Bio-orthogonal metal catalysis

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Chapter 4

Iridium mediated radical addition

Dehydroalanine (Dha) and dehydrobutyrine (Dhb) are remarkably versatile non-canonical amino acids often found in antimicrobial peptides. Here, we present the selective modification of Dha and Dhb in antimicrobial peptides via photocatalytic activation of organoborates under influence of visible light. $Ir(dF(CF_3)ppy)_2(dtbbpy)PF_6$ was used as photoredox catalyst in aqueous solutions for the modification of thiostrepton and nisin. The mild conditions and high selectivity for the dehydrated residues, show photoredox catalysis is a promising tool for modification of peptide derived natural products.

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4.1 - Introduction

Site-selective modification of peptide derived natural products is a promising strategy to obtain new therapeutics. However, potentially interesting targets often are products of sophisticated biological post-translational machineries, and therefore difficult to modify via common bio-orthogonal chemistry,[1-4] or bio-engineering approaches.[5-8] Many of these structures contain unique non-canonical amino acids, which are attractive targets for late-stage chemical modification. Particularly interesting residues are dehydroalanine (Dha) and dehydrobutyrine (Dhb), which are often found in antimicrobial peptides.[9, 10] The unique orthogonal reactivity of the double bond in these dehydrated amino acids is used by nature to introduce for example lanthionine rings, and piperidine moieties. It has been shown that residual dehydrated residues can undergo a variety of chemical modifications,[11-17] however catalytic strategies are scarce. Catalytic activation of an unreactive precursor could provide new strategies for modification of the complex peptides under mild conditions. Here, we present the selective late-stage modification of Dha and Dhb in antimicrobial peptides by photocatalysis, using trifluoroborate salts as radical precursors.

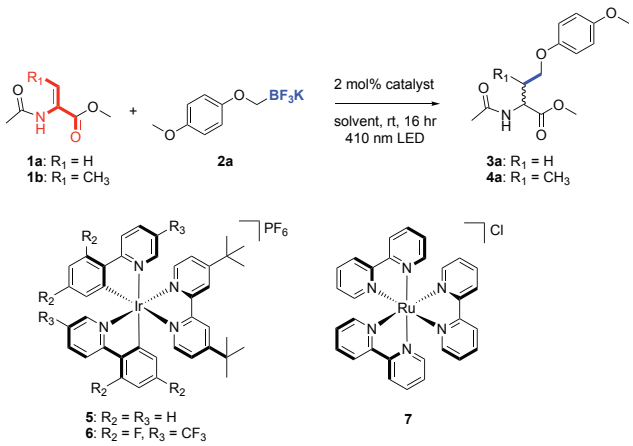
In recent years, photoredox catalysis has emerged as a mild method for visible-light-induced activation of small molecules.[18] Furthermore, photocatalysis is compatible with peptides and proteins as was shown in the photocatalytic induced formation of peptide macrocycles,[19] site-selective modification of cysteine in peptides,[20, 21] trifluoromethylation of peptides,[22] and decarboxylative alkylation of proteins.[23] Typically, cyclometaled polypyridyl iridium complexes or bipyridyl ruthenium complexes generate organic radicals by oxidative or reductive quenching of their excited states. Precursors like organoborates, which are harmless and air- and moisture stable compounds, are known to generate carbon-centered radicals upon oxidation by an excited photocatalyst. These radicals react readily with electron-deficient alkenes.[24-26] Considering the electron-deficient character of Dha, together with the orthogonal reactivity of organoborates, and the mild conditions of visible light irradiation, we envisioned this method could be employed for photocatalytic modification of Dha and Dhb in natural antimicrobial peptides.

Scheme 4.1: Chemical modification of Dha and Dhb in peptides by photoredox catalysis.

4.2 - Results & Discussion

Initial studies focused on the photocatalytic modification of the Dha monomer (**1a**) with potassium (*p*-methoxyphenoxy)methyl-trifluoroborate (**2a**) (see table 4.1). Different commonly used photocatalysts like $Ir(ppy)_2(dtbbpy)PF_6$ (**5**), $Ir(dF(CF_3)ppy)_2(dtbbpy)PF_6$ (**6**) and $Ru(bpy)_3Cl_2$ (**7**)

were evaluated for this reaction in aqueous solution mixed with various amounts of organic co-solvent under the influence of blue light (LED 410 nm) for 16 hours at room temperature. Catalysts **5** and **6** were found to be insoluble in most of the aqueous mixtures, resulting in precipitation of the catalyst and therewith no formation of product **3a** was obtained (table 4.1, entry 1-2). Photocatalyst **7** is water soluble, but no product formation was observed either (entry 3). Only in the case of 50% acetone(aq), 50% 1,4-dioxane(aq) and 100% DMF with catalyst **6** conversion to **3a** was obtained (entries 4-6). In case of the Dhb monomer (**1b**), the corresponding product **4a** was obtained (entry 6). Control reactions in which the reaction was performed in the dark, without



1a: R_1 = H
1b: R_1 = CH_3

2a

3a: R_1 = H
4a: R_1 = CH_3

5: R_2 = R_3 = H
6: R_2 = F, R_3 = CF_3

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Entry	Substrate	Co-solvent	% H_2O	Catalyst	Yield[a]
1	**1a**	-	100	**5**	0
2	**1a**	-	100	**6**	0
3	**1a**	-	100	**7**	0
4	**1a**	acetone	50	**6**	57% (41%)
5	**1a**	1,4-dioxane	50	**6**	full (64%)
6	**1a**	DMF	0	**6**	56% (40%)
7	**1b**	acetone	50	**6**	full (82%)
8	**1a**	methanol	50	**6**	0
9[b]	**1a**	acetone	50	**6**	0
10[c]	**1a**	acetone	50	**6**	0

Table 4.1: Results of photocatalytic reaction of Dha monomer with **2a**; Reaction conditions: a mixture of **1** (10 mM), **2a** (20 mM) and photocatalyst (2 mol%) dissolved or suspended in the degassed solvent mixture, and irradiated with blue LED's for 16 hours at room temperature. [a] Conversion and yield determined by ^{1}H-NMR with 20 mM internal standard 1,3,5-trimethoxybenzene. yield between parentheses; [b] reaction performed in the dark; [c] reaction performed in the presence of TEMPO (10 mM).

catalyst, or with addition of radical scavenger TEMPO resulted in no conversion, indicating the organoborate is indeed converted in a radical species by means of photoredox catalysis (entries 8-10).

The photocatalytic reaction was then tested on Dha and Dhb in a peptide. The antimicrobial peptide thiostrepton is a hydrophobic thiopeptide, soluble in apolar solvents like chloroform, 1,4-dioxane and DMF, which is comparable with the conditions found in the screening. Thiostrepton was therefore mixed with **2a** (6 eq, 1.5 eq per dehydrated amino acid), and 10 mol% **6** in aqueous 1,4-dioxane (9:1 (v/v)). Presence of water was required to fully dissolve the trifluoroborate salt. The reaction mixture was irradiated with blue LED's for one hour, after which an aliquot of the reaction was analysed by LC/MS. Single- and double modified thiostrepton were observed as main products. Elongation of the irradiation time with another two hours gave rise to triple- and quadruple modification of the peptide, which corresponds to the total number of dehydrated amino acids present.

