A Journal of the Gesellschaft Deutscher Chemiker

Angewandte International Edition GDCh Chemie

www.angewandte.org

Accepted Article

Title: Palladium Mediated Direct Disulfide Bond Formation in Proteins Containing S-Acetamidomethyl-Cysteine under Aqueous Conditions

Authors: Ashraf Brik, Shay Laps, Hao Sun, and Guy Kamnesky

To be cited as: *Angew. Chem. Int. Ed.* 10.1002/anie.201900988
Angew. Chem. 10.1002/ange.201900988

Link to VoR: http://dx.doi.org/10.1002/anie.201900988
http://dx.doi.org/10.1002/ange.201900988

WILEY-VCH

10.1002/anie.201900988

COMMUNICATION

WILEY-VCH

Palladium Mediated Direct Disulfide Bond Formation in Proteins Containing S-Acetamidomethyl-Cysteine under Aqueous Conditions

Shay Laps[†], Hao Sun[†], Guy Kamnesky and Ashraf Brik*

Abstract: One of the applied synthetic strategies for correct disulfide bond formation relies on the use of orthogonal Cys protecting groups. Such an approach requires purification before and after the deprotection steps, which prolongs the entire synthetic process and lowers the yield of the reaction. A major challenge in using this approach is to be able to apply one-pot synthesis under mild conditions and aqueous media. In this study, we report on the development of rapid disulfide bond formation employing palladium chemistry and S-acetamidomethyl-cysteine (Cys(Acm)). The oxidation of Cys(Acm) to the corresponding disulfide bond was achieved within minutes and one-pot operation by applying palladium and diethyldithiocarbamate. The utility of this chemistry were exemplified in the synthesis of oxytocin peptide and in the first total chemical synthesis of Thioredoxin-1 protein. Our investigation revealed a critical role of the Acm protecting group in the disulfide bond formation apparently due to the generation of disulfiram in the reaction pathway, which significantly assists the oxidation step.

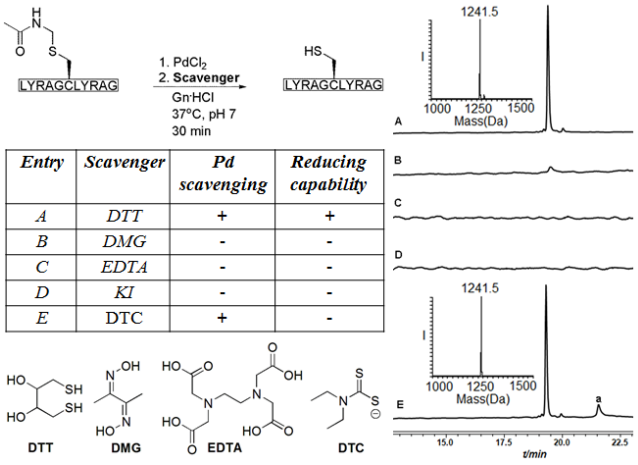
Disulfide bridges are often evolutionarily conserved motifs, which play an important role in the stabilization of the native conformation of various peptides and proteins as well as in the catalysis of known enzymes.[1] Therefore, when attempting the synthesis of these macromolecules, the correct connectivities of the disulfide bonds must be formed in order to obtain functional molecules.[2] Chemical protein synthesis [3] is a useful method to prepare complex protein analogues in high homogeneity and workable quantities.[4] Nevertheless, the generation of proteins bearing disulfide bonds remains a challenging task despite success in interesting cases (e.g. erythropoietin).[1,2, 5]

Two main synthetic strategies are often applied for the formation of disulfide bridges in peptides and proteins.[1, 6] The first one relies on subjecting the reduced peptide to freely oxidative folding conditions using buffer containing redox reagents (e.g. Cys/Cystine) to form the correct connectivity under thermodynamic control.[5a, 5b, 5d, 7] The main limitations of this approach are the slow rate of the reaction (hours-days) and the requirement for careful optimize of the desired ratio between the redox reagents for each system.[6, 8]

The second approach is based on a regioselective disulfide bond formation via stepwise oxidation by employing various orthogonal Cys protecting groups (PGs) such as the S-acetamidomethyl (Acm) and the *tert*-butyl *(t*-butyl).[5c, 8] Although this approach has enabled the chemical syntheses of various interesting peptides and proteins (i.e. insulin),[2, 5c, 6, 9] the harsh removal conditions of the Cys PGs reduce significantly the efficiency of this approach.[8, 10] For example, a widely used method to convert two Cys(Acm) residues to the corresponding disulfide is based on iodine oxidation in acetic acid,[11] however, over-oxidation of the thiol functionality to sulfonic acid could occur as well as other side reactions have been reported when Tyr, Trp and Met are present. [12]

