Chemical Synthesis of Natural Product Peptides: Coupling Methods for the Incorporation of Noncoded Amino Acids into Peptides

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I. Introduction

Marine sponges and tunicates, fungi, bacteria, and other lower animal forms are rich sources of structurally unusual, biologically active peptides.[1,2] These peptides exhibit a variety of activities, including insecticidal, antimicrobial, antiviral, antitumor, tumor promotive, antiinflammatory, and immunosuppressive actions. Some of these compounds have served as drugs or as lead compounds in drug development, while others have proven useful in studies directed toward the elucidation of biochemical pathways.[3] This significant pharmacological diversity is a function of peptide structure and conformation, which are in turn dictated by the constituent amino acids, many of which have structures not found in ribosomally-synthesized polypeptides. These noncoded or nonribosomal amino acids include the "unnatural" D-series, *N*- or *C*-alkylated versions of common amino acids (such as *N*-methyl Ala, Aib, and *β*-methyl Asp), the α,*β*-dehydro amino acids, and structurally elaborate amino acids such as the cyclosporin residue MeBmt,[4−6] the microcystin residue Adda,[7,8] or the theonellamide residue Aboa[9,10] (Chart 1).

Nonribosomal amino acids can have pronounced effects on the conformation of the peptide backbone. For example, *N*-alkyl (or imino acid) residues exhibit reduced preferences for the *trans* conformation normally assumed by secondary amides, and this effect can lead to biologically relevant *β*-turn structures, similar to those often induced by proline residues.[11] The abridgment of intra- and intermolecular hydrogen bonds in *N*-methyl peptides can significantly affect peptide secondary and tertiary structure, and as a result these peptides are useful for structure−activity studies of peptide conformation. *N*-Substituted peptides may also exibit enhanced hydrophobicity and improved stability to proteolytic enzymes, which can increase bioavailability and therapeutic potential. Bioactive natural peptides containing *N*-methyl residues are widespread and commonly occur in bacterial antibiotics, fungal metabolites such as tentoxin and cyclosporine,[12,13] marine natural products such as the dolastatins, jaspamides, geodiamolides, theonellapeptolides, keramamides, motuporins and didemnins, and the cyanobacterial microcystins and nodularin, among others.[1−3]

α,α-Disubstituted amino acids, such as Aib and α-ethylalanine (Chart 18),[14] rigidify the peptide backbone through the formation of helixes and *β*-turns.[15,16] These organized structures are responsible for the interesting biological activity of the peptaibols,[17−21] a group of peptide antibiotics isolated from soil fungi and characterized by a large percentage of Aib residues. In bilayer membranes, the peptaibols form voltage dependent ion channels that are reminiscent of those found at neuronal synapses[22] and, at high cellular concentrations, can cause cell lysis.[19] α,α-Disubstituted residues are also found in nonpeptaibol peptides, such as chlamydocin (Chart 18),[23] where they may have similar conformational effects. The pharmacological importance of these molecules has been discussed elsewhere.[24,25]

Dehydro amino acid (Dhaa) residues also have a rigidifying effect on the peptide backbone, which can increase peptide−receptor affinity by reducing the entropic costs of binding.[26] According to conformational energy calculations, dehydro residues allow conformations that are not permitted with standard saturated residues.[27] While *N*-methylated, dehydro residues induce turn structures, this is not necessarily true for dehydro residues without *N*-alkyl substituents.[28] Another feature of dehydro peptides (Dhp's) includes increased stabilities to degradative enzymes, which has lead to synthetic enzyme inhibitors that act as nonhydrolyzable substrate mimics.[29] Dhaa residues sometimes occur in enzyme active sites and in naturally occurring enzyme inhibitors, where they may serve as electrophiles in nucleophilic addition reactions.[30,31] These features have generated interest in Dhp-based therapeutic agents.[32,33] In some natural product peptides, Dhaa residues are masked by intramolecular Michael addition, giving rise to elaborate macrocyclic structures such as theonellamide F (Chart 2).[10]

With theonellamide F and other cyclic peptides, the absence of polar *C*- and *N*-termini and a high proportion of cis amide bonds and noncoded amino acid residues confer greater stabilities to digestive processes. This stability, when coupled with enhanced membrane permeability, promotes bioavailability.

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John Humphrey was born in Bay City, MI, on August 9, 1967. He received a B.S. in biochemistry from the University of Michigan—Flint in December, 1989, where he did research under the direction of Professors Robert Stach and David O'Keefe. He received a Ph.D. in chemistry from the University of California at Irvine in 1996, where he worked with Professor A. Richard Chamberlin on the synthesis of novel glutamate conformer mimics and on the synthesis of the cyanobacterial cyclopeptide microcystin-LA. John was awarded a postdoctoral fellowship from the National Institutes of Health in 1997 and is currently studying new methods in natural product alkaloid synthesis with Professor Stephen F. Martin at the University of Texas at Austin.

