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Perspective

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On-Demand Complex Peptide Synthesis: An Aspirational (and Elusive?) Goal for Peptide Synthesis

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ABSTRACT: Peptide synthesis is a truly interdisciplinary tool, familiar to a broad group of scientists who do not otherwise overlap scientifically. For this reason, some may perceive even complex peptide synthesis to be a "solved problem", while others might argue that immense opportunity remains untapped or simply inaccessible. At the extreme of complexity, what might a concise assessment of the state-of-the-art in peptide synthesis look like? As one of the most practiced forms of synthetic chemistry by chemists and non-chemists alike, what restrictions remain that constrain access to chemical space? Using popular terminology, what forms of peptide synthesis are appropriately termed "on-demand"? The purpose of this Perspective is to appraise synthetic access to complex peptides, particularly those containing unnatural α-amino amides. Several case studies in complex peptide synthesis are summarized here, each selected to characterize the challenges attendant to unnatural α-amino amide synthesis. As peptidic molecules find increasing value in therapeutic development, especially in clinical applications, their impact will ultimately be determined by efficient preparative methods.

INTRODUCTION

The relentless drive to innovate in synthetic chemistry provides the biomedical and material sciences with increasingly complex chemical tools, specifically pure chemicals whose composition is known with certainty. Innovation results from not only aspirational contemplation but also historical reflection.[1] It is this contextualized analysis, including an awareness of the immediate landscape, that provides a framework for a sobering 360-degree view of the state-of-the-art. Measures of innovation in synthesis most frequently focus on complexity, with molecular size figuring prominently, perhaps due to its ability to transcend the human experience into everyday life. In this vein, the art and business of architecture has long been used to benchmark the sophistication of a community, and size is just one measure of accomplishment. Style, usable area, and accessibility are representative metrics of any structure by which its societal impact might be measured.

Chemical synthesis and architecture are not dissimilar; the parlance of building construction often infiltrates the dialogue of molecular construction. In much the same way that architects would scoff at an attempt to use building height alone to define the frontier of their discipline, attention in chemical synthesis has increasingly focused on metrics of speed, efficiency, and cost for small-molecule synthesis. Moreover, these characteristics transcend molecular size—there are certainly many "smaller" molecules that require more steps to prepare than "larger" relatives. Our forebearers recognized the advantage that the chiral pool offered to chemical synthesis,[2] and in modern terms, the number of "commercially available" [3] starting materials continues to expand at great pace.

Herein we offer a brief analysis that advances a perspective on the current state-of-the-art of peptide synthesis—a segment of chemical synthesis that reaches a broad cross-section of scientists. Practitioners range from experts in small-molecule synthesis, undaunted by the need to use less common α-amino acids, to chemical synthesis non-experts who desire the complexity and accessibility of peptide tools without complicated preparations.[4] Of particular interest is the contrast between complex peptides that are truly readily available—essentially "on-demand"—and those that are only accessible given unusual amounts of time, resources, and, of course, scientific talent. A goal of this Perspective is to scrutinize the synthetic investment required to install unusual α-amino amides into peptides.

This analysis is narrowly focused on the chemical synthesis of peptides with multiple unusual α-amino amide residues. These are referred to by numerous terms, depending on the context, with "unnatural", "nonnatural", "noncanonical", and "non-proteinogenic" representing the most common instances. These four terms were searched for their occurrence within titles, keywords, and article text, including the use of two or more of these terms together, and our findings are summarized by the Venn diagram in Figure 1. "Unnatural" and "nonnatural" appear most frequently, with the former used nearly 3 times as often as the latter. Mixing of terms within a single report occurred but was relatively infrequent.

"On-demand" has been used in contexts ranging from consumer services (cable television) to chemistry and biology.[5,6] It has been applied to fine chemical synthesis to describe both process engineering of a reaction and the strategic approach by which a defined collection of substrates can be connected through iterations of a sequence of reactions. These reactions must be robust, predictable, and promiscuous in order to allow the chemist to reliably prepare a specific intermediate as needed. The chemical synthesis of peptides exhibits these attributes, particularly when performed on resin.[7] Immobilized substrates allow the key reaction to be driven to completion by reactant and reagent excess since purification is streamlined to a series of washes. This approach has been

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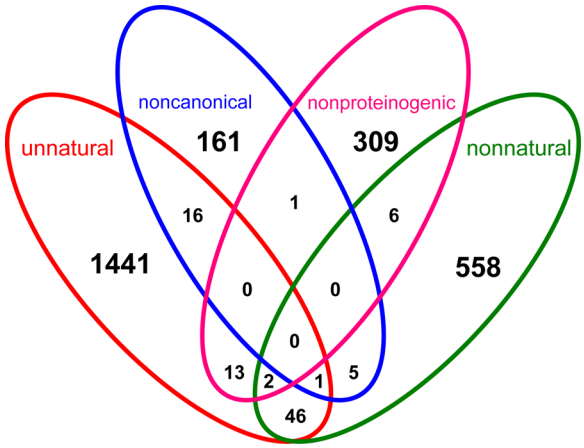

Figure 1. Name game: general descriptors for peptide residues suggestive of the need for chemical synthesis.

leveraged to prepare countless peptides in the half-century since Merrifield's landmark work.[8]

Every approach has its liabilities and limits, whether they are inherent to the method or the result of an outstripped supply chain. Building on the natural repository of α-amino acids, commercial sources now offer a broad range of natural and unnatural α-amino acids endowed with the most common protecting groups. A more subtle liability inherent to most peptide synthesis is the possibility of epimerization at the α-carbon when it is a stereogenic center, as is often the case.[9] The rate of competing epimerization during couplings between *N*-terminal peptides and active esters is most troublesome when the steric demands of the two reactants are high, thereby slowing the nucleophilic addition of amine to the activated, electrophilic carbonyl. Detecting, and especially separating, the epimer (a diastereomer) can be challenging.[10]

Considering the maturity of peptide chemical synthesis, it is not surprising that it has been described as "on-demand",[11] but we questioned whether it is appropriate to use this term in this context. There would almost certainly be consensus that areas of peptide synthesis are an on-demand activity. But where would this term be inappropriately used in the field? In those areas, what aspects of the process fail to meet on-demand criteria? Are these characteristics blemishes or fatal flaws? The most productive question, of course, is what chemical technology—or even a change in approach—might address these shortcomings, thereby bringing more peptides under the umbrella of on-demand peptide chemical synthesis. In order to answer these questions, a rubric for evaluating individual cases is needed. The rubric outlined here is from the perspective of chemical synthesis, a field that weighs cost, time, and diversity equally in the ideal.