Herein we report on the development of a new approach for rapid disulfide bond formation employing Pd chemistry and Cys(Acm) and its utility in various systems. The oxidation of Cys(Acm) to the corresponding disulfide bond was achieved in one-pot by applying Pd and diethyldithiocarbamate (DTC) in a highly efficient manner. We also report on initial mechanistic aspects of this transformation, which revealed an important role of the Acm PG in this process.

We have recently discovered unprecedented reactivity of Pd^{II} complexes for the efficient deprotection of several important side chain PGs.[13] In particular, we have reported the use of $PdCl_2$ under NCL conditions for the rapid Cys(Acm) deprotection. This step was also followed by NCL coupled with desulfurization in a one-pot manner as demonstrated in the total chemical synthesis of various proteins.[13a, 13e, 13h] In order to recover efficiently the peptide, we used excess dithiothreitol (DTT) to scavenge the free and the bound Pd to the peptide side chains. [13a, 13e, 13h] However, DTT is a reducing agent,[14] which cannot be used when in situ oxidation of Cys residues is desired. Therefore, finding an appropriate Pd scavenger as an alternative to DTT is the essential step in performing one-pot Cys deprotection coupled with disulfide formation.



Entry	Scavenger	Pd scavenging	Reducing capability
A	DTT	+	+
B	DMG	-	-
C	EDTA	-	-
D	KI	-	-
E	DTC	+	-

Figure 1. Analytical HPLC and mass analysis of the model **peptide 1** treated with 10 equiv $PdCl_2$ at 37 °C, pH 7 in 6 M Gn·HCl followed by quenching with various scavengers. (A) After 5 min treatment with $PdCl_2$ and 25 min incubation with 25 equiv DTT: the main peak corresponds to unmasked **peptide 1**, with the observed mass 1241.5 ± 0.1 Da (calcd 1241.4 Da, average isotopes). The following tests were done with **peptide 1** after 5 min treatment with $PdCl_2$ and 25 min incubation with 25 equiv: (B) DMG. (C) EDTA. (D) KI. (E) DTC: the main peak corresponds to unmasked **peptide 1**, with the

[*] S. Laps,[†] Dr. H. Sun,[†] Dr. G. Kamnesky, Prof. A. Brik
Schulich Faculty of Chemistry
Technion-Israel Institute of Technology
Haifa, 3200008 (Israel)
E-mail: abrik@technion.ac.il
[†] These authors contributed equally to this work.

Supporting information for this article is given via a link at the end of the document.

observed mass 1241.5 ± 0.1 Da (calcd 1241.4 Da, average isotopes). Peak **a** corresponds to the dimerized **peptide 1** after deprotection, with the observed mass 2480.6 ± 0.1 Da (calcd 2481.0 Da, average isotopes).

For this purpose we prepared the model **peptide 1** (LYRAGC(Acm)LYRAG) and treated with 10 equiv $PdCl_2$ for 5 min for Acm removal[13a] followed by incubation with various potential Pd chelators yet cannot reduce disulfide bonds. This list includes (1) DTT as a reference reaction[13a, 13e, 13h] (2) dimethylglyoxime (DMG),[15] (3) ethylenediaminetetraacetic acid (EDTA),[16] (4) potassium iodide (KI),[17] and (5) DTC.[18] The reactions were analyzed using HPLC and Mass analysis in which we found that the peptide was not recovered upon incubation for 25 min with DMG, EDTA or KI (Figure 1B, C &D), probably due to the inability to release the thiolate group from Pd. However, we were pleased to observe quantitative recovery of our model peptide when using DTC as a scavenger similar to DTT (Figure 1A&E).

