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# Exploring new activating groups for reactive cysteine NCAs

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ABSTRACT

Due to its ability to reversibly crosslink proteins, cysteine has a unique role as an amino acid in nature. For controlled, asymmetric formation of disulfides from two thiols, one thiol needs to be activated. While few activating groups for cysteine have been proposed, they are usually not stable against amines making them unsuitable for solid phase peptide synthesis or amine initiated polymerization of α-amino acid-*N*-carboxy-anhydrides (NCAs). In this Letter we describe a series of new thiol activated cysteines, as well as their NCAs and explore the link between electron deficiency of the leaving group and control over NCA polymerization.



## Introduction

Since their discovery in 1906[1] Leuchs' anhydrides also known as α-amino acid-*N*-carboxy-anhydrides (NCAs) have attracted considerable attention.[2,3] NCAs are mostly used as monomers for the preparation of poly(amino acid)s through ring opening polymerization, but they were also applied as synthons for the stepwise synthesis of peptides.[4,5] In recent years, poly(amino acids) proved to be very successful as carriers or polymeric drugs in the field of nanomedicine. While copaxone is an approved drug with 4.7 billion $ sales in 2013 clinical studies on multiple polypeptide based polymer drug conjugates or drug formulations (OPAXIO, NC-6004, NK 105) are underway.[6] Additionally, polypetide based polymers for drug delivery applications are emerging.[6–9]

However, while disulfides are already widely used in nanomedicine to reversibly attach drugs to carriers or to stabilize nanoparticles, to our knowledge, poly(cysteine) has not been used for this purpose. Neither protected or activated cysteine derivatives have been explored. The reasons for this fact are on the one hand the tendency of poly(cysteine) to adopt a β-sheet conformation which leads to solubility issues and on the other hand the lack of reactive cysteine protecting groups itself. While derivates of 3-nitro-2-pyridinesulfenyl cysteine[10] and 3-(2-pyridyldithio)propionate[11] are frequently employed as thiol reactive moieties, both protecting groups can only be utilized for endgroup modification in Fmoc solid phase peptide coupling[12] or as bifunctional linkers. Further, a series of cysteine NCAs (*S*-allyl-cysteine, *S*-benzyl-cysteine, *S*-benzyloxycarbonyl-cysteine, *S*-benzyloxycarbonyl-methylene-cysteine, *S*-butyl-cysteine, *S*-*tert*-butylmecapto-cysteine, a range of *S*-alkyl-cysteines (dodecyl, hexyl, methyl, ethyl, prolyl, isopropyl), *S*-(2-trimethylsilyl benzyl)-cysteine, *S*-(4-trimethylsilyl benzyl)-cysteine, cystine (bisNCA), *S*-thiophenyl-cysteine) from the early years of NCA polymerization have been collected.[13] However none of them bear thiol reactive protecting groups, which can be directly addressed after polymerization. Newer cysteine NCAs have either not focused on post-polymerization modification of polymers at all[14–16] or have worked with deprotection and modification using thiol-ene chemistry, which leads to the formation of biological non-reversible thioethers.[17–19] Approaches of crosslinking peptidic nanoparticles with disulfides have so far been focused on oxidative disulfide formation[20–24] or polymerization of cystine bis-NCA,[25] while the first method is time-consuming and incomplete the latter hinders control over polymerization and particle formation.

A protective group stable during ROP or standard Fmoc solid phase synthesis of peptides but selectively addressable in post-polymerization modification reactions leading to the formation of a bio-reversible disulfide bond in one step is simply absent. In our search for a reactive cysteine-protecting group that is stable under NCA polymerization conditions[26] we have come across a range of novel reactive cysteine NCAs, more or less suitable for NCA polymerization. In this Letter we report the synthesis and analysis of these NCAs.

