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SHORT COMMUNICATION

Microwave-assisted cleavage of cysteine perfluoroaryl thioethers

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Abstract
The cysteine- perfluoroarene S_NAr reaction allows for the sequence-specific attachment of dyes and affinity tags to peptides and proteins. However, while many methods exist for the desulfuration of native and functionalized cysteine residues, there are no reports of their application to perfluoroarylated cysteines. Herein we report both the hydrogenolysis of a perfluoroarylated cysteine to alanine and elimination to dehydroalanine, reactions that are both accelerated by microwave irradiation.

Keywords Peptides · Cysteine arylation · Hydrogenolysis · Microwave

Introduction

Over the past decade, the cysteine-perfluoroarene S_NAr reaction has emerged as an efficient and selective method for both the functionalization of peptides and proteins and the introduction of side-chain-to-side-chain covalent conformational constraint (Spokoyny et al. 2013; Zou et al. 2014; Zhang et al. 2015; Kalhor-Monfared et al. 2016; Lühmann et al. 2016). While the reactivity and stability of native cysteine residues and their alkylated derivatives have been studied extensively, (Chalker et al. 2009; Gunnoo and Madder 2016; Peraro et al. 2016; Perell et al. 2017) the bulk of work in this area has focused on desulfurization of peptides and proteins derived from native chemical ligation (Jin and Li 2018). To date, the stability and reactivity of these cysteinyl perfluoroaryl thioethers remain unexplored.

Typical methods for desulfurization of cysteine residues involve oxidation and/or sequential elimination/reduction, with the more recent development of a photo-cleavable affinity tag (Chalker et al. 2011; Gunnoo and Madder 2016; Liu et al. 2019). While the hydrogenolysis (Djerassi et al. 1958) of C–S bonds has been established for over 60 years, there are few examples of applications to peptides. Heterogeneous catalysts such as nickel boride (prepared in situ) or Raney nickel have been used for the reduction of aryl sulfides (Truce and Perry 1965). More recently, both Raney nickel and Pd/Al_2O_3 (Yan and Dawson 2001) have been used for the rapid desulfurization of cysteine residues in a cyclic peptide produced via native chemical ligation, with eventual desulfurization of methionine residues upon prolonged reaction times. While the oxidation of bis(thioether) stapled peptides has been studied recently, (Perell et al. 2017) to date, none of these reactions on perfluoroaryl thioethers have been reported. These known reactions are summarized in Scheme 1.

Materials and methods

General

Reagents and solvents were purchased from commercial suppliers and used as received unless otherwise noted. Microwave reactions were performed in a CEM MARS reactor. NMR spectra were acquired on a Bruker Avance DPX 300 MHz spectrometer. Low-resolution mass spectra were acquired on an Agilent 6110 Quadrupole LC/MS system.

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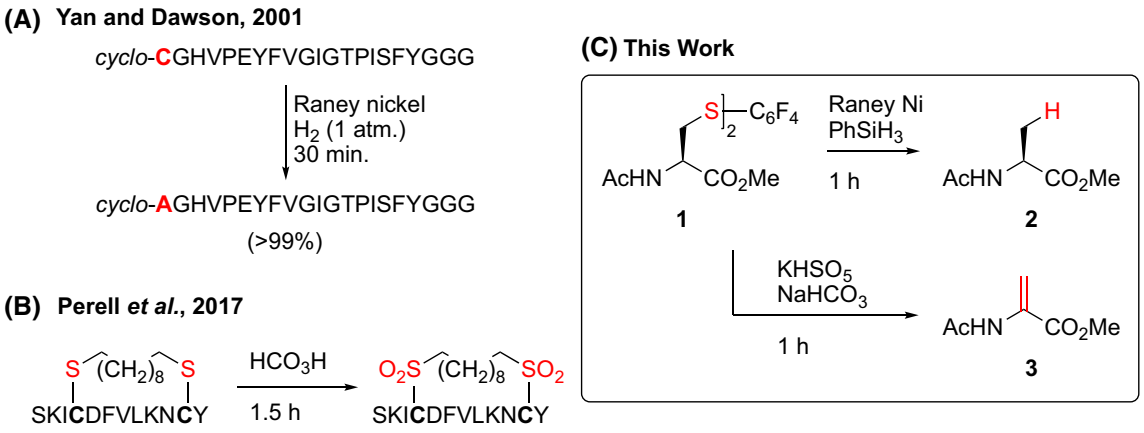


Scheme 1 **a** Desulfuration of cysteine in a cyclic peptide using Raney nickel; (**b**) oxidation of cysteines in a stapled helix using performic acid; (**c**) microwave-assisted desulfuration and oxidative elimination of a cysteine perfluoroarene

Melting points were measured using a Mettler Toledo MP50 melting point system. IR spectra were acquired using an Agilent Cary 630 FTIR.