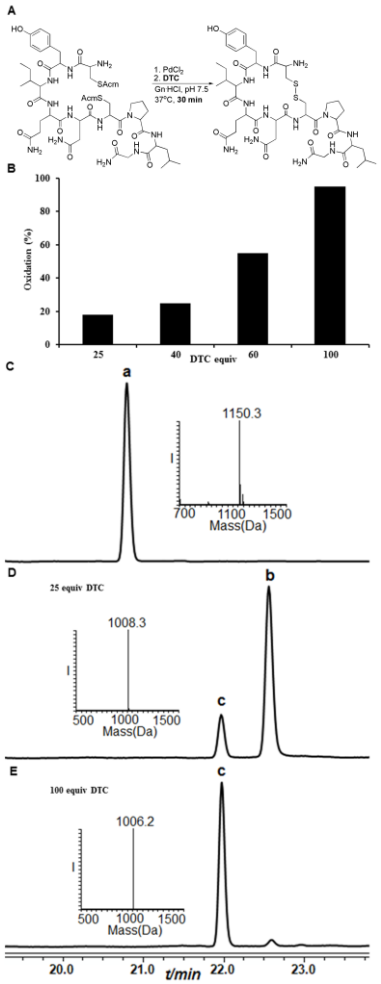


Figure 2. Optimization of one-pot Acm removal and disulfide bond formation in oxytocin. (A) Schematic presentation of Acm removal via $PdCl_2$ followed by treatment with DTC. (B) The effect of DTC amount on disulfide formation after 25 min. (C) Analytical HPLC and mass analysis of purified protected oxytocin, peak **a** with the observed mass 1150.3 ± 0.1 Da (calcd 1151.2 Da, average isotopes). The reaction of protected oxytocin with 10 equiv $PdCl_2$ for 5 min followed by quenching for 25 min with: (D) 25 equiv of DTC, and (E) 100 equiv of DTC. Peak **b** corresponds to the reduced unmasked oxytocin with the observed mass 1108.3 ± 0.1 Da (calcd 1109.2 Da, average isotopes). Peak **c** corresponds to the oxidized unmasked oxytocin with the observed mass 1106.2 ± 0.1 Da (calcd 1107.2 Da, average isotopes).

We then attempted to examine the use of Pd and DTC in one-pot Acm removal and Cys oxidation. We chose as a model system the natural peptide-hormone oxytocin that consists of 9 amino acids (AAs) and two native Cys that forms a disulfide bridge for its active structure. [19] Our strategy was to make the peptide synthetically with two Cys(Acm) and test the Acm removal followed by quenching with DTC and performing in situ oxidation step. After preparing the oxytocin peptide with the Cys(Acm) using Fmoc-SPPS we performed Acm deprotection using 10 equiv of $PdCl_2$ for 5 min[13a] followed by quenching the reaction with 25 equiv DTC for 25 min at pH 7 and 37 °C (Supporting Information, Figure S4B). The reaction was then monitored by HPLC-MS analysis, which indicated the formation of two products including the oxidized oxytocin (15%) and reduced form (85%). After overnight incubation under these conditions the oxidation reaction was completed without the requirement of isolation and oxidation. A control experiment with DTT as the scavenger clearly showed no oxidation even after overnight treatment (Supporting Information, Figure S4D).

At this stage we wondered if changes in the reaction conditions could affect the oxidation rate. Therefore, we initially focused our attention in examining the effect of pH (6-8), which did not show major effect (Supporting Information, Figure S5). We then decided to examine the effect of the DTC amount on the rate of disulfide bond formation. We treated the protected oxytocin with 10 equiv $PdCl_2$ followed by quenching the reactions with increasing amount of DTC up to 100 equiv. Interestingly, the rate of the oxidation was significantly accelerated with increasing the DTC amount. In this case, the oxidation was completed within 30 min when 100 equiv of DTC was used in comparison to requirement for overnight treatment with 25 equiv DTC (Figure 2).

To test the applicability of our new strategy for the total chemical synthesis of proteins containing native disulfide bonds, we chose as a target the *E. coli* thioredoxin-1 (Trx-1) enzyme. The sequence of Trx-1 consists of 109 AAs and bears two Cys residues in positions 33 and 36, which form a disulfide bond as part of its catalytic machinery.[20] Trx-1 acts as thiol disulfide oxidoreductase and catalyzes the reduction of disulfides in various proteins.[20b] Trx-1 is an interesting challenge in terms of synthesis due to the fact that it contains two native Cys in non-strategical positions for NCL. Therefore, the native Ala in position 57 has to be mutated into Cys in order to perform NCL. Subsequently, this Cys must be converted back to Ala after the NCL step via desulfurization.[21] Thus, the two native Cys residues have to be protected with the Acm PG to keep them intact during the desulfurization step.[22] We envisioned here the use of our new approach to enable one-pot ligation/desulfurization steps[23] followed by Acm removal and disulfide bond formation without the need of multiple purification steps and the loss of material.