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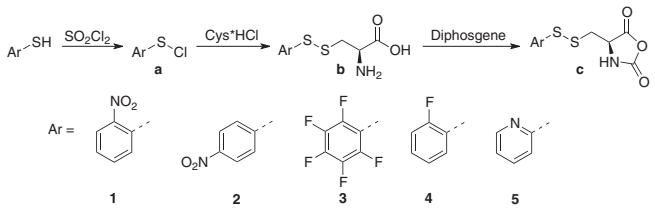
## Results and discussion

As starting point for the synthesis of cysteine NCAs with a thiol reactive protecting group we synthesized thiol reactive groups known in the literature starting with *ortho*-nitrothiophenol.[27] The strong electron withdrawing effect from the nitro group favors a thiol exchange with a more electron rich thiol. According to Phocas et al. *o*-nitrothiophenol sulfenic acid chloride (**1a**), was reacted with cysteine hydrochloride.[27] The product (**1b**) was transformed into the NCA by reaction with diphosgene in the absence of water (**1c**, Scheme 1).

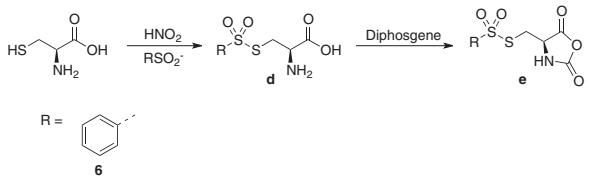
This reaction scheme was used for all disulfide based NCAs. When the sulfenyl chloride was not commercially available, it was synthesized from the thiophenol by chlorination with sulfuryl chloride according to Shang et al.[28] The modified cysteines **1**–**5b** were synthesized with yields ranging from 13% to 98%, while the NCAs **1c**, **3**–**4c** were synthesized with 22–65% yields after 3 recrystallization steps. The NCA of ((*p*-nitrophenyl)disulfanyl)cysteine (**2c**) as well as *S*-(*o*-thiopyridinyl)cysteine (**5c**) could not be purified. While in the case of **2c** cystine impurities could not be removed, **5c** is not even stable, since the pyridine can act as an initiator in the NCA polymerization[2] which was additionally confirmed by polymerizing Lys(Z)NCA with pyridine under the polymerization conditions applied in this study.

The suitability of the NCA **1c** for amine initiated NCA polymerization was investigated by polymerization with neopentylamine in absolute DMF (see Supplementary data).[29,30] The GPC plots corresponding to the molecular weight distribution of polymers were multimodal or broad indicating the existence of multiple side reactions. These side reactions can be attributed to protective group cleavage by the amine chain end and reinitiation by the free thiol (see Fig. S1). After **1c** failed to yield defined polymers we lowered the strength of the electron-withdrawing group (EWG) to decrease the reactivity of the disulfide and prevent a reaction of the disulfide with the primary amine initiator (neopentylamine) or the polymer chain end. The reactivity was reduced to pentafluoro thiophenol and ortho-fluoro thiophenol. With decreasing electron deficiency of the thiol, the quality of the polymers increased ($Đ$ = 2.1, 1.8 and 1.6 for **1c**, **3c,** and **4c** respectively, see SI 2–4) but not to a point which we considered satisfying.

Realizing, that weakening of the EWG would not be enough to facilitate successful NCA polymerization and keep reactivity high enough for postpolymerization modification reactions, we turned our attention to different thiol activating groups. A group that has been used to form asymmetrical disulfides are thiosulfonates.[31,32] Of this class *S*-phenylsulfonates are known to react readily with free thiols achieving complete conversions upon minutes,[33,34] thus a cysteine NCA based on phenylthiosulfonate was synthesized. To achieve this, cysteine was activated by transforming it into *S*-nitroso cysteine, which was reacted with the corresponding sulfinate without purification. The synthesis of thiosulfonates was based on work of Hart et al.,[35,36] which was chosen over the more complex route of Weidner et al.[37] After purification the *S*-thiosulfonylcysteine was transformed into the corresponding NCA using diphosgene (Scheme 2). The *S*-phenylthiosulfonylcysteine **6b** was successfully synthesized with 48% yield,




**Scheme 1.** Synthesis of disulfide-based NCAs.




**Scheme 2.** Synthesis of a thiosulfonyl-based NCA.

while its NCA (**6c**) was synthesized with 77% yield after 3 recrystallization steps to achieve very high purity.

