THE NONCANONICAL CHRONICLE

Content

TRENDING:

1. J&J and Protagonist: Icotrokinra (JNJ-2113) oral peptide selectively blocks the IL-23 receptor (Nov. 2024)
2. PN-881 Il-11 (Protagonist): A potential oral peptide antagonist (Nov. 2024)
3. Viking Therapeutics- VK2735 oral formulation for obesity from Venture phase 2 study (Nov. 2024)
4. Exclusive RF-Diffusion license agreement with Vilya for macrocyclic peptide development (Nov. 2024)
5. Circle Pharma: First-in-class oral macrocycle Cyclin A/B RxL Inhibitor CID-078 (Dec. 2024)

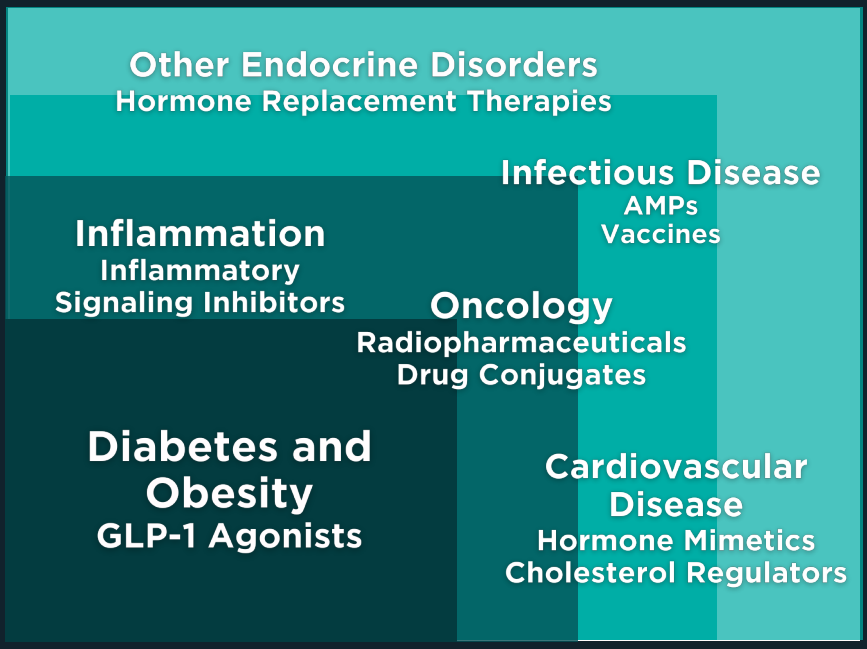
ncAA Fun Fact

Noncanonical amino acids (ncAAs) can be used to create fluorescent proteins that glow in unnatural colors, like infrared or near-UV, expanding the palette of markers for biological imaging [1-2]. Researchers can "hack" the genetic code of an organism to incorporate ncAAs, enabling entirely new functionalities in proteins. For instance, some ncAAs allow proteins to form chemical bonds under UV light, a tool used to study protein interactions dynamically [3]. Of course, canonical amino acids can also be used for imaging with clever labeling strategies like BenzoTag [4] (cover art by Bernadette Mary Dineen [5]).

[1] https://doi.org/10.1002/anie.202216231, [2] https://doi.org/10.1186/s13036-019-0166-3, [3] https://doi.org/10.1002/pro.4637, [4] https://doi.org/10.1039/D4SC05090H, [5] https://www.linkedin.com/in/bernadette-mary-dineen/

