Top Heterocycl Chem (2016) 43: 67–94
DOI: 10.1007/7081_2015_199
© Springer International Publishing Switzerland 2016
Published online: 28 February 2016

Acylbenzotriazoles: New Allies for Short Linear and Cyclic Peptide Constructs

Danniebelle N. Haase

Abstract Peptides and proteins have tremendous potential as medicinal drugs, but their potential has largely been untapped due to several problems such as their fast degradation by enzymes and limited bioavailability. Major advances in the development of peptide synthesis methods, availability of a wide array of coupling reagents and other augments have lowered the barrier to entry for peptide drugs. However, greater access to peptides and proteins is needed in order to realize the possibilities for diagnosing, treating and monitoring critical diseases. Acylbenzotriazoles are an expedient gateway to cyclic and difficult peptides. With this overview, we now describe the use of acylbenzotriazoles as allies for the construction of peptides and peptide conjugates. The efficacy of *N*-(protected-α aminoacyl) benzotriazoles for the preparation of an extensive range of enantiopure peptides with good yields and purities on solid-phase or in solution, as well as challenges encountered and the way forward is addressed in the chapter.

Keywords Acylbenzotriazole • Amino acids • Isopeptide • Microwave • Peptide synthesis • Peptides • Solid-phase

Contents

1 Introduction 68
1.1 Benzotriazole-Based Fmoc-, Boc-, and Alloc-Protected Amino Acids for Chemical Transformations 69
2 Construction of Short Linear Peptides 70
2.1 Introduction 70
2.2 Oligopeptides from *N*-Acylbenzotriazoles 71

D.N. Haase (✉)
The Dow Chemical Company, 400 Arcola Road, Collegeville, PA 19426, USA
e-mail: dhaase@dow.com

2.3 Sterically Hindered Peptides from *N*-(Protected α-Aminoacyl)Benzotriazoles 74
2.4 Difficult Peptides from the Microwave-Assisted Coupling of *N*-(Protected α-Aminoacyl)Benzotriazoles on the Solid Phase 75
3 Cyclic Peptides 77
3.1 Benzotriazole-Mediated Preparation of 2,5-Diketopiperazines 78
3.2 Hetero-2,5-Diketopiperazines from Proline-Containing Cbz-*N*-Protected Dipeptidoyl Benzotriazoles 80
4 *O*- and *S*-Acyl Isopeptide Strategies for the Preparation of Difficult Peptides 82
4.1 Isopeptides and Isodipeptides in Peptide Synthesis 83
4.2 Application of *N*-(Protected α-Aminoacyl)Benzotriazoles in the Synthesis of Peptides via the *O*- and *S*-Acyl Isopeptide Strategy 85
5 Benzotriazole Activation and Coupling of Amino Acids on a Preparative Scale 86
6 Conclusion 86
References 87

1 Introduction

Peptides and proteins are ubiquitous in nature. Peptides and proteins are of interest due to their wide applicability in several areas, including endocrinology, neurology, and hematology. Despite their undeniable utility, peptides and proteins have limited applicability as therapeutics due to their in vivo instability [1]. Since they are composed of naturally occurring amino acids, peptides and proteins suffer from rapid degradation, poor bioavailability, and other problems. Therefore, the synthesis of peptides and proteins is an active area of research as chemists race to develop peptide and proteins with greater in vivo stability and bioavailability and to confirm the structure of proteins and peptides isolated from natural sources [2]. Additionally, there is considerable interest in studying protein–protein interactions and peptide and protein conformations to understand how amino acid sequence controls protein and peptide structure and function in order to understand the role that peptides and proteins play in diseases such as Alzheimer's, Parkinson's, and Creutzfeldt–Jakob [3]. Better understanding of protein and peptide folding, aggregation, or misfolding can facilitate the design of efficacious drugs to monitor and treat a number of diseases. However, useful methods for the preparation of peptides and proteins are necessary.

Notwithstanding more than a century of major breakthroughs in peptide synthesis, in the last four decades, many advances in the efficient preparation of peptides and peptide mimics with greater chemical stability have promoted greater understanding of peptide and protein behavior as well as the development of peptide-based therapeutics [4–6]. This has largely been facilitated by several major developments, including, but not limited to, the availability of improved and efficient coupling reagents, advances in solid-phase peptide synthesis (SPPS), *O*- and *S*-acyl isopeptide synthetic strategies, and the native chemical ligation (NCL) peptide synthesis method [7–10]. This chapter will address the contributions of

acylbenzotriazoles, in particular, *N*-(protected-α aminoacyl)benzotriazoles to the field of solid- and solution-phase peptide synthesis of linear peptides, short difficult peptides, *O*- and *S*-isopeptides, and cyclic peptides.

1.1 Benzotriazole-Based Fmoc-, Boc-, and Alloc-Protected Amino Acids for Chemical Transformations

The preparation and use of *N*-acylbenzotriazoles were widely described in the literature. Reacting carboxylic acids with 1*H*-benzotriazole facilitated the smooth conversion to acylbenzotriazoles, versatile synthetic auxiliaries that have been useful for a host of chemical transformations.

Solution- or solid-phase peptide synthesis requires protection of the amino moiety. The most utilized amino protecting groups are 9-fluorenylmethoxycarbonyl (Fmoc) [11, 12], *tert*-butoxycarbonyl (Boc) [12–17], benzyloxycarbonyl (Cbz) [12, 18, 19], and allyloxycarbonyl (Alloc) [11]. Easy removal of the Fmoc protecting group occurs under mild, basic conditions, while the Boc protecting group requires acidolysis [11]. The Fmoc protection strategy has gained traction over Boc protection as deprotection of the Fmoc group can occur under facile conditions, even in the presence of acid-labile protecting groups on the amino acid side chain. As the Alloc group is stable under both acidic and basic conditions, a selective and facile palladium-catalyzed transfer of the allyl group to nucleophiles is required for its removal [11].

Generally, protection of the amino group is achieved by reacting free amino acids with the haloformate or carbonate of the particular protecting group under Schotten–Baumann conditions [20]. Other protecting group strategies employed include the *N*-(9-fluorenylmethoxycarbonyl-oxy)succinimide (Fmoc-OSu) [21–24], 9-fluorenylmethyl pentafluorophenyl carbonate (Fmoc-Pfp) [23], 9-fluorenylmethyl 1-benzotriazolyl carbonate (Fmoc-OBt) [21, 25, 26] and 5-norbonene-2,3-dicarboximido-Fmoc derivatives [27]. More recently, *N*-Fmoc-or-Alloc-protected amino acids were prepared from Fmoc-or-Alloc-cyanopyridyloxime carbonates by way of a two-step procedure [28–30]. For several of these methods, the starting material is unstable, thus limiting the shelf life. Additionally, the reaction yield is often lowered due to the formation of undesired protected di- and tripeptide side products. Azide-based methodologies could minimize the formation of peptide side products; however, their use in protecting the NH_2 of amino acids is limited due to hazards associated with their use [31–34].

Recently, Fmoc-, Boc-, and Alloc-benzotriazoles have gained prominence as selective protecting groups for the synthesis of the corresponding Fmoc-, Boc-, and Alloc-protected amino acids. *N*-(9-fluorenylmethoxycarbonyl)benzotriazole (Fmoc-Bt) and *N*-(allyloxycarbonyl)benzotriazole (Alloc-Bt) are readily available from the Fmoc-Cl and Alloc-Bt with benzotriazole, while *N*-(*tert*-butoxycarbonyl) benzotriazole (Boc-Bt) can be prepared from Boc-anhydride and benzotriazole (Figs. 1 and 2, Table 1) [11, 14, 35, 36].

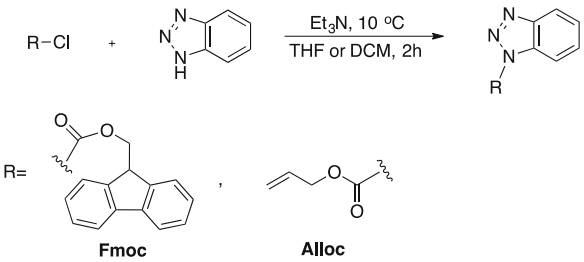


Fig. 1 Preparation of Fmoc- and Alloc-benzotriazoles from 1*H*-benzotriazole [11]

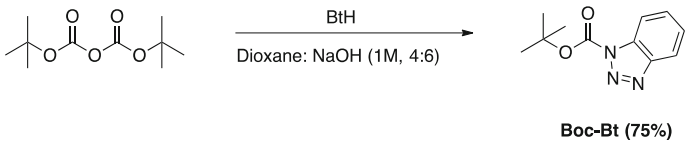


Fig. 2 Preparation of Boc-Bt from Boc-anhydride and 1*H*-benzotriazole

Table 1 Yields of Fmoc-Bt and Alloc-Bt from the corresponding acid chlorides and 1*H*-benzotriazole [11]

Entry	R	Product	mp (°C)	Yield (%)
1	Fmoc	Fmoc-Bt	90–91	88
2	Alloc	Alloc-Bt	105–107[a]	92

[a]Oil in the Lit.

Having successfully prepared the Fmoc-Bt, Alloc-Bt, and Boc-Bt, Katritzky and coworkers used these selective amino protecting reagents in the preparation of 16 Fmoc-, Boc, and Alloc-protected amino acids in yields of 77–94% [11]. The substrate scope is broad and can facilitate the reaction of unprotected serine and tyrosine with the Fmoc-, Alloc-, and Boc-benzotriazole reagents. Additionally, the reaction conditions are mild, with no observable side-product formation. To summarize, Fmoc-, Alloc-, and Boc-benzotriazoles can be used for the selective *N*-acylation of amino acids, which are precursors to *N*-acylbenzotriazoles.

2 Construction of Short Linear Peptides

2.1 Introduction

Short linear peptides are abundant in nature and are quite useful for studying the secondary structure of peptides and proteins. Short linear peptide sequences, frequently referred to as short linear motifs (SLiMs, minimotifs, linear motifs) in structural biology, are typically composed of 3–11 amino acid units (average of 6 amino acid units) [37]. SLiMs facilitate protein–protein interactions and are

integrally involved in recognition, targeting, and other functional properties [38]. As short linear peptides display a wide range of biological activity including antimicrobial, antimalarial, and cytotoxic activity, it is imperative that methods facilitating the preparation of a wide array of peptide and peptide conjugates are available.

Several coupling reagents are available for solid-phase and solution peptide synthesis [39]. Acylbenzotriazoles are efficacious synthetic auxiliaries that have been successfully applied to both the solid- and solution-phase preparation of various short peptide sequences. Now presented are the acylbenzotriazole-mediated syntheses of several classes of peptides.

2.2 *Oligopeptides from* N-*Acylbenzotriazoles*

Peptides containing 2–9 amino acid residues were prepared by coupling *N*-(protected-α aminoacyl)benzotriazoles with (1) monofunctionalized amino acids (Ala, Gly, Leu, Phe, Val), (2) unprotected functionalized amino acids with hydroxy (Ser, Tyr) and sulfanyl (Cys, Met) functionalities, (3) amino acids with dicarboxylic acid moieties (Asp, Glu), and (4) amino- (Lys) and guanidino (Arg)-containing amino acids.