This NCA performed better than the disulfide based NCAs in the amine-initiated polymerization yielding polymers with narrower molecular weight distribution ($Đ$ = 1.16 for **6e**, see SI5). The GPC plots however display the presence of low molecular weight oligomers, which indicates partial protective group cleavage. Although we have developed new reactive cysteine NCA derivatives, further investigation of the influence of the electron deficiency of the substituent with even less electron-deficient *S*-alkylthiosulfonyl-L-cysteine-NCAs seems necessary to achieve protective groups with high stability against amines and combine it with high reactivity toward thiols. Since the thiosulfonate group does only partially engage in side reactions toward amines this class of protective groups needs to be further investigated in future. The next generation of derivatives, however, can be synthesized using the herein reported synthetic pathway.

## Conclusion

We have reported the syntheses of a range of new, activated cysteine amino acids and NCAs based on electron deficient disulfides and thiosulfonates. While especially the more reactive disulfides cannot be polymerized in a controlled manner using primary amines as initiators, they might be polymerized more controlled using organometallic catalyst[38–40] or trimethylsilyl initiators.[41,42] In contrast, the thiosulfonate based monomer can be polymerized in a controlled fashion using primary amines, leading to a new class of reactive polypeptides, showing that higher electron density leads to higher stability against primary amines and thus decreases side reactions during NCA polymerization.

## Experimental

### Materials and methods

All chemicals were purchased from Sigma Aldrich and used as received unless otherwise noted. THF and hexane were distilled from Na/K and ethyl acetate from $CaH_2$. Cysteine was purchased from OPREGEN and diphosgene from Alfa Aesar. Deuterated solvents were purchased from Deutero.

$^{1}H$, $^{19}F$ and $^{13}C$ NMR spectra were recorded on a Bruker AC 400 at a frequency of 400, 376 and 101 MHz, respectively. $^{1}H$ and $^{19}F$ NMR spectra were also recorded on a Bruker Avance III HD 300 at 300 and 282 MHz, respectively. The spectra were recorded at room temperature and calibrated using the solvent signals.[43] Field desorption mass spectrometry (FD-MS) was performed on a FD Finnigan MAT90 spectrometer. Melting points were measured using a Mettler FP62 melting point apparatus at a heating rate of 0.5 °C/min. Preparative reverse phase-HPLC was performed with a Knauer HPLC-System (Berlin, Germany), consisting of two HPLC pumps (Smartline 1000), an UV/vis-detector (Smartline 2500), a RI-detector (Smartline 2400) and a Phenomenex (Torrance, U.S. A.) Luna-column (10 µm, C18(2), 100A, 250 × 30 $mm^2$) run at a flow of 10.0 mL/min and loaded with a 2 mL injection loop. The system was operated and samples analyzed with D-7000

HPLC-System-Manager software (version 4.1). The detector was run at a wavelength of 214 nm.

### ((*o*-Nitrophenyl)disulfanyl)-L-cysteine (Cys(*o*NTP), 1b)

To a suspension of 5.86 g (37.18 mmol) cysteine hydrochloride in 150 mL acetic acid 10 g (52.74 mmol) (commercial) 2-nitrobenzenesulfenyl chloride was added. The suspension was stirred until it got solid, then 100 mL acetic acid was added and the mixture was heated to 70 °C for 3.5 h. The solvent was removed in vacuo, the residue was dissolved in 20 mL DMSO and precipitated with 250 mL $CHCl_3$. After filtration the precipitate was suspended in 200 mL $CHCl_3$, stirred for 30 min, and collected by filtration twice. The product was dried in vacuo yielding 8.63 g (31.45 mmol, 85%) of a yellow solid.

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ [ppm] = 8.85 (s, 3H, N$H_3^+$), 8.28 (dd, $J$ = 9.6, 8.2 Hz, 2H, $H_{Ar}$), 7.89 (dd, $J$ = 8.3, 7.2 Hz, 1H, $H_{Ar}$), 7.55 (dd, $J$ = 8.3, 7.2 Hz, 1H, $H_{Ar}$), 4.20 (t, $J$ = 5.8 Hz, 1H, C$H$NH$_3$), 3.43–3.26 (m, 2H, CH$CH_2$S).