We divided the Trx-1 sequence into two segments, Trx-1(1–56)-thioester (**Trx-1-N**) and Cys-Trx-1(58–109) (**Trx-1-C**).

10.1002/anie.201900988

Trx-1-C was synthesized on a Rink amide resin, while **Trx-1-N** was synthesized on the diaminobenzoic acid (Dbz) linker [24] (Supporting Information, Figure S7-8). Subsequently, we ligated **Trx-1-C** and **Trx-1-N** using 4-mercaptophenylacetic acid (MPAA) and tris(2-carboxyethyl)phosphine (TCEP) as additives in 6 M Gn·HCl, pH 7.3 at 37 °C.[25] The ligation was completed within 10 h and the product was dialyzed against 6 M Gn·HCl to enable one-pot desulfurization, which completed within 2 h (Figure A-C, Supporting Information, Figure S9). The reaction mixture was then dialyzed again, treated with 10 equiv $PdCl_2$ for 5 min and quenched with 100 equiv DTC for 25 min to afford the full length Trx-1 protein with the disulfide bond between Cys33 and Cys36 in one-pot and 37% overall isolated yield (Figure 3D). Circular dichroism (CD) spectroscopy analysis of the purified oxidized Trx-1 exhibited the expected CD signature of the folded protein[26] (Figure 3E). In addition, we confirmed the catalytic activity of the synthetic Trx-1 using established protocol for Trx-1 activity assay[27] (Figure 3F).

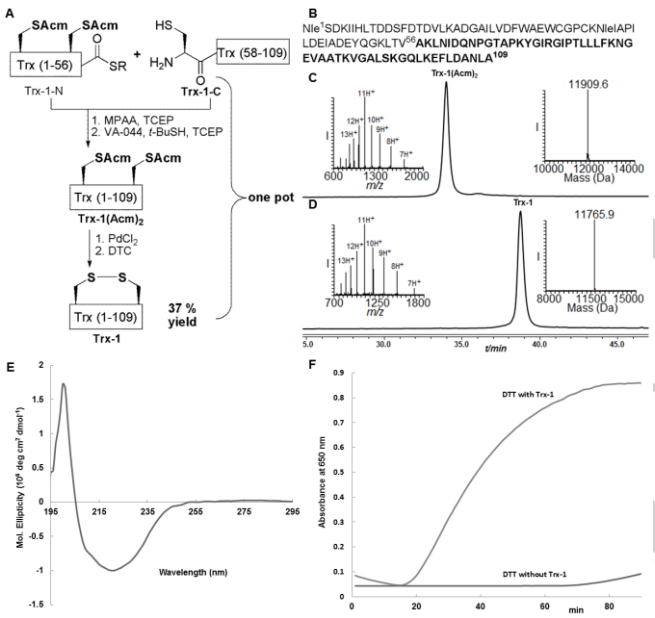


Figure 3. Total chemical synthesis of Trx-1. (A) Schematic presentation of the Trx-1 synthesis. (B) Trx-1 sequence. (C) Analytical HPLC and mass analysis of purified Trx-1$(Acm)_2$ with the observed mass 11909.6 ± 0.4 Da (calcd 11911.5 Da, average isotopes) after one-pot ligation and desulfurization. (D) Analysis of purified full length oxidized Trx-1 with the observed mass 11765.9 ± 0.3 Da (calcd 11767.5 Da, average isotopes). (E) CD analysis of the Trx-1 final product. (F) Activity assay of the reduction of bovine insulin catalyzed by the chemically synthesized Trx-1 with and without DTT. The turbidity of the reaction mixture analyzed via plotting the absorbance at 650 nm versus time.

At this stage of our study we were puzzled about the effect of DTC combined with Pd on the rate of oxidation and were curious to examine if the enhancement of the oxidation reaction could occur in the case of the free and reduced Cys residues. Therefore, we synthesized the oxytocin peptide with unprotected and reduced Cys residues. Subsequently, the peptide was exposed to 10 equiv of Pd and 100 equiv of DTC and the reaction was monitored by analytical HPLC-MS. To our surprise, after 30 min of incubation at 37 °C only 10% oxidized oxytocin was obtained compared to full conversion when the two Cys residues were masked with the Acm (Figure 4B). The oxidation was completed only after overnight. These findings triggered us to further investigate the necessity of the Acm for enhancing the oxidation reaction. To examine this, we prepared another oxytocin variant bearing unprotected N-terminal Cys while Cys6 was protected with the Acm. The peptide was treated under the same conditions where 80% oxidation was observed after 30 min and completed within 2 h (Figure 4C). Together these results indicated that the deprotection step of the Acm under our conditions plays an important role in the oxidation step. Notably, it has been proposed that disulfiram (DSF)- the oxidized form of DTC, is capable to induce glutathione (GSH) oxidation probably via disulfide bonds exchange.[28] In a different study, a complex

