

Recent Advances in Chemoselective Protein Desulfurization: From VA-044 to Emerging Boron, Photochemical, Iron-Catalyzed, and Ultrasound Methods

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	Substrates	Histones	α -Synuclein	Tau	Max	Other substrates
PTMs	Ubiquitination	H2BK34Ub ^[1, 2] ; H2BK120Ub ^[3, 4] ; H3K14Ub ^[5] ; H3K122Ub ^[6] ;	K6Ub ^[7] ; K12Ub ^[8] ; K12Ub ₂ ^[8] ; K12Ub ₄ ^[8] ;			M ₁ /K ₆ /K ₁₁ /K ₂₇ /K ₂₉ /K ₃₃ /K ₄₈ /K ₆₃ -Ub ₂ ^[9] ; K _{11,48} - Ub ₃ /Ub ₄ /Ub ₅ /Ub ₆ ^[10] ; K ₄₈ -Ub ₄ -globin ^[11] ;
	Acetylation	H2AK119Ac ^[12] ; H3K56Ac ^[6]	M1Ac ^[13]	K280Ac ^[14] ; K311Ac ^[15] ; K317Ac ^[15] ; K353Ac ^[15] ; K369Ac ^[15] ;	K31Ac ^[16] ; K57Ac ^[16] ; K31AcK57Ac ^[16, 17] ;	
	Glycosylation	H2BS112GlcNAc ^[3, 18]	T72GlcNAc ^[19] ; T75GlcNAc ^[19] ; T81GlcNAc ^[19] ; S87GlcNAc ^[19]	N359GlcNAc ^[15, 20] ; N359Man ₅ GlcNAc ₂ ^[20] ; N410GlcNAc ^[20] ; N410Man ₅ GlcNAc ₂ ^[20] ;		Antifreeze glycoproteins ^[21] ; 143glycosyl-IL-6 ^[22] ; glycosylated IFN- β ^[23] ; Erythropoietin glycoform 1 ^[24] ; Glycoprotein D ^[25] ;
	Phosphorylation	H2AS1p ^[12] ; H2AY57p ^[26] ;	Y39p ^[27, 28] ; S87p ^[27] ; Y125p ^[29] ; S129p ^[30]	S305p ^[15] ; Y310p ^[14, 15] ; S352p ^[15] ; S356p ^[15] ; S396pS404p ^[14]	S2p ^[31] ; S11p ^[31] ; S2pS11p ^[17, 31] ;	Ub T7/12/14/22/55/66 p ^[32] ; K ₆₃ -Ub ₂ S65p ^[33] ; p19 ^{INK4d} S66pS76p ^[34] IGFBP-2 S107p ^[35]
Mirror-image proteins		^D ASFV pol X ^[36] ; ^D LigA DNA-ligase ^[37] ; ^D T7 RNA-polymerase ^[38] ; ^D IGV ^{TIGIT} ^[39] ; ^D PD1 ₂₁₋₁₆₇ ^[40] ; ^D KRAS(G12V) ^[41] ; ^D IL-6 ^[42] ; ^D Rv1738 ^[43] ; ^D K27-Ub ₃ ^[44] ; ^D monobody-DAM27 ^[45]				
Protein probes	K48/K63-diUb _{photo} ^[46] ; Ubch7 _{Dap} -Ub _{Dha} ^[47] ; H2AK119Ub _{Dha} ^[48]					

Table S1. Representative examples obtained from native chemical ligation coupled with VA-044-based desulfurization.

References

- 1 H. Ai, M. Sun, A. Liu, Z. Sun, T. Liu, L. Cao, L. Liang, Q. Qu, Z. Li, Z. Deng, Z. Tong, G. Chu, X. Tian, H. Deng, S. Zhao, J. B. Li, Z. Lou and L. Liu, H2B Lys34 Ubiquitination Induces Nucleosome Distortion to Stimulate Dot1L Activity. *Nat Chem Biol*, 2022, **18**, 972-980.
- 2 J. Li, Q. He, Y. Liu, S. Liu, S. Tang, C. Li, D. Sun, X. Li, M. Zhou, P. Zhu, G. Bi, Z. Zhou, J. S. Zheng and C. Tian, Chemical Synthesis of K34-Ubiquitylated H2B for Nucleosome Reconstitution and Single-Particle Cryo-Electron Microscopy Structural Analysis. *ChemBiochem*, 2017, **18**, 176-180.