Peptide News

Industry Update



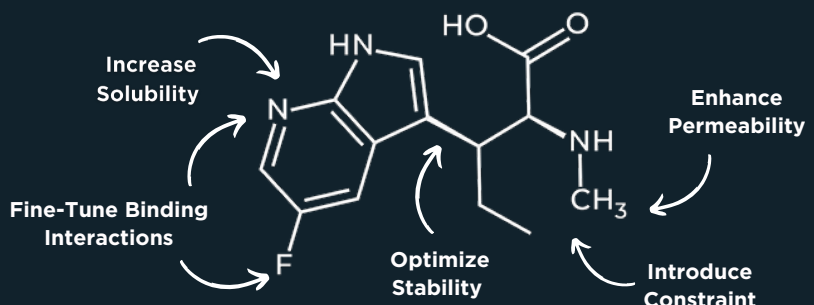
Peptide drug discovery efforts saw substantial growth in 2024, driven by the success of GLP-1 agonists for diabetes and obesity.

Additionally, despite some setbacks to the general biotech industry, peptide-based technology companies are making progress in the clinic, and AI-driven technology is making a big impact on the promise and investment in peptide medicines.

This does not mean the industry does not have layoffs in research. A summary of layoffs in 2024 is nicely tracked by Fierce Biotech.

Several large investments were made from 2023-2024 as outlined and compared by Pharmaceutical Technology. Artificial intelligence accounted for 11 pharmaceutical deals announced in Q3 2024, worth a total value of $1.7bn. Some peptide-based companies have truncated research to invest in development, but there are new VC stealth-backed companies and a host of biotech in series A-B funding.

What Can Noncanonical Amino Acids Do for Your Peptides?



Interesting Reads

ADVANCES IN BIOSYNTHESIS OF NONCANONICAL AMINO ACIDS (NCAAS) AND THE METHODS OF NCAAS INCORPORATION INTO PROTEINS
Chen, L.; Xin, X.; Zhang, Y.; Li, S.; Zhao, X.; Li, S.; Xu, Z.
Molecules 2023, 28, 6745

This study overviews biosynthesis methods for ncAAs and analyzes the difficulties related to biosynthesis. Genetic code expansion (GCE) methods are analyzed for their advantages and disadvantages, followed by a review of the application progress of ncAAs and opportunities for development directions of ncAAs.

ONE-POT CHEMOENZYMATIC SYNTHESES OF NONCANONICAL AMINO ACIDS
Tsung-Han Chao, Xiangyu Wu, Hans Renata
Journal of Industrial Microbiology and Biotechnology, Volume 51, 2024, kuae005

This mini-review highlights several recent case studies that feature the synergistic use of chemical and enzymatic transformations in one pot to synthesize novel noncanonical amino acids. The authors discuss methods of ncAA incorporation via soild-phase peptide synthesis, cell-free protein synthesis, and side-chain modification for application to protein-based materials, therapeutics, vaccines and probes.

EXPLORING FDA-APPROVED FRONTIERS: INSIGHTS INTO NATURAL AND ENGINEERED PEPTIDE ANALOGUES IN THE GLP-1, GIP, GHRH, CCK, ACTH, AND ALPHA-MSH REALMS
Othman Al Musaimi
Biomolecules 2024, 14(3), 264

In this article focused around glucagon-like peptide-1 (GLP-1), growth-hormone-releasing hormone (GHRH), cholecystokinin (CCK), adrenocorticotropic hormone (ACTH), and α-melanocyte stimulating hormone (α-MSH), Dr. Al Musami reviews the discovery, development, and approval of natural and engineered peptides. The biology, chemistry, and history of each peptide therapeutic is summarized along with the approved indications.

Highlights

Peptide Presence at JPM 2025

Bicycle Therapeutics showcased their peptide platform and presented data from the Duravelo-1 Phase 1 clinical trial studying zelenectide to treat Nectin-4 driven tumors.

MBX Biosciences, a clinical-stage company with a peptide delivery platform, Precision Endocrine Peptides (PEPs), presented their pipeline including peptides under investigation to treat obesity, hypoparathyroidism, and post-bariatric hypoglycemia.

AstraZeneca presented data from the Phase II Calypso study of eneboparatide in patients with hypoparathyroidism.

