Non-Canonical Building Blocks Extend the Peptide Alphabet

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As any biochemistry text book will tell you, the “canonical” proteinogenic amino acids harbour much chemical diversity among their side chains. Once amino acids have become bonded together to make a protein, the intrinsic diversity of the side chains may be supplemented with post-translational modifications. New chemical environments can form in folded protein structures when side chains interact. However, for some technological applications of proteins and peptides, this natural diversity may not be enough. In such cases, functions not found in natural systems may be added using synthetic methods. An effective way of installing new functionality is via non-canonical amino acids. These may be natural amino acids that are not normally incorporated into proteins (homocysteine, ornithine, etc.), or they may be entirely unnatural amino acids with new chemistry. Solid-phase peptide synthesis is a flexible way of incorporating non-canonical amino acids into peptides (and, albeit indirectly, into proteins) because the only obvious limitation is the availability of suitably protected building blocks. In this article, I will present selections from the recent literature on the theme of non-canonical amino acids and synthetic peptides.

METHIONINE THAT WON’T GO OFF

My first selection exemplifies how the substitution of a canonical amino acid with a non-canonical analogue can simplify a synthesis. Writing in Journal of Peptide Science, Grob et al. describe the preparation and evaluation of peptide radiopharmaceuticals containing methoxinine (Mox; 1), the oxygen analogue of methionine (Met; 2). Radiopharmaceuticals are compounds that deliver radioactivity to biological targets (e.g., cancer cells) in a targeted fashion; targeting being facilitated by a vector molecule that functions as a ligand for a receptor. In their article, Grob et al. discuss a problem of particular concern when peptides are employed as vectors, namely that Met residues can oxidise during the final step of the synthesis (chelation of a radioactive metal). Others’ earlier attempts to solve the problem had involved the use of norleucine (Nle; 3) as a substitute for Met, but the authors argue that Nle-containing constructs might not recapitulate binding of Met-containing ligands. Given that oxygen and sulfur are in the same group of the periodic table, one might expect Mox and Met to have common properties that are not shared by Nle. Oxygen is smaller and “harder” than sulfur, and is a better hydrogen bond acceptor (Moroder, 2005), but could nevertheless prove to be a better substitute than Nle’s methylene group.

To investigate Mox as an alternative to Nle, the authors synthesised variants of an octapeptide radiopharmaceutical containing Mox, Nle or the native Met (the core octapeptide being “minigastrin”; Fig. 1). Octapeptides 4a-4c were synthesised on Rink amide MBHA resin using Fmoc chemistry, capped with DOTA (a macrocyclic multidentate ligand; Fig. 1), cleaved/deprotected, and finally HPLC-purified. Each peptide was then incubated with a lutetium salt in buffered aqueous solution to afford the corresponding (radio)pharmaceutical. A series of experiments was carried out to compare the analogues: all three were similar in terms of lipophilicity (logD); all were internalised by target cells to a similar degree; all were similarly stable in blood plasma; and all showed similar affinities in a competition assay (displacement of a radioactive reference compound, bound to cultured cells, by non-radioactive variants of the radiopharmaceuticals). These results were consistent with Mox being a viable alternative to Nle for the substitution of Met in minigastrin. Grob and colleagues’ article, along with another recent paper from the same group (Valverde et al., 2016), suggests a possible wider applicability of Met-to-Mox substitutions to the synthesis of other peptide radiopharmaceuticals. In the future, it will be interesting to see how well Mox mimics Met at the atomic level – in a crystal structure of a ligand-receptor complex, for example.
AZIDO BUILDING BLOCKS: DO IT YOURSELF

My second selection concerns azido amino acids, a family (canon!) of non-canonical amino acids used for bioorthogonal ligation chemistry. Organic azides are biologically inert, but will undergo synthetically useful reactions in the presence of certain other reagents. One example is the Staudinger ligation, in which an azide and an acyl donor form an amide linkage via the action of a phosphine (Nilsson et al., 2000). Another example is the now-ubiquitous azide-alkyne “click” reaction, in which a triazole linkage is formed (Tornøe et al., 2002, Rostovtsev et al., 2002).

Also writing in Journal of Peptide Science, Pícha et al. explore the accessibility of Fmoc-protected azido amino acids from a synthetic viewpoint (Pícha et al., 2017). In doing so, the authors address some practical considerations that may not be obvious to the casual reader: potential costs of building blocks, for example; or the time and expertise required to synthesise them in-house. Five Fmoc-protected amino acids were synthesised, all having single azido groups in their side chains (5-8, Fig. 2). In each example, the azido group is connected to the α-carbon by a linker comprising 1-4 methylene groups. Different linker lengths required rather different synthetic routes, but in all cases the chirality came from natural amino acids: 5 from serine or asparagine; 6 from glutamine; 7 from ornithine; 8 from lysine.

For the synthesis of azidoalanine 5, for example, there were several routes to choose from. Routes from serine involved protection of the amino and carboxyl groups and bromination of the hydroxyl group. The bromine atom was then substituted with an azido group, and further protecting group manipulations afforded the Fmoc azido amino acid. Particular attention was paid to the conditions used to introduce the azido group, and to the potential for formation of dehydroalanine in side reactions. Routes from asparagine involved protection of the amino group, followed by Hofmann degradation of the side chain and diazo transfer from triflic azide. The authors conclude that a route via Fmoc-protected asparagine should be the most effective because it is rapid and affordable. As evidence that the preparations were of useable quality, the authors incorporated each building block into a tripeptide (Ac-XFF-NH₂, where X = azido amino acid) and evaluated yields and purities. Pícha and colleagues’ paper should be useful for those wishing to use Fmoc-protected azido amino acids in their research, particularly if in-house synthesis is being considered.

REFERENCES


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