METRICS

As organic chemistry established—over decades—the reasonable belief that any molecule produced by nature might also be prepared using chemical synthesis, attention turned to the process itself. Convergency,[12] atom economy,[13] and step economy[14] have each contributed straightforward expressions of desirable attributes associated with synthesis approach and individual chemical steps. Further scrutiny[15] can include process intensification[16] and its E-factor.[17]

- Convergency:[12] The advantages of parallel synthesis are lost when a synthesis is linear. The dependence of a reaction on all those that precede it injects costs associated with time and throughput. Reactions proceeding in less than quantitative yield challenge throughput on scale, where side products not only detract from yield but also present separation and contamination challenges that only magnify with linearity and scale.
- Atom Economy:[13] Reactions that produce few or no co-products are within the guidelines characterized by atom economy. Isomerization and addition reactions between two or more substrates are particularly powerful, especially when catalyzed by vanishing amounts of a catalyst.
- Step Economy:[14] The economy of length enabled by the development of new reactions can streamline access to small molecules. Innovative approaches that shorten access to common intermediates provide additional direction for catalyst and reagent development.
- Green Chemistry:[15−17] The confluence of perennial chemical needs in industry, and the lack of suitable solutions for them, led industry representatives with a broad range of interests to produce a writ listing key challenges for research to address.[15,16] These priorities not only emphasized common tools of a large number of practicing synthetic chemists but also highlighted the waste associated with those transformations among the most used on a daily basis worldwide. That is, alternatives that provide an improvement, one that might otherwise be considered marginal, would unusually impact the field due to extrapolation of scale. This includes the minimization of peripheral reaction components, such as solvent.

An existing synthesis of a peptide containing noncanonical α-amino amide residues can be evaluated using these criteria, but this is only a first step. Ultimately, need must align properly with availability, and time must be factored into availability. When these are in perfect alignment, the term "on-demand" becomes appropriate. Naturally, the chemical basis for life processes is a key area to find the principles of on-demand synthesis in a healthy balance.

PEPTIDE CHEMICAL SYNTHESIS: A CASE STUDY

Nature achieves an immense level of chemical complexity by approaching its need for storage, translation, and function to moderate the mechanisms leading to life through the use of programmed polymerization reactions and a standard set of monomers. This approach leads to highly selective chemical reagents and catalysts residing and operating within an immensely complex environment. Not surprisingly, this approach has been adapted in vitro to include both unnatural reagents and catalysts that otherwise resemble naturally occurring species.

The goal of on-demand peptide synthesis is to be able to prepare any peptide in pure form, both rapidly and without an abundance of waste. The synergism achieved by coupling SPPS[8] with native chemical ligation[18] has yielded pure, large proteins.[19] The preparation of peptide fragments is enabled by the availability of robust reagents and protocols that produce amides from amines and carboxylic acids, or more contemporary approaches.[20,21] Recent advances in flow synthesis have brought peptide synthesis with natural amino acids closer to on-demand status.[22] While there is room for improvement on this aspect, especially the minimization of waste,[23] it may not be

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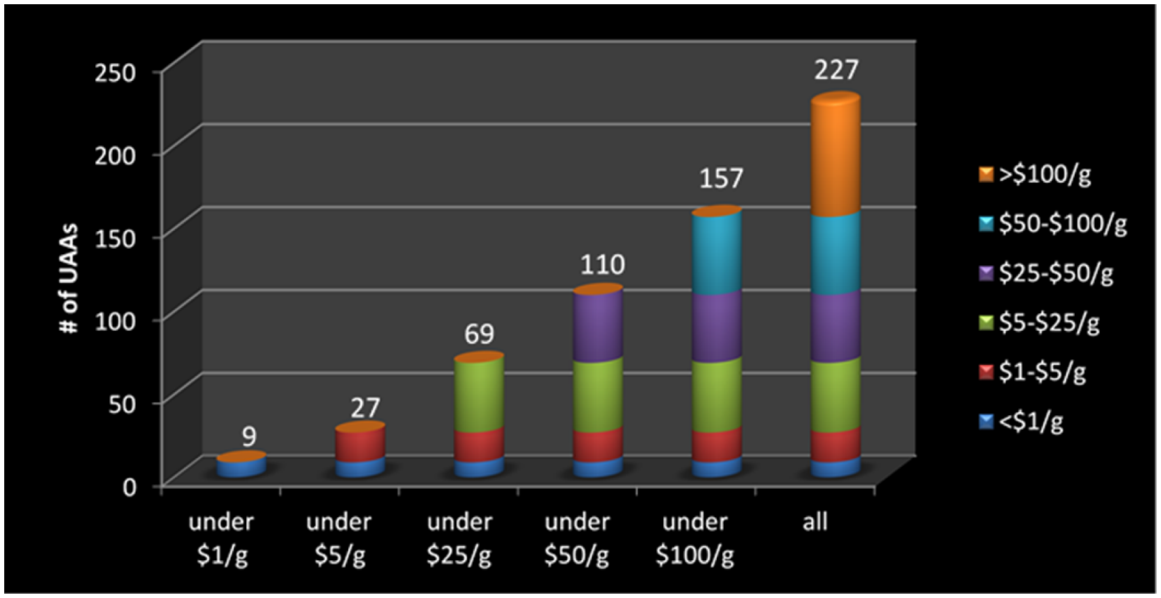

Figure 2. Comparison of unnatural amino acid (UAA) costs using Chem-Impex listings (a total of 227 UAA offerings).