Richard Chamberlin was born in San Francisco, CA, on May 11, 1949. He was raised in northern California and attended Stanford University, where he did research in cardiology. In 1971 he graduated with a Bachelor's degree in Chemistry and took a position at the Stanford Research Institute (SRI International) analyzing new antitumor compounds that were being tested in the then-new "war on cancer". After 3 years, he entered graduate school at the University of California, San Diego, where he earned a Ph.D. degree in Chemistry. He was an NIH postdoctoral fellow at Harvard with E. J. Corey from 1978–80 prior to joining the faculty at the University of California, Irvine, where he is currently Professor and Chair of Chemistry. His research interests include neuronal receptors and transporters, potassium ion channel blockers, protein engineering with noncoded amino acids, and novel phosphatase inhibitors as probes of intracellular signaling pathways.

Cyclic structures reduce peptide conformational freedom and often result in high receptor binding affinities by reducing unfavorable entropic effects. For these reasons, the cyclic peptides often make promising lead compounds for drug discovery.[3,34]

The pharmacological importance of nonribosomal peptides justifies the considerable effort devoted to their synthesis. Chemical synthesis is useful for structure proof and for providing ample quantities of compounds that might not be available in useful amounts from natural sources or through fermentation methods. For peptide-based pharmaceuticals, synthetic manipulation of a lead compound may be required to reduce its toxicity or enhance its activity, selectivity, or bioavailability or in some other way modify its pharmacological profile. An efficient laboratory synthesis of these peptides requires an efficient synthesis of the constituent amino acids (which can be quite complex) and, in the case of the cyclic peptides, an efficient cyclization protocol. Often overlooked, however, is the coupling of these unusual residues, whose structures can complicate amide bond formation by the conventional synthetic methods. Although the incorporation of noncoded amino acids into peptides often requires no special methods, peptide synthesis with members of the three classes mentioned above (the *N*-alkyl amino acids, the α,α-disubstituted amino acids, and the α,β-dehydro amino acids) can be difficult.

In this Account, the common problems encountered with the incorporation of these three types of amino acids into peptides will be covered along with the specialized synthetic methods that have been developed as solutions. Information on segment coupling and macrocyclization will be included, with an emphasis on structural features that may favor or disfavor these challenging operations. Illustrations of cyclic peptides are marked with an arrow to indicate the sites and methods of published cyclization reactions. For linear peptide segments, newly formed peptide bonds will be identified with a double dash (--) when the coupling position is not otherwise obvious. Enzymatic methods of peptide bond formation will not be covered. The synthesis of unusual amino acids will also not be discussed here, since there are numerous publications covering this topic.[35−44] Additionally, Roberts and Vellaccio have prepared a useful compilation of unusual amino acids used in peptide synthesis.[45] The cyclopeptide alkaloids[46−51] and the oxazole/thiazole[3,52−58] containing peptides have also received extensive coverage that will not be reiterated here. Since a number of review articles have appeared on the synthesis and chemistry of Dhaa's and Dhp's,[26,59,60] this section will be limited primarily to the special case of *N*-methyl α,β-dehydro amino acid residues. As part of this discussion, the reactivity of α,β-dehydro residues will be addressed with the aid of specific synthetic examples where possible. Other unsaturated amino acid couplings, such as those involving β,γ-unsaturated or vinylogous amino acids, will not be covered since methods for their incorporation do not deviate significantly from standard protocols. The reader is referred to work of Schreiber et al. and to recent syntheses of the cyclotheonamides for examples of vinylogous amino acid incorporation.[61−67] This review covers material published through 1996.

II. *N*-Methyl Amino Acids[68]

Efficient amide bond formation with *N*-methyl amino acids (Meaa's) can be challenging, because racemization and diketopiperazine formation are common side reactions. *N*-Methyl amino acid esters and peptides such as Z-MeIle-OMe or Z-Ala-MeLeu-OMe racemize or epimerize easily under acidic or basic conditions. This ease of racemization is attributed to the absence of an acidic amide or urethane proton, which would normally ionize first and sup-

Chart 1. Complex Nonribosomal Amino Acids

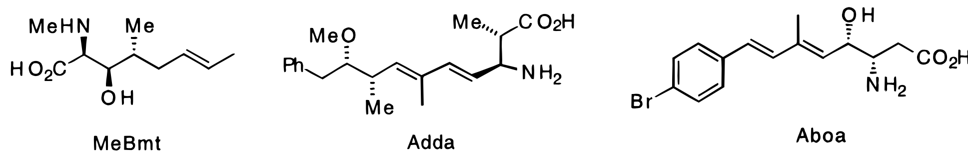
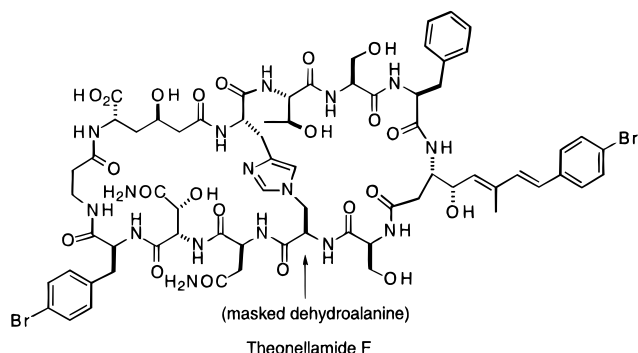