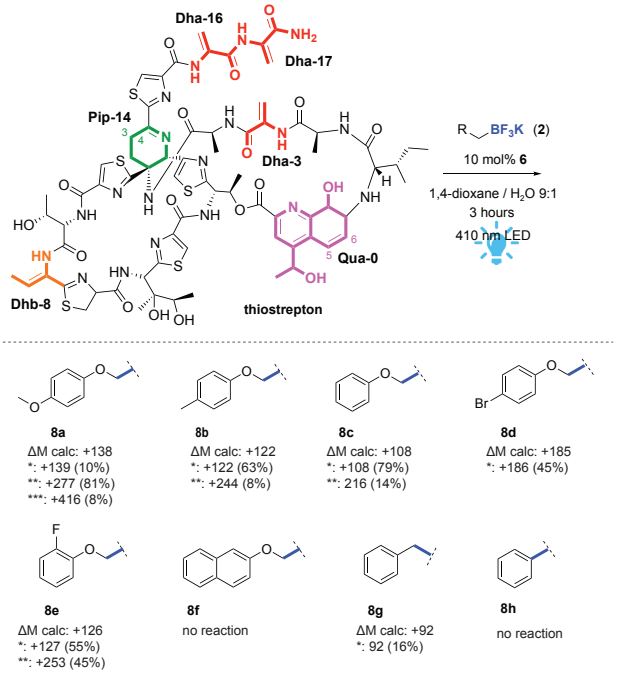


Figure 4.1: Schematic representation of the photocatalytic reaction on thiostrepton. Dha residues are depicted in red and Dhb residue in orange. Scope of trifluoroborate salts for photocatalytic modification of thiostrepton, optimised conditions: thiostrepton (500 µM), trifluoroborate salt (3 mM) and **6** (50 µM) in 400 µL 1,4-dioxane / water (9:1) irradiated with blue LED (410 nm) at room temperature for 3 hours. Single modification (*), double modification (**) and triple modification (***) is observed. In parenthses the conversion is calculated based on integration of the EIC of the corresponding product divided by sum of the areas of all compounds, assuming that ionisation is similar for all products, which are structurally very similar.[27-29]

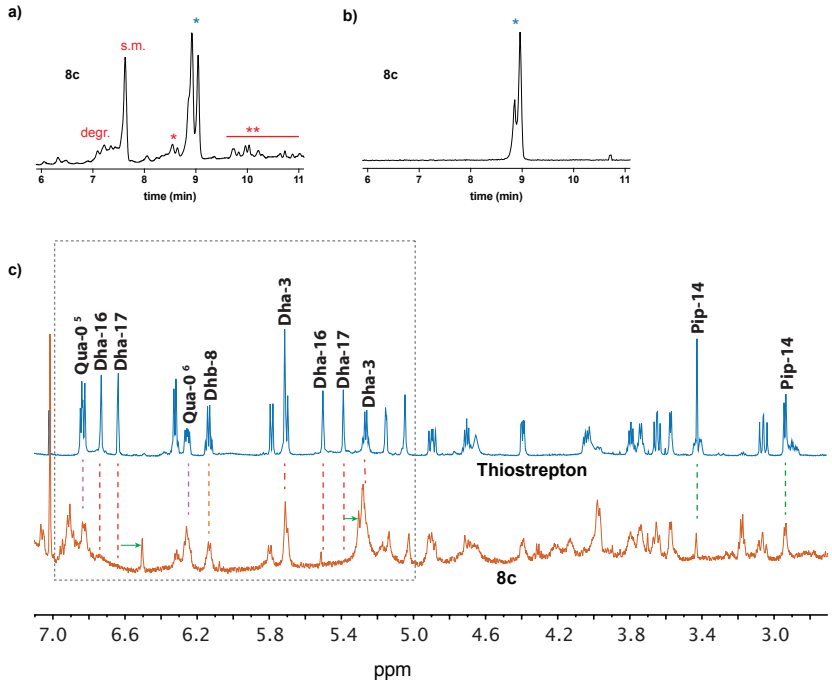


Figure 4.2: Determination of modification site in thiostrepton; a) UPLC chromatogram (280 nm) of crude reaction mixture **8c**: degr. = degraded thiostrepton, s.m. = starting material, * = single modified thiostrepton, mixture of 2 diastereomers, ** = double modified thiostrepton; b) UPLC chromatogram (280 nm) of purified **8c** c) NMR studies on photocatalytically modified thiostrepton (**8c**, orange) compared with unmodified thiostrepton (blue). Zoom in of 5-7 ppm to show signal shifts of Dha-17 and signal disappearance of Dha-16.

The scope of the reaction was investigated by varying the trifluoroborate salts. It was found that the reaction is significantly affected by the substituents present on the aryl ring of the substrate (see figure 4.1). Electron donating groups result in (almost) full conversion of the starting material (**8a-c**), while less electron donating groups on the aryl ring slow down the reaction resulting in mostly single modification and remaining starting material (**8d-f**). This might be due to the nucleophilicity of the generated radicals, or the difference in electrochemical potential to generate radicals from the trifluoroborate salts. Moreover, the oxygen next to the carbon-centered radical turned out to have a beneficial effect on the reaction. By using substrates that lack the heteroatom, the reaction was much slower (**8g**), resulted in no conversion (**8h**), or in degradation of the peptide. In absence of the aryl moiety, only degradation products were obtained.

To determine the site of modification, single modified thiostrepton product **8c** was purified by rp-HPLC, and studied by ^{1}H-NMR. The two peaks indicated with the blue star in the LC chromatogram (figure 4.2a) were established to be two diastereomers of modification at the same position of the peptide, which could be separated by rp-HPLC (figure 4.2b). Comparison

of the ^{1}H-NMR spectrum of purified **8c** with the ^{1}H-NMR spectrum of unmodified thiostrepton shows the disappearance of two singlets at 6.73 and 5.50 ppm and a shift of the singlets at 6.63 and 5.38 ppm (figure 4.2c). These four signals correspond to the four β-protons of Dha-16 and Dha-17, the dehydrated residues in the tail of the peptide. The signals of the other double bonds in the peptide (i.e. Dha-3, Dhb-8, piperidine-14 and quinaldic acid-0) remain unchanged, which indicates the peptide is modified at a Dha in the tail. 2D NMR TOCSY measurements confirmed the modification to be at Dha-16 (figure 4.3). These results show the photocatalytic modification to be selective for the dehydrated amino acids. Moreover, the reaction is chemoselective for Dha-16, which is known to be the most electron deficient dehydrated residue due to it being situated next to a thiazole ring. Single modification at other positions is observed in the UPLC chromatogram of the crude reaction mixture (figure 4.2a), but these products are formed only in low yields, as can be calculated from the low intensity of the peaks of these products.