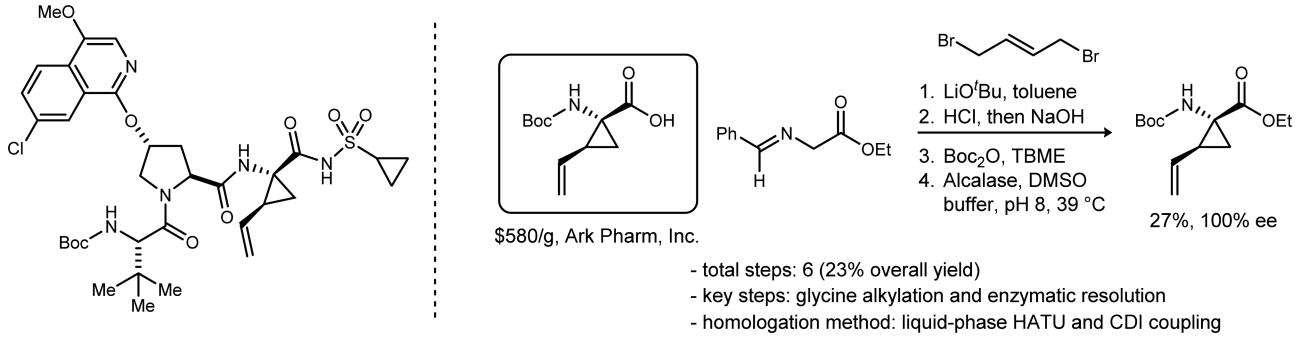

Figure 3. Asunaprevir: synthesis of 1*R*,2*S*-vinyl-ACCA.

the most pressing matter. The current landscape allows only for the rapid incorporation of the 20 canonical amino acids and a few additional residues. In order to move closer to true on-demand synthesis, the barrier to incorporate unnatural amino acids (UAAs) must be lowered. Current methods include genetic encoding,[24] SPPS, and liquid-phase peptide synthesis (LPPS). Genetic encoding allows for the inclusion of a few distinct UAAs in one peptide. This method works well for the synthesis of large peptides/proteins in biological systems. However, it is work-intensive to simply alter the included UAAs. SPPS allows for the production of peptides up to ∼60 amino acids in length with an unlimited number of distinct UAAs.[25] The downside of SPPS is the necessity to use a large excess of the amino acids for each coupling, resulting in a large quantity of waste and difficulty in coupling certain amino acids. In the LPPS method, smaller peptides are traditionally made. Similar to SPPS, LPPS allows for all residues to be UAAs but is significantly more purification-intensive than SPPS.

A major limitation of all of these methods is the accessibility of the UAAs through either purchase or synthesis. Accessibility can be measured by availability—"Is the UAA available for purchase?" or "Is there a known synthetic route?"—and affordability—"How much does it cost to buy or make it?" From the commercial perspective, there are numerous vendors that supply a wide array of UAAs as the free amino acid or with a variety of protecting groups, making them readily available. The affordability aspect is a little more difficult to assess. In an effort to survey the current market for UAAs, one vendor (Chem-Impex International) was selected to analyze the amino acids available for purchase. Only unprotected amino acids bearing unnatural side chains were included in the analysis. In total, 227 UAAs were selected and organized based on $/g in order to gain information about affordability (column graph). As shown in Figure 2, as the price increases, the number of available amino acids increases at a much greater rate. For example, only 27 UAAs are available for under $5/g, while an additional 42 UAAs are available for under $25/g. Significantly, 31% (70 UAAs) of the included UAAs are over $100/g, making them prohibitively expensive for many uses. Application of "on-demand" to peptide synthesis with UAAs is therefore a misnomer due to its expense. Consequently, methods that increase the affordability of UAA-based peptide synthesis are needed.

■ PEPTIDIC DRUGS

The impact of unnatural amino acids continues to increase at an impressive pace, particularly in the area of drug development. Peptide drug development is expected to continue its rapid growth,[26] fueled by treatment of metabolic diseases and oncology, and a global peptide market value approaching U.S. $20B.[27] In the past few years alone, numerous antivirals to treat hepatitis C infection entered the market and featured

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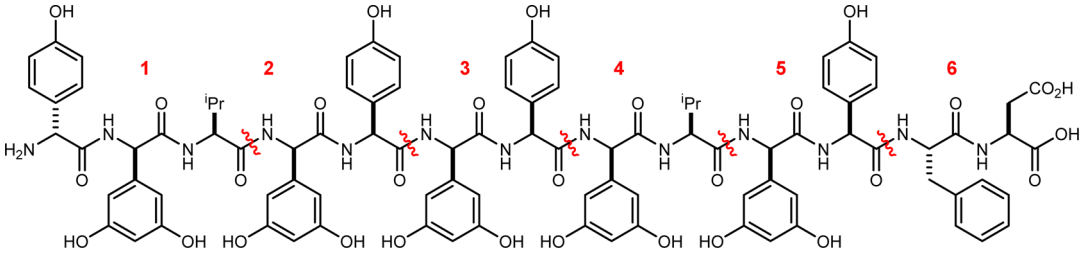

Figure 4. Feglymycin.

structurally unique α-amino amide components.[28] The display of unnatural amino acids in approved drugs, such as asunaprevir, can stem from improvements in not only potency and selectivity but also drug properties (bioavailability, metabolic stability).