$^{13}$C{$^1$H} NMR (101 MHz, DMSO-$d_6$) $\delta$ [ppm] = 169.09 ($C$OO$^-$), 145.26 ($C_{ar}$NO$_2$), 135.31 ($C_{ar}$S, $C_{ar}$), 127.42 ($C_{ar}$), 127.36 ($C_{ar}$), 126.44 ($C_{ar}$), 51.22 ($C_\alpha$), 37.14 ($C_\beta$).

FD-MS: 274.3 (M+H$^+$).

### ((*o*-Nitrophenyl)disulfanyl)-L-cystein-NCA (Cys(*o*NTP)NCA, 1c)

A suspension of 2.015 g (7.35 mmol) of ((2-nitrophenyl)disulfanyl)cysteine and 1.55 mL (9.56 mmol) limonene in 40 mL dry THF was heated to 70 °C. 0.8 mL (4.59 mmol) diphosgene was added in 3 steps every 20 min. The yellow solution was heated for 4 h. Nitrogen was bubbled though the solution for 2 h to remove excess HCl and diphosgene and most of the solvent was removed. The residue was redissolved in 11 mL THF and precipitated by adding 100 mL of cyclohexane very slowly. The precipitate was redissolved in 14 mL THF and insoluble solids were removed by filtration. The product was recrystallized two more times from THF/cyclohexane avoiding contact to air yielding 1.437 g (4.78 mmol; 65%) of a pale yellow powder.

$^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ [ppm] = 9.33 (s, 1H, N$H$), 8.31 (dd, $J$ = 8.2, 1.4 Hz, 1H, $H_{Ar}$), 8.23 (dd, $J$ = 8.2, 1.2 Hz, 1H, $H_{Ar}$), 7.90 (ddd, $J$ = 8.4, 7.2, 1.4 Hz, 1H, $H_{Ar}$), 7.56 (ddd, $J$ = 8.4, 7.3, 1.3 Hz, 1H, $H_{Ar}$), 4.77 (ddd, $J$ = 6.0, 4.8, 1.3 Hz, 1H, C$_\alpha H$), 3.38–3.16 (m, 2H, C$H_2$).

$^1$H NMR (400 MHz, THF-$d_8$) $\delta$ [ppm] = 8.47 (s, 1H, N$H$), 8.30 (td, $J$ = 8.0, 1.2 Hz, 2H, $H_{Ar}$), 7.81 (ddd, $J$ = 8.4, 7.2, 1.4 Hz, 1H, $H_{Ar}$), 7.47 (ddd, $J$ = 8.1, 7.1, 1.1 Hz, 1H, $H_{Ar}$), 4.68 (dd, $J$ = 7.8, 3.7 Hz, 1H, C$_\alpha H$), 3.26 (dd, $J$ = 14.0, 3.9 Hz, 2H, C$H_2$), 3.17 (dd, $J$ = 14.0, 7.8 Hz, 1H, C$H_2$).

$^{13}$C{$^1$H} NMR (101 MHz, THF-$d_8$) $\delta$ [ppm] = 170.27 (C$_\alpha$($C$O)O), 152.57 (NH($C$O)O), 146.98 ($C_{Ar}$NO$_2$), 136.85 ($C_{Ar}$S), 135.53 ($C_{Ar}$), 128.21 ($C_{Ar}$), 127.88 ($C_{Ar}$), 127.14 ($C_{Ar}$), 57.77 ($C_\alpha$), 40.34 ($C$H$_2$).

### ((*p*-Nitrophenyl)disulfanyl)-L-cysteine (Cys(*p*NTP), 2b)

To a suspension of 277 mg (1.76 mmol) cysteine hydrochloride in 2 mL DMF was added 6 mL acetic acid followed by 500 mg (2.64 mmol) commercial 4-nitrobenzenesulfenyl chloride. The suspension was stirred at room temperature for 2 days during which it lost most of its yellow color. The AcOH was removed in vacuo. Then 30 mL $CHCl_3$ was added slowly to precipitate the product. After filtration the precipitate was suspended in 30 mL $CHCl_3$, stirred for 30 min, and collected by filtration three times. The product was dried in vacuo yielding 394 mg (1.44 mmol, 82%) of a pale yellow solid.