Preparation of 1

In a 250 mL round-bottom flask 2.82 mmol N-acetyl-L-cysteine methyl ester was dissolved in 30 mL acetonitrile. $Na_3PO_4 \cdot 6H_2O$ (3.53 mmol) and hexafluorobenzene (1.41 mmol) were then added. The reaction was allowed to stir overnight under argon at ambient temperature. The crude reaction was filtered through a celite plug and concentrated *in vacuo*. This residue was then washed with water (40 mL), dissolved in CH_2Cl_2, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. **1** was obtained as a white solid in 68–92% yield without further purification.

Hydrogenolysis to 2

In a 20 mL scintillation vial (or oven-dried microwave vial), 0.120 mmol **1** was combined with 1,000% v/w Raney nickel slurry. Immediately following Raney nickel addition, 0.5 equivalents of phenylsilane and 2 mL of THF were added. The reaction vessel was sealed under a blanket of argon, and heated to 60 °C in an oil bath (48 h) or in a microwave (400 W pulses, 1 h) with magnetic stirring. The crude mixture was filtered through a celite plug, dried over $MgSO_4$, and concentrated *in vacuo*. **2** was isolated via silica gel column chromatography (EtOAc) and identified by comparison of ^{1}H-NMR with known spectra (Aikawa et al. 2008).

Oxidation-elimination to 3

In an oven-dried microwave vial, 0.120 mmol of **1** was combined with Oxone® (0.30 mmol), $NaHCO_3$ (0.60 mmol), and acetonitrile (4 mL). The sealed reaction was heated to 60 °C in an oil bath (48 h) or in a microwave (400 W pulses, 1 h). The crude reaction was filtered through a plug of celite, concentrated *in vacuo,* and purified via silica gel column chromatography (20% EtOAc/hexane). Purified product was identified by comparison of ^{1}H-NMR with known spectra (Crestey et al. 2006).

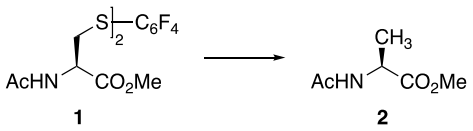
Synthesis of 4

Peptide **4** was synthesized on 38 µmol scale using standard Fmoc conditions on 2-chlorotritylchloride resin. After cleavage, the crude peptide QCW was precipitated using 10 volumes of ice-cold diethyl ether, dried under a stream of argon and dissolved in DMF (2 mL). DIPEA (230 µmol) and hexafluorobenzene (1.60 mmol) were added to this solution, which was allowed to shake at ambient temperature for 4 h, following a published procedure (Zhang et al. 2015). This reaction was then purified by RP-HPLC on a Waters preparative system with a Spherisorb 5 µm ODS2 semi-prep column using a gradient of 20%–80% acetonitrile in water, with 0.1% TFA, and lyophilized to produce a white solid in 48% yield.

Results and discussion

Initial reaction optimization was performed on *p*-$(AcCysOMe)_2C_6F_4$ (**1**), which was readily prepared in millimolar quantity from commercial L-AcCysOMe and C_6F_6. Treatment of **1** with Raney nickel under the reported conditions for cysteine desulfuration (Yan and Dawson 2001) resulted in no observed reduction (Table 1, entry 1). Other catalysts such as Ni_2B or Pd/C (entries 2–3) were unreactive. Switching the solvent to THF resulted in the formation of trace amounts of the product after 48 h (ca. 5% by

Table 1 Reduction of $(AcCysOMe)_2C_6F_4$



1 **2**

Entry	Reductant	Solvent	T (°C)	Time (h)	Yield (%)
1	Raney Ni (100 v/w %), H_2	4:1 H_2O:AcOH	r.t	24	nr
2	Ni_2B, H_2	9:1 MeOH:AcOH	r.t	24	nr
3	Pd/C, H_2	9:1 MeOH: AcOH	r.t	24	nr
4	Raney Ni (200 v/w %), H_2	THF	r.t	48	< 5[a]
5	Raney Ni (200 v/w %)	THF	60	48	13
6	Raney Ni (1,000 v/w %), $PhSiH_3$ (0.5 equiv.)	THF	60	48	63

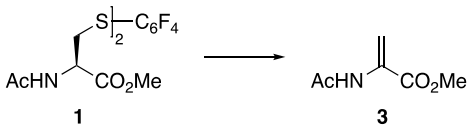
[a]NMR conversion

NMR, entry 4). Heating the reaction to 60 °C in a sealed vial improved yield to 13%, even in the absence of H_2 (entry 5). Increasing the amount of catalyst to 1000 w/v% and adding 0.5 equiv. $PhSiH_3$ ultimately resulted in a 63% yield of **2** (entry 6). Other alkylsilanes (Et_3SiH, $(EtO)_3SiH$, iPr_3SiH) and hydride sources such as $NaBH_4$ failed to produce measurable amounts of **2** (data not shown).