Asunaprevir (Figure 3), an NS3 protease inhibitor, was developed by Bristol-Myers Squibb as an oral treatment for hepatitis C infection.[29] Structurally, asunaprevir is a tripeptide featuring three unnatural amino acids: L-*tert*-Leu, *R*-hydroxy-L-Pro, and 1*R*,2*S*-1-amino-2-vinylcyclopropanecarboxylic acid (vinyl-ACCA). A key aspect of the synthesis of asunaprevir was the preparation 1*R*,2*S*-vinyl-ACCA in 6 steps starting from benzaldehyde and glycine ethyl ester hydrochloride. A condensation reaction provided the glycine Schiff base as the starting material for the dialkylation step. Dialkylation with dibromobutene, followed by imine hydrolysis and Boc protection, yielded vinyl-ACCA in 53% yield in racemic form. Subsequent enzymatic resolution with Alcalase provided 1*R*,2*S*-vinyl-ACCA ethyl ester in 50% yield (with 50% of the enantiomer acid). Hydrolysis of the ethyl ester furnished the free acid ready for peptide coupling in 23% overall yield. After *C*-terminal amidation with CDI and cyclopropyl sulfonamide, iterative peptide synthesis followed, using materials prepared from commercially available *R*-hydroxyl-L-Pro and L-*tert*-Leu. Overall, asunaprevir was prepared in a 12-step longest linear sequence (19 steps overall) and 16% overall yield.

The synthesis of asunaprevir highlights the importance that commercial availability plays in an efficient synthesis. Two of the three necessary amino acid residues were available, limiting the overall number of steps needed for the synthesis. Conversely, the preparation of 1*R*,2*S*-vinyl-ACCA highlights the need for improved synthetic methods for the preparation of enantioenriched UAAs, as the synthesis accounts for half of the longest linear sequence, and the use of an enzymatic resolution results in an inherent 50% sacrifice of material.

EARLY-STAGE THERAPEUTIC DEVELOPMENT

Moderate to large peptides displaying numerous UAAs have been prepared by chemical synthesis with varying degrees of overall synthetic efficiency. Among complex peptide syntheses[30] reported in recent years are those of feglymycin and polytheonamide B. Feglymycin was first prepared by Süssmuth in 2009.[31] This tridecapeptide provides an interesting case study against the backdrop of the vancomycin class of heptapeptides that attracted an extensive following.[32] Unlike the highly cross-linked and glycosylated vancomycin, feglymycin instead presents a unique stereochemical challenge in addition to its larger size. In a size category of its own, polytheonamide B was prepared synthetically by Inoue.[33] Each of these landmark achievements is described below with detail appropriate to assess the level of accessibility that each synthesis provides, and the logistical and chemical challenges associated with their synthesis. In their own ways, feglymycin and polytheonamide B admirably represent the current frontier of peptide targets, and their individual preparations illustrate the state-of-the-art in peptide chemical synthesis by measures of size and complexity.

Case Study 1: The Süssmuth Synthesis of Feglymycin. Feglymycin is a peptide natural product isolated from *Streptomyces* sp. DSM 11171[34] and found to have novel biological activity. It was found to inhibit the formation of HIV syncytia and also showed antibacterial activity against Gram-positive bacteria.[35] Structurally, feglymycin consists of 13 amino acids almost entirely alternating between *S* and *R*, 9 of which are aryl glycines (Figure 4). There are four 4-hydroxyphenylglycines (Hpg's) and five 3,5-dihydroxyphenylglycines (Dpg's). The Süssmuth synthesis uses a convergent strategy in which LPPS was used to form the necessary peptide bonds. The two main challenges were the preparation of enantiopure Dpg and the minimization of racemization during peptide coupling with the electron-rich aryl glycines.

Dpg was prepared in high enantiopurity in 4 steps starting from 3,5-dibenzyloxybenzaldehyde. A Wittig reaction transformed the aldehyde into the necessary styrene for the key reaction, a Sharpless aminohydroxylation. The amino alcohol was obtained in 52% yield and 98% enantiomeric excess (ee) (Figure 5). A subsequent two-step oxidation led to Dpg with

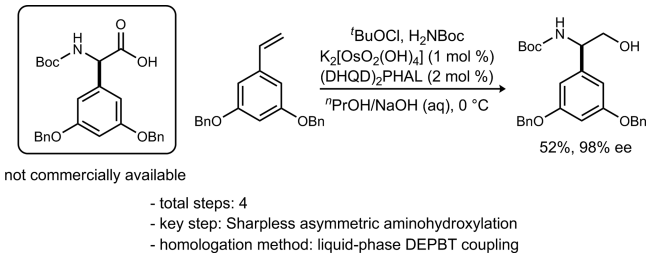

Figure 5. Synthesis of 3,5-dihydroxyphenylglycine (Dpg).

the desired protecting groups for the peptide coupling in 46% overall yield. A traditional iterative method for peptide coupling was not feasible due to the ease with which the hydroxyphenyl glycines epimerize. A convergent strategy was adopted in which only Phe, Hpg, and Val were activated in peptide couplings, avoiding the activation of Dpg which is known to be highly prone to epimerization. Retrosynthetically, the 13-mer was disconnected into five dipeptides (**2**–**6**) and one tripeptide (**1**). DEPBT was used to couple dipeptide **2** with dipeptide **3** followed by coupling with the tripeptide **1** to provide a heptapeptide. Dipeptides **4**–**6** were iteratively coupled to provide a hexapeptide. Lastly, the heptapeptide and hexapeptide were coupled to provide feglymycin (Figure 6).