Chart 2



press α-deprotonation.[69] Racemization during the activation of *N*-protected Meaa's can also occur readily, proceeding through enolization or tautomerization of the oxazolonium (or 5-oxo-Δ^2-oxazolonium) ion to give pseudoaromatic structures (Chart 3).[70] This epimerization pathway is analogous to oxazolone formation during the activation of standard (non-*N*-methyl) amino acids and is especially common in segment couplings, which employ *N*-acyl (rather than urethane) "protection" of the α-nitrogen.[71] For this reason, the use of stepwise coupling procedures, which allow for oxazolone-suppressing urethane protection of the acid components during activation, is usually a better strategy (Chart 4). Epimerization via oxazolones or oxazolonium ions can also be reduced through the use of additives such as *N*-hydroxysuccinimide (HOSu) or hydroxybenzotriazole (HOBt) at 0 °C.[12,70,72] Without urethane protection, however, HOBt additives are ineffective, and the HOBt esters of acylated *N*-methyl residues racemize easily, possibly through intramolecular proton abstraction by a triazole nitrogen. For these cases, and especially when the segment coupling proceeds slowly, the additive HOSu is a better choice.[73]

Due to slow coupling rates, oxazolone or oxazolonium ion formation is more prevalent when acylating *N*-methylamines than primary amines. This poses a special problem with Boc-protected amino acids during activation, because the Boc-oxazolonium ions decompose to *N*-carboxyanhydride derivatives (via loss of the *tert*-butyl cation) much more readily than do the Cbz- or Fmoc-protected derivatives (Chart 5). Regardless of the epimerization that also accompanies oxazolone formation, the low yields that result from this decomposition make Boc protection a poor choice for acylating secondary amines. For example, the peptides Z-Val-MeVal-OMe and Z-MeVal-MeVal-OMe were formed using PyBOP activation in 90% and 87% yield, respectively, whereas the corresponding BOC derivatives were generated in only 44% and 33% respective yields under similar conditions.[74]