- 3 M. Seenaiah, M. Jbara, S. M. Mali and A. Brik, Convergent versus sequential protein synthesis: the case of ubiquitinated and glycosylated H2B. *Angew Chem Int Ed Engl*, 2015, **54**, 12374-12378.
- 4 R. K. McGinty, J. Kim, C. Chatterjee, R. G. Roeder and T. W. Muir, Chemically ubiquitylated histone H2B stimulates hDot1L-mediated intranucleosomal methylation. *Nature*, 2008, **453**, 812-816.
- 5 M. Sun, Y. Du, Z. Li, A. Aderjiang, M. Xin and H. Ai, Chemically Synthesized H3K14Ub Unveils Clr4's IDR-Mediated Multivalent Nucleosome Recognition in H3K9 Methylation. *Angew Chem Int Ed Engl*, 2026, **65**, e20817.
- 6 Y. K. Qi, Q. Q. He, H. S. Ai, J. Guo and J. B. Li, The convergent chemical synthesis of histone H3 protein for site-specific acetylation at Lys56 and ubiquitination at Lys122. *Chem Commun (Camb)*, 2017, **53**, 4148-4151.
- 7 M. Hejjaoui, M. Haj-Yahya, K. S. Kumar, A. Brik and H. A. Lashuel, Towards elucidation of the role of ubiquitination in the pathogenesis of Parkinson's disease with semisynthetic ubiquitinated α -synuclein. *Angew Chem Int Ed Engl*, 2011, **50**, 405-409.
- 8 M. Haj-Yahya, B. Fauvet, Y. Herman-Bachinsky, M. Hejjaoui, S. N. Bavikar, S. V. Karthikeyan, A. Ciechanover, H. A. Lashuel and A. Brik, Synthetic polyubiquitinated α -Synuclein reveals important insights into the roles of the ubiquitin chain in regulating its pathophysiology. *Proc Natl Acad Sci U S A*, 2013, **110**, 17726-17731.
- 9 K. S. Kumar, L. Spasser, L. A. Erlich, S. N. Bavikar and A. Brik, Total chemical synthesis of di-ubiquitin chains. *Angew Chem Int Ed Engl*, 2010, **49**, 9126-9131.
- 10 S. Tang, L. J. Liang, Y. Y. Si, S. Gao, J. X. Wang, J. Liang, Z. Mei, J. S. Zheng and L. Liu, Practical Chemical Synthesis of Atypical Ubiquitin Chains by Using an Isopeptide-Linked Ub Isomer. *Angew Chem Int Ed Engl*, 2017, **56**, 13333-13337.
- 11 H. Sun, S. M. Mali, S. K. Singh, R. Meledin, A. Brik, Y. T. Kwon, Y. Kravtsova-Ivantsiv, B. Bercovich and A. Ciechanover, Diverse fate of ubiquitin chain moieties: The proximal is degraded with the target, and the distal protects the proximal from removal and recycles. *Proc Natl Acad Sci U S A*, 2019, **116**, 7805-7812.
- 12 G. Hayashi, T. Sueoka and A. Okamoto, In vitro and in cell analysis of chemically synthesized histone H2A with multiple modifications. *Chem Commun (Camb)*, 2016, **52**, 4999-5002.
- 13 B. Fauvet, M. B. Fares, F. Samuel, I. Dikiy, A. Tandon, D. Eliezer and H. A. Lashuel, Characterization of semisynthetic and naturally N α -acetylated α -synuclein in vitro and in intact cells: implications for aggregation and cellular properties of α -synuclein. *J Biol Chem*, 2012, **287**, 28243-28262.
- 14 M. Haj-Yahya and H. A. Lashuel, Protein Semisynthesis Provides Access to Tau Disease-Associated Post-translational Modifications (PTMs) and Paves the Way to Deciphering the Tau PTM Code in Health and Diseased States. *J Am Chem Soc*, 2018, **140**, 6611-6621.
- 15 W. C. Powell, M. Nahum, K. Pankratz, M. Herlory, J. Greenwood, D. Poliyenko, P. Holland, R. Jing, L. Biggerstaff, M. H. B. Stowell and M. A. Walczak, Post-Translational Modifications Control Phase Transitions of Tau. *ACS Cent Sci*, 2024, **10**, 2145-2161.
- 16 R. V. Nithun, Y. M. Yao, O. Harel, S. Habiballah, A. Afek and M. Jbara, Site-Specific Acetylation of the Transcription Factor Protein Max Modulates Its DNA Binding Activity. *ACS Cent Sci*, 2024, **10**, 1295-1303.
- 17 R. V. Nithun, S. Khoury and M. Jbara, One-Pot Total Synthesis of a Post-translationally