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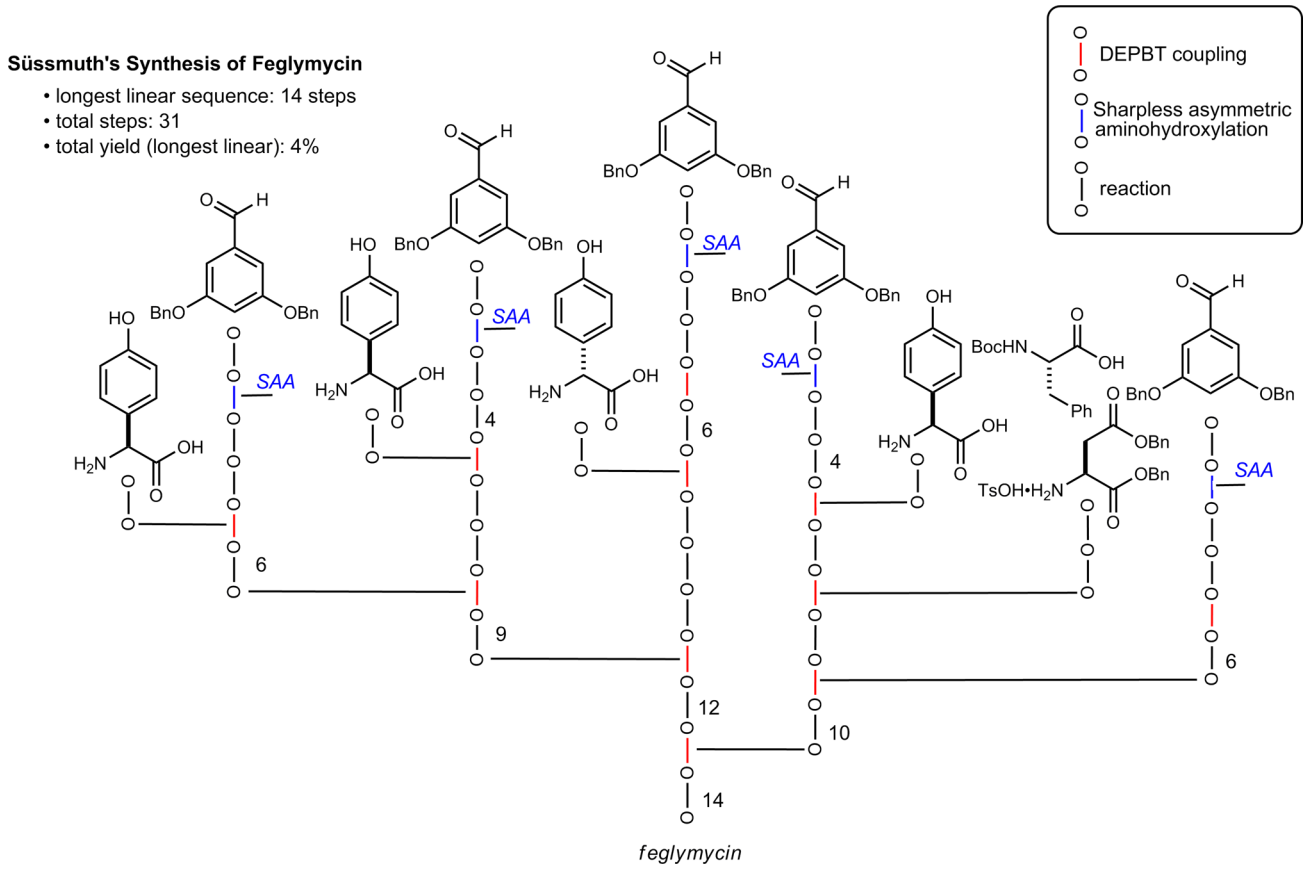

Figure 6. Feglymycin synthesis: a schematic representation that highlights coupling points, longest linear sequence, and starting materials.

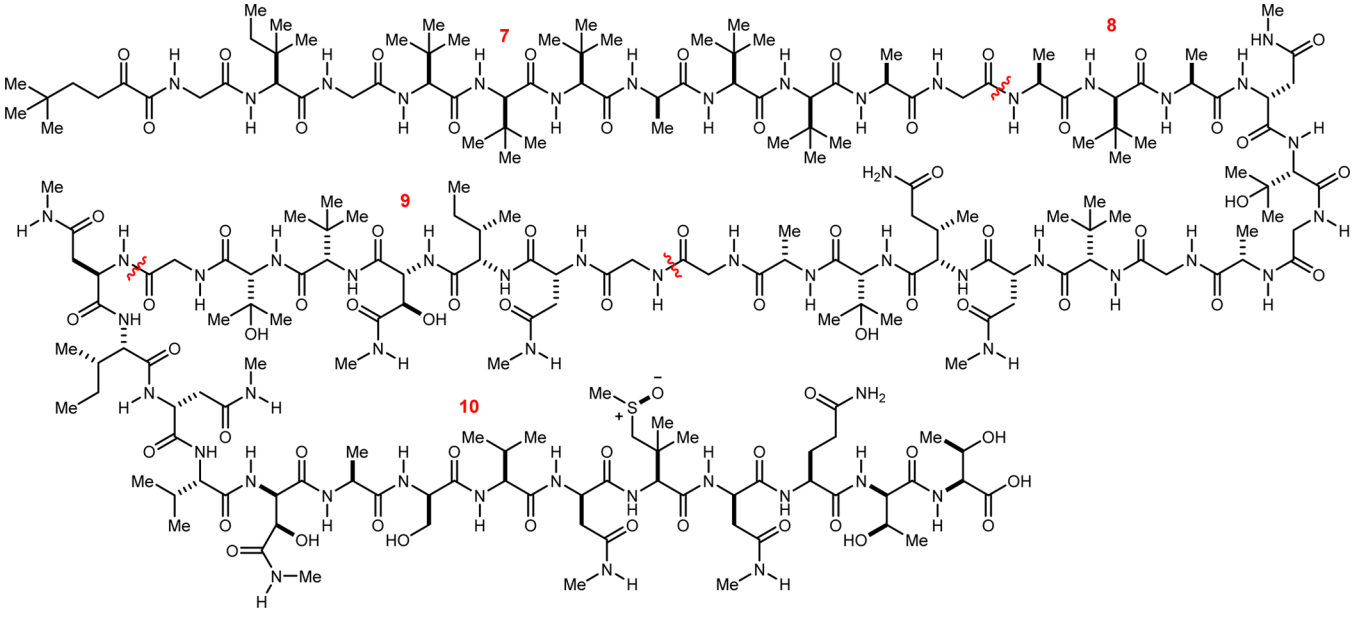

Figure 7. Polytheonamide B.