Chart 3. Racemization through Oxazolones

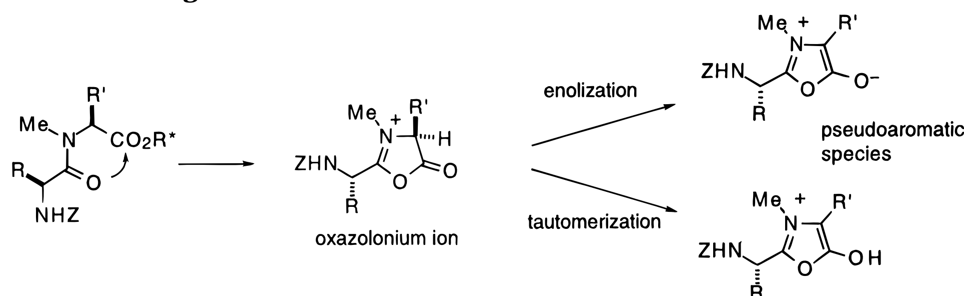


Chart 4. *N*-Methyl Amino Acid Couplings: Segment vs Stepwise Strategies

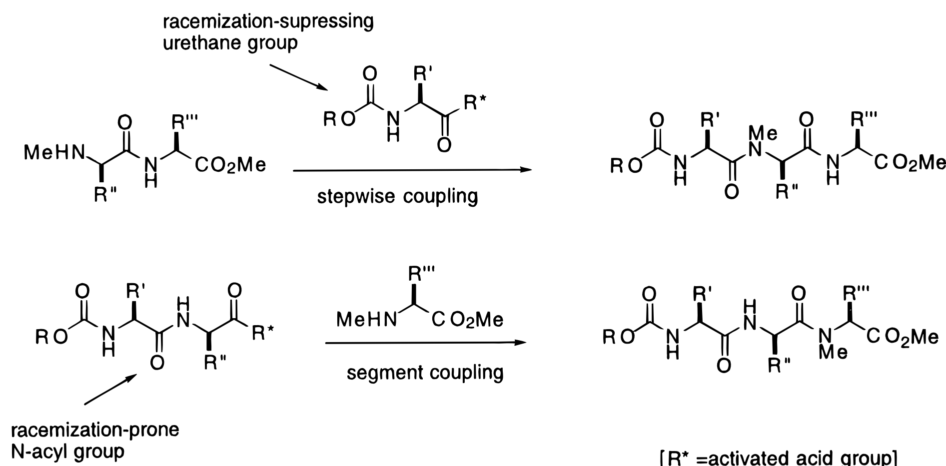


Chart 5. *N*-Carboxyanhydride Formation from Boc-Oxazolidinones

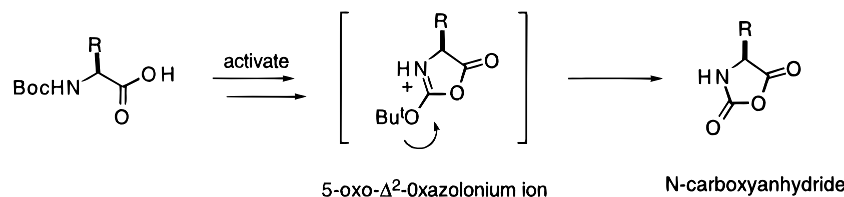


Chart 6. Diketopiperazine Cyclization

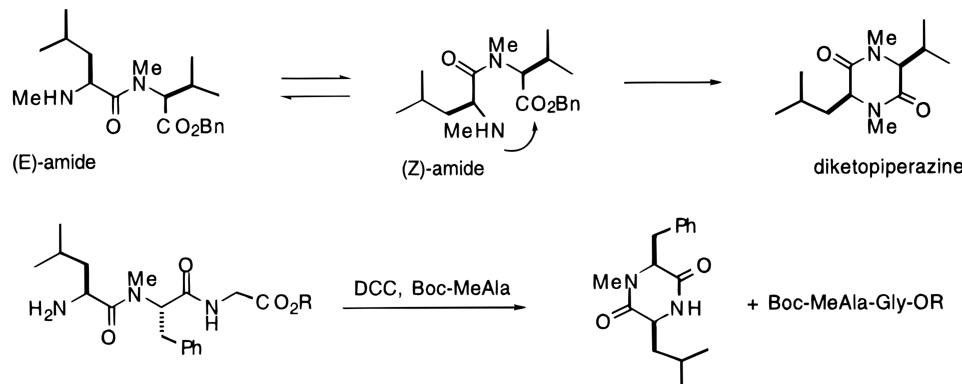
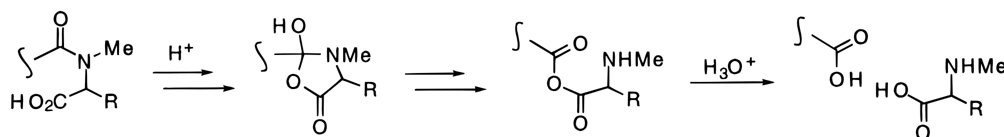
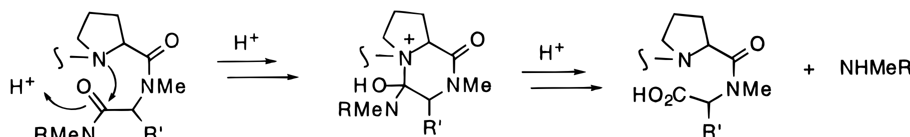


Chart 7. Acid Catalyzed Cleavage of Imino Acid Sequences

Cleavage of a C-terminal imino acid residue:



Cleavage of the third residue of an imino acid triad:



For dipeptide esters containing *N*-methyl or prolyl-type amide linkages, chain extension in the *N*-terminal direction can be hampered by spontaneous diketopiperazine formation.[75–77] For example, the dipeptide Boc-MeLeu-MeVal-OBn cyclizes spontaneously upon liberation of the amine group (Chart 6), although in this case the reaction could be prevented by switching to the *tert*-butyl ester.[12] In a similar study, Ala-MeLeu-OBn was also prone to cyclization, whereas the corresponding *tert*-butyl ester derivative could be coupled without difficulty.[70] Diketopiperazine formation is avoidable by extending such dipeptides in the carboxyl direction, but racemization through the oxazolone route then becomes a concern. Tripeptides containing *N*-methyl residues should also be treated with caution because of this side reaction. In one case, an attempt to link Boc-MeAla to Leu-MePhe-Gly-OR with DCC during a synthetic study on the plant fungus tentoxin (Chart 36) gave predominantly the diketopiperazine *cyclo*-(-Leu-MePhe-) through displacement of the carboxy terminal Gly residue by the leucyl nitrogen (Chart 6).[75] Larger populations of the Z-amide conformer in *N*-alkyl peptides (relative to non-*N*-alkylated structures) contribute to this cyclization reaction.