The peptides were prepared using several methods, including solution- and solid-phase peptide synthesis strategies [40]. Solid- and solution-phase methods display several advantages, and both have a unique niche in the field of peptide synthesis. The solution-phase preparation of peptides is known for its versatility, especially in the preparation of peptides containing unnatural amino acids and peptide conjugates. Convergent synthesis can easily be performed when solution-phase methods are employed, and side products are quickly detected. However, this method is time-consuming and rigorous, as the growing peptide must be purified after each step in the sequence [41]. Today, solid-phase peptide synthesis (SPPS) strategies are more popular than solution-phase methods because the peptide can be rapidly prepared and more easily separated from reagents. As such, in SPPS, coupling reactions can often be driven to completion by using a large excess of reagents. In SPPS, side products are not readily detected and/or separated from the product during the process, and this ultimately leads to lower yields than solution-phase methods [41].

2.2.1 Syntheses of Dipeptides

Solution-phase preparation of dipeptides was achieved by reacting free L- and D-amino acids with *N*-(protected-α aminoacyl)benzotriazoles in aqueous acetonitrile (MeCN: H_2O, 2:1) for 1 h at 20°C in the presence of triethylamine. Separation of the dipeptides from the BtH byproduct by washing the mixture with 4N HCl

afforded the enantiopure products in yields of 78–98% without further purification [42–48].

Diastereomeric mixtures of select dipeptides were prepared with DL-amino acids to confirm the enantiopurity of the synthesized peptides via HPLC and NMR spectroscopy. All LL- and LD-dipeptides showed no detectable racemization (<1%).

2.2.2 Preparation of Tri- and Tetrapeptides via Solution- and Solid-phase Assembly

Tripeptides can be prepared via the stepwise synthetic route. Typically, the solution-phase, *N*-acylbenzotriazole-mediated, stepwise assembly of tripeptides begins with the coupling of a free amino acid and a *N*-(protected α-aminoacyl) benzotriazoles (see Fig. 3). The resulting *N*-protected dipeptide is easily converted to the corresponding *N*-(protected dipeptidoyl)benzotriazoles, after which, the *N*-(protected dipeptidoyl)benzotriazoles are reacted with a free amino acid to afford tripeptides in good yields and with minimal racemization. Examples of tripeptides prepared by the solution-phase, stepwise method are illustrated in Fig. 4 and Table 1.

Alternatively, Katritzky and coworkers demonstrated the utility of *N*-(protected α-aminoacyl)benzotriazoles in the microwave-assisted, solid-phase preparation of tripeptides (Fig. 5). Peptide amides were synthesized in fair yields with excellent purity by anchoring the growing peptide to the Rink amide resin. Following the stepwise or fragment coupling of *N*-acylbenzotriazoles, global deprotection and cleavage of the peptide from the resin using trifluoroacetic acid (TFA) gave the tripeptides. An example of tripeptides made via the stepwise and segment condensation routes is presented in Table 2 [49, 50].

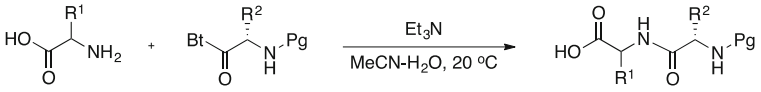


Fig. 3 General solution-phase synthesis of dipeptides from *N*-(protected-α aminoacyl) benzotriazoles

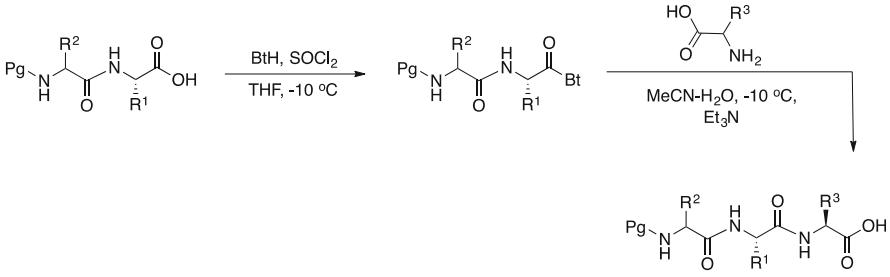


Fig. 4 Synthesis of tripeptides starting from *N*-(protected α-aminoacyl)benzotriazoles

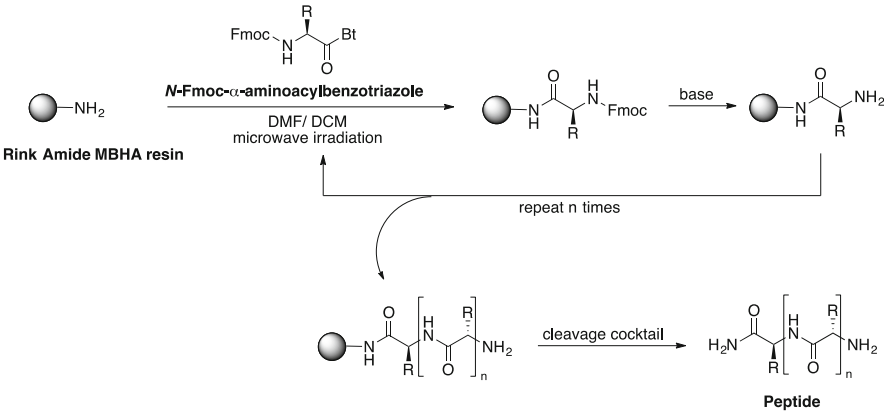


Fig. 5 General scheme for the preparation of peptides via solid-phase peptide synthesis

Table 2 Tripeptides synthesized by solid-phase peptide synthesis [49, 50]

Sequence (N-C terminus)	t_R (min)	Crude product		Product after preparative HPLC	
		% purity	% yield	% purity	% yield
H-L-Ala-L-Phe-L-Ala-NH_2[a]	5.17	90	77[b]	>99	45
H-L-Leu-L-Ala-L-Leu-NH_2[c]	8.49	n.r.	n.r	99	68

[a]Tripeptide from stepwise solid-phase peptide synthesis
[b]Yield quoted is calculated based on the amount of product obtained multiplied by the % purity obtained from HPLC
[c]Tripeptide from segment condensation on solid phase
n.r. not reported

Table 3 Examples of tetrapeptides from the solution- and solid-phase peptide syntheses

Sequence (N-C terminus)	Method	% purity	% yield[a]	References
H-L-Pro-L-Trp-L-Met-L-Trp-NH_2	Solid phase, stepwise	>95	39	[49]
H-L-Trp-L-Ala-L-Met-L-Ala-NH_2[b]	Solid phase, fragment	>99	26	[50]
Z-L-Ala-L-Phe-Gly-L-Leu-OH	Solution phase, fragment	>99	85	[42]
Z-L-Phe-L-Ala-Gly-L-Leu-OH	Solution phase, fragment	>99	86	[42]

[a]Isolated yields
[b]Crude yields and purity are 73% and 82%, respectively

Tetrapeptides can be prepared by (1) the stepwise coupling of amino acid residues in either solution or solid phase as previously described (Table 3) or (2) the solution- or solid-phase coupling of fragments (Table 3). The fragments included free dipeptides and *N*-(protected dipeptidoyl)benzotriazoles. As expected, solution-phase peptide affords higher yields than solid-phase peptide synthesis.

Table 4 Solid-phase peptide synthesis of oligopeptides containing 4–7 amino acid residues from *N*-(protected α-aminoacyl)benzotriazoles and *N*-(protected dipeptidoyl)benzotriazoles

Sequence (N-C terminus)	Solid-phase method	% purity[a]	% yield[b]	t_R (min)	HRMS $[M+1]^+$	References
H-L-Leu-L-Met-Gly-L-Phe-L-Ala-NH_2[c]	Stepwise	>99	24	11.67	537.2626	[49]
H-L-Pro-L-Leu-L-Met-Gly-L-Phe-L-Ala-NH_2[c]	Stepwise	>99	31	12.42	634.3678	[49]
H-L-Pro-L-Met-L-Phe-Gly-L-Leu-L-Ala-L-Phe-NH_2[c]	Stepwise	>99	27	15.97	781.3959	[49]
H-L-Trp-L-Phe-L-Met-L-Leu-L-Ala-NH_2	Segment condensation	99	21[d]	15.45	666.3432	[50]
H-L-Leu-L-Ala-L-Met-L-Phe-L-Phe-L-Met-NH_2	Segment condensation	72	20	16.43	758.3707	[50]
H-L-Leu-L-Ala-L-Met-L-Ala-L-Phe-L-Ala-L-Gly-NH_2	Segment condensation	99	30	11.29	679.3589	[50]

[a]Purity after HPLC purification
[b]Isolated yield after HPLC purification
[c]See reference for crude yields and purities
[d]Isolated yield of the major peak and an impurity not derived from racemization

With solid-phase peptide synthesis, stepwise coupling offers higher yields than segment condensation.

2.2.3 Penta-, Hexa-, and Heptapeptides via Solid-Phase Peptide Synthesis

Stepwise and fragment coupling procedures were used to prepare penta-, hexa-, and heptapeptides. Following the established *N*-acylbenzotriazole protocol, the enantiopure peptide were obtained in good yields. Several examples are shown in Table 4.

2.3 Sterically Hindered Peptides from N-*(Protected α-Aminoacyl)Benzotriazoles*

The coupling reactions of α,α-disubstituted amino acids are of interest due to their unique conformation [51] and *N*-methyl peptides are useful in the drug discovery process as they can improve the pharmacokinetic properties of biologically active peptides [52]. On this premise, Katritzky and coworkers synthesized di- and tripeptides featuring a sterically hindered amino acid residue such as *N*-methyl

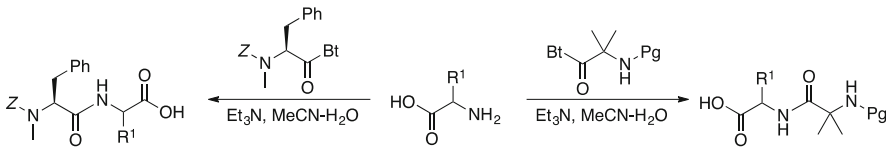


Fig. 6 Preparation of dipeptides containing a C-activated sterically hindered amino acid residue

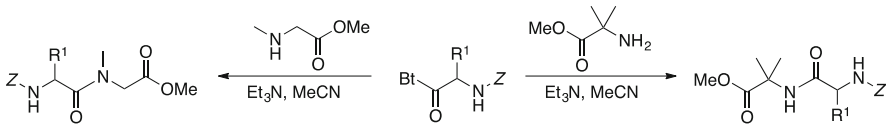


Fig. 7 Preparation of dipeptides by linking the amino group of a sterically hindered amino acid with *N*-(*Z*-α-aminoacyl)benzotriazoles

amino acids *N*(Me)-Phe and *N*(Me)-Gly, as well as the α,α-disubstituted amino acid, α-aminoisobutyric acid (Aib) (Figs. 6 and 7).