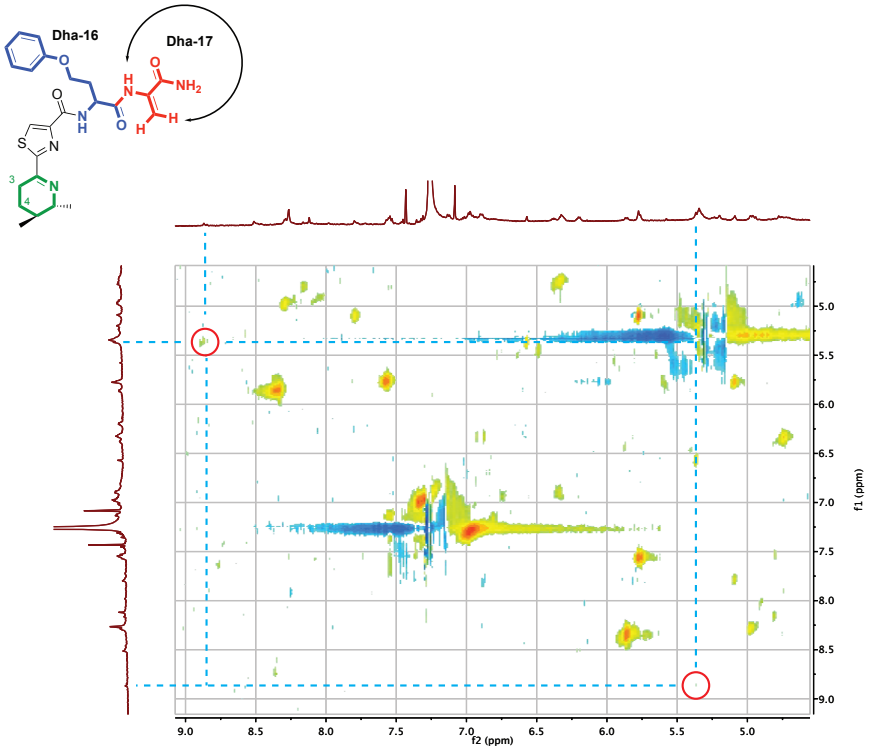


Figure 4.3: TOCSY spectrum of **8c**. Signals in red circles indicate coupling of NH-17 with protons of Dha-17 indicating modification has taken place at Dha-16.

To show the versatility of our approach, the lantipeptide nisin was subjected to the photoredox catalysis. Nisin is less hydrophobic than thiostrepton. Hence, the photocatalytic reaction on nisin was performed in 1,4-dioxane or acetone with 50% water containing 0.1% AcOH(aq). Nisin was reacted with **2a** (4.5 eq, 1.5 eq per dehydrated residue), catalysed by 10 mol% **6**. After irradiation with blue LEDs for 1 hour almost full conversion to triple modified nisin was obtained as can be

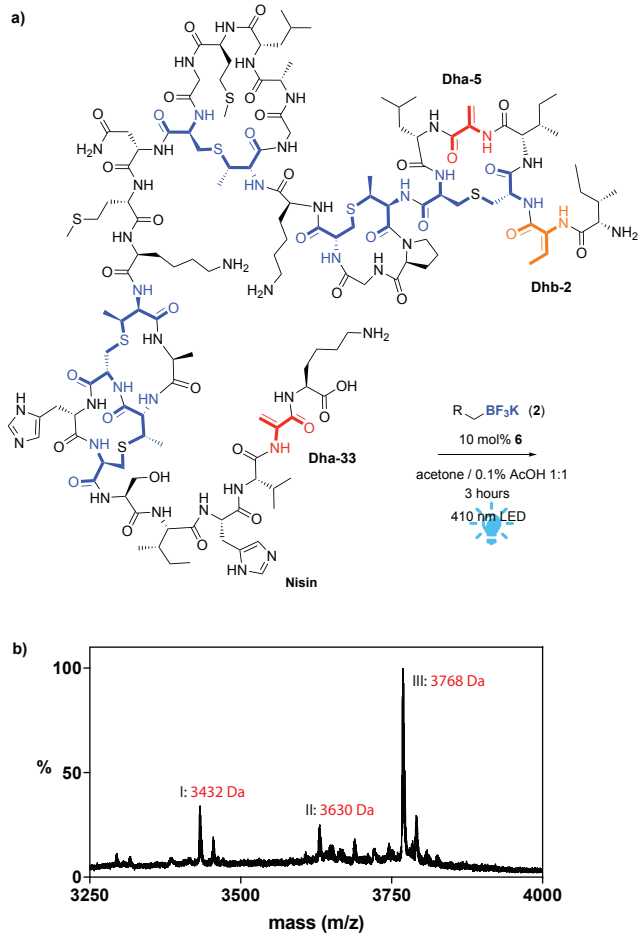


Figure 4.4: a) Schematic representation of the photocatalytic modification of nisin; b) MALDI-TOF measurement of the crude product of the photocatalytic modification of nisin with **2a** I) degraded Nisin($CH_2OPhOMe)_2$, II) Nisin($CH_2OPhOMe)_2$, III) Nisin($CH_2OPhOMe)_3$.

seen from the MALDI-TOF spectrum (figure 4.4b). Exploration of the scope of the reaction on nisin showed a similar trend as in the case of thiostrepton (see table 4.2 in experimental section). The best results were obtained when both the aryl ring, as well as the heteroatom adjacent to the carbon-centered radical are present (**9a-c**). Less donating substituents on the phenyl ring result in lower conversion and mainly single modified product (**9b-c**). Organoborates with less electron donating substituents like halogens resulted in no conversion at all (**9d-e**). Addition of TEMPO as radical scavenger gave unmodified starting material confirming the involvement of radical species and showing that the peptide is stable under the conditions of the photocatalytic reaction.[29]

To determine the selectivity of the photocatalytic modification of nisin, triple modified product **9a** was studied by NMR. The ^{1}H-NMR spectrum of this product revealed that the peaks of Dha-

5 (5.35 and 5.48 ppm), Dha-33 (5.60 ppm) and Dhb-2 (6.51 ppm) had disappeared (see figure 4.5a). Hence, the photocatalytically generated radicals react selectively with the dehydrated amino acids in the peptide, yielding an *O*-phenylhomoserine (OPhHse) residue. To confirm the presence of this newly formed residue, modified nisin (**9c**) was hydrolysed in a microwave oven in 6 M HCl(aq) to study the amino acids present. The hydrolysate was reacted with Marfey's reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA)).[30] Analysis with LC/MS and comparison with FDAA derivatised OPhHse confirmed the presence of OPhHse in **9c**. (see figure 4.5b).