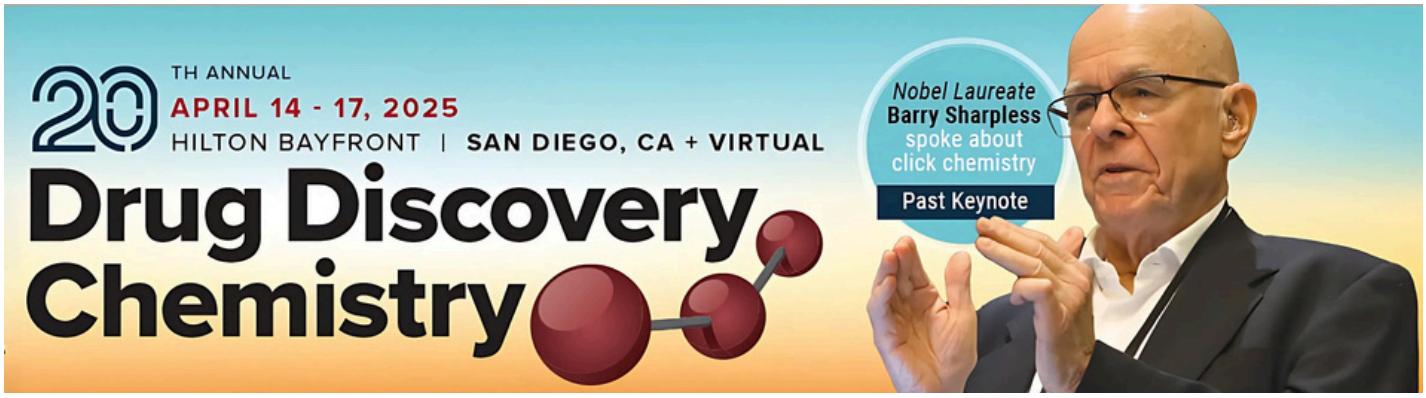
Innovent Biologics highlighted mazdutide, a GLP-1 and glucagon receptor dual agonist under investigation for the treatment of obesity, type 2 diabetes, and obstructive sleep apnea.

Protagonist Therapeutics presented data from Phase II trials of hepcidin mimetic, rusfertide, in polycythemia vera and Phase III results of the orally available IL-23R antagonist, icotrokinra.

News Bite: AbbVie Acquires Nimble Therapeutics' Massively Parallel Peptide Platform and Immunology Asset

Nimble Therapeutics' platform harnesses light-directed chemical synthesis to construct massively parallel peptide libraries that exploit vast amino acid chemical space for the discovery of novel peptide therapeutics. AbbVie's acquisition strengthens the company's capability to discover and develop oral peptides. In addition to the platform, the deal includes Nimble's orally available IL-23R inhibitor targeting autoimmune disease.

Upcoming Events



Look for us at these events!

Wendy Hartsock
Director of Strategic Partnerships

Tina Boville
Co-founder and Chief Executive Officer

Jimmy Mann
Account Executive

Noncanonical Insights

The Case for Noncanonical Tryptophan

Indole is a privileged structure [1], and nature has encoded tryptophan, a 3 substituted indole ring system as one of the 20 canonical alpha amino acid building blocks for incorporation into proteins. There is only one codon (UGG), so, on average, only 1.1% of amino acids in proteins are tryptophan. Despite its low frequency in proteins, tryptophan is often involved in key interactions and ligand binding sites. The indole ring of Trp can participate in a wide-range of interactions that stabilize structure and peptide-protein interfaces including hydrophobic, pi-stacking, hydrogen-bonding, and pi-cation interactions among others [2-5].

