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Efficient Microwave-Assisted Synthesis of Unsymmetrical Disulfides

Kristina Görmer, Herbert Waldmann, and Gemma Triola*

Department of Chemical Biology, Max Planck Institute of Molecular Physiology, Otto Hahn Strasse 11, 44227 Dortmund, Germany, and Department of Chemistry, TU Dortmund, Otto Hahn Strasse 6, 44227 Dortmund, Germany

gemma.triola@mpi-dortmund.mpg.de

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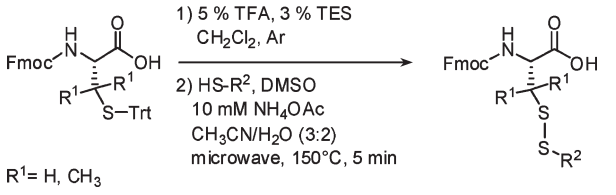

An efficient synthesis of unsymmetrical disulfides exemplified for cysteines and penicillamines is described. The use of dimethyl sulfoxide mediated oxidation accelerated by microwave irradiation afforded various unsymmetrical disulfides in one step and in high yields.

Disulfide bonds are present in numerous proteins, where they play an important role in stabilizing their tertiary structure.[1,2]

Apart from this biological relevance, unsymmetrical disulfides have also emerged as invaluable tools in biochemistry and medicinal and biological chemistry due to their selective formation in the presence of other functional groups and reversibility of their formation under reducing conditions. Hence, disulfide bond formation has been used for facilitating protein refolding and for the synthesis of prodrugs with increased hydrophobicity or cell permeability. Inside the cell the disulfide bond is then conveniently reduced as a result of the high glutathione levels, thus releasing the active compound.[3−7]

Furthermore, unsymmetrical disulfides serve as protecting groups, especially in peptide chemistry. For instance, the *tert*-butyl disulfide is a widely applied, acid- and base-stable, yet reduction-sensitive blocking group for the nucleophilic and easy oxidizable cysteine thiol.[8−12]

Although several methods are known,[13] options for the synthesis of unsymmetrical cysteine disulfides are fairly limited,[14−17] typically involving multistep sequences or highly pH-dependent conditions.[18]

To overcome these limitations we investigated the synthesis of Fmoc-protected penicillamine, which can be regarded as a sterically highly demanding derivative of cysteine and therefore also a challenging candidate compound for method development.

Initial investigation of Tesser's method, which had been successfully applied to the synthesis of cysteine disulfides before,[16] met with failure. Thus treatment of Fmoc-penicillamine with methoxycarbonylsulfenyl chloride and subsequent thiol-mediated heterolytic fragmentation in the presence of base led to the formation of the activated thiocarbonate, but the subsequent thiolysis failed, probably because of steric hindrance (Scheme 1).

In the light of this failure, an alternative method for the preparation of unsymmetrical disulfides that could be applied to sterically demanding thiols such as penicillamine was sought.

Oxidation of thiols by dimethyl sulfoxide (DMSO) as an oxidizing agent has occasionally been used for the formation of disulfide bridges in peptides, proteins,[19] and small molecules.[13] This method is applicable over a wide pH range (pH 3−8) in contrast to air oxidation and is not prone to side reactions with other nucleophilic amino acids such as Met, Trp, or Tyr (common problems encountered with other stronger oxidizing agents such as iodine).[20] However, its application to the synthesis of unsymmetrical disulfides has proven difficult because of mixed product formation, long reaction times, and reduced reactivity in particular with tertiary thiols.[13]

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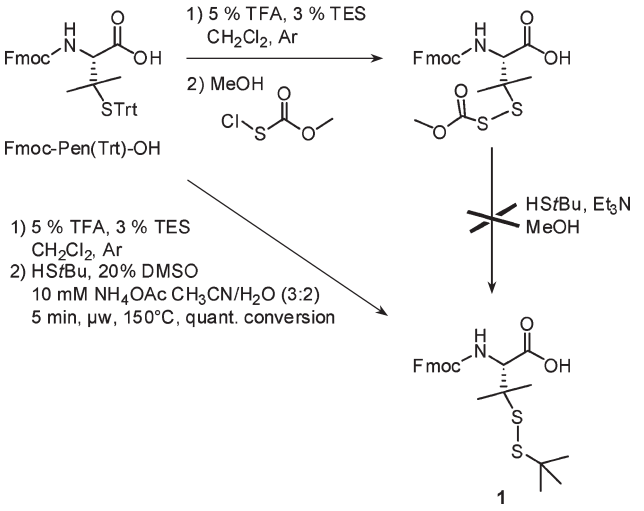
SCHEME 1. Synthesis of Unsymmetrical Disulfide 1: Unsuccessful Attempt via Activation as Thiocarbonate and Successful Formation of 1 by Dimethyl Sulfoxide Mediated Oxidation