C-terminal *N*-methyl residues possessing a free carboxyl group are prone to cleavage by acid.[78] This process also proceeds via the *Z*-amide isomer (Chart 7) but is generally too slow to cause serious problems during standard acidolytic Boc or *tert*-butyl ester deprotections. In a related reaction, the third residue of a chain of three imino acids is readily cleaved by acid. This process may proceed via the diketopiperazinium ion as shown in Chart 7.[79]

Peptide bond formation is generally not difficult when only the acid component of a coupling pair is *N*-methylated, and these reactions proceed well under standard coupling conditions (i.e., DCC/HOBt, BOP,[80] etc.), although as always one must be aware of the potential for racemization and diketopiperazine formation. On the other hand, serious problems with coupling rates arise when the amine component or when both the amine and the acid components are *N*-methylated.[81] Although the reactivity of secondary amines compares with that of primary amines in S_N2 reactions, this is not the case with α-amino acid coupling reactions, where the steric bulk of a secondary amine outweighs its enhanced nucleophilicity. Low coupling rates in these cases can lead to extended reaction times, undesired side reactions, and

Chart 8. HOBt-Mediated Couplings of Secondary Amines

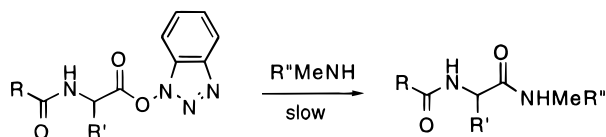
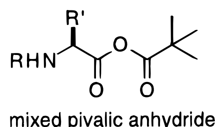


Chart 9



extensive racemization.[82] In the early examples, dipeptides such as Z-MeVal-MeAla-OMe were prepared by DCC protocols, but the yields were only modest to poor, and reactions were plagued by epimerization.[83,84] The traditional HOBt-based reagents (HBTU,[85] BOP, etc.) and other methods such as DCC/HOBt or *N*-carboxy anhydride activation tend to give suboptimal or inconsistent results.[12,86−92] That the unsuccessful cases often give benzotriazole active esters as major products[93] underscores the low reactivity of the benzotriazole ester toward hindered secondary amines (Chart 8),[81] a problem that is compounded in solid phase synthesis because incomplete couplings under these conditions give rise to deletion sequences.[94] DCC-mediated couplings of Meaa's are surprisingly more effective in the absence of HOBt[81] (or other additives[86,95]) than in its presence, and the employment of hydroxybenzotriazole for the acylation of hindered amines is now considered to be ill-advised.[81]

In spite of strong evidence implicating HOBt in reduced coupling rates of Meaa's, some successful examples have been reported. In Konopelski's synthesis of the depsipeptide jasplakinolide (Chart 50), the DCC/HOBt-mediated acylation of a D-MeTrp residue with Boc-Ala was efficient (90% yield), although the HOBt was used only in catalytic quantities.[96] Grieco's synthesis of geodiamolide B (Chart 50) features a similar DCC/HOBt-mediated acylation with Boc-Ala, which in this case proceeded in 90% yield with one full equivalent of HOBt.[97] The HOBt-based BOP reagent[80] furnished Schreiber and Valentekovich with 88% yield for the acylation of a MeThr residue with Boc_2-D-Glu-OMe,[98] and White's synthesis of geodiamolide A (Chart 50) provides another successful example: the acylation of an *N*-methyliodotyrosine unit with Boc-Ala was mediated by DCC/HOBt in 76% yield.[99] Reasons for the success of HOBt in some cases but not in others are not clear, and good results should be considered the exception rather than the rule.

The difficulties with *N*-methyl amino acid couplings have been addressed by several groups, and a number of specialized reagents and methods are now available to facilitate the acylation of *N*-methyl amino acid derivatives. Wenger, among the first to couple these residues in fair yields and with only moderate racemization, employed a modified mixed pivalic anhydride protocol (Chart 9)[100] through much of his synthesis of the cyclic undecapeptide cyclosporine (Chart 10).[12] Cyclosporine, with seven *N*-methyl residues, is the classic example of this class of peptide and is a formidable synthetic challenge in view of the particularly ominous *N*-methyl sequence MeLeu-MeLeu-MeVal-MeBmt. Wenger's disconnection at D-Ala-Ala (position 7−8, Chart 10) and at MeBmt-MeVal (position 1−11, Chart 10) gave the target peptides Boc-D-Ala-MeLeu-MeLeu-MeVal (**1**) and MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn (**2**). For fragment **1**, Wenger employed a nontraditional segment coupling strategy designed to evade diketopiperazine cyclization of MeLeu-MeVal-OBn, which would have been inevitable through a stepwise coupling route. This pivalic anhydride method of activation and segment coupling gave moderate to fair yields (generally 60−70%) and 5−20% epimerization throughout the synthesis but nevertheless allowed the construction of this challenging target. For example, the highly hindered MeLeu-MeVal linkage of fragment **1** was formed in 60% yield,