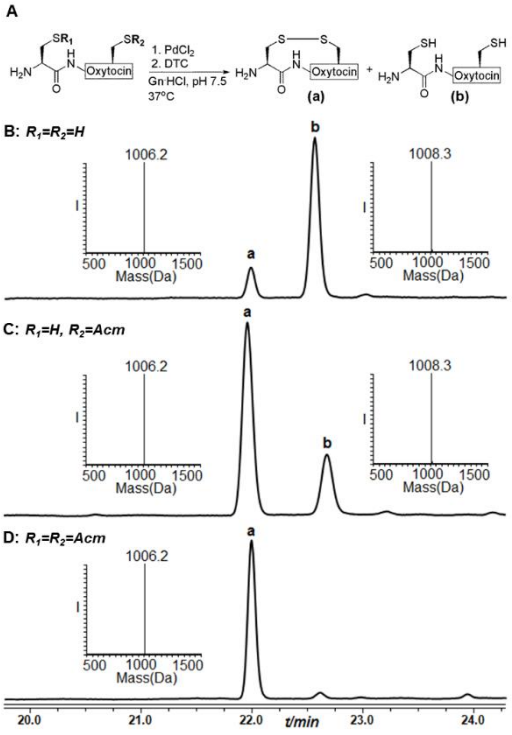


Figure 4. The effect of Acm on the Pd/DTC mediated disulfide bonds formation: (A) Schematic presentation of the reaction of different analogues of oxytocin with $PdCl_2$ followed by treatment with DTC in 6 M Gn·HCl, pH 7.5, 37 °C. Incubation of oxytocin analogues with 10 equiv $PdCl_2$ for 5 min followed by quenching with 100 equiv DTC for additional 25 min: (B) the case of unprotected oxytocin; peak **a** corresponds to the oxidized oxytocin with the observed mass 1106.2 ± 0.1 Da (calcd 1107.2 Da, average isotopes). Peak **b** corresponds to the reduced and unmasked oxytocin with the observed mass 1108.3 ± 0.1 Da (calcd 1109.2 Da, average isotopes). (C) The case of oxytocin analogue bearing one Cys(Acm). (D) The case of oxytocin bearing two Cys(Acm).

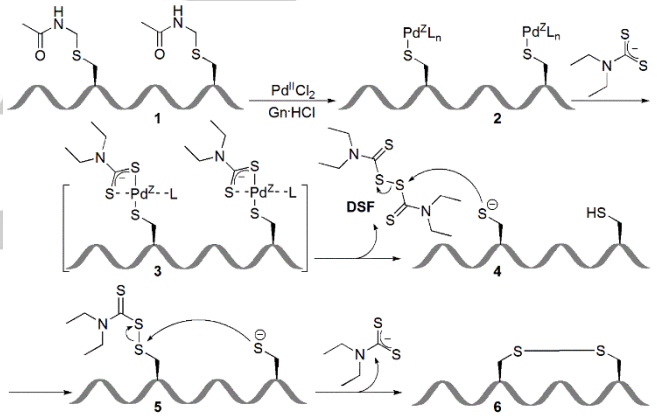
of Cu^{II} and DTC as a ligand was reported to promote GSH oxidation possibly via a redox cycle of the copper.[29] Although the exact mechanism is unclear, the generation of DSF through a redox cycle of $Cu^{II}(DTC)_4$ to $Cu^{I}(DTC)_2$ has been proposed.[29]

These previous findings prompted us to examine whether in our reaction DSF could also be a key element in the oxidation step, which could be formed via a redox cycle involving Pd. To examine this assumption we first exposed the unprotected oxytocin to our optimized conditions in the presence of commercially available DSF (2 equiv). A complete oxidation was observed within 5 min compared to overnight treatment in the absence of DSF. When this peptide was incubated with DSF only and without Pd and DTC, the same results were obtained. In addition, when oxytocin bearing the two Cys(Acm) was treated with 10 equiv of $PdCl_2$, 25 equiv of DTC and 2 equiv of DSF we observed Cys deprotection and oxidation in situ within total 10 min, compared to 30 min when using 100 equiv DTC and Pd. These results support possible formation of DSF during the Acm deprotection step and a critical role of DSF in the rapid disulfide bond formation. Notably, external addition of acetamidomethyl alcohol (2 equiv), which is one of the products of the deprotection step, [13a] to the reaction mixture had no effect on the oxidation rate. To further support the requirement of Cys(Acm) for the generation of DSF assisted by Pd, we treated unprotected oxytocin peptide with external peptide containing Cys(Acm) in the presence of $PdCl_2$ and DTC, which led to a complete disulfide bond formation within 1.5 h compared to overnight treatment in the absence of Cys(Acm) (Supporting Information, Figure S14).