This synthesis not only highlights the convergency associated with solution-phase peptide synthesis but also emphasizes the pivotal role that commercial availability plays for each Hpg enantiomer. Although the preparation of Dpg was relatively high yielding (46% over 4 steps), it required the use of a Wittig reaction as well as two stoichiometric oxidations resulting in poor atom economy. Current methods for peptide synthesis frequently suffer from poor step economy due to the necessity of protecting groups. The synthesis of feglymycin is no different in that 17 of the 31 total steps are protection/deprotection steps compared to the 11 steps utilized to build structure. The remaining 3 steps are functional group manipulations en route to Dpg. The extensive, but ultimately successful search for a coupling reagent that delivers the desired amide with little or

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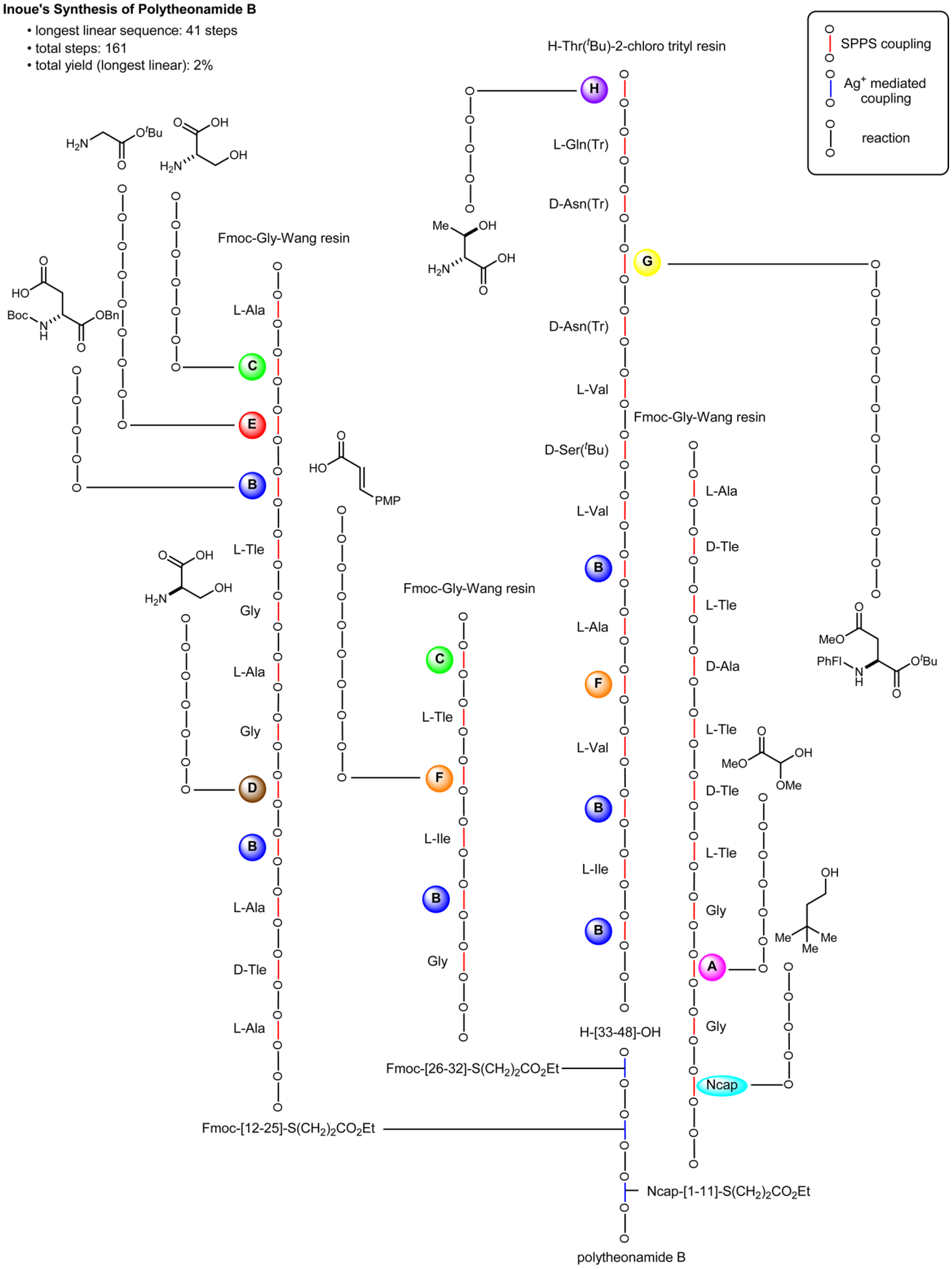

Figure 8. Polytheonamide B synthesis: a schematic representation that highlights coupling points, longest linear sequence, and starting materials.