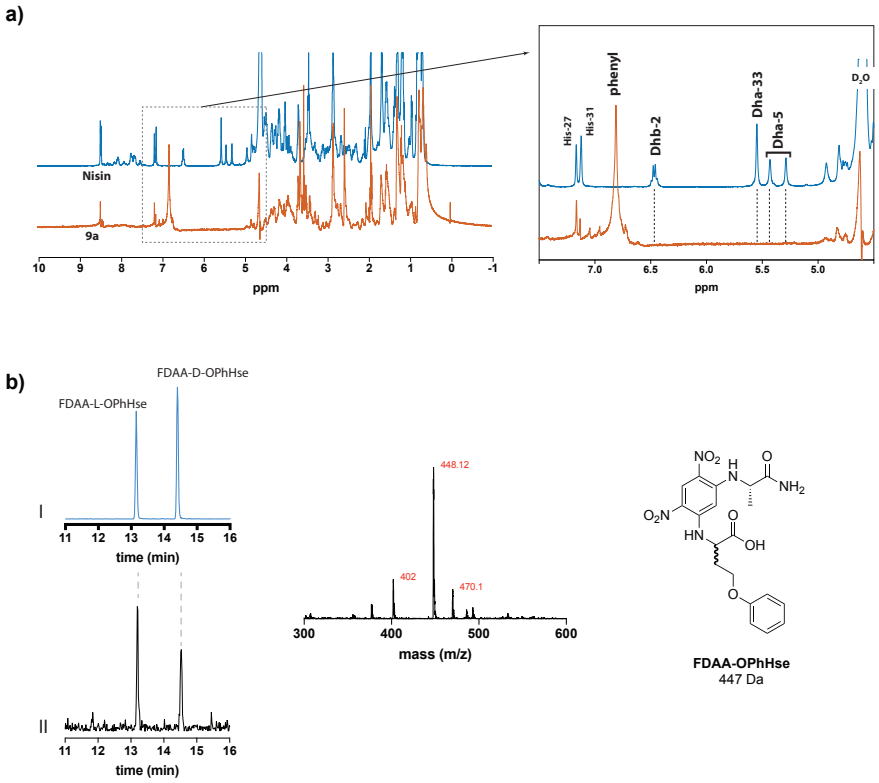


Figure 4.5: Analysis of the site selectivity of photocatalytic modified nisin. a) NMR studies on photocatalytically modified nisin (**9a**, orange) compared with unmodified nisin (blue). Zoom in of 4.5-7.5 ppm to show signal disappearance of Dhb-2, Dha-5 and Dha-33; b) Analysis of introduced *O*-PhenylHomoserine using Marfey's method: (I) Extracted Ion Chromatogram (EIC) of [M+H] = 448 Da corresponding to D/L-OPhHse derivatised with FDAA; (II) EIC of the hydrolysate of **9c** derivatised with FDAA; Mass spectrum of FDAA-L-OPhHSe from graph (I).

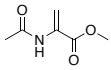
4.3 - Conclusion

In conclusion, we have demonstrated that visible-light-driven photoredox catalysis is an efficient and mild catalytic method for the selective late-stage modification of dehydrated amino acids in antimicrobial peptides. Dha and Dhb react selectively with the carbon-centered radicals generated from organoborates with only 10 mol% catalyst loading in aqueous conditions. This study illustrates the potential of photoredox catalysis for the late-stage modification of complex active natural products and is therefore a promising tool in the quest for new antibiotics.

4.4 - Experimental

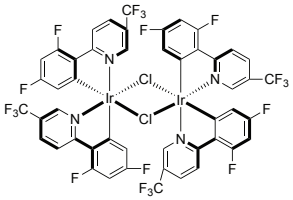
General remarks

Chemicals were purchased from TCI Europe, Sigma-Aldrich, Acros, Strem Chemical, Handary or Chem-Impex, solvents from Lab-Scan and were all used without further purification. Column chromatography was performed by hand on silica gel (Aldrich, 230-400 mesh) or automated on a Grace Reveleris Flash X1 Chromatography system. Solvents were removed under reduced pressure at 40 °C (water bath). ^{1}H-NMR and ^{13}C-NMR spectra were recorded with Varian Mercury Plus 400, Agilent Technologies 400/54 Premium Shield, Varian VXR 300 or Bruker 600 MHz at ambient temperature. HRMS ESI mass spectra of small organic molecules were recorded with Thermo Fisher Scientific Orbitrap XL. Melting points were recorded on a Büchi B-545 melting point apparatus. Elemental analysis were determined on a EuroVector S.P.A. model Euro EA 3000. HPLC separation was achieved with an XBridge C8 3.5 um 4.6x250mm column and a linear gradient of 80% -> 30% water (0.1%FA) in ACN (0.1%FA) in 30 min. UPLC/MS analysis was done on Waters Acquity Ultra Performance LC with Acquity TQD detector. Separation was achieved with an Acquity UPLC BEH C8 1.7 um 2.1x150 mm column and a linear gradient of 90% -> 50% water (0.1%FA) in ACN (0.1%FA) in 10 minutes for nisin and 70% -> 30% water (0.1%FA) in ACN (0.1%FA) in 10 minutes for thiostrepton. Charge density spectra were deconvoluted with the algorithm MagTran.[31]



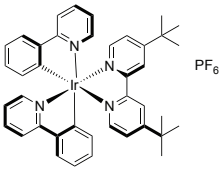
Methyl 2-acetamidoacrylate (1)

Prepared as described by Crestey *et al.*:[32] Acetamide (1000 mg, 16.9 mmol), methyl pyruvate (1.3 mL, 15.2 mmol) and 30 mL toluene were added to a round-bottom-flask equipped with magnetic stirrer and Dean-Stark-trap. A catalytic amount of *p*-toluenesulfonice acid (0.001 eq) and *p*-methoxyphenol (0.001 eq) were added. After heating under reflux for 24 hours, the solvent was evaporated. The crude yellow oil was redissolved in dichloromethane, washed with saturated $NaHCO_3$(aq) and water. Drying over $MgSO_4$, removal of the solvent and purification by column chromatography (SiO_2, pet ether / ethyl acetate 3:1, R_f=0.71 in EtOAc) gave **1** (805 mg, 37%) as a white solid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.13 (s, 3H), 3.84 (s, 3H), 5.88 (s, 1H), 6.60 (s, 1H), 7.71 (br, 1H) ppm; ^{13}C-NMR ($CDCl_3$, 101 MHz) δ 24.8, 53.1, 108.9, 131.1, 164.7, 169.0 ppm; Elemental analaysis Calc: C: 50.35, H: 6.34, N:9.79, Found: C: 50.27, H: 6.35, N: 9.66. MS (ESI, HCOOH) m/z 144.0654 ([M+H]$^+$, calc: 144.0655) mp: 51.4-52.3 °C



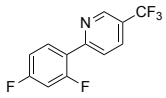
[(dF(CF₃)ppy)₂-Ir-μ-Cl]₂ (10)

Prepared as described by Molander *et al.*:[33] **11** (501 mg, 1.93 mmol) and iridium(III)chloride.hydrate (261 mg, 0.87 mmol) were suspended in 14 mL 2-ethoxyethanol and 4.7 mL water. The mixture was heated at 120 °C overnight. After cooling to room temperature, the mixture was diluted with 20 mL water. The yellow precipitate was collected by filtration, washed with water and ether to give 10 (457 mg, 70%) as yellow solid. ^{1}H-NMR δ ($CDCl_3$, 400 MHz) 5.08 (2H, m) 6.42 (2H, m) 8.03 (2H, m), 8.47 (2H, m), 9.51 (2H, m) ppm.



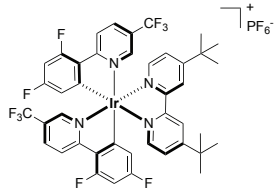
Ir(ppy)₂(tbbpy) (5)

Prepared as described by Malliaras *et al.*:[34] **10** (75 mg, 0.07 mmol) and 4,4-di-tert-butyl-2,2-dipyridyl (41 mg, 0.153 mmol) were dissolved in 3 mL 1,2-ethanediol. The mixture was heated to 150 °C overnight. After cooling to room temperature 45 mL water was added. The aqueous layer was washed with ether and heated to 60 °C for 10 minutes to remove traces of ether. Ammonium hexafluorophosphate (342 mg, 2.1 mmol) was added and the mixture was cooled to 5°C. The precipitate was collected by filtration and washed with ether to give **5** (80 mg, 63%) as yellow solid. ^{1}H-NMR (acetone-d_6, 400 MHz) δ 1.41 (s, 18H), 6.35 (d, J=7.3, 2H), 6.91 (t, J=7.50, 2H), 7.03 (t, J=7.01, 2H), 7.13 (t, J=6.77, 2H), 7.71 (d, J=5.32, 2H), 7.78 (d, J=5.32, 2H), 7.90-7.99 (m, 6H), 8.25 (d, J=8.22, 2H), 8.87 (s, 2H) ppm; ^{13}C-NMR (acetone-d_6, 101 MHz) δ 32.2, 38.2, 66.0, 122.5, 124.6, 125.0, 126.1, 127.6, 128.2, 133.0, 134.2, 141.2, 146.7, 151.7, 152.8, 153.6, 158.6, 166.6, 170.6 ppm; MS (ESI, HCOOH) m/z 769.289 ($[M\text{-}PF_6]^+$, calc: 769.288; Calcd for $C_{40}H_{40}F_6IrN_4P.3H_2O$: C: 49.63, H: 4.79, N: 5.79, Found: C: 49.93, H: 4.60, N: 5.67.



