Biochemistry

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Residue-Specific Peptide Modification: A Chemist's Guide

Fully compatible | Compatible | Progress made | Preliminary | Unanswered challenge

Fully compatible
- protein or antibody substrates
- aqueous, mild conditions
- fully chemoselective
- multiple modes of reactivity
- reproducible (many examples)

Unanswered challenge
- no peptide examples
- methods unavailable or single amino acids only
- poised for innovation

*See supporting information for details

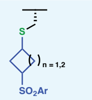
AMINO ACID SIDE-CHAIN MODIFICATION REPORT CARD

Method \ Residue	Ala	Cys	Asp/Glu	Phe	Gly	His	Ile	Lys	Leu	Met	Asn/Gln	Pro	Arg	Ser	Thr	Val	Trp	Tyr	Sec	Dha
(A) Alkylation	Unanswered challenge	Fully compatible	Progress made	Progress made	Preliminary	Progress made	Unanswered challenge	Fully compatible	Unanswered challenge	Fully compatible	Progress made	Unanswered challenge	Progress made	Preliminary	Unanswered challenge	Unanswered challenge	Fully compatible	Fully compatible	Fully compatible	Unanswered challenge
(B) Arylation	Preliminary	Fully compatible	Unanswered challenge	Preliminary	Preliminary	Preliminary	Preliminary	Compatible	Preliminary	Unanswered challenge	Unanswered challenge	Preliminary	Unanswered challenge	Unanswered challenge	Unanswered challenge	Preliminary	Progress made	Progress made	Compatible	Unanswered challenge
(C) Acylation	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Preliminary	Unanswered challenge	Fully compatible	Unanswered challenge	Unanswered challenge	Preliminary	Unanswered challenge	Progress made	Preliminary	Preliminary	Unanswered challenge	Preliminary	Progress made	Unanswered challenge	Unanswered challenge
(D) Halogenation	Unanswered challenge	Preliminary	Unanswered challenge	Preliminary	Preliminary	Preliminary	Unanswered challenge	Unanswered challenge	Preliminary	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Preliminary	Preliminary	Unanswered challenge	Preliminary
(E) Oxidation	Preliminary	Fully compatible	Unanswered challenge	Preliminary	Preliminary	Preliminary	Preliminary	Progress made	Preliminary	Fully compatible	Unanswered challenge	Progress made	Unanswered challenge	Preliminary	Preliminary	Preliminary	Compatible	Compatible	Compatible	Unanswered challenge
(F) 1,4-Addition	Unanswered challenge	Fully compatible	Unanswered challenge	Unanswered challenge	Unanswered challenge	Preliminary	Unanswered challenge	Progress made	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Fully compatible	Fully compatible
(G) Condensation	Unanswered challenge	Unanswered challenge	Compatible	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Fully compatible	Unanswered challenge	Unanswered challenge	Preliminary	Unanswered challenge	Fully compatible	Progress made	Preliminary	Unanswered challenge	Compatible	Compatible	Unanswered challenge	Unanswered challenge
(H) Cross-Coupling	Preliminary	Compatible	Progress made	Preliminary	Unanswered challenge	Preliminary	Preliminary	Preliminary	Preliminary	Unanswered challenge	Unanswered challenge	Preliminary	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Progress made	Fully compatible	Unanswered challenge	Unanswered challenge
(I) Pericyclic Reaction	Unanswered challenge	Preliminary	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Progress made	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Progress made	Compatible	Unanswered challenge	Progress made
(J) Photo Reaction	Unanswered challenge	Fully compatible	Preliminary	Preliminary	Preliminary	Preliminary	Unanswered challenge	Unanswered challenge	Preliminary	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Progress made	Progress made	Unanswered challenge	Unanswered challenge
(K) Radical Reaction	Unanswered challenge	Fully compatible	Progress made	Preliminary	Preliminary	Unanswered challenge	Unanswered challenge	Unanswered challenge	Preliminary	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Unanswered challenge	Fully compatible	Compatible	Compatible	Unanswered challenge
(L) Transition-Metal Functionalization	Preliminary	Fully compatible	Progress made	Progress made	Preliminary	Progress made	Preliminary	Preliminary	Preliminary	Unanswered challenge	Progress made	Preliminary	Progress made	Preliminary	Unanswered challenge	Preliminary	Fully compatible	Fully compatible	Compatible	Fully compatible