The coupling reaction of C-activated sterically hindered and free amino acids proceeded smoothly under the typical conditions for benzotriazole-mediated amide link formation (see Sect. 2.2.1, Figs. 3 and 5). However, the coupling of the amino group of sterically hindered amino acids requires different conditions to that of proteinogenic amino acids. Using the general conditions for benzotriazole-mediated amide bond formation and modified conditions (different reaction times, temperatures, solvent systems, additives and bases), the coupling reactions at the N-terminus of sterically hindered free amino acids with *N*-(*Z*-α-aminoacyl) benzotriazoles resulted largely in the hydrolysis of the *N*-(*Z*-α-aminoacyl) benzotriazoles [48]. Thus, water insoluble hindered amino acid esters were coupled with the *N*-(*Z*-α-aminoacyl)benzotriazoles in the presence of anhydrous THF or MeCN at 20°C for 24–36 h to yield the corresponding dipeptide (Fig. 7). Employing microwave irradiation was found to reduce the reaction time to approximately 1 h. In all cases, enantiopure dipeptides were obtained in fair to excellent yields of 67–93% [48].

2.4 *Difficult Peptides from the Microwave-Assisted Coupling of* N-*(Protected α-Aminoacyl)Benzotriazoles on the Solid Phase*

Solution- and solid-phase syntheses of biologically active peptides and proteins are of particular importance due to their potential use as medicines. Katritzky and coworkers have demonstrated the effectiveness of the *N*-(protected α-aminoacyl)

benzotriazole auxiliaries in the fast assembly of peptides in solution- and solid-phase syntheses by preparing over 215 distinct peptides using diverse methods [42–48, 50, 53–55].

When compared to solution-phase syntheses, solid-phase syntheses of peptides offer several advantages. Formation of the amide linkage via solid-phase methodologies affords significant enhancements in reaction yields and purities, and this is often associated with a concomitant decrease in reaction times [40, 56, 57]. In solid-phase peptide synthesis (SPPS), reactions are driven to completion by utilizing a large excess of reagents, and the completion of each coupling reaction can be conveniently and rapidly monitored by the qualitative Kaiser (ninhydrin) test. Additionally, SPPS eliminates the majority of product losses associated with conventional solution-phase methodologies (filtration, recrystallization, etc.), and it offers fast and easy separation of the product from excess reagents [40, 56, 57]. With better coupling reagents, improved yields, and shorter coupling times, SPPS has become the dominant method for the rapid assembly of peptides over the last 35 years [39].

Microwave irradiation has revolutionized the preparation of a wide variety of organic compounds [58]. The use of microwave irradiation in organic synthesis is primarily known for accelerating reaction times. As such, microwave-assisted synthesis of organic compounds has been applied in SPPS to minimize coupling times (6–20 min) and to improve peptide product yields [59–62].

One challenge in the syntheses of peptides is the preparation of difficult sequences. The primary reason for the occurrence of difficult peptide sequences are incomplete aminoacylation and/or deprotection during the assembly of the peptide. In particular, challenges in amide link formation arise due to steric effects when the two amino acids being linked contain β-branched side chains, for example, isoleucine, valine, and threonine. Secondly, difficult sequences arise when intra- and interchain hydrogen bonding promote the formation of secondary structures. These synthesized peptides may be partially racemized and/or contaminated with deletion sequences, aspartimides, and related side products [56, 63]. Several strategies to alleviate the problems associated with the preparation of difficult sequences have been described in the literature [56, 57].

Having demonstrated wide applicability of the benzotriazole-based auxiliaries in peptide synthesis, Katritzky and coworkers further expanded the scope of this methodology in preparing several difficult peptides. The difficult peptide sequences were assembled on the Rink amide resin via the microwave-accelerated coupling of *N*-(Fmoc-α-aminoacyl)benzotriazoles (Fig. 5). Examples of the difficult peptides, both model and biologically relevant peptides, are illustrated in Table 5 [57].

Table 5 Difficult peptides from the microwave-assisted, benzotriazole-mediated SPPS

Sequence (N-C terminus)	t_R (min)	Crude		After preparative HPLC		HRMS $[M+1]^+$
		% purity	% yield[a]	% purity	% yield	
H-Val-Val-Ser-Val-Val-NH_2	7.57	60	63	>99	7	501.3385
H-Val-Val-Val-Ser-Val-Val-NH_2	9.79	60	34	nd[b]	nd[b]	600.4058
H-Val-Ile-Val-Ile-Gly-OH[c]	10.82	70	40	>99	24	500.3643
H-Thr-Val-Thr-Val-Thr-Val-NH_2	8.70	50	35	89	16	618.3864
H-Val-Lys-Asp-Gly-Tyr-Ile-NH_2	8.31	30	68	>99	24	693.3916
H-Val-Lys-Asp-Val-Tyr-Ile-NH_2	9.10	37	64	>99	29	735.4419
H-Tyr-Gly-Gly-Phe-Leu-NH_2	11.54	80	86	>99	37	555.2948
H-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-NH_2	13.82	28	42	89	22	857.5277

[a]Calculated from the amount of product obtained multiplied by the purity from HPLC
[b]*nd* not determined
[c]Peptide amide hydrolyzed to the corresponding carboxylic acid on purification

3 Cyclic Peptides

Peptides, peptide analogues, and proteins play significant roles in numerous biochemical processes and continue to be attractive targets for novel therapeutics and biomaterials [64, 65]. Recent advances in the preparation of linear peptides, peptide analogues, and proteins have to some extent circumvented several barriers to their utility [39, 56, 66, 67]. Therefore, there has been tremendous growth in the number of previously unattainable peptides and related compounds available in the medicinal chemistry toolbox. However, even with major synthetic breakthroughs, the portion of peptide-based drugs on the market has lagged behind. The primary reason for this underutilization of peptide-based drugs is their lack of in vivo stability [68].

On the contrary, their cyclic counterparts have attracted considerable interest given their tunable lipophilicity and resistance to enzymatic degradation. Cyclic peptides display a wide array of biological activity, including but not limited to immunosuppressive, anti-tumor, and antibacterial properties. Additionally, cyclic peptides that contain bioactive hormone sequences are suitable models for studying conformation–activity relationships [69].

Several synthetic methods are available for the preparation of cyclic peptides. Commonly used methods are (1) the intramolecular head-to-tail condensation between the amino and carboxyl groups at the N- and C-termini of the matching linear peptide, (2) intermolecular coupling of two symmetrical peptides and (3) methods devoid of peptide coupling [64, 65].

In exploiting several areas of expertise, Katritzky and coworkers have synthesized a plethora of cyclic oligopeptides. Now presented are the benzotriazole-mediated preparations of cyclic peptides.

3.1 Benzotriazole-Mediated Preparation of 2,5-Diketopiperazines

2,5-Diketopiperazines (2,5-DKPs) represent the simplest class of naturally occurring, bioactive cyclic peptides and are obtained from the condensation of two α-amino acids [69–71]. These cyclodipeptides, also known as 2,5-dioxopiperazines or anhydride dipeptides, are biosynthesized by several organisms, for example, bacteria, fungi, plants, and mammals. The 2,5-DPK motifs can be found alone or as a part of larger, more complex structures. Additionally, they are frequently made from the degradation of polypeptides in processed goods and are observed as undesired side products resulting from the synthesis of peptide construct [69–71].

2,5-DPKs have garnered widespread attention as their particular heterocyclic subunit makes them a promising source for new drug candidates. Diversity can be introduced at all six positions of these small, conformationally restricted heterocycles with stereocontrol at up to four positions [69–71]. They are stable to proteolysis and have the ability to bind to multiple types of receptors. As a result of these characteristics, they possess cytotoxic, neuroprotective, antifungal, antimalarial, antibacterial, antiviral, plant retardation, and other properties [72–85]. They have been used as scaffolds for the construction of combinatorial libraries, and recent breakthroughs in SPPS have made them even more attractive drug targets.

2,5-DKPs are readily available from the head-to-tail condensation of α-amino acids. However, this reaction is challenging as the cyclic dimer can be formed exclusively or in conjunction with the desired cyclic monomer [64]. Gottesfeld and coworkers found that carrying out the reaction under dilute conditions could reduce the formation of the cyclic dimer [86]. A difficulty that poses more of a challenge is being able to bring the N- and C-termini in close proximity by prompting a β-turn in the peptide. However, amino acid residues such as Pro and Gly, D-amino acids, *N*-methyl amino acids, and other unnatural amino acids or non-proteinogenic are known to form *cis* peptide linkages and can promote β-turns in peptide sequences [71, 87–90].

Head-to-tail condensation of dipeptides typically requires long reaction times and harsh conditions [91, 92]. Generally, the 2,5-DKPs are formed in low yields with low enantiopurity and are often contaminated with other side products [93–99]. Historically, traceless Staudinger ligation has been a useful tool for the preparation of medium and large cyclic peptides until Katritzky and coworkers pioneered the application of the traceless Staudinger ligation for the synthesis of small cyclopeptides, for example, 2,5-DKPs [69].

Table 6 Optimization of the Staudinger cyclization for the preparation of cyclo(glycyl-L-leucyl)

	Conditions					
Entry	X_3	Solvent	Equiv. of H_2O	rt or MW	Time (min)	Yield (%)
1	Bu	CH_2Cl_2	–	rt	720	55
2[a,b]	Bu	CH_2Cl_2	–	MW	30	66
3[b]	Bu	CH_2Cl_2	5	MW	5	78
4[b]	Bu	THF	5	MW	5	70
5[b]	Bu	MeCN	5	MW	5	65
6[b]	Bu	Toluene	5	MW	5	63
7[b]	Ph	CH_2Cl_2	5	MW	5	58

[a]Reaction was quenched with water
[b]Reaction was carried out at 50°C and 50 W

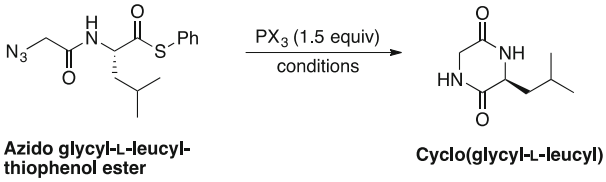


Fig. 8 Head-to-tail cyclization of azido glycyl-L-leucyl thiophenyl ester

Katritzky and coworkers have utilized the traceless Staudinger ligation for the preparation of 2,5-DKPs via solid- and solution-phase methods. This atom-economical yet inexpensive approach features mild reaction conditions for the fast and expedient tandem deprotection and cyclization steps [69].

By modifying established procedures, the starting material, azido glycyl-L-leucyl thiophenyl ester, was prepared in 65% yield over three steps from chloroacetyl chloride and L-Leu. Further reaction of the azido-protected dipeptide thioester with phosphines under microwave acceleration afforded the cyclic peptide (Table 6) [69]. The pure cyclic peptide was separated from the reaction mixture by filtration and required no further purification. Examining the effect of reaction time, solvent, and phosphine on the product yield and purity assisted in the optimization of the Staudinger-mediated cyclization. In addition, the reaction was carried out in the presence or absence of water and microwave irradiation (Table 6). The highest yields and shortest reaction times were obtained when tributylphosphine was reacted with the azido thioester for 5 min. in the presence of dichloromethane, five equivalents of water and under microwave irradiation (Fig. 8) [69].