- Modified Max Transcription Factor Sheds Light on Ser-Phosphorylation and Lys-Acetylation Crosstalk in DNA Binding. *Org Lett*, 2025, **27**, 3760-3764.
- 18 Z. Deng, S. Tao, Y. Du, Y. Li, L. Zhang, Q. Shi, X. Du, M. Sun, Z. Tong, M. Pan, L. Liu and H. Ai, Allosteric activation of RNF20/RNF40-RAD6A-mediated H2BK120 monoubiquitylation by H2BS112 GlcNAcylation. *Nat Chem Biol*, 2026, **22**, 740-750.
- 19 P. M. Levine, A. Galesic, A. T. Balana, A. L. Mahul-Mellier, M. X. Navarro, C. A. De Leon, H. A. Lashuel and M. R. Pratt, α -Synuclein O-GlcNAcylation alters aggregation and toxicity, revealing certain residues as potential inhibitors of Parkinson's disease. *Proc Natl Acad Sci U S A*, 2019, **116**, 1511-1519.
- 20 W. C. Powell, R. Jing, M. Herlory, P. Holland, D. Poliyenko, C. C. Ebmeier, M. H. B. Stowell and M. A. Walczak, Chemical Synthesis Reveals Pathogenic Role of N-Glycosylation in Microtubule-Associated Protein Tau. *J Am Chem Soc*, 2025, **147**, 6995-7007.
- 21 B. L. Wilkinson, R. S. Stone, C. J. Capicciotti, M. Thaysen-Andersen, J. M. Matthews, N. H. Packer, R. N. Ben and R. J. Payne, Total synthesis of homogeneous antifreeze glycopeptides and glycoproteins. *Angew Chem Int Ed Engl*, 2012, **51**, 3606-3610.
- 22 Y. Liu, Y. Maki, R. Okamoto, A. Satoh, Y. Todokoro, Y. Kanemitsu, K. Otani and Y. Kajihara, Uncovering a Latent Bioactive Interleukin-6 Glycoform. *Angew Chem Int Ed Engl*, 2024, **63**, e202411213.
- 23 I. Sakamoto, K. Tezuka, K. Fukae, K. Ishii, K. Taduru, M. Maeda, M. Ouchi, K. Yoshida, Y. Nambu, J. Igarashi, N. Hayashi, T. Tsuji and Y. Kajihara, Chemical synthesis of homogeneous human glycosyl-interferon- β that exhibits potent antitumor activity in vivo. *J Am Chem Soc*, 2012, **134**, 5428-5431.
- 24 P. Wang, S. Dong, J. H. Shieh, E. Peguero, R. Hendrickson, M. A. S. Moore and S. J. Danishefsky, Erythropoietin derived by chemical synthesis. *Science*, 2013, **342**, 1357-1360.
- 25 J. Zhao, X. Liu, J. Liu, F. Ye, B. Wei, M. Deng, T. Li, P. Huang and P. Wang, Chemical Synthesis Creates Single Glycoforms of the Ectodomain of Herpes Simplex Virus-1 Glycoprotein D. *J Am Chem Soc*, 2024, **146**, 2615-2623.
- 26 M. Jbara, S. K. Maity, M. Morgan, C. Wolberger and A. Brik, Chemical Synthesis of Phosphorylated Histone H2A at Tyr57 Reveals Insight into the Inhibition Mode of the SAGA Deubiquitinating Module. *Angew Chem Int Ed Engl*, 2016, **55**, 4972-4976.
- 27 S. Zhang, R. Zhu, B. Pan, H. Xu, M. F. Olufemi, R. J. Gathagan, Y. Li, L. Zhang, J. Zhang, W. Xiang, E. M. Kagan, X. Cao, C. Yuan, S. J. Kim, C. K. Williams, S. Magaki, H. V. Vinters, H. A. Lashuel, B. A. Garcia, E. James Petersson, J. Q. Trojanowski, V. M. Lee and C. Peng, Post-translational modifications of soluble α -synuclein regulate the amplification of pathological α -synuclein. *Nat Neurosci*, 2023, **26**, 213-225.
- 28 K. Zhao, Y. J. Lim, Z. Liu, H. Long, Y. Sun, J. J. Hu, C. Zhao, Y. Tao, X. Zhang, D. Li, Y. M. Li and C. Liu, Parkinson's disease-related phosphorylation at Tyr39 rearranges α -synuclein amyloid fibril structure revealed by cryo-EM. *Proc Natl Acad Sci U S A*, 2020, **117**, 20305-20315.
- 29 M. Hejjaoui, S. Butterfield, B. Fauvet, F. Vercautse, J. Cui, I. Dikiy, M. Prudent, D. Olschewski, Y. Zhang, D. Eliezer and H. A. Lashuel, Elucidating the role of C-terminal post-translational modifications using protein semisynthesis strategies: α -synuclein phosphorylation at tyrosine 125. *J Am Chem Soc*, 2012, **134**, 5196-5210.
- 30 L. M. Gatzemeier, F. Meyer, U. Diederichsen and T. F. Outeiro, Chemical Synthesis of Alpha-