Aralez Bio has recognized the value of tryptophan and has developed an enzyme technology platform to provide a variety of noncanonical amino acids (ncAAs) in a sustainable and scalable manner. In fact, the Aralez catalog has more than just tryptophan-substituted ncAAs. Peptide drug hunters can take advantage of ncAAs to optimize their peptides and peptidomimetics. The properties of the Trp can be tuned to enhance existing interactions or create additional interactions not available with natural Trp [6,7]. These ncAAs can be protected to be compatible with commonly employed automated Fmoc-solid-phase peptide synthesis (SPPS) and other synthesis techniques.
This short insight will be a brief overview of the general considerations to incorporate tryptophan and other noncanonical indole-privileged structures into peptide drugs.

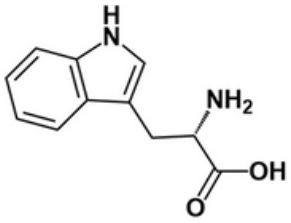
General:
Every atom of the indole ring system of tryptophan is capable of reacting selectively under appropriate conditions and is sensitive to oxidation and photodegradation. An awareness of this reactivity during synthesis and storage will help to diagnose and circumvent unwanted side reactions. While the indole of Trp is often Boc-protected to avoid side reactions, a common side reaction is incomplete deprotection of the indole nitrogen [8]. Fortunately, unprotected Trp can be incorporated into peptides without a loss in purity due to side reactions but must be carefully optimized for scale and depends on SPPS coupling conditions (vide infra). A detailed discussion of protecting group strategies is described by Yang and team listing non-essential (Nδ,Nω,Nω′-Arg, the carboxamide group from Asn/Gln, Oβ-Ser/Thr, the phenolic group from Tyr, N(im)-His, and N(indole)-Trp) and essential protecting groups (-Nα, Lys-Nε,Orn-Nδ, Dab (2,4-diaminobutyric acid)-Nγ, Dap (2,3-diaminopropionic acid)-Nβ, COOHβ-Asp, COOHγ-Glu, Sβ-Cys, and COOH-C-terminal in the case of amino acid side-chain immobilization) [9]. Cabri and team have also demonstrated successful minimal-protection SPPS with unprotected Tyr, His, and Arg which ultimately leads to improved atom economy [10].

Synthesis:
Incorporation of tryptophan can be challenging due to steric hindrance and the possibility of side reactions, including racemization, especially when adjacent to other bulky amino acids.

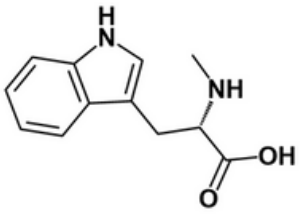
Using highly efficient coupling reagents like HATU or PyBOP can improve the coupling efficiency. There are a number of useful reviews on the vast arsenal of coupling reagents, but Fernando Albericio's detailed paper in Chemical Reviews is a good option [11]. To control racemization, low temperatures and buffering to control pH can help. Specific optimized conditions should be developed.

Cleavage:
Upon cleavage, acidic cleavage conditions using TFA (trifluoroacetic acid) are typical, but the presence of antioxidant scavengers like thioanisole or ethanedithiol can help protect tryptophan from oxidation and alkylation. Inert atmospheres can also avoid unwanted oxidation.

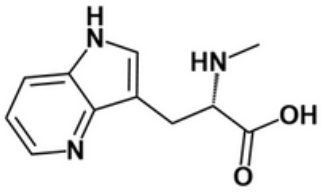
Purification:
Tryptophan is a very bulky and hydrophobic amino acid that increases hydrophobicity, resulting in a tendency to aggregate and making purification difficult. Another analytical challenge to be aware of is the inherent fluorescence and absorbance. Fortunately, several noncanonicals modifications, such as azatryptophans and indazole rings, have been developed and are available in the Aralez catalog. In our list, we have calculated* the hydrophobicity of our ncAAs to help you with tuning your peptide.