Two additional examples of 2,5-DKPs were prepared using the optimized reaction conditions described above (Fig. 9, Table 7). Following the successful Staudinger-mediated synthesis of small cyclic peptides in solution, Katritzky and coworkers demonstrated the scope of the method by applying it to the solid-phase preparation of several cyclic peptides (Fig. 9, Table 7) [69]. Using standard DCC coupling procedures, *N*-(Boc-protected aminoacyl)benzotriazoles were added to the 3-thiopropanoic acid linker of a modified aminomethyl (AM) resin. Boc

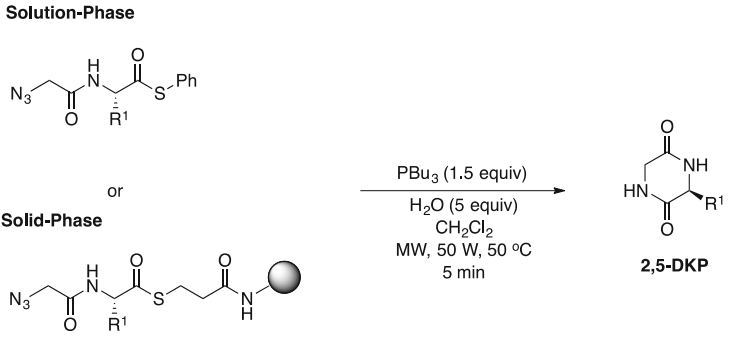


Fig. 9 Solution- and solid-phase preparation of 2,5-diketopiperazines (2,5-DKPs)

Table 7 Examples of 2,5-diketopiperazines prepared by solution- and solid-phase Staudinger cyclization methods

Entry	R^1	Method	Yield (%)
1	*i*-Bu	Solution	78
2[a]	Bn	Solution	74
3	Me	Solid	78
4	*i*-Bu	Solid	79
5	Bn	Solid	72
6	Me	Solid	81
7[a]	Bn	Solid	82

[a]Linker-AM resin was recovered and reused

deprotection and successive coupling with azido glycyl benzotriazole afforded the solid supported linear sequence. The cleavage and subsequent cyclization to the corresponding 2,5-DKP was achieved by subjecting the solid supported material to the optimized solution-phase conditions (Table 6, entry 3) [69]. Filtration, treatment of the residue with hot MeOH, and then recrystallization afforded the enantiopure 2,5-DKP. When compared with the solution-phase Staudinger ligation, the solid-phase method gave similar or slightly better yields. Furthermore, the modified AM resin could be recovered and reused in subsequent cyclization with no significant loss in activity [69].

3.2 *Hetero-2,5-Diketopiperazines from Proline-Containing Cbz-*N*-Protected Dipeptidoyl Benzotriazoles*

The majority of naturally occurring 2,5-diketopiperazines (2,5-DKPs) are obtained from L-α-amino acids and therefore possess a *cis* configuration (Fig. 10) [70, 71]. In addition to the previously described Staudinger ligation method for the preparation of cyclic peptides, Katritzky and coworkers have developed a *cis*-selective tandem deprotection/cyclization sequence for the synthesis of *cis*-2,5-diketopiperazines [71].

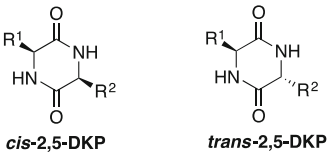


Fig. 10 *cis*- and *trans*-2,5-DKP (diketopiperazine)

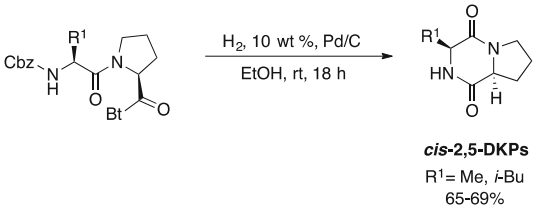


Fig. 11 Preparation of *cis*-DKPs by tandem deprotection/cyclization

Starting with Cbz-dipeptidoyl benzotriazoles, the one-pot, two-step, condensation–deprotection sequence proceeded smoothly to afford the *cis*-DKPs. L-Proline-containing dipeptidoyl benzotriazoles were converted to *cis*-DKPs in the presence of Pd/C (10 wt%) and hydrogen gas in 65–69% yields (Fig. 11) [71]. The stereochemistry of the *cis*-DKPs was confirmed via optical rotation and NMR spectroscopy.

Over the last couple of years, *trans*-diketopiperazines (*trans*-DKPs) have been gaining increasing attention due to their potential as anti-HIV, anticancer, and antibiotic agents [100–102]. The works of several researchers indicate that *trans*-DKPs can serve as foldamer building blocks [103]. Peptide drugs that have *trans*-DKPs incorporated into the backbone may be more potent due to the slower enzymatic degradation of DKPs [104]. Also, peptide drugs with a *trans*-DKPs backbone may be more selective for specific substrates [97].

Several strategies have been used in the synthesis of *trans*-DKPs. The most straightforward method requires the use of unnatural amino acid, D-proline [101, 105–107]. Alternatively, access to *trans*-DKPs can be obtained by epimerization of the corresponding *cis*-DKPs, although epimerization is frequently untended and occurs upon cyclization of the *cis*-DKP precursor or upon further functionalization of the *cis*-DKP [108–111]. Unintentional epimerization is inefficient and leads to *cis*/*trans*-DKP mixtures. Ultimately, the *trans*-DKPs are obtained in low yields.

Katritzky and coworkers introduced a more flexible and inexpensive method for the preparation of *trans*-DKPs. The sequence began with the synthesis of dipeptides from *N*-(Cbz-α-aminoacyl)benzotriazoles and L-proline via established procedures. The dipeptides were then smoothly converted to the corresponding benzotriazole derivatives [71]. In the presence of 1 equiv. of Et_3N, the Cbz-L-aa-L-Pro-Bt derivatives were transformed to the protected *trans*-DKPs in 69–75% yields. As a proof of concept, a protected *trans*-DKP was deprotected with hydrogen in the presence of Pd/C to afford the trans-DKPs (Fig. 12) [71].

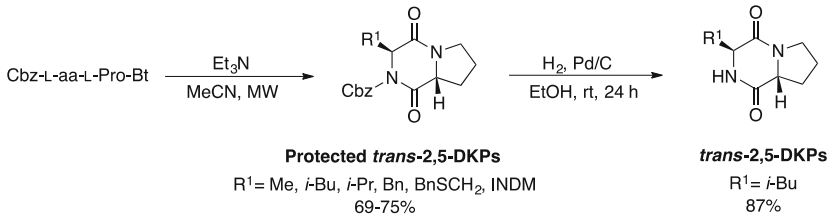


Fig. 12 Tandem cyclization/epimerization of dipeptidoyl benzotriazoles to afford *trans*-DKPs

Computational studies provided insight into the possible mechanisms for the transformations. Two possible mechanisms were proposed: The first was a unimolecular (unassisted) mechanism, in which the triazole leaving group functions as a base. The second scenario involves a bimolecular assisted mechanism where the amine base (Me_3N used as a model for Et_3N) abstracted a proton and was used to induce cyclization. Regardless of the pathway, as expected, the most stable conformation of the dipeptidoyl benzotriazole was twisted into a ready-to-cyclize conformation [71]. However, the bimolecular pathway had a lower activation barrier than the unassisted unimolecular pathway. Generally, the formation of the *trans*-DKP is thermodynamically favored over the *cis*-DKP. The formation of the *trans*-DKP from the dipeptidoyl benzotriazole is thought to occur via enolization and an ensuing protonation on the *Si*-face of the DKPs. In the case of the *cis*-DKP, it was found that the Cbz protecting group played a significant role in lowering the energy difference between the *cis*-DKP and the corresponding enol [71].

Having explored the preparation of DKPs, Katritzky and coworkers expanded the Staudinger-mediated ring closure to the synthesis of difficult medium-sized cyclic peptides. Several, novel 7- and 8-membered cyclic dipeptides and 10-membered cyclic tripeptides were prepared from azido peptide thioesters in yields of 48–75% [112]. Additionally, computational studies provided insight into the reactivity as a function of ring size [112].

4 *O*- and *S*-Acyl Isopeptide Strategies for the Preparation of Difficult Peptides

Since Emil Fisher's [113] pioneering work in the field of peptide synthesis at the turn of the twentieth century, tremendous progress has been made in the syntheses of peptides [4–6, 39]. Despite the plethora of peptide coupling reagents, utilization of microwave acceleration, and other advances in solution- and solid-phase peptide synthesis, the preparation of complex peptides with high purity and yields still poses a challenge. Typically, convergent peptide synthesis methods feature a reduction in the number of synthetic steps and as such are popular due to increased yields and easy purification. Such exploitation of chemoselectivity during peptide

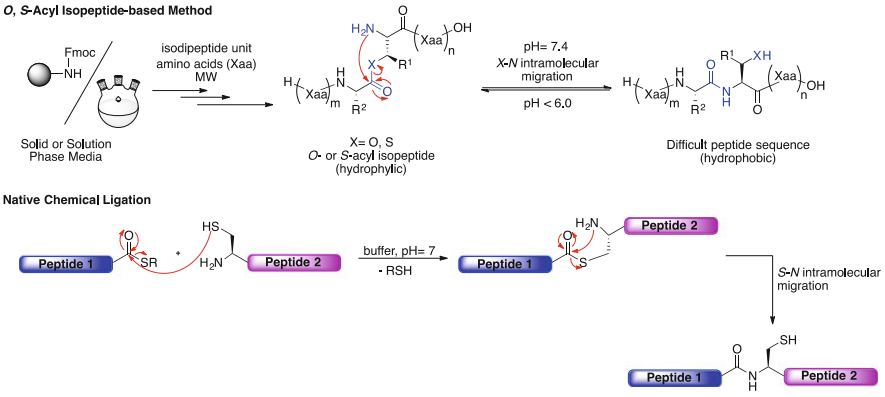


Fig. 13 Representations of *O*- and *S*-acyl isopeptide method and native chemical ligation (NCL)

synthesis affords the rapid assembly of enantiopure peptides under facile conditions that reduces the need for protecting groups. Chemoselective peptide synthesis strategies have focused on the use of naturally occurring amino acids cysteine (Cys, C), serine (Ser, S), and threonine (Thr, T). Cysteine possesses the nucleophilic β-sulfhydryl side chain, while both serine and threonine feature a hydroxyl side group. Over the last 30 years, extensive research effort on chemoselective transformations involving these three amino acid residues has led to the development of two primary methods for easier peptide chain extensions. The methods are the *O*- and *S*-acyl isopeptide-based strategy and native chemical ligation (NCL) [114–120]. When the *O*- and *S*-acyl isopeptide-based strategy for peptide synthesis are used, peptides are prepared using fully or partially protected amino acids and conventional peptide synthesis; however, native chemical ligation involves the condensation of unprotected peptide fragments (Fig. 13). Ultimately, the *O*- and *S*-acyl isopeptide-based strategies and native chemical ligation (NCL) feature an *X*-to-*N*-acyl transfer (X=*O*, *S*) leading to the formation of peptides that possess all native amide linkages [121–126]. This section will cover the *O*- and *S*-acyl isopeptide-based strategies. For further details regarding native chemical ligation, see [127].