Chart 10. Cyclosporine

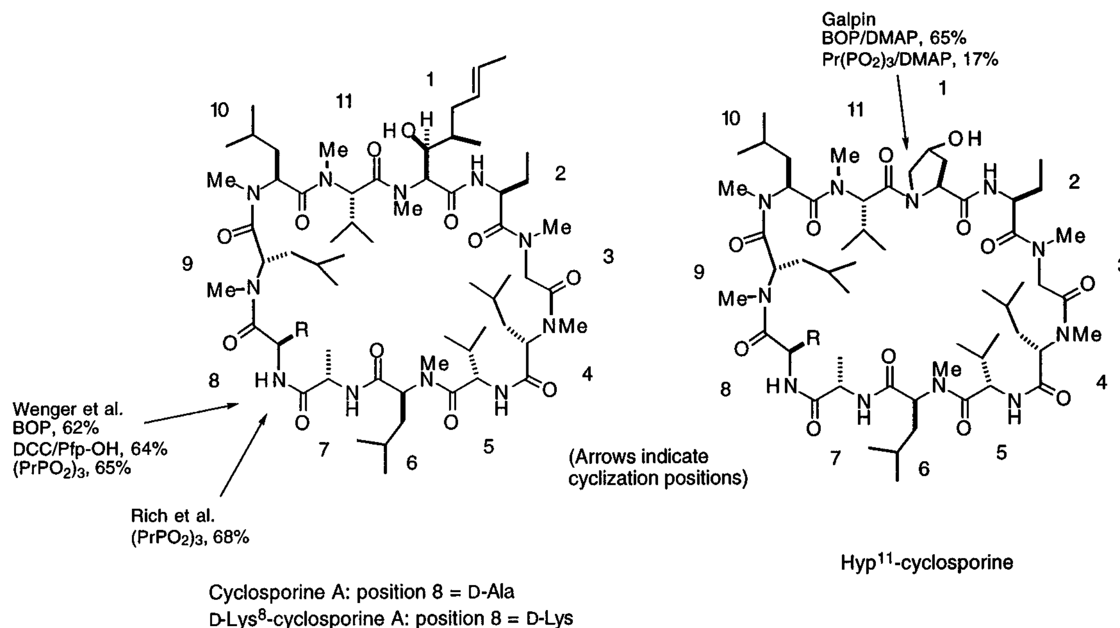
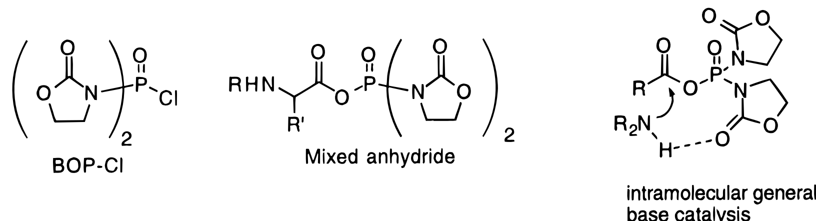


Chart 11. Coupling Reactions with BOP-Cl



although the product tetrapeptide was contaminated by 10% of the MeLeu epimer. Interestingly, epimerization by this method proceeds through enolization of the intermediate mixed anhydride rather than through the oxazolone, which might have led to significantly greater epimerization.

For fragment **2**, Wenger again used the pivalic anhydride method; however, the use of reliable stepwise (rather than segment) coupling protocols furnished higher yields and virtually eliminated problems with epimerization. In one example, the difficult acylation of MeLeu-Ala-OBn with the hindered Boc-Val proceeded in 88% yield. Although the mixed pivalic anhydride supplies the powerful activation needed for *N*-methyl amino acid couplings, drawbacks of this reagent include a requirement for the low temperature preactivation of the acid component (−20 °C, 2−6 h) and coupling reaction periods of up to several days at this low temperature. The preactivation step is critical because excessive preactivation gives more epimerization, and insufficient preactivation gives lower yields. Unfortunately, the optimal preactivation conditions are substrate dependent and must be determined separately for each case.

In later work on cyclosporine analogues, Rich et al. prepared the Fmoc/*tert*-butyl ester derivative of Wenger's tetrapeptide fragment **1** (Fmoc-D-Ala-MeLeu-MeLeu-MeVal-O^{t}Bu, **3**) en route to the important D-Lys[8] derivative (Chart 10).[95,101] In this case, the *tert*-butyl ester was chosen over benzyl ester protection to preclude diketopiperazine formation from the MeLeu-MeVal-OR fragment and to allow for reliable BOP-Cl[102,103] mediated stepwise couplings (Chart 11), which led to yield improvements and racemization reduction. The high efficiency of BOP-Cl for mediating Meaa couplings was demonstrated in the union of Fmoc-D-Lys(Boc)-OH with MeLeu-MeLeu-MeVal-O^{t}Bu in 94% yield; the tetrapeptide **3** was itself efficiently prepared via BOP-Cl in 66−73% overall yield from the starting amino acids.[104]

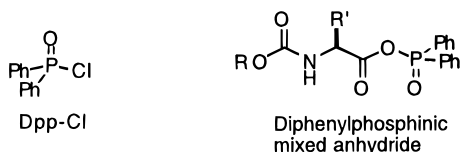
The effectiveness of BOP-Cl for these couplings, in contrast to other phosphorous-based reagents, is attributed to intramolecular general base catalysis by the oxazolidinone carbonyl of the phosphorous mixed anhydride active species (Chart 11).[88] BOP-Cl reacts faster with carboxylate anions than with secondary amines and allows for selective one-pot coupling protocols in these cases.[106] However, BOP-Cl mediated couplings should be limited to acylations of secondary amines, because primary amines sometimes compete with carboxylate anions for the reagent.[95] In one such example, Rich et al. prepared the hindered cyclosporine segment Fmoc-MeLeu--Val-MeLeu-Ala-OBn (**4**) via BOP-Cl in only 25% yield (this yield was improved to 65% with Boc-MeLeu).[95]

For BOP-Cl mediated acylations of primary amines, fair results can be achieved through low temperature preactivation of the acid component, but this process is hampered by the poor organic solubility of BOP-Cl. Other reagents (DCC/HOBt, BOP, acid halides, etc.) are better suited for standard primary amine couplings.