We have now found that in the formation of cysteine and penicillamine disulfides these limitations can be overcome by means of microwave-mediated rate acceleration. To explore unsymmetrical disulfide formation of Fmoc-protected penicillamine **1**, *S*-trityl protected Fmoc penicillamine was *S*-deprotected and treated with buffered ammonium acetate containing acetonitrile, 20% DMSO and an excess of 2-methylpropane-2-thiol at room temperature. Under these conditions compound **1** was indeed formed, but conversion did not reach more than 50% even after long reaction times (10 days). Encouraged by these initial results, we tried to accelerate the reaction and to enhance the product formation by applying microwave irradiation. Therefore, an analogous reaction mixture was irradiated for 5 min at 150 °C with 150 W using a single-mode microwave instrument. Under these conditions the highly sterically hindered unsymmetrical disulfide **1** was quantitatively formed and isolated in 81% yield after column chromatography (Scheme 1) (Table 1, entry 1).

Although dimethyl sulfoxide oxidation mainly leads to homodimer formation and subsequently leads to complex product mixtures,[13] no penicillamine homodimer formation was observed.

In light of these promising results, we explored the scope of this fast and high-yielding transformation for the synthesis of unsymmetrical disulfides of Fmoc-protected cysteine and penicillamine. The synthesis of protected Fmoc-Cys-(S*t*-Bu)-OH (**2**) also proceeded efficiently, giving the desired disulfide **2** in quantitative yield (Table 1, entry 2), proving the method as an advantageous alternative for the synthesis of this costly protected cysteine derivative. Since cysteines are prone to racemization,[21] we investigated if any epimerization occurred during the reaction.

Therefore, the same reaction was performed with Fmoc-D-cysteine and both disulfides (**2a**, **2b**) were separately carboxymethylated. Analysis of both enantiomers by normal phase chiral HPLC showed that the reaction proceeds without racemization (Supporting Information).

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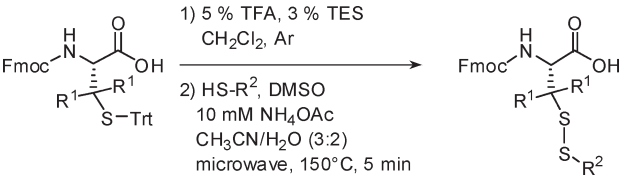
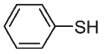
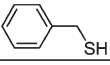
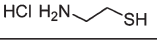
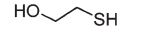
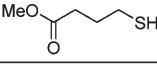
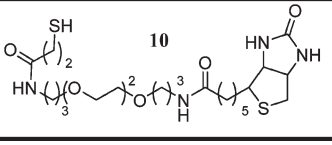
TABLE 1. Scope of Dimethyl Sulfoxide Mediated and Microwave-Enhanced Synthesis of Unsymmetrical Disulfides of Cysteine and Penicillamine



Entry	R^1	R^2-SH	Cp	Yield
1	CH_3		**1**	81 %
2	H		**2**	98 %
3	CH_3		**3**	85 %
4	H		**4**	65 %
5	CH_3		**5**	99 %
6	H		**6**	92 %
7	H		**7**	80 %
8	H		**8**	75 %
9	H		**9**	86 %
10	H		**11**	44 %

To demonstrate the general applicability of this method, a set of thiols was submitted to the described conditions to afford the corresponding unsymmetrical disulfides of Fmoc-cysteine and -penicillamine.

Microwave-assisted disulfide formation proceeded in high yields with aromatic thiols such as phenylthiol and Fmoc-penicillamine or Fmoc-cysteine (Table 1, entries 3 and 4, respectively) or even quantitative with benzylthiol (Table 1, entry 5). Aliphatic thiols were also converted in high yields (Table 1, entries 6−9). Unsymmetrical disulfides of Fmoc-cysteine with aliphatic thiols containing additional unprotected functional groups such as amines (Table 1, entry 7), alcohols (Table 1, entry 8) and esters (Table 1, entry 9) were also obtained in high yield; thus demonstrating the broad scope of the reaction. Although initial experiments were performed with 7−10 equiv of thiol, eventually 5 equiv proved sufficient without reducing the yield.