$^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ [ppm] = 10.02–8.36 (br, 3H, N$H_3^+$), 8.24 (d, $J$ = 8.9 Hz, 2H, $H_{Ar}$), 7.84 (d, $J$ = 9.0 Hz, 2H, $H_{Ar}$), 4.15 (t, $J$ = 5.9 Hz, 1H, C$_\alpha H$), 3.36 (dd, $J$ = 6.0, 2.4 Hz, 2H, C$H_2$).

### Pentafluorophenylsulfenyl chloride (PFTPCl, 3a)

A solution of 5 mL pentafluorothiophenol (7.505 g, 37.5 mmol) in 75 mL DCM was cooled to 0 °C. 4.56 mL sulfuryl chloride (7.59 g, 56.3 mmol) was added and the solution was stirred overnight. DCM and excess sulfuryl chloride were removed in vacuo at 40 °C yielding 8.35 g (4.97 mL, 35 mmol, 93%) of an orange liquid.

$^{19}$F NMR (400 MHz, $CDCl_3$) $\delta$ [ppm] = −128.57 (m, 2F, *o*-F), −145.49 (tt, 1F, *p*-F), −160.17 (m, 2F, *m*-F).

### *S*-(Pentafluorothiophenyl)-L-cysteine (Cys(PFTP), 3b)

To a suspension of 1.35 g (8.59 mmol) cysteine hydrochloride in 35 mL acetic acid, 3.02 g (13 mmol) pentafluorophenylsulfenyl chloride was added. The suspension solidified after 5 min and another 23 mL of acetic acid was added, yielding a yellow solution, which was stirred at 70 °C for 3.5 h. During the reaction the yellow color of the solution got weaker. The solvent was removed in vacuo and the slightly yellow residue was extracted twice with 58 mL $CHCl_3$, yielding 2.7 g (8.46 mmol, 98%) of a colorless solid.

$^1$H NMR (400 MHz, $CD_3CN$ + TFA-*d*) $\delta$ [ppm] = 4.43 (dd, $J$ = 8.5, 4.3 Hz, 1H, C$_\alpha H$), 3.51 (dd, $J$ = 15.3, 4.3 Hz, 1H,CH$_2$), 3.35 (dd, $J$ = 15.3, 8.6 Hz, 1H, CH$_2$).

$^{19}$F NMR (400 MHz, $CD_3CN$ + TFA-d) $\delta$ [ppm] = −134.66 (m, 2F, *o*-*F*), −153.01 (tt, $J$ = 20.0, 3.9 Hz, 1F, *p*-*F*), −163.35 (m, 2F, *m*-*F*).

$^{13}$C{$^1$H} NMR (101 MHz, $CD_3CN$ + TFA-*d*) $\delta$ [ppm] = 168.95 ($C$OO), 147.61 (ddd, $J$ = 246.4, 11.6, 4.4 Hz, *o*-$C_{Ar}$), 144.73–140.78 (m, *p*-$C_{Ar}$), 139.72–135.61 (m, *m*-$C_{Ar}$), 110.06 (t, $J$ = 20.6 Hz, $C_{Ar}$S), 53.23 ($C$H$_2$), 39.31 ($C_\alpha$).

FD-MS: 319.1 (M+H$^+$).

### *S*-(Pentafluorothiophenyl)-L-cysteine NCA (3c)

2.35 g (7.35 mmol) *S*-(pentafluorphenyl)cysteine was suspended in 40 mL abs. THF and the suspension was heated to 70 °C. Then 0.8 mL (6.63 mmol) of diphosgene was added over 1 h in which the solid dissolved. The solution was stirred for 1.5 more hours. Nitrogen was bubbled through the solution overnight to remove HCl and diphosgene. The slightly yellow solid was then dissolved in 11 mL abs. THF and precipitated by slowly adding 130 mL abs. hexane. The precipitate was collected by filtration without contact to air and recrystallized 3 times from THF/hexane yielding 568 mg (1.64 mmol, 22%) of the *S*-(pentafluorphenyl)cysteine NCA.