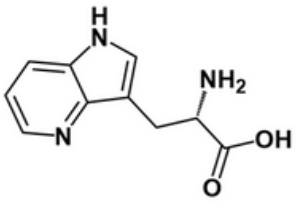
L-tryptophan
cLogP: 1.12
PSA: 79.11 Å²



N-methyl-L-tryptophan
cLogP: 1.38
PSA: 65.12 Å²



N-methyl-4-aza-L-tryptophan
cLogP: 0.78
PSA: 78.01 Å²



4-aza-L-tryptophan
cLogP: 0.52
PSA: 92 Å²

*cLogP and PSA calculated using RDKit

In conclusion, tryptophan is a privileged structure selected by evolution to achieve specific biological roles, but it creates several synthetic challenges. To improve peptide therapeutics, Aralez Bio has harnessed directed evolution to expand the noncanonical toolbox to mine the chemical space of peptide therapeutics and overcome synthesis issues. Much of the work on the specific conditions for protection of amino acids during SPPS has been done with canonical Tryptophan. Collectively, we should remain aware of the effects on noncanonical Tryptophan analogs and share our collective insights on the impact of our traditional know-how in SPPS. Aralez provides both protected and non-protected noncanonical analogs to help enable novel medicines and greener processes.

About the Author

Charles Johannes, Ph.D., is currently Chief Scientist at EPOC Scientific LLC and former VP of Exploratory Chemistry at Fog Pharma (now Parabilis Medicines). Charles has deep expertise in peptides, small molecules, and chemistry platforms in biotech and pharma and has applied over 25 years of experience in the development of new modalities with an interest in targeted protein degradation, molecular glues, and ML/AI.

For more contemporary and detailed issues with the incorporation of ncAAs and peptide synthesis technologies, we encourage you to explore the Peptide Drug Hunting Consortium (PDHC) initiatives and engage with the Entrepreneurial Advisory Board and Leadership Team. Aralez Bio is a proud member of the PDHC Entrepreneurial Business Network.

https://www.linkedin.com/company/peptide-drug-hunting/

Did you know that Aralez Bio has an e-store?

ARALEZ BIO

https://aralezbio-store.com/

Noncanonical Insights References
1. Wan, Y.; Li, Y.; Yan, C.; Yan, M.; Tang, Z. Indole: A Privileged Scaffold for the Design of Anti-Cancer Agents. Eur. J. Med. Chem. 2019, 183, 111691; 2. Meyer EA, Castellano RK, Diederich F. Interactions with aromatic rings in chemical and biological recognition. Angew Chem Int Ed Engl. 2003 Mar 17;42(11):1210-50; 3. Gallivan JP, Dougherty DA. Cation-pi interactions in structural biology. Proc Natl Acad Sci U S A. 1999 Aug 17;96(17):9459-64; 4. Newberry RW, Raines RT. Secondary Forces in Protein Folding. ACS Chem Biol. 2019 Aug 16;14(8):1677-1686; 5. Salonen LM, Ellermann M, Diederich F. Aromatic rings in chemical and biological recognition: energetics and structures. Angew Chem Int Ed Engl. 2011 May 16;50(21):4808-42; 6. Tobola F, Lelimousin M, Varrot A, Gillon E, Darnhofer B, Blixt O, Birner-Gruenberger R, Imberty A, Wiltschi B. Effect of Noncanonical Amino Acids on Protein-Carbohydrate Interactions: Structure, Dynamics, and Carbohydrate Affinity of a Lectin Engineered with Fluorinated Tryptophan Analogs. ACS Chem Biol. 2018 Aug 17;13(8):2211-2219; 7. Yang DT, Gronenborn AM, Chong LT. Development and Validation of Fluorinated, Aromatic Amino Acid Parameters for Use with the AMBER ff15ipq Protein Force Field. J Phys Chem A. 2022 Apr 14;126(14):2286-2297; 8. Isidro-Llobet, A., Álvarez, M., & Alberício, F. (2009). Amino acid-protecting groups. Chemical Reviews, 109(6), 2455-2504; 9. Yang, Y. T., Hansen, L., & Ryberg, P. (2022). Side-chain unprotected fmoc-arg/his/tyr-oh couplings and their application in solid-phase peptide synthesis through a minimal-protection/green chemistry strategy. Organic Process Research &Amp; Development, 26(5), 1520-1530; 10. Fantoni, T. et al. Solid phase peptide synthesis using side-chain unprotected arginine and histidine with Oxyma Pure/TBEC in green solvents. Green Chem., 2024, 26, 10929-10939; 11. El-Faham, A.; Albericio, F. Peptide Coupling Reagents, More than a Letter Soup. Chem. Rev.2011, 111 (11), 6557–6602.

