. Frentzel, H. Figler, I. R. Corrêa, B. Baker, C. Ramspacher, M. Marshall, S. Dasa, J. Linden, C. J. Noren and K. A. Kelly, *Bioconjug. Chem.*, 2015, **26**,

View Article Online
DOI: 10.1039/C7OB01635B

529–536.

15 S. Ng, M. R. Jafari, W. L. Matochko and R. Derda, *ACS Chem. Biol.*, 2012, **7**, 1482–1487.

16 P. I. Kitov, D. F. Vinals, S. Ng, K. F. Tjhung and R. Derda, *J. Am. Chem. Soc.*, 2014, **136**, 8149–8152.

17 K. F. Tjhung, P. I. Kitov, S. Ng, E. N. Kitova, L. Deng, J. S. Klassen and R. Derda, *J. Am. Chem. Soc.*, 2016, **138**, 32–35.

18 R. J. Spears and M. A. Fascione, *Org. Biomol. Chem.*, 2016, **14**, 7622–7638.

19 F. Tian, M.-L. Tsao and P. G. Schultz, *J. Am. Chem. Soc.*, 2004, **126**, 15962–15963.

20 O. Boutureira and G. J. L. Bernardes, *Chem. Rev.*, 2015, **115**, 2174–2195.

21 C. P. Ramil and Q. Lin, *Chem. Commun.*, 2013, **49**, 11007–11022.

22 C. D. Spicer and B. G. Davis, *Nat. Commun.*, 2014, **5**, 4740–4754.

23 R. K. V. Lim, N. Li, C. P. Ramil and Q. Lin, *ACS Chem. Biol.*, 2014, **9**, 2139–2148.

24 K. M. Lum, V. J. Xavier, M. J.-H. Ong, C. W. Johannes and K.-P. Chan, *Chem. Commun.*, 2013, **49**, 11188–11190.

25 M.-J. Han, D.-C. Xiong and X.-S. Ye, *Chem. Commun.*, 2012, **48**, 11079–11081.

26 D. McLeod and J. McNulty, *European J. Org. Chem.*, 2012, **2012**, 6127–6131.

27 Z. J. Gartner, B. N. Tse, R. Grubina, J. B. Doyon, T. M. Snyder and D. R. Liu, *Science (80-.).*, 2004, **305**, 1601–1605.

28 S. Ng, M. R. Jafari and R. Derda, *ACS Chem. Biol.*, 2012, **7**, 123–138.

29 S. Krishnan, R. M. Miller, B. Tian, R. D. Mullins, M. P. Jacobson and J. Taunton, *J. Am. Chem. Soc.*, 2014, **136**, 12624–12630.

View Article Online
DOI: 10.1039/C7OB01635B

30 I. M. Serafimova, M. A. Pufall, S. Krishnan, K. Duda, M. S. Cohen, R. L. Maglathlin, J. M. McFarland, R. M. Miller, M. Frödin and J. Taunton, *Nat. Chem. Biol.*, 2012, **8**, 471–476.

31 R. M. Miller, V. O. Paavilainen, S. Krishnan, I. M. Serafimova and J. Taunton, *J. Am. Chem. Soc.*, 2013, **135**, 5298–5301.

32 D. C. Rideout and R. Breslow, *J. Am. Chem. Soc.*, 1980, **102**, 7816–7817.

33 S. Otto, G. Boccaletti and J. B. F. N. Engberts, *J. Am. Chem. Soc.*, 1998, **120**, 4238–4239.

34 M. J. Gattner, M. Ehrlich and M. Vrabel, *Chem. Commun.*, 2014, **50**, 12568–12571.

35 A.-C. Knall and C. Slugovc, *Chem. Soc. Rev.*, 2013, **42**, 5131–5142.

36 R. Robiette, J. Richardson, V. K. Aggarwal and J. N. Harvey, *J. Am. Chem. Soc.*, 2005, **127**, 13468–13469.

37 J. M. J. Tronchet and B. Gentile, *Helv. Chim. Acta*, 1979, **62**, 2091–2098.

38 J. Dambacher, W. Zhao, A. El-Batta, R. Anness, C. Jiang and M. Bergdahl, *Tetrahedron Lett.*, 2005, **46**, 4473–4477.