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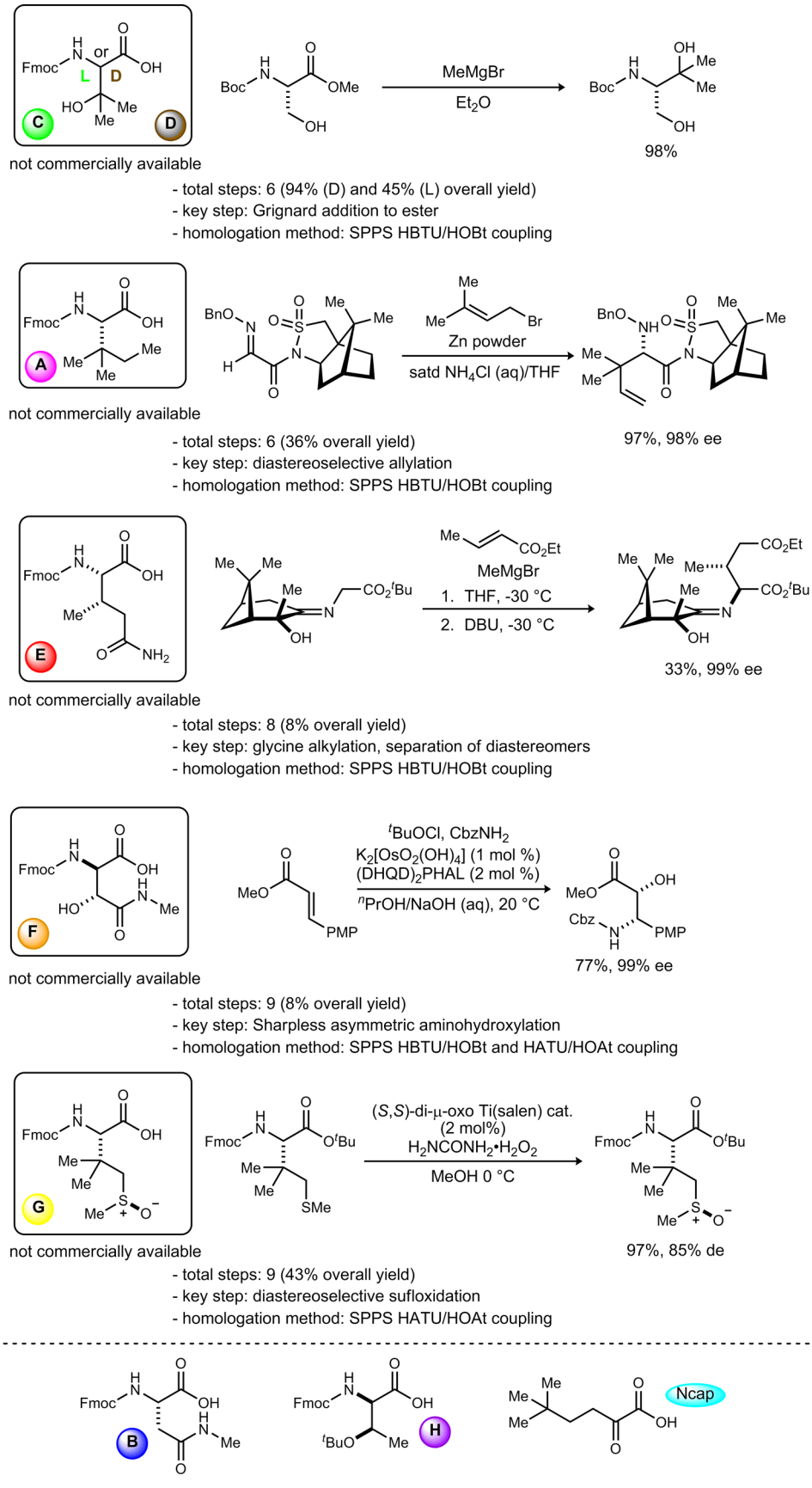

Figure 9. Preparation of unnatural amino acids for the total synthesis of polytheonamide B.

no epimerization suggests that situations like this will continue to present themselves and slow, if not prevent in some cases, progress toward a peptide target. More salient is the availability of α-amino acids with alternative protecting groups.

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Case Study 2: The Inoue Synthesis of Polytheonamide B. Polytheonamide B (Figure 7) was isolated from a marine sponge *Theonella swinhoei* and found to have extraordinary cytotoxicity toward mouse leukemia P388 cells.[36] With a linear structure of 48 amino acid residues, it is the largest non-ribosomal peptide presently known. Polytheonamide B contains 19 distinct amino acids, 13 of which are unnatural residues. The absolute stereochemistry of the residues alternates between L and D, with eight glycines as the only exceptions. In 2009, Inoue and co-workers reported the first total synthesis of polytheonamide B using predominantly SPPS.[33] Of the 13 unnatural amino acids present in polytheonamide B, five of them (D-Ala, D-Ser, D-Tle, L-Tle, and D-Asn) were commercially available (currently all under $20/g[37]). The remaining eight were synthesized in enantiopure form using a variety of different methods.

Retrosynthetically, polytheonamide B was dissected into four different fragments of 7, 11, 14, and 16 amino acids, as SPPS only reliably provided linear chains of up to 16 amino residues. Three of the four fragments have a glycine residue at the C-terminus, which avoids the issue of epimerization during the late-stage couplings. Each fragment was synthesized using Wang resin Fmoc-SPPS with either HBTU/HOBt or HATU/HOAt for their coupling steps. Once all four fragments were prepared, the three non-C-terminus fragments were converted to a thioester using HOBt and DCC. Fragment **10** was then coupled to fragment **9** using $AgNO_3$ and HOBt. Deprotection followed by Ag(I)-mediated coupling with fragment **8** provided 37 of the desired 48 amino acids. Subsequent deprotection and coupling with fragment **7** under the same conditions and global deprotection provided polytheonamide B in 161 overall steps (Figure 8). Overall, 0.5 mg of polytheonamide B was prepared, and the structural identity was confirmed by comparison to the natural source (^{1}H NMR, HPLC, 2D NMR).