- Synuclein Proteins via Solid-Phase Peptide Synthesis and Native Chemical Ligation. *Chemistry*, 2023, **29**, e202300649.
- 31 R. V. Nithun, Y. M. Yao, X. Lin, S. Habiballah, A. Afek and M. Jbara, Deciphering the Role of the Ser-Phosphorylation Pattern on the DNA-Binding Activity of Max Transcription Factor Using Chemical Protein Synthesis. *Angew Chem Int Ed Engl*, 2023, **62**, e202310913.
- 32 B. Wang, C. Li, F. Zhao, L. Shi, X. Li, Y. Liu, S. Sun, L. Yuan, M. Sun, Y. Zhang, J. Shi and L. J. Liang, Examining the Role of Threonine Phosphorylation in Ubiquitin's Function Using Chemical Protein Synthesis. *JACS Au*, 2025, **5**, 2148-2158.
- 33 S. Bondalapati, W. Mansour, M. A. Nakasone, S. K. Maity, M. H. Glickman and A. Brik, Chemical synthesis of phosphorylated ubiquitin and diubiquitin exposes positional sensitivities of e1-e2 enzymes and deubiquitinases. *Chem Eur J*, 2015, **21**, 7360-7364.
- 34 M. Msallam, H. Sun, R. Meledin, P. Franz and A. Brik, Examining the role of phosphorylation of p19(INK4d) in its stability and ubiquitination using chemical protein synthesis. *Chem Sci*, 2020, **11**, 5526-5531.
- 35 B. Premdjee, A. S. Andersen, M. Larance, K. W. Conde-Frieboes and R. J. Payne, Chemical Synthesis of Phosphorylated Insulin-like Growth Factor Binding Protein 2. *J Am Chem Soc*, 2021, **143**, 5336-5342.
- 36 Z. Wang, W. Xu, L. Liu and T. F. Zhu, A synthetic molecular system capable of mirror-image genetic replication and transcription. *Nat Chem*, 2016, **8**, 698-704.
- 37 J. Weidmann, M. Schnölzer, P. E. Dawson and J. D. Hoheisel, Copying Life: Synthesis of an Enzymatically Active Mirror-Image DNA-Ligase Made of D-Amino Acids. *Cell Chem Biol*, 2019, **26**, 645-651.e643.
- 38 Y. Xu and T. F. Zhu, Mirror-image T7 transcription of chirally inverted ribosomal and functional RNAs. *Science*, 2022, **378**, 405-412.
- 39 B. Zhang, Y. Zheng, G. Chu, X. Deng, T. Wang, W. Shi, Y. Zhou, S. Tang, J. S. Zheng and L. Liu, Backbone-Installed Split Intein-Assisted Ligation for the Chemical Synthesis of Mirror-Image Proteins. *Angew Chem Int Ed Engl*, 2023, **62**, e202306270.