4.1 Isopeptides and Isodipeptides in Peptide Synthesis

Isopeptides are frequently referred to as click peptides, switch peptides, or depsipeptides and are characterized by an isopeptide bond [128]. An isopeptide bond is formed when a peptide chain extension occurs at a lateral amino acid side chain (e.g. Ser, Thr, Cys) as opposed to the α-amide backbone of the growing peptide [129]. While Ohfune and coworkers established the basis for *O*- and *S*-acyl isopeptide strategies [130] in the 1990s, it wasn't until the close of the twentieth

century that *O*- and *S*-acyl isopeptides emerged as a solution for the syntheses of difficult peptides [131–135]. When compared to native peptides, isopeptides tend to have greater solubility and are therefore less prone to aggregation. The introduction of the (thio)ester linkage into the growing peptide interrupts the usual hydrogen-bonding network and allows additional degrees of freedom [131–135]. As a result, isopeptide linkages have been broadly utilized in the preparation of difficult peptides. Additionally, isopeptide rearrangements have found great utility in polymer science and have provided insight into protein folding and aggregation and the relationship between peptide conformation and catalytic activity.

In the early part of the twenty-first century, Carpino, Kiso, and Mutter elegantly illustrated the application of *O*-isopeptide strategies [131–135]. Generally, the peptide is assembled using standard Fmoc-based SPPS. On completion of the sequence, the isopeptide is cleaved from the resin and stored as the TFA salt as no *O*-to-*N*-acyl shift is anticipated with protonated Ser, Thr, or Cys residues. Subsequent adjustment of the pH to 6.2–7.4 at ambient temperature promotes the rapid, quantitative *O*-to-*N*-acyl transfer (Fig. 13) [116, 136, 137]. At a pH of <6.0, the reverse reaction, the *N*-to-*O*-acyl transfer, will occur [138]. In addition to the pH-triggered *O*-to-*N*-acyl transfer, photolabile protecting groups, for example, 6-nitroveratryloxycarbonyl (Nvoc) introduced by Patchornik, Amit, and Woodward, have been shown to promote the clean *O*-to-*N*-acyl rearrangement in the presence of UV irradiation [139–142].

Generally, *O*-to-*N*-acyl transfers occur more rapidly with Thr as opposed to Ser amino acid residues [143, 144]. More information regarding the kinetics of the process was uncovered by Stella and coworkers [145, 146]. Although advantageous, the classical *O*-to-*N*-acyl strategy was plagued by several obstacles. From Carpino's work, it was apparent that the early inclusion of the isopeptide resulted in an easier synthesis of the difficult peptide; however, racemization could occur in up 23% of the peptide [135]. Along with epimerization, other side reactions included the formation of deletion sequences and 2,5-diketopiperazines (DKPs) (Fig. 14). However, Kiso and coworkers' subsequent improvement to the *O*-to-*N*-acyl strategy via incorporation of an isodipeptide eliminated or substantially reduced side reactions [139, 144]. Of note, using the *O*-acyl isodipeptide method can result in deletion of the Ser or Thr residue in the presence of a base. This reaction appears to be solvent dependent, and it can be avoided via changes in the solvents and/or activation method.

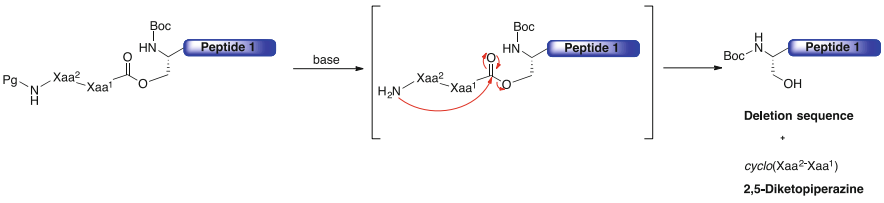


Fig. 14 2,5-Diketopiperazine and deletion sequence formation under classical *O*-acyl isopeptide conditions

Kiso and coworkers further extended the *O*-acyl isopeptide strategies to *S*-acyl isopeptides [147]. Unlike their oxygen counterparts, *S*-acyl isopeptides display a lower tendency for aggregation; therefore, they are easier to prepare and purify. Building on their improvements to the *O*-acyl isopeptide methods, they added to the *S*-acyl isopeptide method by using the allyl-based protective Alloc group for the synthesis of *S*-acyl isopeptides [148].

4.2 *Application of* N-*(Protected α-Aminoacyl)Benzotriazoles in the Synthesis of Peptides via the* O- *and* S-*Acyl Isopeptide Strategy*

Further contributions by Katritzky and coworkers expanded the *O*-to-*N*-isodipeptide methodology. In general, *O*-to-*N*-acyl rearrangements occur via a 5-membered cyclic transition state. However, Katritzky and coworkers prepared peptides of high yields and purities, facilitated by rearrangements involving 8- and 11-membered transition states [129]. These transformations occurred at 50°C in DMF in the presence of microwave irradiation (50 W). For example, the native peptide yields obtained from the rearrangement of the *O*-acyl isopeptide via the 8-membered transition state were quite notable and ranged from 57 to 78%, indicating that the nature of the amino acid residue acting as the nucleophile and not the nature of the amino acid esterifying the *O*-acyl isopeptide had a tremendous effect on the yield. Thus, the preorganization of the initial *O*-acyl isopeptide significantly affected the success of these long-range rearrangements [129]. In contrast to the *O*-to-*N*-acyl rearrangements, *S*-to-*N*-acyl transfers were facilitated by 17–20-membered macrocyclic transition states [149]. In fact, the formation of 8-, 11-, and 14 membered cyclic transition states posed a challenge. The yields and rates of the *S*-to-*N*-acyl rearrangements were strongly correlated to the size of the macrocyclic transition state [150–153]. As with the *O*-acyl isopeptide, the preorganization of the starting oligo *S*-acyl isopeptide had a large influence on the long-range acyl transfers [154]. Generally, analysis of the experimental data can be problematic as the ligation product may be contaminated by a range of side products [150, 152, 153, 155]. Thus, to facilitate the interpretation of results, a computational study of the long-range rearrangements in *S*-acyl isopeptides was designed [149]. Enthalpic effects were found to be the driving force for reactions involving 5–20-membered transition states. Again, the nature of the amino acid substituent was negligible. Finally, it was determined that the introduction of turn inducers may lower the activation barrier for long-range *S*-to-*N*-acyl rearrangements by promoting hydrogen-bonding interactions [149].

5 Benzotriazole Activation and Coupling of Amino Acids on a Preparative Scale

The widespread use of benzotriazole-mediated methods in the synthesis of peptides and their analogues hinges on the ability to scale up these processes. Frequently, microwave irradiation is required to accelerate the coupling steps, so the large-scale preparation of peptides and peptide conjugates from *N*-(protected-α aminoacyl) benzotriazoles can be difficult. Until recently there was no literature precedence for the use of *N*-(protected-α aminoacyl)benzotriazoles in conjunction with continuous flow methods. Now, as a proof of concept, Stevens and coworkers have reported the large-scale preparation of a library of (α-aminoacyl)amino-substituted heterocycles via a two-step continuous flow process [156]. The first step involves the benzotriazole activation of *N*-(Cbz-α-aminoacyl)benzotriazoles under continuous flow conditions. No racemization of the products was observed, and yields of 71–99% were obtained under optimized reaction conditions (solvent, time, temperature, reagents) [156]. The second step, the reaction of the *N*-(Cbz-α-aminoacyl) benzotriazole derivatives and heterocyclic amines in the presence of DMSO afforded (α-aminoacyl)amino-substituted heterocycles in yields of 40–99% [156].

Additionally, Stevens and coworkers explored telescoping the coupling step with the benzotriazole activation; however, the inconvenient work-up associated with the tandem process resulted in product losses and difficulties with product purification. In addition to increased yields and easier purification, the two-step, continuous flow method facilitates free solvent selection for each step, thereby enabling the maximum possible product yields [156].

6 Conclusion

N-(Protected-α aminoacyl)benzotriazoles are useful reagents for the preparation of a wide range of peptides via solution- and solid-phase peptide synthesis methods. These reagents have made important contributions to the field of peptide synthesis by facilitating the rapid assembly of peptides and peptide conjugates under mild conditions, with good to excellent yields and purities. Importantly, the prepared peptide and peptide conjugates are enantiopure. Microwave-assisted, benzotriazole-mediated synthesis of peptides has provided greater and more rapid access to cyclic and difficult peptides. Additionally, the *O*- and *S*-acyl isopeptide strategies have been an instrumental gateway to large difficult peptides, which are usually prone to aggregation. In order for the benzotriazole-based peptide synthesis platform to be widely applicable, the reagents and methods must be amenable to scale up. Recently, Stevens and coworkers demonstrated the scalability of such processes by preparing a library of (α-aminoacyl)amino-substituted heterocycles.

Despite significant improvements in accessing important peptides and peptide conjugates, our knowledge of protein–protein interactions and our ability to

elucidate protein structure, there is still more to do in order to reduce the barrier to drug development for many of the world's critical diseases. Exploiting acylbenzotriazoles as allies for the construction of peptides and peptide conjugates and the development of such methods is a step in the right direction.

References

1. Bruno BJ, Miller GD, Lim CS (2013) Basics and recent advances in peptide and protein drug delivery. Ther Deliv 4(11):1443–1467
2. Dunn BM (ed) (2015) Peptide chemistry and drug design. Wiley, New Jersey
3. Fischer G, Rossmann M, Hyvönen M (2015) Alternative modulation of protein–protein interactions by small molecules. Curr Opin Biotechnol 35:78–85
4. Ramakers BEI, van Hest JCM, Löwik DWPM (2014) Molecular tools for the construction of peptide-based materials. Chem Soc Rev 43(8):2743–2756
5. Palomo JM (2014) Solid-phase peptide synthesis: an overview focused on the preparation of biologically relevant peptides. RSC Adv 4(62):32658–32672
6. Tang W, Becker ML (2014) "Click" reactions: a versatile toolbox for the synthesis of peptide-conjugates. Chem Soc Rev 43(20):7013–7039
7. Chen M, Heimer P, Imhof D (2015) Synthetic strategies for polypeptides and proteins by chemical ligation. Amino Acids 47(7):1283–1299
8. Vanier GS (2013) Microwave-assisted solid-phase peptide synthesis based on the Fmoc protecting group strategy (CEM). Methods Mol Biol 1047:235–249
9. Chow HY, Li X (2015) Development of thiol-independent peptide ligations for protein chemical synthesis. Tetrahedron Lett 56:3715–3720
10. Malins LR, Payne RJ (2015) Synthetic amino acids for applications in peptide ligation–desulfurization chemistry. Aust J Chem 68(4):521–537
11. Ibrahim TS, Tala SR, El-Feky SA, Abdel-Samii ZK, Katritzky AR (2011) Benzotriazole reagents for the syntheses of Fmoc-, Boc- and Alloc-protected amino acids. Synlett 14:2013–2016
12. Ibrahim TS, Tala SR, El-Feky SA, Abdel-Samii ZK, Katritzky AR (2012) Cysteinoyl- and cysteine-containing dipeptidoylbenzotriazoles with free sulfhydryl groups: easy access to N-terminal and internal cysteine peptides. Chem Biol Drug Des 80(2):194–202
13. Butula I, Lj Č, Proštenik MV, Vela V, Zorko F (1977) Reaktion mit 1-chlorocarbonylbenzotriazol; II[1]. Synthese von Carbamidsäure-estern und verwandten. Synthesis 1977(10):704–706
14. Paio A, Zaramella A, Ferritto R, Conti N, Marchioro C, Seneci P (1999) Solid-supported benzotriazoles: synthetic auxiliaries and traceless linkers for the combinatorial synthesis of amine libraries. J Comb Chem 1(4):317–325
15. Dener JM, Rice KD, Newcomb WS, Wang VR, Young WB, Gangloff AR, Kuo EY-L, Cregar L, Putnam D, Wong M (2001) Dibasic inhibitors of human mast cell tryptase. Part 3: identification of a series of potent and selective inhibitors containing the benzamidine functionality. Bioorg Med Chem Lett 11:1629–1633
16. Kumareswaran R, Hassner A (2001) Asymmetric syntheses of *N*-acetyl-(*R*)-coniine and *N*-Boc-(2*R*,6*R*)-solenopsin A via ring-closing metathesis. Tetrahedron Asymmetry 12:2269–2276
17. Wang J, Liang Y-L, Qu J (2006) Boiling water-catalyzed neutral and selective *N*-Boc deprotection. Chem Commun 5144–5146
18. Wünsch E, Graf W, Keller O, Keller W, Wersin G (1986) On the synthesis of benzyloxycarbonyl amino acids. Synthesis 1986(11):958–960