Links for your convenience:

Fun Fact
1. https://doi.org/10.1002/anie.202216231
2. https://doi.org/10.1186/s13036-019-0166-3
3. https://doi.org/10.1002/pro.4637
4. https://doi.org/10.1039/D4SC05090H
5. https://www.linkedin.com/in/bernadette-mary-dineen/

Trending
1. https://www.jnj.com/media-center/press-releases/icotrokinra-delivered-an-industry-leading-combination-of-significant-skin-clearance-with-demonstrated-tolerability-in-a-once-daily-pill-in-phase-3-topline-results
2.https://www.protagonist-inc.com/protagonist-announces-nomination-of-pn-881-a-potential-best-in-class-oral-peptide-il-17-antagonist-development-candidate
3.https://ir.vikingtherapeutics.com/2024-11-04-Viking-Therapeutics-Reports-New-Data-from-VK2735-Obesity-Program-at-ObesityWeek-R-2024
4.https://www.globenewswire.com/news-release/2024/11/21/2985270/0/en/Vilya-Announces-Exclusive-License-Agreement-of-RFdiffusion-based-Generative-Model-from-the-University-of-Washington-for-the-Design-of-Macrocyclic-Peptides.html
5.https://circlepharma.com/circle-pharma-presents-promising-preclinical-data-on-first-in-class-oral-macrocycle-cyclin-a-b-rxl-inhibitor-cid-078-at-the-san-antonio-breast-cancer-symposium-2024
6. https://www.fiercepharma.com/pharma/lilly-unwraps-detailed-data-showing-tirzepatide-kept-nearly-99-pre-diabetic-patients

Peptide News
https://www.fiercebiotech.com/biotech/fierce-biotech-layoff-tracker-2024
https://www.pharmaceutical-technology.com/dashboards/deals-dashboards/ma-activity-artificial-intelligence-pharmaceutical-industry/

Interesting Reads
1. https://doi.org/10.3390/molecules28186745
2. https://doi.org/10.1093/jimb/kuae005
3. https://doi.org/10.3390/biom14030264

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AbbVie Acquires Nimble Therapeutics

ARALEZ BIO

ncINSIGHTS

1. Wan, Y.; Li, Y.; Yan, C.; Yan, M.; Tang, Z. Indole: A Privileged Scaffold for the Design of Anti-Cancer Agents. Eur. J. Med. Chem. 2019, 183, 111691. https://doi.org/10.1016/j.ejmech.2019.111691.

2. Meyer EA, Castellano RK, Diederich F. Interactions with aromatic rings in chemical and biological recognition. Angew Chem Int Ed Engl. 2003 Mar 17;42(11):1210-50. doi: 10.1002/anie.200390319. Erratum in: Angew Chem Int Ed Engl. 2003 Sep 15;42(35):4120. PMID: 12645054.

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7. Yang DT, Gronenborn AM, Chong LT. Development and Validation of Fluorinated, Aromatic Amino Acid Parameters for Use with the AMBER ff15ipq Protein Force Field. J Phys Chem A. 2022 Apr 14;126(14):2286-2297. doi: 10.1021/acs.jpca.2c00255. Epub 2022 Mar 30. PMID: 35352936; PMCID: PMC9014858.

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