Based on these observations we propose a mechanism in which after the rapid Acm deprotection step the bound Pd to the unprotected thiolate undergoes complexation with the added DTC. Such a complex could contain Pd and a DTC ligand on each Cys of the unprotected peptide. Indeed, we were able to isolate such an intermediate in the case of Trx-1 and characterize it by mass spectrometry, which matches the mass of the Trx-1 in addition to two Pd and two DTC ligands (**3**, Scheme 1). Moreover, this isolated intermediate in presence of excess Pd and DTC was converted to the oxidized Trx-1 product within 30 min (Supporting Information, Figure S12). Therefore, we also propose that such an intermediate in presence of excess DTC collapses rapidly to the free peptide (**4**, Scheme 1) and generate DSF, similar to the Cu^{II} case, in yet unclear mechanism.[29] Subsequently, the free Cys react with DSF to facilitate rapid disulfide bond formation (Scheme 1), which is the most stable disulfide bond of which DTC cannot further reduce.

Due to the quantitative appearance of the free thiols immediately after adding the DTC to the bound Pd-peptide (**4**, Scheme 1) as we detected by HPLC-MS analysis (Supporting Information, Figure S11), it seems that the generation of DSF after the removal of the Acm is a rate-limiting step. Hence, the additional 25 min that was required to obtain the product is likely the time, which is needed to generate enough DSF for the rapid oxidation step. We also observed a clear correlation between the amount of DSF and the oxidation rate, as the addition of 1 equiv of DSF led to a relatively slower oxidation of oxytocin, which completed within 45 min compared to 5 min with 2 equiv. This is also consistent with our study on the oxytocin peptide bearing one Cys(Acm) where the oxidation step was slower and required 2 h compared to 30 min in the case of oxytocin with two Cys(Acm), (Figure 4). Based on these results we developed an optimized protocol in which Cys(Acm) deprotection is performing by treatment with 10 equiv $PdCl_2$ for 5 min followed by the addition of 25 equiv DTC and 2 equiv DSF for another 5 min to furnish the desired oxidized product (Supporting Information, S23).

In summary we have established the use of Pd chemistry, for first time, in the context of disulfide bridge formation within peptides and proteins under mild and aqueous conditions. Our results revealed unprecedented enhanced oxidation of Cys(Acm) residues to the corresponding disulfide mediated by Pd and DTC in a highly efficient manner and was found to be compatible with one-pot total chemical synthesis of proteins as exemplified with Trx-1. In this process, the generation of DSF appears to be the key reagent for the rapid disulfide bond formation. Our study also shows the first utilization of DSF as an efficient and mild reagent for rapid disulfide bond formation in peptide and protein synthesis under aqueous media. This approach has the potential to be further expanded to generate multiple disulfide bonds in peptides and proteins in a regioselective manner and one-pot operation



Scheme 1. Proposed mechanism for disulfide bond formation via Pd mediated Acm removal and DSF formation.

in light of our recent findings regarding the on demand deprotection of various Cys PGs with Pd.[13h] These exciting directions and others are currently under investigation in our laboratory.

Acknowledgements

A. Brik holds The Jordan and Irene Tark Academic Chair. H. S. is supported in part at the Technion by a Technion-Guangdong Fellowship.

Keywords: desulfurization • disulfide bonds • palladium • protein synthesis • S-acetamidomethyl-cysteine

[1] M. Gongora-Benitez, J. Tulla-Puche, F. Albericio, *Chem. Rev.* **2014,** *114*, 901.
[2] M. A. Hossain, J. D. Wade, *Acc. Chem. Res.* **2017,** *50*, 2116.
[3] S. B. H. Kent, *Chem. Soc. Rev.* **2009,** *38*, 51.