Finally, an unsymmetrical disulfide composed of a cysteine and biotin derivative (**10**) was synthesized under the described conditions and biotin-tagged Fmoc-cysteine **11** was isolated in 44% yield. Given the substantial effort required to synthesize thiol **10**, only 3 equiv was used, which might have led to the reduced yield.

In conclusion, we have developed an efficient method for the preparation of unsymmetrical cysteine and penicillamine disulfides. Application of microwave irradiation reduces the

reaction time dramatically to 5 min, avoids racemization, is widely applicable to aromatic as well as aliphatic thiols, and tolerates the presence of different unprotected functionalities such as amines, alcohols, and esters. The experimental procedure is operationally easy and leads to high yields in short reaction time without using toxic reagents. The method allows the synthesis of several differently protected cysteines and penicillamines and can also be used for selective attachment of structurally more demanding biomolecules, such as biotin, to cysteine. In particular, the methodology gives access to sterically highly demanding disulfides such as the Fmoc-penicillamine-disulfide **1**.

Experimental Section

Microwave irradiation was performed in a single-mode Discovery system coupled to an Explorer system (CEM). Reactions were carried out under stirring in a 10 mL closed reaction vessel and temperature was controlled by an IR temperature sensor.

(L)-2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-3-(*tert*-butyldisulfanyl)-3-methyl Butanoic Acid. (1). Fmoc-Pen(Trt)-OH (435 mg, 0.71 mmol) was deprotected following reported conditions.[22] The residue was dissolved in 2.5 mL of buffer containing 10 mM NH_4OAc in acetonitrile/water (3:2). 2-Methyl-2-propanethiol (0.8 mL, 7.13 mmol) and 0.75 mL of dimethyl sulfoxide were added, and the reaction mixture was irradiated using a single-mode microwave instrument (P = 150 W, t = 2 min ramp, 5 min hold, T_{max} = 150 °C). After TLC had indicated complete consumption of the starting material, the reaction mixture was concentrated and extracted with brine and CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash chromatography using CH_2Cl_2/MeOH (97:3) as eluent to yield the desired product (266 mg, 81%). Analytical data: ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.90 (s, 1H), 7.89 (d, J = 7.5 Hz, 2H), 7.83 (d, J = 9.2 Hz, 1H), 7.77 (d, J = 5.4 Hz, 2H), 7.42 (t, J = 7.3 Hz, 2H), 7.32 (t, J = 7.3 Hz, 2H), 4.37−4.13 (m, 4H), 1.37 (s, 3H), 1.33 (s, 3H), 1.26 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.4, 156.1, 143.7, 140.6, 127.6, 127.0, 125.5, 125.4, 120.1, 66.0, 61.3, 50.5, 46.6, 46.5, 30.2, 25.9, 24.2; IR $\tilde{\nu}$ = 2962, 2921, 2896, 2860, 1714; LC-MS (ESI) calcd for $C_{24}H_{30}NO_4S_2$ 460.16108 [M + H]$^+$, found 459.67 [M + H]$^+$, t_R = 10.54 min; HR-MS m/z calcd for $C_{24}H_{30}NO_4S_2$ 460.16108 [M + H]$^+$, found 460.16073 [M + H]$^+$; $[\alpha]^{20}_D$ = 0.28 ($CHCl_3$, c 5).