For tetrapeptide **4**, the amino acid chloride Fmoc-MeLeu-Cl[105] mediated the acylation of the hindered valine component in 85% yield.[95] Others have recommended amino acid chlorides for coupling hindered residues,[106,107] but these reagents are not well suited for Meaa acylations under the usual conditions because slow coupling rates favor the formation of oxazolones from the highly reactive acid chlorides. Oxazolone formation is reduced in Fmoc-amino acid chloride-mediated reactions by substituting the usual organic base DIEA with aqueous $NaHCO_3$ in a two-phase reaction mixture as described by Carpino[108] or with KOBt according to the method of Sivanandaiah et al.[109] The latter conditions were applied to the preparation of the cyclosporine segments 4−7 and 8−11 in low to moderate yield (ca. 50−70%), as expected for HOBt-mediated couplings of *N*-substituted amino acids.

For BOP-Cl-mediated acylations of Meaa's, steric congestion in the form of side chain branching of the acid component (i.e., with Val and Ile residues) is detrimental to the coupling reaction, especially with bulky carbamate protecting groups.[87,88] For example, the dipeptide MeLeu-Ala-OBn was acylated with Boc-Val in only 67% yield (57% in the presence of HOBt), and the corresponding Fmoc derivative of this tripeptide was likewise prepared in low yield.[87] The poor performance of BOP-Cl in these cases can be improved by employing less bulky urethane protection: Z-MeVal-MeVal-O^{t}Bu was formed via BOP-Cl activation in 89% yield with no racemization. The substitution of Boc or Fmoc protecting groups with the smaller Alloc or Teoc groups can also result in 10−20% yield improvements.[87] Similar observations have been noted with other methods of activation: the substitution of Alloc-Val-Cl for Fmoc-Val-Cl gave significant yield improvements for the acylation of MeLeu-Ala-OBn.[95] During Rich's work on cyclosporine, the predominant side product in some of the less successful coupling reactions was the amino acid symmetrical anhydride, which is reportedly nonreactive toward *N*-methylamines under standard conditions (see below for exceptions). Symmetrical anhydride formation could be prevented in this case by dropwise addition of the acid component and a tertiary amine base to a solution of BOP-Cl and the secondary amine component.[95] Other dipeptides consisting of various combinations of less hindered secondary amino acids such as Pro and SPip were

Chart 12. Coupling with Diphenylphosphinic Chloride



generally prepared efficiently by the BOP-Cl method.[88]

Efforts to employ high purity BOP-Cl are generally rewarded with higher yields because the presence of BOP-Cl degradation products or other impurities can give poor or inconsistent results. BOP-Cl purity can vary significantly among commercial sources, but high quality reagent can be prepared from aminoethanol, diethylcarbonate, and PCl_5 according to the published procedure.[103] Potential side products from BOP-Cl meditated couplings include amidoacylation and the formation of urethanes, oxazolones, oxazolonium salts, *N*-carboxy anhydrides, and symmetric anhydrides.[88]

Dpp-Cl (Chart 12), which is structurally related to BOP-Cl, mediates hindered couplings via the diphenylphosphinic mixed anhydride (Chart 12). Galpin et al. used this method to prepare 11 cyclosporine analogues that differed from natural cyclosporine by the substituents at positions 1 and 2 (Chart 10).[72,110] The linear undecapeptides were assembled in good yield and without racemization via stepwise protocols. Dpp-Cl requires low temperature preactivation of the acid component for good results. The acid component must be urethane protected to avoid significant racemization, so the method is not amenable to segment condensations.

In response to the ineffectiveness of the HOBt-based BOP reagent in Meaa couplings,[111] Coste et al. developed the highly reactive BroP reagent (Chart 13).[112] The active intermediate of the BroP-induced coupling reaction is presumed to be either the acyloxyphosphonium salt or the acyl bromide, reactive intermediates that outperform the more sluggish -OBt esters in secondary amine acylations. The effectiveness of BroP was demonstrated in the difficult coupling of two MeVal residues, giving Z-MeVal-MeVal-OMe in 70% yield, whereas BOP gave only 5% of the desired product. One disadvantage to the use of BroP is the production of the carcinogen HMPA as a side product of the coupling reaction.