19. Akamatsu M, Fujimoto Y, Kataoka M, Suda Y, Kusumoto S, Fukase K (2006) Synthesis of lipid A monosaccharide analogues containing acidic amino acid: exploring the structural basis for the endotoxic and antagonistic activities. Bioorg Med Chem 14:6759–6777
20. Smith MB, March J (2007) 20 Advanced organic chemistry: reactions mechanism and structure, 6th edn. Wiley, Hoboken
21. Paquet A (1982) Introduction of 9-fluorenylmethyloxycarbonyl, trichloroethoxycarbonyl, and benzyloxycarbonyl amine protecting groups into O-unprotected hydroxyamino acids using succinimidyl carbonates. Can J Chem 60(8):976–980
22. Ten Kortenaar PBW, Van Dijk BG, Peeters JM, Raaben BJ, Adams PJHM, Tesser GI (1986) Rapid and efficient method for the preparation of Fmoc-amino acids starting from 9-fluorenylmethanol. Int J Pept Protein Res 27(4):398–400
23. Lapatsanis L, Milias G, Froussios K, Kolovos M (1983) Synthesis of *N*-2,2,2-(Trichloroethoxycarbonyl)-L-amino acids and *N*-(9-fluorenylmethoxycarbonyl)-L-amino acids involving succinimidoxy anion as a leaving group in amino acid protection. Synthesis 1983 (8):671–673
24. Chinchilla R, Dodsworth DJ, Najera C, Soriano JM (2001) A new polymer-supported reagent for the Fmoc-protection of amino acids. Tetrahedron Lett 42(43):7579–7581
25. Bogdan FM, Chow CS (1998) The synthesis of allyl- and allyloxycarbonyl-protected RNA phosphoramidites. Useful reagents for solid-phase synthesis of RNAs with base-labile modifications. Tetrahedron Lett 39(14):1897–1900
26. Hayakawa Y, Wakabayashi S, Kato H, Noyori R (1990) The allylic protection method in solid-phase oligonucleotide synthesis. An efficient preparation of solid-anchored DNA oligomers. J Am Chem Soc 112(5):1691–1696
27. Henklein P, Heyne H-U, Halatsch W-R, Niedrich H (1987) 5-Norbornene-2,3-dicarboximido carbonochloridate. A new stable reagent for the introduction of amino-protecting groups. Synthesis 1987(2):166–167
28. Hlebowicz E, Andersen AJ, Andersson L, Moss BA (2005) Identification of Fmoc-β-Ala-OH and Fmoc-β-Ala-amino acid-OH as new impurities in Fmoc-protected amino acid derivatives. Synthesis 65(1):90–97
29. Zalipsky S (1998) Alkyl succinimidyl carbonates undergo Lossen rearrangement in basic buffers. Chem Commun 1:69–70
30. Isidro-Llobet A, Just-Baringo X, Ewenson A, Alvarez M, Albericio F (2007) Fmoc-2-mercaptobenzothiazole, for the introduction of the Fmoc moiety free of side-reactions. Biopolymers 88(5):733–737
31. Carpino LA, Han GY (1972) 9-Fluorenylmethoxycarbonyl amino-protecting group. J Org Chem 37(22):3404–3409
32. Grzonka Z, Lammek B (1974) A simple method of preparation of *t*-butoxycarbonyl amino acids. Synthesis 1974(9):661–662
33. Tessier M, Albericio F, Pedroso E, Grandas A, Eritja R, Giralt E, Granier C, Van Rietschoten J (1983) Amino-acids condensations in the preparation of *N*α-9-fluorenylrnethyloxycarbonylamino-acids with 9-fluorenylmethylchloroformate. Int J Pept Protein Res 22(1):125–128
34. Cruz LJ, Beteta NG, Ewenson A, Albericio F (2004) "One-Pot" preparation of *N*-carbamate protected amino acids via the azide. Org Process Res Dev 8(6):920–924
35. Katritzky AR, Fali CN, Li J, Ager DJ, Prakash I (1997) Synthesis of 1-(*T*-butoxycarbonyl) benzotriazole and 1-(*p*-methoxybenzyloxycarbonyl)benzotriazole and their use in the protection of amino acids. Synth Commun 27(9):1623–1630
36. Katritzky AR, Zhang G-F, Fan W-Q, Wu J, Pernak J (1993) Studies on the thermal decarboxylation of 1-alkoxycarbonylbenzotriazoles. J Phys Org Chem 6(10):567–573
37. Yang C-W (2012) A comparative study of short linear motif compositions of the influenza A virus ribonucleoproteins. PLoS One 7(6), e38637
38. Searle MS, Williams DH, Packman LC (1995) A short linear peptide derived from the N-terminal sequence of ubiquitin folds into a water-stable non-native β-hairpin. Nat Struct Biol 2:999–1006

39. El-Faham A, Albericio F (2011) Peptide coupling reagents, more than a letter soup. Chem Rev 111:6557–6602
40. Katritzky AR, Angrish P, Todadze E (2009) Chiral acylation with N-(protected α-aminoacyl) benzotriazoles for advantageous syntheses of peptides and peptide conjugates. Synlett 2009 (15):2392–2411
41. Chandrudu S, Simerska P, Toth I (2013) Chemical methods for peptide and protein production. Molecules 18(4):4373–4388
42. Katritzky AR, Suzuki K, Singh SK (2004) Highly diastereoselective peptide chain extensions of unprotected amino acids with N-(Z-α-aminoacyl)benzotriazoles. Synthesis 2004 (16):2645–2652
43. Katritzky AR, Angrish P, Suzuki K (2006) The efficient preparation of di- and tripeptides by coupling N-(Cbz- or Fmoc-α-aminoacyl)benzotriazoles with unprotected amino acids. Synthesis 2006(3):411–424
44. Katritzky AR, Meher G, Angrish P (2006) Peptides by extension at the N- or C-termini of lysine. Chem Biol Drug Des 68(6):326–333
45. Katritzky AR, Todadze E, Cusido J, Angrish P, Shestopalov AA (2006) Selective peptide chain extension at the N-terminus of aspartic and glutamic acids utilizing 1-(N-protected-α-aminoacyl)benzotriazoles. Chem Biol Drug Des 68(1):37–41
46. Katritzky AR, Todadze E, Shestopalov AA, Cusido J, Angrish P (2006) Selective peptide chain extension at the C-terminus of aspartic and glutamic acids utilizing N-protected (α-aminoacyl)benzotriazoles. Chem Biol Drug Des 68(1):42–47
47. Katritzky AR, Meher G, Narindoshvili T (2008) Efficient synthesis of peptides by extension at the N- and C-termini of arginine. J Org Chem 73(18):7153–7158
48. Katritzky AR, Todadze E, Angrish P, Draghici B (2007) Efficient peptide coupling involving sterically hindered amino acids. J Org Chem 72(15):5794–5801
49. Katritzky AR, Khashab NM, Yoshioka M, Haase DN, Wilson KR, Johnson JV, Chung A, Haskell-Luevano C (2007) Microwave-assisted solid-phase peptide synthesis utilizing N-Fmoc-protected (α-aminoacyl)benzotriazoles. Chem Biol Drug Des 70(5):465–468
50. Katritzky AR, Yoshioka M, Narindoshvili T, Chung A, Khashab NM (2008) N-Fmoc-protected(α-dipeptidoyl)benzotriazoles for efficient solid-phase peptide synthesis by segment condensation. Chem Biol Drug Des 2(3):182–188
51. Isao A, Rolf A, Stefan K, Julius R Jr (1995) Synthesis and coupling reactions of α, α-dialkylated amino acids with nucleobase side chains. Proc Natl Acad Sci 92 (26):12013–12016
52. Chatterjee J, Laufer B, Kessler H (2012) Synthesis of *N*-methylated cyclic peptides. Nat Protoc 7(3):432–444
53. Katritzky AR, Wang M, Yang H, Zhang S, Akhmedov NG (2002) 1-(α-Boc-aminoacyl) benzotriazoles: stable chiral α-aminoacylation reagents. Arkivoc (viii):134–142
54. Katritzky AR, Vincek AS, Suzuki K (2005) Microwave-assisted synthesis of peptidyl phosphorus ylides. Arkivoc (v):116–126
55. Katritzky AR, Angrish P (2006) Efficient microwave assisted access to chiral O-(α-protected-aminoacyl)steroids. Steroids 71(8):660–669
56. Chan WC, White PD (2000) Basic principles. In White PD (ed) Fmoc solid phase peptide synthesis, a practical approach. University Press, New York
57. Katritzky AR, Haase DN, Johnson JV, Chung A (2009) Benzotriazole-assisted solid-phase assembly of Leu-enkephalin, amyloid β segment 34–42, and other "Difficult" peptide sequences. J Org Chem 74(5):2028–2032
58. Monroc S, Feliu L, Planas M, Bardaji E (2006) Microwave-assisted cyclization of peptides on SynPhase™ lanterns. Synlett 2006(9):1311–1314
59. Erdélyia M, Gogoll A (2002) Rapid microwave-assisted solid phase peptide synthesis. Synthesis 2002(11):1592–1596
60. Gorske BC, Jewell SA, Guerard EJ, Blackwell HE (2005) Expedient synthesis and design strategies for new peptoid construction. Org Lett 7(8):1521–1524