2-(2,4-difluorophenyl)-5-(trifluoromethyl)pyridine (11)

Prepared as described by Weaver *et al.*:[35] 2-chloro-5-(trifluoromethyl)pyridine (317 mg, 1.75 mmol), 2,4-difluorophenylboronic acid (331 mg, 2.1 mmol), triphenylphosphine (45 mg, 0.175 mmol) and potassium carbonate (649 mg, 4.7 mmol) were dissolved in 1,2-dimethoxyethane (2 mL). After degassing by N_2 bubbling for 15 minutes palladium(II)acetate (10 mg, 0.043 mmol) is added and the mixture was degassed by N_2 bubbling for another 15 minutes. After refluxing overnight the mixture was cooled to room temperature and diluted with dichloromethane. The organic layer was washed with water and brine. Dryinig over Na_2SO_4, removal of the solvent and purification by column chromatography (SiO_2, heptane / ethyl actate 0% —> 3%, R_f=0.57) gave **11** (322 mg, 71%) as white solid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.95 (m, 1H), 7.05 (m, 1H), 7.91 (m, 1H), 8.01 (m, 1H), 8.11 (m, 1H), 8.96 (m, 1H) ppm; ^{13}C-NMR ($CDCl_3$, 101 MHz) δ 105.5, 112.3, 123.7, 125.0, 132.5, 133.9, 146.6, 155.8, 159.9, 162.3, 162.7, 165.2 ppm; MS (ESI, HCOOH) m/z 260.050 ($[M+H]^+$, calc: 260.050); Calcd for $C_{12}H_6F_5N$ C: 55.61, H: 2.33, N: 5.40, Found: C: 55.60, H: 2.34, N: 5.24.

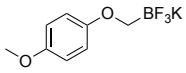


Ir(dF(CF₃)ppy)₂(dtbbpy)]PF₆ (6)

Prepared as described by Stephenson *et al*.:[36] Iridium(III)chloride.hydrate (52 mg, 0.17 mmol) and **11** (360 mg, 1.39 mmol) were suspended in 5 mL ethyleneglycol in a microwave tube. The mixture was stirred for 1 minute at ambient conditions and then heated by microwave irradiation to 200 °C for 50 minutes at ambient atmosphere in a microwave oven. The mixture is cooled to room temperature and 4,4'-tertbutyl-2,2-dipyridil (70 mg, 0.26 mmol) was added. The mixture was heated by microwave irradiation at 200 °C for another 30 minutes. After cooling to room temperature the mixture was diluted with water and brine and extracted to ethyl acetate. The organic layer was dried over Na_2SO_4 and concentrated. The residue was suspended in water and ammonium hexafluorophosphate solution (2 g in 20 mL water) was added. The mixture was sonicated to initiate precipitation. The formed precipitate is collected by filtration, washed extensively with cold water and ether to give **6** (133 mg, 70%) as yellow solid. ^{1}H-NMR ($(CD_3)_2CO$, 400 MHz) δ 1.45 (s, 18H), 5.96 (m, 2H), 2.68 (m, 2H), 7.80 (m, 2H), 8.16 (d, J=5.83, 2H) 8.42 (m, 2H), 8.61 (m, 2H), 9.13 (s, 2H) ppm; ^{13}C-NMR ($CDCl_3$, 101 MHz) δ 33.0, 38.9, 102.5, 116.4, 126.2, 126.3, 126.5, 128.9, 129.0, 139.2, 147.4, 152.5, 157.7, 157.8, 158.3, 169.3, 170.6, 170.7 ppm; MS (ESI, HCOOH) m/z 977.224 ([M-PF6]$^+$, calc: 977.225); Elemental analysis calcd for $C_{42}H_{34}F_{10}IrN_4$: C: 44.96, H: 3.05, N: 4.99, Found: C: 45.19, H: 3.05, N: 5.14.

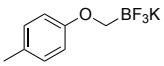
General preparation of phenoxymethyltrifluoroborate salts

Potassium tert-butoxide (4 eq) or sodium hydride (60% dispersed on oil, 3 eq) was suspended in 10 mL dry THF under nitrogen. The mixture was cooled to 0 °C and a solution of phenol in dry THF (1M) was added dropwise. The mixture was allowed to warm up to room temperature. After stirring for minimal 1 hour potassium(bromomethyl) trifluoroborate (1 eq) is added as solid in one portion. After stirring at 45 °C for 16 hours the reaction is quenched by addition of KHF_2(aq) (4.5M, 2 eq). After stirring for 30 minutes, the solvent was removed and the remaining solid was thoroughly washed with ether and DCM. The crude solid was dissolved in boiling acetonitrile with activated charcoal to remove remaining color. Hot filtration, removal of the solvent and a final wash with DCM provided the trifluoroborate salt. N.B.: Due to coupling with the boron atom, the carbon adjacent to the boron is never observed in carbon NMR.



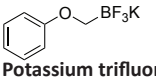
Potassium trifluoro((4-methoxyphenoxy)methyl)borate (2a)

Prepared via the general method from 4-methoxyphenol (931 mg, 7.5 mmol), sodium hydride (60% dispersed on oil, 300 mg, 7.5 mmol) and potassium (bromomethyl)trifluoroborate (502 mg, 2.5 mmol) to give **2a** (171 mg, 30%) as a white solid. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 2.85 (m, 2H), 3.64 (s, 3H), 6.74 (m, 4H) ppm; ^{13}C-NMR (DMSO-d_6, 101 MHz) δ 58.4, 117.3, 117.4, 155.0, 159.3 ppm; MS (ESI, NH_4OH) m/z 205.064984 ([M-K]$^-$, calc: 205.06422).



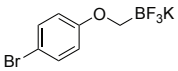
Potassium trifluoro((p-tolyloxy)methyl)borate (2b)

Prepared via the general method from 4-methylphenol (324 mg, 3 mmol), potassium tert-butoxide (335 mg, 3 mmol) and potassium(bromomethyl)trifluoroborate (200 mg, 1 mmol) to give **2b** (220 mg, 96%) as a beige solid. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 2.18 (s, 3H), 2.87 (m, 2H), 6.69 (d, 2H, J=8.2), 6.96 (d, 2H, J=8.2) ppm; ^{13}C-NMR (DMSO-d_6, 101 MHz) δ 23.2, 116.7, 129.8, 132.5, 163.1 ppm; MS (ESI, NH_4OH) m/z 189.070 ([M-K]$^-$, calc: 189.069).