$^1$H NMR (400 MHz, THF-$d_8$) $\delta$ [ppm] = 4.77 (dd, $J$ = 7.8, 3.6 Hz, 1H, C$_\alpha H$), 3.41 (dd, $J$ = 14.4, 3.6 Hz, 1H, C$H_2$), 3.22 (dd, $J$ = 14.3, 7.6 Hz, 1H, C$H_2$).

$^{19}$F NMR (400 MHz, THF-$d_8$) $\delta$ [ppm] = −134.64 (m, 2F, *o*-*F*), −152.94 (tt, $J$ = 20.7, 3.5 Hz, 1F, *p*-*F*), −163.28 (m, 2F, *m*-*F*).

$^{13}$C{$^1$H} NMR (101 MHz, THF-$d_8$) $\delta$ [ppm] = 168.99 (C$_\alpha$($C$O)O), 151.50 (NH($C$O)O), 147.47 (ddq, $J$ = 247.0, 11.8, 4.2 Hz, *o*-$C_{Ar}$), 142.75 (dtt, $J$ = 256.2, 13.8, 5.3 Hz, *p*-$C_{Ar}$),139.08–136.55 (*m*-$C_{Ar}$), 111.68–110.23 (m, $C_{Ar}$S), 56.59 (C$H_2$), 40.94 ($C_\alpha$).

### *o*-Fluorophenylsulfenyl chloride (*o*FTPCl, 4a)

5 mL (46 mmol) *o*-fluorothiophenol was dissolved in 92 mL DCM and cooled to 0 °C. 5.65 mL (70 mmol) sulfurylchloride was added and the solution was stirred for 16 h at room temperature. DCM and excess sulfuryl chloride were removed in vacuo, yielding 7.39 g (45 mmol, 98%) of an orange–red liquid. That was used without further purification.

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ [ppm] = 7.74 (td, 1H, $J$ = 1.7, 5.8 Hz, 1H, $H_{Ar}$), 7.51–7.46 (m, 1H, $H_{Ar}$), 7.26–7.17 (m, 2H, $H_{Ar}$).

$^{19}$F NMR (400 MHz, $CDCl_3$) $\delta$ [ppm] = −105.82 (m, 1F, $F_{Ar}$).

### *S*-(*o*-Fluorothiophenyl)-L-cysteine (Cys(*o*FTP), 4b)

7.94 g (49 mmol) *o*-fluorophenylsulfenyl chloride was mixed with 3 mL TFA and cooled in an ice-bath. 3.68 g (23 mmol) cysteine hydrochloride was dissolved in 40 mL TFA and added to the solution at a rate of 15 mL/h under constant stirring. The TFA was removed in vacuo at 45 °C and 100 mL $CHCl_3$/MeOH (1:1) was added to the resulting solid to extract the product (residue: cystine, side product). The residue was filtered off and the filtrate was concentrated in vacuo. 150 mL $Et_2O$ was added und the product was collected by filtration. This was repeated one more time yielding 0.99 g (2.88 mmol, 13%) a slightly yellow/brownish solid (TFA salt).

$^1$H NMR (400 MHz, MeOD) $\delta$ [ppm] = 7.73 (td, $J$ = 7.6, 1.7 Hz, 1H, $H_{Ar}$), 7.50–7.38 (m, 1H, $H_{Ar}$), 7.3–7.13 (m, 2H, $H_{Ar}$), 4.40 (dd, $J$ = 8.3, 4.2 Hz, 1H, $C_\alpha H$), 3.39 (dd, $J$ = 14.9, 4.2 Hz, 1H, $CH_2$), 3.21 (dd, $J$ = 14.8, 8.3 Hz, 1H, $CH_2$).

$^{19}$F NMR (400 MHz, MeOD) $\delta$ [ppm] = −112.16 (ddd, $J$ = 9.4, 7.5, 5.1 Hz, 1F, $F_{Ar}$).