61. Yu HM, Chen ST, Wang KT (1992) Enhanced coupling efficiency in solid-phase peptide synthesis by microwave irradiation. J Org Chem 57(18):4781–4784
62. Matsushita T, Hinou H, Fumoto M, Kurogochi M, Fujitani N, Shimizu H, Nishimura S-I (2006) J Org Chem 71(8):3051–3063
63. Mergler M, Dick F, Sax B, Stähelin C, Vorherr TJ (2003) The aspartimide problem in Fmoc-based 56 Part II. J Peptide Sci 9(8):518–526
64. Humphrey JM, Chamberlin AR (1997) Chemical synthesis of natural product peptides: coupling methods for the incorporation of noncoded amino acids into peptides. Chem Rev 97(6):2243–2266
65. Han S-Y, Kim Y-A (2004) Recent development of peptide coupling reagents in organic synthesis. Tetrahedron 60(11):2447–2467
66. Pourvali A, Cochrane JR, Hutton CA (2014) A new method for peptide synthesis in the N→C direction: amide assembly through silver-promoted reaction of thioamides. Chem Commun 50(100):15963–15966
67. Bray BL (2003) Large-scale manufacture of peptide therapeutics by chemical synthesis. Nat Rev Drug Discov 2:587–593
68. Avan I, Hall CD, Katritzky AR (2014) Peptidomimetics *via* modifications of amino acids and peptide bonds. Chem Soc Rev 43(10):3575–3594
69. Beagle LK, Hansen FK, Monbaliu J-CM, DesRosiers MP, Phillips AM, Stevens CV, Katritzky AR (2012) Efficient synthesis of 2,5-diketopiperazines by staudinger-mediated cyclization. Synlett 23(16):2337–2340
70. Borthwick AD (2012) 2,5-Diketopiperazines: synthesis, reactions, medicinal chemistry, and bioactive natural products. Chem Rev 112(7):3641–3716
71. Monbaliu J-CM, Hansen MK, Beagle LK, Panzner MK, Steel PJ, Todadze E, Stevens CV, Katritzky AR (2012) A new benzotriazole-mediated stereoflexible gateway to hetero-2,5-diketopiperazines. Chem Eur J 18(9):2632–2638
72. Balboni G, Guerrini R, Salvadori S, Tomatis R, Bryant SD, Bianchi C, Attila M, Lazarus LH (1997) Opioid diketopiperazines: synthesis and activity of a prototypic class of opioid antagonists. Biol Chem 378(1):19–29
73. Bryant SD, Balboni G, Guerrini R, Salvadori S, Tomatis R, Bianchi C, Attila M, Lazarus LH (1997) Opioid diketopiperazines: refinement of the δ opioid antagonist pharmacophore. Biol Chem 378(2):107–114
74. Crescenzi O, Fraternali F, Picone D, Tancredi T, Balboni G, Guerrini R, Lazarus LH, Salvadori S, Temussi PA (1997) Design and solution structure of a partially rigid opioid antagonist lacking the basic center — models of antagonism. Eur J Biochem 247(1):66–73
75. Hayashi Y, Orikasa S, Tanaka K, Kanoh K, Kiso Y (2000) Total synthesis of anti-microtubule diketopiperazine derivatives: phenylahistin and aurantiamine. J Org Chem 65(24):8402–8405
76. Edmondson S, Danishefsky SJ, Sepp-Lorenzino L, Rosen N (1999) Total synthesis of spirotryprostatin A, leading to the discovery of some biologically promising analogues. J Am Chem Soc 121(10):2147–2155
77. Kanoh K, Kohno S, Katada J, Takahashi J, Uno I, Hayashi Y (1999) Synthesis and biological activities of phenylahistin derivatives. Bioorg Med Chem 7(7):1451–1457
78. Graz CJM, Grant GD, Brauns SC, Hunt A, Jamie H, Milne PJ (2000) Cyclic dipeptides in the induction of maturation for cancer therapy. J Pharm Pharmacol 52(1):75–82
79. Boger DL, Fink BE, Hedrick MP (2000) A new class of highly cytotoxic diketopiperazines. Bioorg Med Chem Lett 10(10):1019–1020
80. Prakash KRC, Tang Y, Kozikowski AP, Flippen-Anderson JL, Knoblach SM, Faden AI (2002) Bioorg Med Chem 10(9):3043–3048
81. Sugie Y, Hirai H, Inagaki T, Ishiguro M, Kim YJ, Kojima Y, Sakakibara T, Sakemi S, Sugiura A, Suzuki Y, Brennan L, Duignan J, Huang LH, Sutcliffe J, Kojima N (2001) A new antibiotic CJ-17,665 from *Aspergillus ochraceus*. J Antibiotics 54(11):911–916

82. Stevens BW, Joska TM, Anderson AC (2006) Progress toward re-engineering non-ribosomal peptide synthetase proteins: a potential new source of pharmacological agents. Drug Dev Res 66(1):9–18
83. Houston DR, Synstad B, Eijsink VGH, Stark MJR, Eggleston IM, van Aalten DMF (2004) Structure-based exploration of cyclic dipeptide chitinase inhibitors. J Med Chem 47 (23):5713–5720
84. Nilanonta C, Isaka M, Kittakoop P, Saenboonrueng J, Rukachaisirikul V, Kongsaeree P, Thebtaranonth Y (2003) New Diketopiperazines from the Entomopathogenic Fungus Verticillium hemipterigenum BCC 1449. J Antibiotics 56(7):647–651
85. Sinha S, Srivastava R, De Clercq E, Singh RK (2004) Synthesis and antiviral properties of arabino and ribonucleosides of 1,3-dideazaadenine, 4-nitro-1,3-dideazapurine and diketopiperazine. Nucleosides Nucleotides Nucleic Acids 23(12):1815–1824
86. Hu F, Chou CJ, Gottesfeld JM (2009) Design and synthesis of novel hybrid benzamide–peptide histone deacetylase inhibitors. Bioorg Med Chem Lett 19(14):3928–3931
87. Fairweather KA, Sayyadi N, Luck IJ, Clegg JK, Jolliffe KA (2010) Synthesis of all-L cyclic tetrapeptides using pseudoprolines as removable turn inducers. Org Lett 12(14):3136–3139
88. Singh EK, Ravula S, Pan C-M, Pan P-S, Vasko RC, Lapera SA, Weerasinghe SVW, Pflum MKH, McAlpine SR (2008) Synthesis and biological evaluation of histone deacetylase inhibitors that are based on FR235222: a cyclic tetrapeptide scaffold. Bioorg Med Chem Lett 18(8):2549–2554
89. Singh EK, Nazarova LA, Lapera SA, Alexander LD, McAlpine SR (2010) Histone deacetylase inhibitors: synthesis of cyclic tetrapeptides and their triazole analogs. Tetrahedron Lett 51(33):4357–4360
90. Nolasco L, Gonzalez MP, Caggiano L, Jackson RFW (2009) Application of negishi cross-coupling to the synthesis of the cyclic tripeptides OF4949-III and K-13. J Org Chem 74 (21):8280–8289
91. Pérez-Picaso L, Escalante J, Olivo HF, Yolanda Rios M (2009) Efficient microwave assisted syntheses of 2,5-diketopiperazines in aqueous media. Molecules 14(8):2836–2849
92. Cini E, Botta CB, Rodriguez M, Taddei M (2009) Microwaves enhance cyclisation of tetrapeptides. Tetrahedron Lett 50(51):7159–7161
93. Kopple KD, Ghazarian HG (1968) Convenient synthesis of 2,5-piperazinediones. J Org Chem 33(2):862–864
94. Ueda T, Saito M, Kato T, Izumiya N (1983) Facile synthesis of cyclic dipeptides and detection of racemization. Bull Chem Soc Jpn 56(2):568–572
95. Lin Q, Blackwell HE (2006) Rapid synthesis of diketopiperazine macroarrays via Ugi four-component reactions on planar solid supports. Chem Commun 27:2884–2886
96. Tullberg M, Grøtli M, Luthman K (2006) Efficient synthesis of 2,5-diketopiperazines using microwave assisted heating. Tetrahedron 62(31):7484–7491
97. Houston DR, Eggleston I, Synstad B, Eijsink VGH, van Aalten DMF (2002) The cyclic dipeptide CI-4 [cyclo-(l-Arg-d-Pro)] inhibits family 18 chitinases by structural mimicry of a reaction intermediate. Biochem J 368(1):23–27
98. Montero A, Beierle JM, Olsen CA, Ghadiri MR (2009) Design, synthesis, biological evaluation, and structural characterization of potent histone deacetylase inhibitors based on cyclic α/β-tetrapeptide architectures. J Am Chem Soc 131(8):3033–3041
99. Saxon E, Armstrong JI, Bertozzi CR (2000) A "Traceless" staudinger ligation for the chemoselective synthesis of amide bonds. Org Lett 2(14):2141–2143
100. Jain HD, Zhang C, Zhou S, Zhou H, Ma J, Liu X, Liao X, Deveau AM, Dieckhaus CM, Johnson MA, Smith KS, Macdonald TL, Kakeya H, Osada H, Cook JM (2008) Synthesis and structure–activity relationship studies on tryprostatin A, an inhibitor of breast cancer resistance protein. Bioorg Med Chem 16(8):4626–4651
101. Pérez-Balado C, de Lera AR (2010) Concise total synthesis and structural revision of (+)-pestalazine B. Org Biomol Chem 8(22):5179–5186

102. Kwon OS, Park SH, Yun B-S, Pyun YR, Kim C-J (2001) Cyclo (D-Pro-L-Val), a specific. BETA.-glucosidase inhibitor produced by *Aspergillus* sp. F70609. J Antibiot 54(2):179–181
103. Gupta S, Macala M, Schafmeister CE (2006) Synthesis of structurally diverse bis-peptide oligomers. J Org Chem 71(23):8691–8695
104. Zhao S, Smith KS, Deveau AM, Dieckhaus CM, Johnson MA, Macdonald TL, Cook JM (2002) Biological activity of the tryprostatins and their diastereomers on human carcinoma cell lines. J Med Chem 45(8):1559–1562
105. Fischer PM (2003) Diketopiperazines in peptide and combinatorial chemistry. J Pept Sci 9 (1):9–35. doi:10.1002/psc.446
106. Lim HJ, Gallucci JC, RajanBabu TV (2010) Annulated diketopiperazines from dipeptides or Schöllkopf reagents via tandem cyclization−intramolecular N-arylation. Org Lett 12 (9):2162–2165
107. Moir EM, Yoshiizumi K, Cairns J, Cowley P, Ferguson M, Jeremiah F, Kiyoi T, Morphy R, Tierney J, Wishart G, York M, Baker J, Cottney JE, Houghton AK, McPhail P, Osprey A, Walker G, Adam JM (2010) Design, synthesis, and structure–activity relationship study of bicyclic piperazine analogs of indole-3-carboxamides as novel cannabinoid CB1 receptor agonists. Bioorg Med Chem Lett 20(24):7327–7330
108. Fdhila F, Vázquez V, Sánchez JL, Riguera R (2003) dd-Diketopiperazines: antibiotics active against vibrio anguillarum isolated from marine bacteria associated with cultures of *Pecten maximus*. J Nat Prod 66(10):1299–1301. doi:10.1021/np030233e
109. Eguchi C, Kakuta A (1974) Cyclic dipeptides. I Thermodynamics of the cis-trans isomerization of the side chains in cyclic dipeptides. J Am Chem Soc 96(12):3985–3989
110. Pichowicz M, Simpkins NS, Blake AJ, Wilson C (2008) Studies towards complex bridged alkaloids: regio- and stereocontrolled enolate chemistry of 2,5-diketopiperazines. Tetrahedron 64(17):3713–3735
111. Sanz-Cervera JF, Stocking EM, Usui T, Osada H, Williams RM (2000) Synthesis and evaluation of microtubule assembly inhibition and cytotoxicity of prenylated derivatives of cyclo-l-Trp-l-Pro. Bioorg Med Chem 8(10):2407–2415
112. Ha K, Monbaliu J-CM, Williams BC, Pillai GG, Ocampo CE, Zeller M, Stevens CV, Katritzky AR (2012) A convenient synthesis of difficult medium-sized cyclic peptides by Staudinger mediated ring-closure. Org Biomol Chem 10:8055–8058
113. Fischer E, Fourneau E (1901) Ueber einige Derivate des Glykocolls. Ber Dtsch Chem Ges 34 (2):2868–2879
114. Payne RJ, Wong C-H (2010) Advances in chemical ligation strategies for the synthesis of glycopeptides and glycoproteins. Chem Commun 46:21–43
115. Rohde H, Seitz O (2010) Ligation-desulfurization: a powerful combination in the synthesis of peptides and glycopeptides. Biopolymers 94(4):551–559
116. Coin I (2010) The depsipeptide method for solid-phase synthesis of difficult peptides. J Pept Sci 16(5):223–230
117. Kent SBH (2009) Total chemical synthesis of proteins. Chem Soc Rev 38:338–351
118. Hackenberger CPR, Schwarzer D (2008) Chemoselective ligation and modification strategies for peptides and proteins. Angew Chem Int Ed 47(52):10030–10074
119. Dirksen A, Dawson PE (2008) Expanding the scope of chemoselective peptide ligations in chemical biology. Curr Opin Chem Biol 12(6):760–766
120. Coltart DM (2000) Peptide segment coupling by prior ligation and proximity-induced intramolecular acyl transfer. Tetrahedron 56(22):3449–3491
121. Deakyne CA, Ludden AK, Roux MV, Notario R, Demchenko AV, Chickos JS, Liebman JF (2010) Energetics of the lighter chalcogen analogues of carboxylic acid esters. J Phys Chem B 114(49):16253–16262
122. Castro EA (2009) Kinetics and mechanism of the aminolysis of thioesters and thiocarbonates in solution. Pure Appl Chem 81(4):685–696
123. Erben MF, Boese R, Della Védova CO, Oberhammer H, Willner H (2006) Toward an intimate understanding of the structural properties and conformational preference of