- 40 H. Zhou, H. Wu, Z. Zhong, P. Blasco, J. Zeng, J. Wang, H. Liu and X. Li, Discovery of d-Miniprotein Inhibitors of PD-1/PD-L1 Interaction via Mirror-Image Phage Display against Synthetic d-PD-1. *JACS Au*, 2025, **5**, 6200-6209.
- 41 A. M. Levinson, J. H. McGee, A. G. Roberts, G. S. Creech, T. Wang, M. T. Peterson, R. C. Hendrickson, G. L. Verdine and S. J. Danishefsky, Total Chemical Synthesis and Folding of All-l and All-d Variants of Oncogenic KRas(G12V). *J Am Chem Soc*, 2017, **139**, 7632-7639.
- 42 Y. Ren, Y. Liu, D. Han and L. J. Liang, An Integrated Solubilizing-Tag-Free Strategy for Chemical Synthesis of Mirror-Image Interleukin-6. *Org Lett*, 2025, **27**, 10760-10765.
- 43 R. D. Bunker, K. Mandal, G. Bashiri, J. J. Chaston, B. L. Pentelute, J. S. Lott, S. B. Kent and E. N. Baker, A functional role of Rv1738 in Mycobacterium tuberculosis persistence suggested by racemic protein crystallography. *Proc Natl Acad Sci U S A*, 2015, **112**, 4310-4315.
- 44 M. Pan, S. Gao, Y. Zheng, X. Tan, H. Lan, X. Tan, D. Sun, L. Lu, T. Wang, Q. Zheng, Y. Huang, J. Wang and L. Liu, Quasi-Racemic X-ray Structures of K27-Linked Ubiquitin Chains Prepared by Total Chemical Synthesis. *J Am Chem Soc*, 2016, **138**, 7429-7435.
- 45 N. Schmidt, A. Kumar, L. Korf, A. V. Dinh-Fricke, F. Abendroth, A. Koide, U. Linne, M. Rakwalska-Bange, S. Koide, L. O. Essen, O. Vázquez and O. Hantschel, Development of mirror-image monoclonal antibodies targeting the oncogenic BCR::ABL1 kinase. *Nat Commun*, 2024, **15**,

10724.

- 46 J. Liang, L. Zhang, X. L. Tan, Y. K. Qi, S. Feng, H. Deng, Y. Yan, J. S. Zheng, L. Liu and C. L. Tian, Chemical Synthesis of Diubiquitin-Based Photoaffinity Probes for Selectively Profiling Ubiquitin-Binding Proteins. *Angew Chem Int Ed Engl*, 2017, **56**, 2744-2748.
- 47 L. J. Liang, G. C. Chu, Q. Qu, C. Zuo, J. Mao, Q. Zheng, J. Chen, X. Meng, Y. Jing, H. Deng, Y. M. Li and L. Liu, Chemical Synthesis of Activity-Based E2-Ubiquitin Probes for the Structural Analysis of E3 Ligase-Catalyzed Transthiolation. *Angew Chem Int Ed Engl*, 2021, **60**, 17171-17177.
- 48 M. Jbara, S. Laps, M. Morgan, G. Kamnesky, G. Mann, C. Wolberger and A. Brik, Palladium prompted on-demand cysteine chemistry for the synthesis of challenging and uniquely modified proteins. *Nat Commun*, 2018, **9**, 3154.