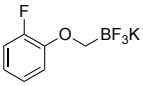
Potassium trifluoro(phenoxymethyl)borate (2c)

Prepared via the general method from phenol (1411 mg, 15 mmol), sodium hydride (600 mg, 60% on oil, 15 mmol) and postassium(bromomethyltrifluorborate (1000 mg, 5 mmol) to give **2c** (1001 mg, 93%) as a white solid. ^{1}H-NMR (DMSO-d$_6$, 400 MHz) δ 2.92 (m, 2H), 6.74 (t, J=7.06, 1H), 6.83 (d, J=8.18, 2H), 7.17 (m, 2H) ppm; ^{13}C-NMR (DMSO-d$_6$, 101 MHz) δ 116.9, 121.6, 132.1, 165.1 ppm; MS (ESI neg, 0.1% NH$_4$OH) m/z 175.05454 ([M-K]$^-$, calc: 175.05366)



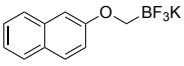
Potassium ((4-bromophenoxy)methyl)trifluoroborate (2d)

Prepared via the general method from 4-bromophenol (519 mg, 3 mmol), potassium *tert*-butoxide (335 mg, 3 mmol) and potassium(bromomethyl)trifluoroborate (200 mg, 1 mmol) to give **2d** (196 mg, 67%) as a white solid. ^{1}H-NMR (DMSO-d$_6$, 400 MHz) δ 2.93 (m, 2H), 6.80 (m, 2H), 7.29 (m, 2H) ppm. ^{13}C-NMR (DMSO-d$_6$, 101 MHz) δ 112.7, 119.3, 134.7, 164.5 ppm; MS (ESI, NH$_4$OH) m/z 252.0965 ([M-K]$^-$, calc: 252.964).



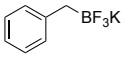
Potassium trifluoro((2-fluorophenoxy)methyl)borate (2e)

Prepared via the general method from 2-fluorphenol (0.2 mL, 2 mmol), sodium hydride (240 mg, 60% on oil, 6 mmol) and potassium(bromomethyl)trifluorborate (150 mg, 0.75 mmol) to give **2e** (55 mg, 32%) as a white solid. ^{1}H-NMR (DMSO-d$_6$, 400 MHz) δ 3.01 (m, 2H), 6.73 (m, 2H), 7.03 (m, 3H) ppm; ^{13}C-NMR (DMSO-d$_6$, 101 MHz) δ 113.5, 115.2 (d), 118.4 (d), 124.4 (d), 150.5, 152.9 ppm; MS (ESI, NH$_4$OH) m/z 193.045 ([M-K]$^-$, calc: 193.044).



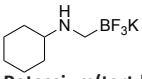
Potassium trifluoro((naphthalen-2-yloxy)methyl)borate (2f)

Prepared via the general method from 2-naphtol (432 mg, 3 mmol), potassium *tert*-butoxide (336 mg, 3 mmol) and potassium(bromometyl)trifluoroborate (200 mg, 1 mmol) to give **2f** (155 mg, 58%) as a beige solid. ^{1}H-NMR (DMSO-d$_6$, 400 MHz) δ 3.06 (m, 2H), 7.05-7.07 (m, 1H), 7.18 (m, 1H), 7.25 (m, 1H), 7.36 (m, 1H), 7.68 (m, 3H) ppm; ^{13}C-NMR (DMSO-d$_6$, 101 MHz) δ 108.4, 122.6, 125.6, 128.9, 129.6, 130.4, 130.9, 131.6, 137.8, 163.3 ppm; MS (ESI, NH$_4$OH) m/z 225.069 ([M-K]$^-$, calc: 225.071).



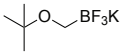
Potassium benzyltrifluoroborate (2g)

Benzylbromide (1 mL, 8.6 mmol) was dissolved in dry ether (0.5M). Magnesium turnings are added. After refluxing for 30 minutes, the mixture was cooled to -78 °C. Trimethylborate (1.5 mL, 13 mmol) was added dropwise. After stirring at -78°C for 30 minutes, the mixture was allowed to warm up to room temperature and was stirred for another hour. The mixture is cooled to 0 °C and KHF_2(aq) (4.5M, 11 mL) dropwise. The mixture was left stirring at room temperature for another hour. Evaporation of the solvent and extraction to acetone by soxhlet extraction gave **2g** (613 mg, 36%) as a white solid. ^{1}H-NMR (CD$_3$OD, 400 MHz) δ 1.69 (m, 2H), 6.89-6.92 (m, 1H), 7.05-7.11 (m, 4H) ppm. ^{13}C-NMR (DMSO-d$_6$, 101 MHz) δ 125.0, 130.0, 131.7, 150.0 ppm; MS (ESI, NH$_4$OH) m/z 159.05976 ([M-K]$^-$, calc: 159.05874).



Potassium(tert-butoxymethyl)trifluoroborate (2j)

Potassium(bromomethyl)trifluoroborate (100 mg, 0.5 mmol) was added to neat cyclohexylamine (2 mL, 17 mmol). The mixture was heated to 80 °C for 30 minutes. After removal of the solvent, the crude was taken up in 15 mL acetone with $KHCO_3$ (69 mg). After stirring for 20 minutes, the insolubles were filtered off. Removal of the solvent and washing with ether afforded **2j** (60 mg, 54%) as a white solid. ^{1}H-NMR ($(CD_3)_2CO$, 400 MHz) δ 1.18-126 (m, 1H), 1.31-1.41 (m, 2H), 1.44-1.54 (m, 2H), 1.67-1.71 (m, 1H), 1.84-1.88 (m, 2H), 1.98-2.05 (s, 2H), 2.24 (m, 2H), 3.13 (m, 1H), 6.79 (br, 1H) ppm; ^{13}C-NMR (DMSO-d_6, 101 MHz) δ 27.2, 28.0, 31.3, 60.3 ppm; MS (ESI, NH_4OH) m/z 180.118 ($[M-K]^-$, calc: 180.117).



Potassium(tert-butoxymethyl)trifluoroborate (2k)

Prepared via the general method from *tert*-butoxide (252 mg, 2.25 mmol) and potassium(bromomethyl) trifluoroborate (150 mg, 0.75 mmol) to give **2k** (73 mg, 50%) as a white solid. ^{1}H-NMR (CD_3OD, 400 MHz) 1.15 (s, 9H), 2.66 (m, 2H) ppm. ^{13}C-NMR (DMSO-d_6, 101 MHz) δ 30.4, 73.9 ppm. MS (ESI, NH_4OH) m/z 155.086 ($[M-K]^-$, calc: 155.085).

General procedure of photocatalysis on Dha-monomer

Catalysis was performed in 4 mL solvent with a final concentration of 33 mM Dha, 33 mM organoborate and 675 µM catalyst. A typical catalysis reaction was set up as follows: **1a** (19 mg, 0.135 mmol), **2a** (33 mg, 0.135 mmol) and **5** (3 mg, 0.0027 mmol) were dissolved in 4 mL solvent in a schlenk tube equipped with a stir bar. The mixture was degassed by three repetitive freeze-pump-thaw-cycles and exposed to blue LED's for 16 hours at room temperature. The mixture was diluted with water and extracted to DCM. Drying over Na_2SO_4 and removal of the solvent gave the crude product which was analysed by NMR directly with 1,3,5-TMB as internal standard for determination of the yield.

Analytically pure samples were obtained via purification by column chromatography (SiO_2, heptane / ethyl acetate 0->50%).

3a: white solid; R_f = 0.19 (Heptane / EtOAC 1:1 (v/v)); ^{1}H-NMR ($CDCl_3$, 400 MHz), 2.02 (s, 3H), 2.30 (m, 2H) 3.76 (s, 3H), 3.76 (s, 3H), 3.97 (m, 2H), 4.74 (m, 1H), 6.36 (br, 1H), 6.81 (m, 4H) ppm. ^{13}C-NMR ($CDCl_3$, 101 MHz) 23.2, 31.3, 50.4, 52.4, 55.7, 64.9, 114.7, 115.5, 152.4, 154.1, 169.9, 172.5 ppm; MS (ESI, HCOOH) m/z 282.16 ($[M+H]^+$, calc: 282.13).