While disappointing, the modest reactivity of **1** is not entirely unsurprising. Desulfurization of homocysteine-containing peptides with Raney nickel was reported to result in demethylthiolation of methionine only after "prolonged exposure," giving ~ 50% conversion in 4 h and complete removal only after 14 h, compared to 80% in 30 min for cysteine (Yan and Dawson 2001). In a similar system, the hydrogenolysis of naphthyl ethers using $Pd(OH)_2$ and H_2 (3 atm.) proceeded selectively in the presence of alkylthioethers (12 h), phenylthioglycosides (2–16 h), and a methionine residue (7 h) (Adero et al. 2017). It is anticipated that the combined steric and inductive electron-withdrawing properties of the perfluoroaryl substituent led to the lower reactivity of **1** for heterogeneous hydrogenolysis of the C–S bond.

Conditions for the elimination of **1** to **3** were also examined, based on methods for the alkylation/elimination and oxidation/elimination of cysteine and methionine (Chalker et al. 2011). Once again, **1** showed no reactivity at ambient temperature (Table 2, entry 1). Alkylation with bromoacetic acid at elevated temperature resulted in 17% conversion after 48 h (entry 2). Use of iodoacetamide under the same conditions resulted in quantitative conversion to the protected dehydroalanine **3** (entry 3). Oxidation with potassium peroxymonosulfate (Oxone) in acetonitrile resulted in a sluggish reaction, with only 42% conversion to **3** after 48 h (entry 4).

Table 2 Elimination of $(AcCysOMe)_2C_6F_4$

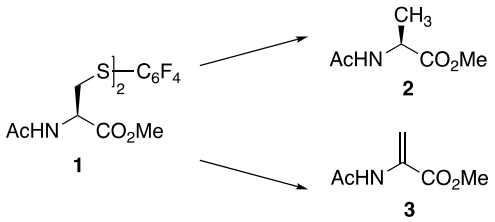


1 **3**

Entry	Conditions	Solvent	T (°C)	Time (h)	Yield (%)
1	bromoacetic acid, DIPEA	9:1 THF:H_2O	r.t	48	nr
2	bromoacetic acid, $NaHCO_3$	9:1 THF:H_2O	60	48	17[a]
3	iodoacetamide, $NaHCO_3$	9:1 THF:H_2O	60	48	100[a]
4	$KHSO_5$ (2.5 equiv.), $NaHCO_3$ (5.7 equiv.)	MeCN	65	48	42[a]

[a]NMR conversion

Table 3 Microwave-assisted cleavage of 1



Entry	Conditions	Solvent	T (°C)	Time (h)	Yield (%)
1	Raney Ni (1500 v/w %), $PhSiH_3$ (0.5 equiv.)	THF	60[b]	1	78
2	Raney Ni (750 v/w %), $PhSiH_3$ (0.5 equiv.)	THF	60[b]	1	60[a]
3	Raney Ni (375 v/w %), $PhSiH_3$ (0.5 equiv.)	THF	60[b]	1	36[a]
4	Raney Ni (188 v/w %), $PhSiH_3$ (0.5 equiv.)	THF	60[b]	1	15[a]
5	bromoacetic acid, $NaHCO_3$	THF	60[b]	1	nr
6	iodoacetamide, $NaHCO_3$	THF	60[b]	1	nr
7	$KHSO_5$ (2.5 equiv.), $NaHCO_3$ (5.7 equiv.)	MeCN	60[b]	1	29 (100[a])

[a]NMR conversion

[b]1600 W Microwave, 400 W pulses

Microwave acceleration of perfluoroaryl thioether cleavage

Microwave irradiation, which has previously been reported to accelerate the heterogenous, nickel-catalyzed depolymerization of lignin, (Toledano et al. 2014) was explored as a method to optimize the hydrogenolysis of **1**–**2**. Using the previously optimized conditions (Table 1, entry 6) in a microwave reactor accelerate this reaction drastically, resulting in 78% yield of **2** in only 1 h (Table 3, entry 1). Reduction of catalyst loading (entries 8–10), unfortunately, resulted in correspondingly lower conversion to **2**. Microwave irradiation did not accelerate either of the alkylation/elimination reactions, with no observable product after 1 h with bromoacetic acid or iodoacetamide (entries 5–6). The microwave-accelerated oxidative elimination conditions, however, did result in quantitative conversion to **3** after 1 h (29% isolated yield, entry 7).

Application to a model peptide

To determine the utility of this reaction, these optimized conditions were then applied to a short, perfluoroarylated peptide Gln-Cys(C_6F_5)-Trp (**4**), designed to test as much functional group tolerance (free N- and C-termini; amide side-chain) as possible in a short peptide. Using the optimized microwave hydrogenolysis conditions, **4** was fairly unreactive, resulting in the consumption of 61% of **4** after 2 h, but failing to produce obersevable amounts expected peptide Gln-Ala-Trp by LC/MS. Side-reactions to produce multiple, unidentified products were previously observed with prolonged reaction times in the hydrogenolysis of cysteine-containing peptides (Yan and Dawson 2001).

Conclusion

Known chemistry for the reduction and elimination of cysteine can readily be applied to protected, perfluoroarylated cysteines. While these derivatives were less reactive compared to what had been reported for unsubstituted cysteine, microwave irradiation was found to accelerate these reactions. Although perfluoroaryl groups are readily cleaved from a simple, protected cysteine, these reactions will require further optimization for application to peptides or proteins.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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