[4] a) S. S. Kulkarni, J. Sayers, B. Premdjee, R. J. Payne, *Nat. Rev. Chem.* **2018,** *2*, 0122; b) S. Bondalapati, M. Jbara, A. Brik, *Nat. Chem.* **2016,** *8*, 407. c) L. Raibaut, N. Ollivier, O. Melnyk, *Chem. Soc. Rev.* 2012, *41*, 7001.

[5] a) T. M. Hackeng, J. A. Fernández, P. E. Dawson, S. B. H Kent, J. H. Griffin, *Proc. Natl. Acad. Sci. U. S. A.* **2000,** *97*, 14074.; b) S. H. Liu, B. L. Pentelute, S. B. H. Kent, *Angew. Chem. Int. Ed.* **2012,** *51*, 993; c) F. Z. Wu, J. P. Mayer, V. M. Gelfanov, F. Liu, R. D. DiMarchi, *J. Org. Chem.* **2017,** *82*, 3506; d) G. N. Boross, S. Shimura, M. Besenius, N. Tennagels, K. Rossen, M. Wagner, J. W. Bode, *Chem. Sci.* **2018,** *9*, 8388.

[6] I. Annis, B. Hargittai, G. Barany, *Method. Enzymol.* **1997,** *289*, 198.

[7] M. Murakami, R. Okamoto, M. Izumi, Y. Kajihara, *Angew. Chem. Int. Ed.* **2012,** *51*, 3567.

[8] M. Mochizuki, S. Tsuda, K. Tanimura, Y. Nishiuchi, *Org. Lett.* **2015,** *17*, 2202.

[9] a) F. Liu, E. Y. Luo, D. B. Flora, J. P. Mayer, *Org. Lett.* **2013,** *15*, 960; b) Z. Dekan, M. Mobli, M. W. Pennington, E. Fung, E. Nemeth, P. F. Alewood, *Angew. Chem. Int. Ed.* **2014,** *53*, 2931.

[10] M. Jbara, S. K. Maity, A. Brik, *Angew. Chem. Int. Ed.* **2017,** *56*, 10644.

[11] a) B. Kamber, A. Hartmann, K. Eisler, B. Riniker, H. Rink, P. Sieber, W. Rittel, *Helv. Chim. Acta.* **1980,** *63*, 899. b) S. B. Gunnoo, A. Iyer, W. Vannecke, K. W. Decoene, T. Hebbrecht, J. Gettemans, M. Laga, S. Loverix, I. Lastersc, A. Madder, *Chem. Commun.* **2018,** *54*, 11929. c) D. G. Mullen, B. Weigel, G. Barany, M. D. Distefano, *J. Pept. Sci.* **2010,** *16*, 219.

[12] a) P. Sieber, B. Kamber, B. Riniker, W. Rittel, *Helv. Chim. Acta.* **1980,** *63*, 2358; b) K. Akaji, T. Tatsumi, M. Yoshida, T. Kimura, Y. Fujiwara, Y. Kiso, *J. Am. Chem. Soc.* **1992,** *114*, 4137. c) N. Fufii, A. Otaka, S. Funakoshi, K. Bessho, T. Watanabe, K. Akaji, H. Yajima, *Chem. Pharm. Bull.* **1987,** *35*, 2339.

[13] a) S. K. Maity, M. Jbara, S. Laps, A. Brik, *Angew. Chem. Int. Ed.* **2016,** *55*, 8108; b) M. Jbara, S. K. Maity, M. Seenaiah, A. Brik, *J. Am. Chem. Soc.* **2016,** *138*, 5069; c) M. Jbara, S. Laps, S. K. Maity, A. Brik, *Chem. Eur. J.* **2016,** *22*, 14851; d) S. K. Maity, M. Jbara, G. Mann, G. Kamnesky, A. Brik, *Nat. Protoc.* **2017,** *12*, 2293; e) S. Bondalapati, E. Eid, S. M. Mali, C. Wolberger, A. Brik, *Chem. Sci.* **2017,** *8*, 4027; f) S. K. Maity, G. Mann, M. Jbara, S. Laps, G. Kamnesky, A. Brik, *Org. Lett.* **2016,** *18*, 3026; g) M. Jbara, E. Eid, A. Brik, *Org. Biomol. Chem.* **2018,** *16*, 4061; h) M. Jbara, S. Laps, M. Morgan, G. Kamnesky, G. Mann, C. Wolberger, A, Brik, *Nat. Commun.* **2018,** DOI: 10.1038/s41467-018-05628-0.

[14] W. W. Cleland, *Biochemistry* **1964,** *3*, 480.