Figure 9 summarizes the unnatural amino acids prepared by chemical synthesis, detailing the overall length and yield, as well as the key enantioselective step. For example, L- and D-β-methyl threonine (**C**, **D**) were prepared from serine in 94% (D) and 45% (L) overall yield in 6 steps each. The key step was a double methyl Grignard addition that was carried out in high yield (Figure 9). L-β-Methyl isoleucine (**A**) was synthesized in 6 steps with 36% overall yield starting from methyl 2-hydroxy-2-methoxyacetate featuring a diastereoselective allylation (Figure 9). D-*N*-Methyl asparagine (**B**) was prepared from commercially available D-Asn from a simple amide coupling with methylamine and protecting group manipulations in 72% yield over 4 steps. β-Methyl glutamine (**E**) was synthesized in 8% yield over 8 steps. The key reaction in the sequence was the alkylation of a chiral glycine equivalent, followed by separation of diastereomers, resulting in 33% yield and 99% ee (Figure 9). Starting from *p*-methoxycinnamic acid, *N*-methyl-β-hydroxy asparagine (**F**) was prepared in 9 steps with an 8% overall yield. Both the amine and hydroxyl stereocenters were established in a single step through a Sharpless asymmetric aminohydroxylation, which provided the desired adduct in 77% yield and >99% ee (Figure 9). The final unnatural residue, sulfoxide **G**, was prepared in 9 steps with a 43% overall yield. Starting from fully protected aspartic acid, a sequence of dimethylation, reduction, and S_N2 displacement with NaSMe led to the thioether. Subsequent diastereoselective sulfoxidation provided sulfoxide **G** in 97% yield and 85% diastereomeric excess (de) (Figure 9). The de was then increased to 96% via SiO_2 purification.

The synthesis of polytheonamide B is a remarkable achievement due to its size and number of UAAs. Although not as convergent as the synthesis of feglymycin, this synthesis is completed in only 41 longest linear steps out of 161 total steps. The use of SPPS provides sufficient yield and material throughput allowing for the completion of the synthesis, but it also requires large excess of amino acids as well as coupling reagents resulting in significant waste. Unnatural amino acid availability, and lack thereof, is highlighted by this synthesis. Of the 161 total steps, 58 steps are required for the preparation of the monomers (eight amino acids and the *N*-terminus ketoacid). Of those 58 steps, 34 are protection/deprotection steps, highlighting the need for new, more efficient methods of amino acid synthesis.

NEXT STEPS: UNNATURAL α-AMINO ACID TOOLS FOR PEPTIDE DESIGN AND LABELING

The syntheses of feglymycin and polytheonamide B reveal contrasting degrees of access to the precursors needed to homologate noncanonical α-amino amides. The need for chemical access stems from nature's creation of these peptides, their limited availability, and the correlation of their structure to interesting biological activity. Synthesis of the natural product is a first step, and in each of these cases, the total synthesis was followed by preparation of simplified derivatives and their biological evaluation.[38] The preparation of derivatives, however, is increasingly directed toward conformational stabilization, often using α-amino amide side chain cross-linking strategies. Unnatural α-amino amides are often required.

One such application is the stapling of peptides to increase α-helix stability. In 2004, Korsmeyer and co-workers developed an all-hydrocarbon peptide staple to stabilize the α-helix of the BH3 domain from the BID protein.[39] BID is a pro-apoptotic protein that belongs to the BCL-2 protein family that contains only the BH3 domain. They found that by incorporating an all hydrocarbon chain into a protein mimicking the BH3 domain of BID, the α-helicity in solution dramatically increased from 16% up to 87%. Along with increased α-helicity came improved proteolytic stability, cell permeability, and in vitro and in vivo activity toward leukemia cells. The strategy employed was to introduce two α,α-disubstituted amino acid residues bearing terminal olefins into the peptide sequence and then connect them together using a ruthenium-catalyzed olefin metathesis. The necessary amino acid was synthesized in high enantiopurity in 11 steps featuring a diastereoselective glycine alkylation (Figure 10). The peptide was synthesized using SPPS, incorporating the unsaturated amino acid at two positions separated by three residues. Subsequent olefin metathesis provided the desired stapled peptide.

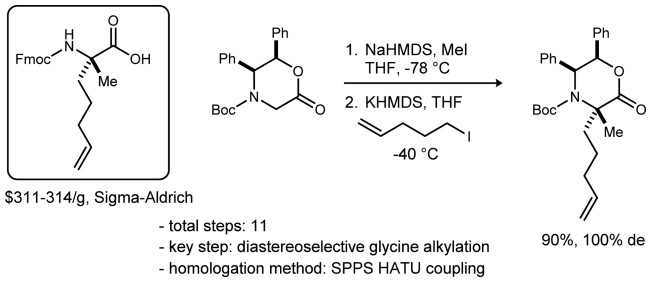

Figure 10. Synthesis of α,α-disubstituted amino acid for use in peptide stapling.

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CONCLUSION

In summary, several examples in peptide synthesis were analyzed, with particular attention focused on the availability or synthesis of unnatural α-amino acids. These examples are drawn from state-of-the-art synthetic efforts chosen for their relevance to therapeutic development. Among recently developed antiviral drugs, asunaprevir's vinylcyclopropane-containing α-amino amide residue required a late-stage enzymatic resolution. Despite the relatively small size of feglymycin (13-mer), this aryl glycine-rich natural product posed stereochemical challenges typical of aryl glycinamides that were surmounted by a strategic approach and careful application of peptide coupling reagents. Polytheonamide B offered fewer coupling-related stereochemical challenges but instead posed obstacles to the concise preparation of relatively complex unnatural amino acids. Again, synthetic expertise prevailed in this campaign, with the benefit that compelling biological activity of the peptide could be pursued.[40] Therapeutic development is also the driving force behind new techniques to stabilize peptide conformation by stapling. This requires the use of unnatural α-amino amides carefully positioned within a peptide chain and illustrates the challenge often presented for the synthesis of α,α-disubstituted α-amino acids.

These case studies illustrate the synthetic challenge that peptides containing unnatural amino amides present, the expanse between existing on-demand peptide synthesis, and the application of this term to the efforts detailed in the case studies described here. Innovation in α-amino amide synthesis can, and will, close this gap, as will advances that streamline the enantioselective synthesis of the precursors needed for peptide homologation requiring unnatural α-amino amide precursors. The benefits that result from success in addressing this challenge, of course, are immeasurable.

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The authors declare no competing financial interest.

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