oxoesters and thioesters: gas and crystal structure and conformational analysis of dimethyl monothiocarbonate, CH3OC(O)SCH3. J Org Chem 71(2):616–622
124. Yang W, Drueckhammer DG (2001) Understanding the relative acyl-transfer reactivity of oxoesters and thioesters: computational analysis of transition state delocalization effects. J Am Chem Soc 123(44):11004–11009
125. Yang W, Drueckhammer DG (2000) Computational studies of the aminolysis of oxoesters and thioesters in aqueous solution. Org Lett 2(26):4133–4136
126. Castro EA (1999) Kinetics and mechanisms of reactions of thiol, thiono, and dithio analogues of carboxylic esters with nucleophiles. Chem Rev 99(12):3505–3524
127. Albers T, Watkins DL, Gameiro AF, Povstyanoy V, Povstyanoy MV, Lebedyeva IO (2015) Benzotriazole-based strategies toward peptidomimetics, conjugates, and other peptide derivatives. Top Heterocycl Chem. doi:10.1007/7081_2015_182
128. Monbaliu J-CM, Katritzky AR (2012) Recent trends in Cys- and Ser/Thr-based synthetic strategies for the elaboration of peptide constructs. Chem Commun 48:11601–11622
129. El Khatib M, Elagawany M, Jabeen F, Todadze E, Bol'shakov O, Oliferenko A, Khelashvili L, El-Feky SA, Asiri A, Katritzky AR (2012) Traceless chemical ligations from *O*-acyl serine sites. Org Biomol Chem 10(25):4836–4838
130. Horikawa M, Shigeri Y, Yumoto N, Yoshikawa S, Nakajima T, Ohfune Y (1998) Syntheses of potent Leu-enkephalin analogs possessing β-hydroxy-α, α-disubstituted-α-amino acid and their characterization to opioid receptors. Bioorg Med Chem Lett 8(15):2027–2032
131. Mutter M, Chandravarkar A, Boyat C, Lopez J, Dos Santos S, Mandal B, Mimna R, Murat K, Patiny L, Saucède L, Tuchscherer G (2004) Switch peptides in statu nascendi: induction of conformational transitions relevant to degenerative diseases. Angew Chem Int Ed 43 (32):4172–4178
132. Sohma Y, Sasaki M, Hayashi Y, Kimura T, Kiso Y (2004) Novel and efficient synthesis of difficult sequence-containing peptides through *O–N* intramolecular acyl migration reaction of *O*-acyl isopeptides. Chem Commun 2004:12–125
133. Sohma Y, Sasaki M, Hayashi Y, Kimura T, Kiso Y (2004) Design and synthesis of a novel water-soluble Aβ1-42 isopeptide: an efficient strategy for the preparation of Alzheimer's disease-related peptide, Aβ1-42, via *O–N* intramolecular acyl migration reaction. Tetrahedron 45(31):5965–5968
134. Sohma Y, Hayashi Y, Skwarczynski M, Hamada Y, Sasaki M, Kimura T, Kiso Y (2004) O-N intramolecular acyl migration reaction in the development of prodrugs and the synthesis of difficult sequence-containing bioactive peptides. Biopolymers 76(4):344–356
135. Carpino LA, Krause E, Sferdean CD, Schümann M, Fabian H, Biernert M, Beyermann M (2004) Synthesis of "difficult" peptide sequences: application of a depsipeptide technique to the Jung-Redemann 10- and 26-mers and the amyloid peptide A(1-42). Tetrahedron Lett 45 (40):7519–7523
136. Bozso Z, Penke B, Simon D, Laczkó I, Juhász G, Szegedi V, Kasza A, Soós K, Hetényi A, Wéber E, Tóháti H, Csete M, Zarándi M, Fülöp L (2010) Controlled in situ preparation of Aβ (1–42) oligomers from the isopeptide "iso-Aβ(1–42)", physicochemical and biological characterization. Peptides 31(2):248–256
137. Sohma Y, Yoshiya T, Taniguchi A, Kimura T, Hayashi Y, Kiso Y (2007) Development of *O*-acyl isopeptide method. Biopolymers 88(2):253–262
138. Carpino LA, Krause E, Sferdean CD, Schümann M, Biernert M, Beyermann M (2005) Dramatically enhanced N→O acyl migration during the trifluoroacetic acid-based deprotection step in solid phase peptide synthesis. Tetrahedron Lett 46(8):1361–1364
139. Vila-Perelló M, Hori Y, Ribó M, Muir TW (2008) Activation of protein splicing by protease- or light-triggered O to N acyl migration. Angew Chem Int Ed 47(40):7764–7767
140. Taniguchi A, Sohma Y, Kimura M, Okada T, Ikeda K, Hayashi Y, Kimura T, Hirota S, Matsuzaki K, Kiso Y (2006) "Click Peptide" based on the "O-Acyl Isopeptide Method": control of Aβ1−42 production from a photo-triggered Aβ1−42 analogue. J Am Chem Soc 128(3):696–697

141. Sohma Y, Kiso Y (2006) "Click Peptides"—chemical biology-oriented synthesis of Alzheimer's disease-related amyloid β peptide (Aβ) analogues based on the "*O*-Acyl Isopeptide Method". ChemBioChem 7(10):1549–1557
142. Shigenaga A, Hirakawa H, Yamamoto J, Ogura K, Denda M, Yamaguchi K, Tsuji D, Itoh K, Otaka A (2011) Design and synthesis of caged ceramide: UV-responsive ceramide releasing system based on UV-induced amide bond cleavage followed by O-N acyl transfer. Tetrahedron 67(22):3984–3990
143. Sohma Y, Taniguchi A, Skwarczynski M, Yoshiya T, Fukao F, Kimura T, Hayashi Y, Kiso Y (2006) *O*-Acyl isopeptide method' for the efficient synthesis of difficult sequence-containing peptides: use of '*O*-acyl isodipeptide unit'. Tetrahedron Lett 47(18):3013–3017
144. Dos Santos S, Chandravarkar A, Mandal B, Mimna R, Murat K, Saucède L, Tella P, Tuchscherer G, Mutter M (2005) Switch-peptides: controlling self-assembly of amyloid β-derived peptides in vitro by consecutive triggering of acyl migrations. J Am Chem Soc 127(34):11888–11889
145. Oliyai R, Stella VJ (1995) Structural factors affecting the kinetics of O, N-acyl transfer in potential O-peptide prodrugs. Bioorg Med Chem Lett 5(22):2735–2740
146. Oliyai R, Siahaan TJ, Stella VJ (1995) The importance of structural factors on the rate and the extent of N, O-acyl migration in cyclic and linear peptides. Pharm Res 12(3):323–328
147. Yoshiya T, Ito N, Kimura T, Kiso Y (2008) Isopeptide method: development of *S*-acyl isopeptide method for the synthesis of difficult sequence-containing peptides. J Pept Sci 14 (11):1203–1208
148. Yoshiya T, Hasegawa Y, Kawamura W, Kawashima H, Sohma Y, Kimura T, Kiso Y (2010) *S*-acyl isopeptide method: Use of allyl-type protective group for improved preparation of thioester-containing *S*-acyl isopeptides by Fmoc-based SPPS. Biopolymers 96(2):228–239
149. Monbaliu J-CM, Dive G, Stevens CV, Katritzky AR (2013) Governing parameters of long-range intramolecular S-to-N acyl transfers within (S)-acyl isopeptides. J Chem Theory Comput 9(2):927–934
150. Ha K, Chahar M, Monbaliu J-CM, Todadze E, Hansen FK, Oliferenko AA, Ocampo CE, Leino D, Lillicotch A, Stevens CV, Katritzky AR (2012) Long-range intramolecular S → N acyl migration: a study of the formation of native peptide analogues via 13-, 15-, and 16-membered cyclic transition states. J Org Chem 77(6):2637–2648
151. Hansen FK, Ha K, Todadze E, Lillicotch A, Frey A, Katritzky AR (2011) Microwave-assisted chemical ligation of *S*-acyl peptides containing non-terminal cysteine residues. Org Biomol Chem 9(20):7162–7167
152. Katritzky AR, Tala SR, Abo-Dya NE, Ibrahim TS, El-Feky SA, Gyanda K, Pandya KM (2011) Chemical ligation of S-acylated cysteine peptides to form native peptides via 5-, 11-, and 14-membered cyclic transition states. J Org Chem 76(1):85–96
153. Katritzky AR, Abo-Dya NE, Tala SR, Abdel-Samii ZK (2010) The chemical ligation of selectively *S*-acylated cysteine peptides to form native peptides via 5-, 11- and 14-membered cyclic transition states. Org Biomol Chem 8:2316–2319
154. Oliferenko AA, Katritzky (2011) Alternating chemical ligation reactivity of S-acyl peptides explained with theory and computations. Org Biomol Chem 9:4756–4759
155. Haase C, Seitz O (2009) Internal cysteine accelerates thioester-based peptide ligation. Eur J Org Chem 2009(13):2096–2101
156. Seghers S, Van Waes FEA, Cukalovic A, Monbaliu J-CM, De Visscher J, Thybaut JW, Heugebaert TSA, Stevens CV (2015) Efficient continuous flow benzotriazole activation and coupling of amino acids. J Flow Chem 5(4):220–227

http://www.springer.com/978-3-319-31552-2

The Chemistry of Benzotriazole Derivatives
A Tribute to Alan Roy Katritzky
Monbaliu, J.-C.M. (Ed.)
2016, XI, 289 p. 431 illus., 19 illus. in color., Hardcover
ISBN: 978-3-319-31552-2