[15] A. Rios, M. Valcarcel, *Analyst* **1982,** *107*, 737.

[16] W. M. MacNevin, O. H. Kriege, *J. Am. Chem. Soc.* **1955,** *77*, 6149.

[17] F. E. Beamish, J. Dale, *Ind. Eng. Chem., Anal. Ed.* **1938,** *10*, 697.

[18] L. G. Zhu, L. Qin, T. N. Parac, N. M. Kostic, *J. Am. Chem. Soc.* **1994,** *116*, 5218.

[19] a) Z. R. Donaldson, L. J. Young, *Science* **2008,** *322*, 900; b) J. P. Rose, C. K. Wu, C. D. Hsiao, E. Breslow, B. C. Wang, *Nat. Struct. Biol.* **1996,** *3*, 163.

[20] a) C. V. Goemans, F. Beaufay, K. Wahni, I. Van Molle, J. Messens, J. F. Collet, *J. Biol. Chem.* **2018,** *293*, 3839; b) B. Ezraty, A. Gennaris, F. Barras, J. F. Collet,, *Nat. Rev. Microbiol.* **2017,** *15*, 385. c) A. Holmgren, B. O. Soderberg, H. Eklund, C. I. Branden, *Proc. Natl. Acad. Sci. U. S. A.* **1975,** *72*, 2305.

[21] L. Z. Yan, P. E. Dawson, *J. Am. Chem. Soc.* **2001,** *123*, 526.

[22] a) B. L. Pentelute, S. B. H. Kent, *Org. Lett.* **2007,** *9*, 687; b) A. Brik, S. Ficht, Y. Y. Yang, C. S. Bennett, C. H. Wong, *J. Am. Chem. Soc.* **2006,** *128*, 15026. c) P. S. Reddy, S. Dery, N. Metanis, *Angew. Chem. Int. Ed.* **2016,** *128*, 1004.

[23] a) P. Siman, O. Blatt, T. Moyal, T. Danieli, M. Lebendiker, H. A. Lashuel, A. Friedler, A. Brik, *ChemBioChem* **2011,** *12*, 1097; b) T. Moyal, H. P. Hemantha, P. Siman, M. Refua, A. Brik, *Chem. Sci.* **2013,** *4*, 2496.

[24] J. B. Blanco-Canosa, P. E. Dawson, *Angew. Chem. Int. Ed.* **2008,** *47*, 6851.

[25] E. C. B. Johnson, S. B. H. Kent, *J. Am. Chem. Soc.* **2006,** *128*, 6640.

[26] H. Reutimann, B. Straub, P. L. Luisi, A. Holmgren, *J. Biol. Chem.* **1981,** *256*, 6796.

[27] A. Holmgren, *J. Biol. Chem.* **1979,** *254*, 9627.

[28] J. H. Stromme, *Biochem. Pharmacol.* **1963,** *12*, 157.

[29] M. J. Burkitt, H. S. Bishop, L. Milne, S. Y. Tsang, G. J. Provan, C. S. I Nobel, S. Orrenius, A. F. G Slater, *Arch. Biochem. Biophys.* **1998,** *353*, 73.

10.1002/anie.201900988

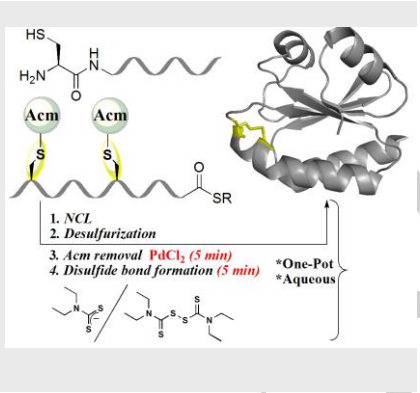
WILEY-VCH

Entry for the Table of Contents

Layout 1:

COMMUNICATION

S-Acetamidomethyl protects and connects: The chemistry of palladium and diethyldithiocarbamate was applied for the efficient and rapid conversion of S-Acetamidomethyl-Cysteine to the corresponding disulfide, in which disulfiram is suggested to play a key role in this process. This approach enabled one-pot synthesis of disulfide bond contaiting pepide/protein under mild and fully aquous conditions.



*Shay Laps[†], Hao Sun[†], Guy Kamnesky, Ashraf Brik**

Page No. – Page No.

Palladium Mediated Direct Disulfide Bond Formation in Proteins Containing S-Acetamidomethyl-Cysteine under Aqueous Conditions