(L)-2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-3-(ethyldisulfanyl)propanoic Acid. (6). After deprotection of Fmoc-Cys-(Trt)-OH (510 mg, 0.87 mmol) under acidic conditions, the reaction was performed under the described conditions for compound **1** in the presence ethyl mercaptan (0.45 mL, 6.12 mmol). After purification by column chromatography using CH_2Cl_2/MeOH (97:3), compound **6** was isolated (325 mg, 92%). Analytical data: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (d, J = 7.5 Hz, 2H), 7.72 (d, J = 7.4 Hz, 2H), 7.57 (d, J = 6.6 Hz, 1H), 7.41 (t, J = 7.4 Hz, 2H), 7.32 (t, J = 7.4 Hz, 2H), 4.37−4.10 (m, 4H), 3.16 (dd, J = 13.4, 3.9 Hz, 1H), 2.94 (dd, J = 13.3, 9.9 Hz, 1H), 2.70 (q, J = 7.3 Hz, 2H), 1.21 (t, J = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.6, 155.9, 143.8, 140.7, 127.6, 127.1, 125.3, 120.1, 65.6, 53.8, 46.6, 40.1, 31.6, 14.3; IR $\tilde{\nu}$ = 3339, 3043, 3017, 2962, 2925, 2870, 1713, 1691; LC-MS (ESI) calcd for $C_{20}H_{22}NO_4S_2$: 404.09848 [M + H]$^+$, found 403.75 [M + H]$^+$, t_R = 10.16 min; HR-MS m/z calcd for $C_{20}H_{22}NO_4S_2$ 404.09848 [M + H]$^+$, found 404.09832 [M + H]$^+$; $[\alpha]^{20}_D$ = −25.22 ($CHCl_3$, c 5).

(L)-2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-3-((2-aminoethyl)disulfanyl)propanoic Acid. (7). After deprotection of Fmoc-Cys(Trt)-OH (280 mg, 0.48 mmol) under acidic conditions, the reaction was performed under the described conditions for compound **1** in the presence of cystamine hydrochloride (463 mg, 4.08 mmol). After purification of the reaction mixture by preparative HPLC, compound **7** was isolated after lyophilization (194 mg, 80%). Analytical data: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01 (br, 2H), 7.89 (d, J = 7.5 Hz, 2H), 7.82 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 7.4 Hz, 2H), 7.42 (t, J = 7.4 Hz, 2H), 7.33 (t, J = 7.4 Hz, 2H), 4.39−4.17 (m, 4H), 3.23−3.04 (m, 3H), 2.96−2.92 (m, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.1, 156.1, 143.8, 140.7, 127.7, 127.1, 125.2, 120.1, 65.8, 53.0, 46.6, 39.3, 37.7, 33.9; IR $\tilde{\nu}$ = 3320, 3042, 2947, 1686, 1530; LC-MS (ESI) calcd for $C_{20}H_{23}N_2O_4S_2$ 419.10938 [M + H]$^+$, found 419.10 [M + H]$^+$, t_R = 7.67 min; HR-MS m/z calcd for $C_{20}H_{23}N_2O_4S_2$ 419.10938 [M + H]$^+$, found 419.10891 [M + H]$^+$; $[\alpha]^{20}_D$ = −1.209 (MeOH, c 2).

(L)-1-(9*H*-Fluoren-9-yl)-3,11-dioxo-2,12-dioxa-7,8-dithia-4-azatridecane-5-carboxylic Acid. (9). After deprotection of Fmoc-Cys(Trt)−OH (510 mg, 0.87 mmol) under acidic conditions, the reaction was performed under the described conditions for compound **1**, in the presence of methyl-3-mercaptopropionate (0.66 mL, 6.12 mmol). After purification of the reaction mixture by column chromatography using CH_2Cl_2/MeOH (97:3) as eluent, compound **9** was isolated (346 mg, 86%). Analytical data: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (d, J = 7.5 Hz, 2H), 7.72 (d, J = 7.4 Hz, 2H), 7.53 (br, 1H), 7.41 (t, J = 7.4 Hz, 2H), 7.32 (t, J = 7.4 Hz, 2H), 4.40−4.09 (m, 4H), 3.59 (s, 3H), 3.18 (m, 1H), 3.01−2.84 (m, 3H), 2.68 (t, J = 6.8 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.5, 171.6, 155.9, 143.8, 140.7, 127.6, 127.1, 125.3, 120.1, 65.7, 53.9, 51.5, 46.6, 40.7, 33.3, 32.6; IR $\tilde{\nu}$ = 3323, 2950, 2923, 2853, 1717; LC-MS (ESI) calcd for $C_{22}H_{24}NO_6S_2$ 462.10396 [M + H]$^+$, found 461.86 [M + H]$^+$, t_R = 9.86 min; HR-MS m/z calcd for $C_{22}H_{24}NO_6S_2$ 462.10396 [M + H]$^+$, found 462.10362 [M + H]$^+$; $[\alpha]^{20}_D$ = −17.28 ($CHCl_3$, c 5).

Supporting Information Available: Detailed experimental and NMR data of all products and analytic chiral HPLC data. This material is available free of charge via the Internet at http://pubs.acs.org.

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