### *S*-(*o*-Fluorothiophenyl)-L-cysteine NCA (Cys(*o*FTP)NCA, 4c)

0.99 g (2.88 mmol) *S*-(*o*-fluorphenyl)cysteine was suspended in 30 mL abs. THF and the suspension was heated to 70 °C. Then 0.35 mL (2.88 mmol) of diphosgene was added over 1 h in which the solid dissolved. The solution was stirred for 1.5 more hours. Nitrogen was bubbled through the solution overnight to remove HCl and diphosgene. The slightly yellow solid was then dissolved in 11 mL abs. AcOEt and precipitated by slowly adding 60 mL abs. hexane. The precipitate was collected by filtration without contact to air and recrystallized 3 times from AcOEt/hexane yielding 332 mg (1.21 mmol, 42%) of the *S*-(*o*-fluorphenyl)cysteine NCA.

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ [ppm] = 9.31 (s, 1H, N$H$), 7.73 (td, $J$ = 7.8, 1.8 Hz, 1H, $H_{Ar}$), 7.45 (dddd, $J$ = 8.7, 7.2, 5.3, 1.7 Hz, 1H, $H_{Ar}$), 7.37–7.21 (m, 2H, $H_{Ar}$), 4.82 (ddd, $J$ = 6.0, 4.6, 1.3 Hz, 1H, $C_\alpha H$), 3.32–3.15 (m, 2H, $CH_2$).

$^{19}$F NMR (376 MHz, DMSO-$d_6$) $\delta$ [ppm] = −111.70 (ddd, $J$ = 10.2, 7.8, 5.2 Hz, 1F, $F_{Ar}$).

$^{13}$C{$^1$H} NMR (101 MHz, DMSO-$d_6$) $\delta$ [ppm] = 170.48 ($C_\alpha$(CO)O), 160.53 (d, $J$ = 244.3 Hz, $C_{Ar}$F), 152.21 (NH(*C*O)O), 131.93 ($C_{Ar}$), 131.16 (d, $J$ = 7.9 Hz, $C_{Ar}$), 125.95 (d, $J$ = 3.5 Hz, $C_{Ar}$), 122.96 (d, $J$ = 17.0 Hz, $C_{Ar}$), 116.62 (d, $J$ = 21.5 Hz, $C_{Ar}$), 57.14 ($C_\alpha$), 39.51 ($CH_2$).

### *o*-Pyridinylsulfenyl chloride hydrochloride (*o*TPCl, 5a)

See *o*-fluorophenylsulfenyl chloride. Yield: 97%.

$^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ [ppm] = 14.50 (s, 1H, $N_{Ar}H^+$), 8.49 (ddd, $J$ = 4.9, 1.9, 0.9 Hz, 1H, $H_{Ar}$), 7.84 (ddd, $J$ = 8.1, 7.5, 1.8 Hz, 1H, $H_{Ar}$), 7.65 (dt, $J$ = 8.1, 1.0 Hz, 1H, $H_{Ar}$), 7.31 (ddd, $J$ = 7.5, 4.9, 1.0 Hz, 1H, $H_{Ar}$).

### *S*-(*o*-Thiopyridinyl)-L-cysteine (Cys(*o*TP), 5b)

7.90 g (54.25 mmol) *o*-pyridinylsulfenyl chloride and 5.70 g (36.17 mmol) cysteine hydrochloride were heated in 50 mL acetic acid for 30 min. The solution turned bright yellow. The reaction mixture was kept in the fridge overnight. The solid was collected by filtration and washed with acetic acid. The solid was further washed by suspending it in chloroform, stirring for 1 h and filtering of the chloroform 3 times, yielding a bright yellow powder. The powder was further purified by HPLC yielding 4.01 g (17.41 mmol, 48%) of a yellow powder.

$^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ [ppm] = 8.52 (ddd, $J$ = 4.8, 1.8, 0.9 Hz, 1H, $H_{Ar}$), 7.84 (ddd, $J$ = 8.1, 7.4, 1.9 Hz, 1H, $H_{Ar}$), 7.73 (dt, $J$ = 8.1, 1.0 Hz, 1H, $H_{Ar}$), 7.32 (ddd, $J$ = 7.4, 4.9, 1.1 Hz, 1H, $H_{Ar}$), 4.21 (dd, $J$ = 7.5, 5.0 Hz, 1H, $C_\alpha H$), 3.48–3.22 (m, 2H, $CH_2$).