4a: yellow solid; R_f = 0.26 (Heptane / EtOAC 1:1 (v/v)); ^{1}H-NMR ($CDCl_3$, 400 MHz) 1.08 (d, 3H, J=6.72), 2.04 (s, 3H), 2.63 (m, 1H), 3.73 (s, 3H), 3.76 (s, 3H), 3.85 (m, 2H) 4.74 (dd, 1H, J1= 4.34, J2=8.54), 6.32 (br, 1H), 6.80 (m, 4H) ppm; ^{13}C-NMR ($CDCl_3$, 101 MHz) 14.3, 23.6, 35.7, 52.3, 54.8, 55.7, 70.4, 114.7, 115.5, 152.4, 154.2, 170.3, 172.2 ppm; MS (ESI, HCOOH) m/z 296.16 ([M+H]+, calc: 296.15).

General procedure of photocatalysis on thiostrepton

Catalysis was performed in dioxane / H_2O (9:1) with a final concentration of 500 µM peptide, 2 mM organoborate and 50 µM catalyst. A typical catalysis reaction was set up as follows: Thiostrepton (0.2 mmol in 316 µL dioxane) and 80 µL of a 10 mM organoborate stock solution (dioxane/H_2O 1:1) were combined. 4 µL of 5 mM catalyst stock solution in DMF was added in a schlenk-vial. The mixture was degassed by three repeated freeze-pump-thaw-cycles. The schlenk was filled with nitrogen and exposed to blue LED's for 3 hours at room temperature. The reaction mixture was analysed by UPLC/MS TQD directly.

NMR studies on Thiostrepton

'Big scale' catalysis was performed in dioxane / H_2O (9:1) with a final concentration of 500 µM peptide, 2 mM **2c** and 50 µM catalyst. The reaction was set up as follows: **2c** (6.4 mg, 30 µmol) was dissolved in 13 mL 1,4-dioxane

#	R	calcd.[a]	calcd.[b]	calcd.[c]	measured[a]	measured[b]	measured[c]
8a	**2a** (CO-Ph-4-OMe)	1802	1940	2078	1903 (10%)	1941 (81%)	2080 (8%)
8b[d]	**2b** (CO-Ph-4-Me)	1786	1908	2030	1786 (63%)	1908 (8%)	-
8c	**2c** (CO-Ph)	1772	1880	1988	1772 (79%)	1880 (14%)	-
8d[d]	**2d** (CO-Ph-4-Br)	1850	2036	2222	1851 (45%)	-	-
8e[e]	**2e** (CO-Ph-2-F)	1790	1916	2042	1791 (55%)	1917 (45%)	-
8f[d]	**2f** (CO-naphtyl)	1822	1980	2138	-	-	-
8g[d]	**2g** (C-Ph)	1756	1848	1940	1756 (16%)	-	-
8h[f]	**2h** (Ph)	1741	1818	1895	-	-	-
8i[e]	**2i** (cyclohexane)	1746	1828	1910	-	-	-
8j[e]	**2j** (C-NH-cyclohexane)	1777	1890	2003	-	-	-
8k[e]	**2k** (CO-tBu)	1752	1840	1928	-	-	-
8l[e]	**2l** (C-NH-Boc)	1795	1926	2057	-	-	-

Table 4.2: Scope of BF3K-salts in the photoredox catalysed reaction on thiostrepton. [a] single modification (conversion); [b] double modification (conversion); [c]: triple modification (conversion); [d]: starting material was still present; [e]: the peptide had degraded; [f]: no reaction observed.

#	R	calcd.[a]	calcd.[b]	calcd.[c]	measured[a]	measured[b]	measured[c]
9a	**2a** (CO-Ph-4-OMe)	3492	3630	3768	-	3648 (59%)	3768 (40%)
9b	**2b** (CO-Ph-4-Me)	3476	3598	3720	3475 (43%)	3598 (2%)	-
9c	**2c** (CO-Ph)	3462	3570	3678	3462 (64%)	3570 (15%)	-
9d	**2d** (CO-Ph-4-Br)	3539	3724	3909	-	-	-
9e[e]	**2e** (CO-Ph-2-F)	3480	3606	3732	-	-	-
9f	**2f** (CO-naphtyl)	3512	3670	3828	-	-	-
9g[d]	**2g** (C-Ph)	3446	3538	3630	3446 (35%)	3536 (23%)	3630 (4%)
9h[f]	**2h** (Ph)	3432	3508	3584	-	-	-
9i[e]	**2i** (cyclohexane)	3438	3522	3606	-	-	-
9j[e]	**2j** (C-NH-cyclohexane)	3467	3580	3693	-	-	-
9k[e]	**2k** (CO-tBu)	3442	3530	3618	-	-	-
9l[e]	**2l** (C-NH-Boc)	3485	3616	3747	-	-	-
9m[g]	**2a** (CO-Ph-4-OMe)	3354	-	-	3354	-	-

Table 4.3: Scope of BF_3K-salts in the photoredox catalysed reaction on nisin. [a] single modification; [b] double modification; [c]: triple modification; [d]: starting material was still present; [e]: the peptide had degraded; [f]: reaction observed; [g]: addition of TEMPO.

and 1.5 mL water in a Schlenck vial. Thiostrepton (5 µmol in 500 µL 1,4-dioxane) and **6** (100 µL of 5 mM stock solution in DMF) were added. The mixture was degassed by three repeated freeze-pump-thaw-cycles. The Schlenk was filled with nitrogen and exposed to blue LED's for 3 hours at room temperature. The mixture was filtered over celite and the solvent was removed. The crude was taken up in 2 mL 2,2,2-trifluoroethanol and diluted with 2 mL water. Purification was done by rp-HPLC separation on an XBridge C8 3.5 um 4.6x250mm column and a linear gradient of 50% -> 5% water (0.1%FA) in ACN (0.1%FA) in 30 min. The fractions with single modified peptide were combined and lyophilised. The purified product was dissolved in $CDCl_3$ and analysed on a Bruker 600 MHz NMR.

General procedure of photocatalysis on nisin

Catalysis was performed in acetone / 0.1% AcOH(aq) (1:1) with a final concentration of 1 mM peptide, 10 mM organoborate and 100 µM catalyst. A typical catalysis reaction was set up as follows: Organoborate (1.3 mg, 5.3 µmol) was dissolved in 254 µL acetone in a Schlenk vial. The mixture was diluted with 132 µL 0.1% AcOH(aq) and 132 µL nisin stock solution (4 mM in 0.1% AcOH(aq)). 10 µL of the catalyst stock solution (5 mM in acetone) was added. The mixture was degassed by three repeated freeze-pump-thaw-cycles. The Schlenk was filled with nitrogen and exposed to blue LED's for 3-16 hours at room temperature. After 16 hours the reaction mixture was diltues 2x with 0.1% AcOH and filtered over 0.45 µm filters prior to analysis by UPLC/MS TQD.

NMR studies on Nisin

The crude reaction mixture of **9a** was diluted 2x with 0.1% AcOH(aq) and filtered over 0.45 µm to remove precipitated catalyst. Purification from other reagents was done by size exclusion chromatography over a NAP-10 column. The mixture was concentrated by freezedrying and redissolved in 0.1%CD_3COOD in D_2O for NMR analysis with water suppression.