SELECTED METHODS: 2007–2017

CYSTEINE

entry | AA compatibility/comments

1


Strain-release alkylation (Ref. 1 and 2)
H, K, N/[Q], [S], T, W, Y, α-acid, α-amine
- organic/aqueous media
- mild conditions, short reaction times
- installation of high-value small, strained ring system

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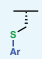

Pd-catalyzed arylation (Ref. 4)
D/[E], H, N/[Q], R, S/T, Y
- stable, high-value products
- demonstrated use in stapling
- no N-terminal protection required
- Pd-complexes are easy to handle

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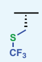

Electrophilic trifluoromethylation (Ref. 6)
D/[E], K, T, W, α-acid, α-amine
- *moderate yields*
- *low temperatures required*

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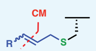

Aqueous cross-metathesis (Ref. 9)
protein
- efficient allylation followed by aqueous cross-metathesis (CM) reactions

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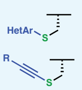

Pd-catalyzed arylation/alkynylation (Ref. 11)
N/Q, S, W, Y, α-acid, α-amine, Ab
- cross-coupling of either free Cys *or* one-pot disulfide reduction/coupling
- installation of HetAr, Ar, alkynyl, and alkenyl groups

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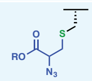

Conj. addition to 2-azidoacrylates (Ref. 3)
S, R, protein
- dual functionalization
  - facile reactivity with functionalized alkynes
- acceptor may possess high-value appendages, including fluorescents

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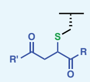

Thiol-Michael addition to carbonylacrylic reagents (Ref. 5)
protein, Ab
- stable adduct formed
- simple, stoichiometric acceptors

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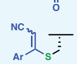

3-arylpropiolonitrile as a maleimide replacement (Ref. 7 and 8)
Ab
- stable adduct formed
- installation of heterobifunctional handle
- acceptors are commercial

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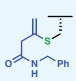
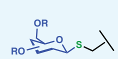


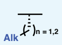
Allenamide reagents for alkylation (Ref. 10)
D/[E], H, K, M, R, S, W, Y, protein
- short reaction time, high yielding
- *unconjugated product observed*
- stable adduct formed

10


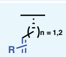
Disulfide desulfurization (Ref. 12)
protein
- proceeds through desulfurization of disulfide-linked glycosyl amino acids
- protected or unprotected sugars
  - retention of anomeric carbon
- *epimerization of α-center*

ASPARTIC ACID and GLUTAMIC ACID

entry | AA compatibility/comments

11


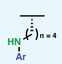
Decarboxylative alkyl–alkyl cross-coupling (Ref. 13)
(on-resin)
- dual functionalization of Asp/Glu
- installation of high-value synthetic handles

12


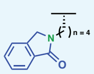
Decarboxylative alkenylation (Ref. 14)
[N]/[Q], Y
- α-chirality maintained, no erosion of *ee*
- stereospecific olefin installation

LYSINE

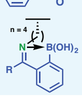
entry | AA compatibility/comments

13


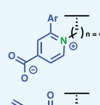
Pd-mediated arylation (Ref. 15)
D/E, H, M, S, T, W, Y, α-acid
- demonstrated use in stapling, macrocyclization
- bench-stable preformed Pd-complexes (in most cases)