ESI-MS: 231.0 ($M+H^+$).

The *S*-(*o*-thiopyridinyl)cysteine NCA (**5c**) could not be completely purified. It is not stable, as the pyridine can act as an initiator in the NCA polymerization, which was confirmed by polymerizing Lys(Z)NCA with pyridine.

### *S*-Phenylthiosulfonyl-L-cysteine (Cys($SO_2$Ph), 6d)

3 g (17.08 mmol) cysteine hydrochloride monohydrate was dissolved in 17 mL 2 M hydrochloric acid and cooled to 5 °C in an ice bath. 1.18 g (17.08 mmol) Sodium nitrite was dissolved in 10 mL milli-Q water, cooled and added to the cysteine solution via a dropping funnel. The solution turned red. After stirring for 80 min at 5 °C, 5.61 g (34.16 mmol) sodium benzylsulfinate was added. After 2 h discoloration of the solution was observed and a precipitate formed. Another 1.40 g (8.54 mmol) sodium benzylsulfinate was added and the mixture was stirred for 2 more hours and stored at 4 °C overnight. The solid was collected by filtration and washed two times with ice-cold milli-Q water. The brown solid was recrystallized from water, washed with ethanol and diethyl ether and dried in vacuo to yield 2.14 g (8.20 mmol, 48%) of a slightly yellowish solid.

$^1$H NMR (400 MHz, $D_2O$ + TFA-$d$) $\delta$ [ppm] = 7.90–7.81 (m, 2H, $H_{Ar}$), 7.69–7.61 (m, 1H), 7.58–7.48 (m, 2H, $H_{Ar}$), 4.27 (dd, $J$ = 7.3, 4.6 Hz, 1H, $C_\alpha H$), 3.48 (dd, $J$ = 15.8, 4.6 Hz, 1H, $CH_2$), 3.39 (dd, $J$ = 15.8, 7.3 Hz, 1H, $CH_2$).

$^{13}$C{$^1$H} NMR (101 MHz, $D_2O$) $\delta$ [ppm] = 171.52 (*C*OOH), 144.52 ($C_{Ar}$S), 137.88 ($C_{Ar}$), 132.51 ($C_{Ar}$), 129.69 ($C_{Ar}$), 54.71 ($CH_2$), 36.97 ($C_\alpha$).

### *S*-Phenylthiosulfonyl-L-cysteine-NCA (Cys($SO_2$Ph)NCA, 6e)

In an inert gas atmosphere 5 g (19.13 mmol) phenyl cysteine thiosulfonate was suspended in 50 mL abs. THF and heated to 70 °C. Diphosgene (2.08 mL, 17.22 mmol) was added in three steps every 20 min and the suspension was stirred for another 2 h, in which all solid dissolved. The solution was allowed to cool down and THF was removed, by passing dry nitrogen through the solution overnight. The crude product was dried in vacuo and recrystallized 3 times by dissolving in THF and precipitating with hexane, yielding 4.20 g (14.73 mmol, 77%) of a slightly yellow powder.

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ [ppm] = 9.23 (s, 1H, N$H$), 7.95 (d, $J$ = 7.6 Hz, 2H, $H_{Ar}$), 7.81 (t, $J$ = 7.4 Hz, 1H, $H_{Ar}$), 7.71 (t, $J$ = 7.7 Hz, 2H, $H_{Ar}$), 4.73 (t, $J$ = 5.6 Hz, 1H, $C_\alpha H$), 3.49 (d, $J$ = 5.6 Hz, 2H, $CH_2$).

$^{13}$C{$^1$H} NMR (101 MHz, DMSO-$d_6$) $\delta$ [ppm] = 169.29 ($C_\alpha$(*C*O)O), 151.47 (NH(*C*O)O), 143.59 ($C_{Ar}$S), 134.69 ($C_{Ar}$), 130.02 ($C_{Ar}$), 126.69 ($C_{Ar}$), 56.52 ($CH_2$), 36.04 ($C_\alpha$).

FD-MS: 287.0 ($M^+$).

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### Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.01.104.

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