39 S. Tiwari and A. Kumar, *Chem. Commun.*, 2008, 4445–4447.

40 J. McNulty and P. Das, *Tetrahedron Lett.*, 2009, **50**, 5737–5740.

41 P. Das and J. McNulty, *Tetrahedron Lett.*, 2010, **51**, 3197–3199.

42 R. Robiette, J. Richardson, V. K. Aggarwal and J. N. Harvey, *J. Am. Chem. Soc.*, 2006, **128**, 2394–2409.

43 G. Aksnes and J. Songstad, *Acta Chem. Scand.*, 1964, **18**, 655–661.

44 A. El-Batta, C. Jiang, W. Zhao, R. Anness, A. L. Cooksy and M. Bergdahl, *J. Org. Chem.*,

View Article Online
DOI: 10.1039/C7OB01635B

2007, **72**, 5244–5259.

45 X. M. Zhang and F. G. Bordwell, *J. Am. Chem. Soc.*, 1994, **116**, 968–972.

46 A. Kohyama, M. Fukuda, S. Sugiyama, H. Yamakoshi, N. Kanoh, C. Ishioka, H. Shibata and Y. Iwabuchi, *Org. Biomol. Chem.*, 2016, **14**, 10683–10687.

47 C. Avonto, O. Taglialatela-Scafati, F. Pollastro, A. Minassi, V. Di Marzo, L. De Petrocellis and G. Appendino, *Angew. Chemie Int. Ed.*, 2011, **50**, 467–471.

48 B. Bernardim, P. M. S. D. Cal, M. J. Matos, B. L. Oliveira, N. Martínez-Sáez, I. S. Albuquerque, E. Perkins, F. Corzana, A. C. B. Burtoloso, G. Jiménez-Osés and G. J. L. Bernardes, *Nat. Commun.*, 2016, **7**, 13128–13136.

49 M. I. Chemistry, 1995, **36**, 2645–2648.

50 K. Ayub and R. Ludwig, *RSC Adv.*, 2016, **6**, 23448–23458.

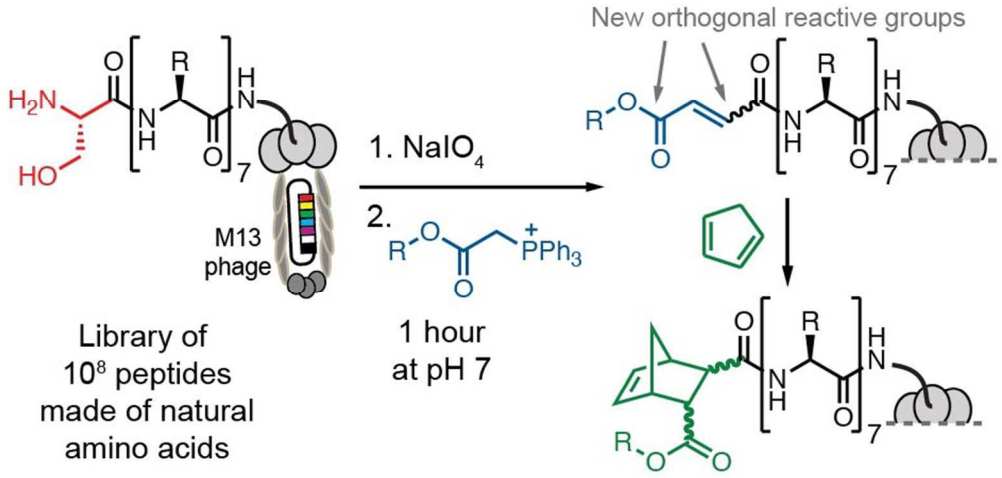
51 P. A. Byrne and D. G. Gilheany, *Chem. Soc. Rev.*, 2013, **42**, 6670–6696.

52 E. Vedejs and T. J. Fleck, *J. Am. Chem. Soc.*, 1989, **111**, 5861–5871.

53 E. Vedejs, T. Fleck and S. Hara, *J. Org. Chem.*, 1987, **52**, 4637–4639.

54 P. A. Byrne, Y. Ortin and D. G. Gilheany, *Chem. Commun.*, 2015, **51**, 1147–1150.

View Article Online
DOI: 10.1039/C7OB01635B



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