15


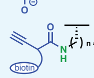
Orthophthaldehyde–amine condensation (Ref. 17)
protein, α-amine (Pro)
- can be used for protein immobilization
- can be used for PEGylation (substitution at the benzo-moiety)

17


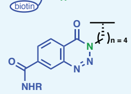
Iminoboronate formation (Ref. 19)
protein
- potentially reversible
  - readily decomposes in the presence of glutathione
- proceeds in aqueous buffer

14


6π-azaelectrocyclization (Ref. 16)
protein, mAb
- reversible, non-destructive labeling
- selective for surface-exposed Lys

16


Kinetically-controlled amidation with NHS esters (Ref. 18)
protein
- *peptide must be used in 2-fold excess*
- installation of bifunctional handle

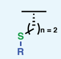
18


Amine capture with diazonium terephthalates (Ref. 20)
protein
- stable adduct formed
- *α-amine may react*
- *most accessible Lys reacts preferentially*

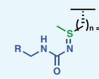
See: "Residue-Specific Peptide Modification: A Chemist's Guide" (DOI: 10.1021/acs.biohcem.7b00536) and associated content for more information

METHIONINE

entry | AA compatibility/comments

19


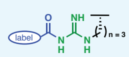
Alkylation–demethylation (Ref. 21)
C, H, K, R, α-amine (Pro)
- provides stable, functionalized homoCys derivatives
- *lability of R < Me for efficient demethylation*

20


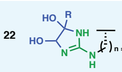
Sulfur imidation using oxaziridines (Ref. 22)
protein
- installation of high-value synthetic handles
- high selectivity, mild conditions

ARGININE

entry | AA compatibility/comments

21


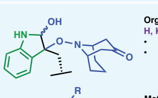
Acylation with activated esters (Ref. 23)
E, H, S/T, W, Dha, α-amine
- installation of carboxyfluorescein, biotin, and diazirin lables
- chemically stable, HPLC isolation
- *intolerant of Cys, free amines*

22


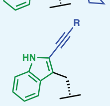
Condensation with glyoxal reagents (R = Me, Ref. 24; R = Ar, Ref. 25)
protein
- R = Me, irreversible reaction leads to Arg selectivity (Lys, Cys react reversibly)
- R = Ar, reversible reaction
  - enables traceless PEGylation

TRYPTOPHAN

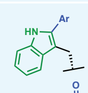
entry | AA compatibility/comments

23


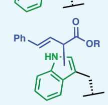
Organoradical conjugation (Ref. 26)
H, K, M, S, Y, disulfide, protein, mAb
- transition-metal free, aqueous media
- carbonyl can be further modified by oxime formation

25


Metal-catalyzed alkynylation (Mn, Ref. 30; Au, Ref. 31)
[W (unprotected)]
- R = SiR_3, Ar, Alk, alkenyl
- demonstrated use in macrocyclization
- high functional group tolerance
- *2-pym indole PG required (for Mn)*

24


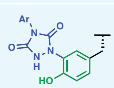
Metal-catalyzed C–H arylation (Ref. 27–29)
- Pd- and Ru-catalyzed reactions
- high functional group tolerance
- installation of high-value synthetic handles
  - bifunctional arenes allow for ligation

26


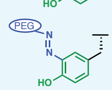
Rh-carbenoid labeling at mild pH (Ref. 32–34)
K, [N]/Q, R, S/T, protein
- mild temperature, pH, aqueous conditions
- *both N–H insertion and C-2 alkylation products observed*

TYROSINE

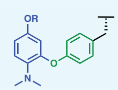
entry | AA compatibility/comments

27


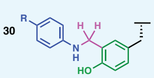
Ene-like reaction with triazoline-diones (Ref. 35 and 36)
H, K, R, S, W, protein
- stable adduct formed
  - tolerates extreme pH, temperature
- installation of high-value synthetic handles

29


Coupling with polymeric diazonium salts (Ref. 38)
E, H, K, Q, R, S/T, α-acid, α-amine
- allows for ^{15}N isotopic labeling
- conditions optimized for high Tyr selectivity