Marfey Analysis of Nisin

The crude reaction mixture of **9c** was diluted 2x with 0.1% AcOH(aq) and filtered over 0.45 µm to remove precipitated catalyst. Purification from other reagents was done by size exclusion chromatography over a NAP-10 column. The mixture was concentrated by lyophilisation and redissolved in 300 µL 6 M HCl(aq) and transferred to a microwave tube equipped with stir bar. The sample was exposed to microwave irradiation for 10 minutes at 160 °C, with maximum 50 Watt power. The mixture was transferred to an eppendorf vial and concentrated to dryness. The residue was taken up in 100 µL 1 M $NaHCO_3$(aq). 12 µL of 1% Marfey's reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA)) in acetone is added. After shaking for 1 hour at 40 °C, the sample was neutralised with 60 µL 2 M HCl(aq), diluted with 800 µL methanol, and analysed directly by UPLC/MS TQD. Signals obtained at 340 nm absorption were assigned to the corresponding FDAA derivatised amino acids.

4.5 - Bibliography

[1] J. A. Prescher, C. R. Bertozzi, *Nat. Chem. Biol.* **2005**, *1*, 13.

[2] C. P. Ramil, Q. Lin, *Chem. Commun*. **2013**, *49*, 11007.

[3] T. Koopmans, T. M. Wood, P. t Hart, L. H. Kleijn, A. P. Hendrickx, R. J. Willems, E. Breukink, N. I. Martin, *J. Am. Chem. Soc.* **2015**, *137*, 9382.

[4] J. C. Slootweg, S. van der Wal, H. C. Quarles van Ufford, E. Breukink, R. M. Liskamp, D. T. Rijkers, *Bioconjug. Chem.* **2013**, *24*, 2058.

[5] Y. Shi, X. Yang, N. Garg, W. A. van der Donk, *J. Am. Chem. Soc.* **2011**, *133*, 2338.

[6] L. Zhou, J. Shao, Q. Li, A. J. van Heel, M. P. de Vries, J. Broos, O. P. Kuipers, *Amino Acids* **2016**, *48*, 1309.

[7] X. Just-Baringo, F. Albericio, M. Alvarez, *Angew. Chem. Int. Ed.* **2014**, *53*, 6602.

[8] L. M. Repka, J. R. Chekan, S. K. Nair, W. A. van der Donk, *Chem. Rev.* **2017**, 5457.

[9] D. E. Palmer, C. Pattaroni, K. Nuami, R. K. Chadba, M. Goodman, T. Wakamiya, K. Fukase, S. Horimot, M. Kitazawa, H. Fujita, A. Kubo, T. Shiba, *J. Am. Chem. Soc.* **1992**, *114*, 5645.

[10] P. J. Knerr, W. A. van der Donk, *Annu. Rev. Biochem.* **2012**, *81*, 479.

[11] 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, *Chem. Sci.* **2011**, *2*, 1666.
[12] A. M. Freedy, M. J. Matos, O. Boutureira, F. Corzana, A. Guerreiro, P. Akkapeddi, V. J. Somovilla, T. Rodrigues, K. Nicholls, B. Xie, G. Jimenez-Oses, K. M. Brindle, A. A. Neves, G. J. L. Bernardes, *J. Am. Chem. Soc.* **2017**, *139*, 18365.
[13] M. R. Aronoff, B. Gold, R. T. Raines, *Org. Lett.* **2016**, *18*, 1538.
[14] T. H. Wright, et. al., *Science* **2016**, *354*, aag1465.
[15] A. Yang, S. Ha, J. Ahn, R. Kim, S. Kim, Y. Lee, J. Kim, D. Söll, H. Y. Lee, H. S. Park, *Science* **2016**, *354*, 623.
[16] J. G. Gober, S. V. Ghodge, J. W. Bogart, W. J. Wever, R. R. Watkins, E. M. Brustad, A. A. Bowers, *ACS Chem. Biol.* **2017**, *12*, 1726.
[17] H. M. Key, S. J. Miller, *J. Am. Chem. Soc.* **2017**, *139*, 15460.
[18] M. H. Shaw, J. Twilton, D. W. MacMillan, *J. Org. Chem.* **2016**, *81*, 6898.
[19] S. J. McCarver, J. X. Qiao, J. Carpenter, R. M. Borzilleri, M. A. Poss, M. D. Eastgate, M. M. Miller, D. W. MacMillan, *Angew. Chem. Int. Ed.* **2017**, *56*, 728.
[20] C. Bottecchia, M. Rubens, S. B. Gunnoo, V. Hessel, A. Madder, T. Noël, *Angew. Chem. Int. Ed.* **2017**, *56*, 12702.
[21] B. A. Vara, X. Li, S. Berritt, C. R. Walters, E. J. Petersson, G. A. Molander, *Chem. Sci.* **2018**, *9*, 336.
[22] N. Ichiishi, J. P. Caldwell, M. Lin, W. Zhong, X. Zhu, E. Streckfuss, H. Y. Kim, C. A. Parish, S. W. Krska, *Chem. Sci.* **2018**, *9*, 4168.
[23] S. Bloom, C. Liu, D. K. Kolmel, J. X. Qiao, Y. Zhang, M. A. Poss, W. R. Ewing, D. W. C. MacMillan, *Nat. Chem.* **2018**, *10*, 205.
[24] G. Molander, D. L. Sandrock, *Curr. Opin. Drug Discov. Devel.* **2009**, *12*, 811.
[25] Y. Yasu, T. Koike, M. Akita, *Adv. Synth. & Catal.* **2012**, *354*, 3414.
[26] K. Miyazawa, Y. Yasu, T. Koike, M. Akita, *Chem. Commun.* **2013**, *49*, 7249.
[27] L. M. Hicks, S. E. O'Connor, M. T. Mazur, C. T. Walsh, N. L. Kelleher, *Chem. Biol.* **2004**, *11*, 327.
[28] W. Wang, H. Zhou, H. Lin, S. Roy, T.A. Shaler, L. R. Hill, S. Norton, p. Kumar, M. Anderle, C.H. Becker, *Anal. Chem.*, **2003**, *75*, 4818.
[29] C. J. Thibodeaux, T. Ha, W. A. van der Donk, *J. Am. Chem. Soc.* **2014**, *136*, 17513.
[30] P. Marfey, *Carlsberg Res. Commun.* **1984**, *49*, 591.
[31] Z. Zhang, A.G. Marshall, *J. Am. Soc Mass. Spec.* **1998**, *9*, 225.
[32] F. Crestey, V. Collot, S. Stiebing, S. Rault, *Synthesis* **2006**, 3506-3514.
[33] P. John, D.N. Primer, C. Tellis, Gary A. Molander, *Science* **2014**, *345*, 433.
[34] J.D. Slinker, A.A. Gorodetsky, M.S. Lowry, J. Wang, S. Parker, R. Rohl, S. Bernhard, G.G. Malliaras, *J. Am. Chem. Soc.* **2004**, *126*, 2763.
[35] A. Singh, K. Teegardin, M. Kelly, K. S. Prasad, S. Krishnan, J. D. Weaver, *J. Organomet. Chem.* **2015**, *776*, 51-59.
[36] T. M. Monos, A. C. Sun, R. C. McAtee, J. J. Devery, 3rd, C. R. Stephenson, *J. Org. Chem.* **2016**, *81*, 6988-6994.