28


O-arylation via aniline coupling (Ref. 37)
D/[E], H, K, Q, R, S/T
- enables efficient coupling of electron-rich aniline derivatives
- *limited to N,N-dialkyl anilines*
- *free Cys may be oxidized*
- *cannot readily differentiate between Tyr/Trp*

30


Three-component Mannich-type reaction (Ref. 39 and 40)
D/E, K, N/Q, R, S/T, W, Y, protein
- method developed for attachment of pre-functionalized peptide building blocks
- aniline of peptide coupling partner can be readily installed at C- or N-terminus

DEHYDROALANINE

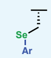
entry | AA compatibility/comments

31


Radical-based conj. addition (Ref. 41 and 42)
protein
- compatible with 1°, 2°, 3° alkyl halides
- installation of hydrophobic and charged/polar protic side-chains, including PTMs

SELENOCYSTEINE

entry | AA compatibility/comments

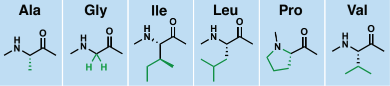
32


Cu-catalyzed, umpolung-based arylation (Ref. 43)
D/[E], H, K, N/[Q], S/[T], R, Y, α-amine
- no reducing agent, no O_2 exclusion required
- High functional group tolerance for aryl component
  - HetAr, –OH, $–NO_2$, Cl/F, CN, etc.

UNANSWERED CHALLENGES

ALIPHATIC SIDE-CHAINS

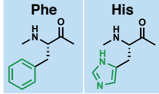
Ala | Gly | Ile | Leu | Pro | Val



While there are very few methods available for the derivatization of aliphatic residues, recent advances in C–H functionalization have enabled the direct modification of hydrocarbon side-chains in small peptide systems.[44-46] Although not yet broadly applicable to complex peptides, these proof-of-principle demonstrations are expected to herald a new era of diverse peptide modifications.

AROMATIC SIDE-CHAINS

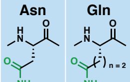
Phe | His



Relatively few methods exist for the functionalization of Phe and His. This unmet challenge is particularly fascinating for His, given the prevalence of imidazole substitution reactions and the privileged biological role of the His side-chain. Under physiological conditions, the protonation state of His is split between the imidazole and imidazolium forms, leading to the epithet, "Nature's proton shuttle", and in metalloproteins, His serves as a common coordinating ligand—a property which may be fine-tuned through targeted modifications. New methods are needed to unlock the potential of both His and Phe as fully diversifiable residues in complex peptides.

POLAR, NON-IONIZABLE SIDE-CHAINS

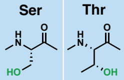
Asn | Gln



Aside from a few isolated examples, targeted modifications of Asn and Gln residues within peptides and proteins are exceedingly rare. However, the preponderance of enzyme-mediated post-translational modifications targeting these residues both justifies and inspires further examination of selective side-chain carboxamide functionalizations. Indeed, the first example of Asn and Gln selective modifications, described by Popp and Ball,[32] exploited the enzyme-like specificity of a proximity-driven reaction with dirhodium metallopeptides. Further developments in the area will likely require similar molecular recognition strategies to achieve the desired chemoselectivity.

POLAR, IONIZABLE SIDE-CHAINS

Ser | Thr



In contrast to many of the other underrepresented residues, both Thr and Ser possess a well-defined functional handle seemingly poised for site-selective modification. In practice, however, the superior nucleophilicity of other residues (e.g., Cys and Lys) has largely precluded chemoselective modifications of the side-chain hydroxyl group. Of the few existing methods, most are proximity-driven, or are highly sequence-dependent—relying on the local peptide environment to enhance the reactivity of the target hydroxyl side-chain. The realization of broadly applicable, side-chain specific functionalizations thus remains a significant challenge.

REFERENCES

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