A number of BroP and HBTU congeners, PyBroP, PyCloP, and PyClU (Chart 13), have since been developed by Coste et al. and applied to these difficult couplings.[81] These reagents, which give results that are comparable to those obtained from BroP, are improvements in that their use does not involve HMPA. In one case, PyBroP furnished Z-MeVal-MeVal-OMe in 87% yield, in contrast to the -OBt version PyBOP,[92] which gives only moderate yields of extensively epimerized products. Furthermore, PyBroP, PyCloP, and PyClU each provided near-quantitative yields of the hindered dipeptide Z-Val-MeVal-OMe, while HOBt-based reagents gave only moderate yields.[81] With PyBroP and other coupling

Chart 13

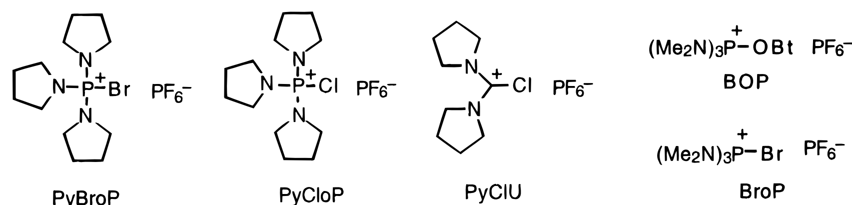
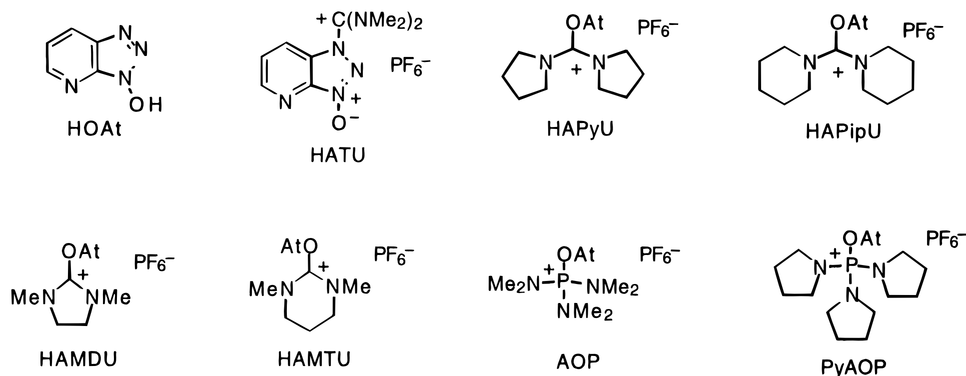


Chart 14. HOAt-Based Coupling Reagents



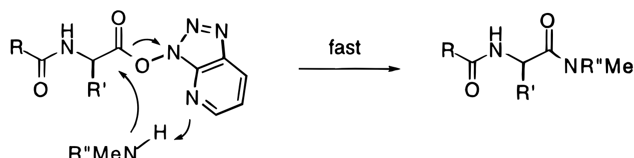
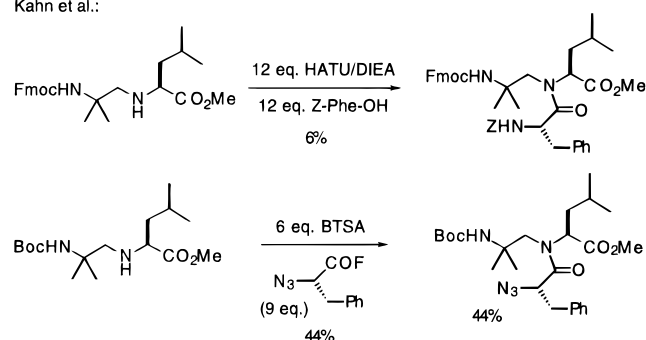


Chart 15



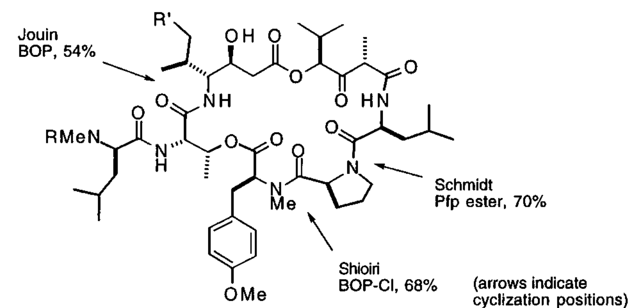
agents, the amount of the requisite tertiary amine base can have a notable yet undefined effect on the coupling yields, causing dramatic increases in some cases and reducing the yields in others.[94] These halogenated reagents are crystalline, stable, and in some cases commercially available.

In solid phase peptide synthesis (SPPS), a resin-bound secondary amine suffers lower reactivity toward activated acids than when in solution, which renders the reagents PyBroP and BOP-Cl less effective for solid phase Meaa acylations.[94] Better SPPS results are obtained with the HOAt-based reagents (i.e., DCC/HOAt, HATU, PyAOP, HAPyU, etc., Chart 14) developed by Carpino et al.[90,113-115] HOAt is superior to HOBt for improving reaction rates and reducing racemization. Unlike -OBt esters, -OAt active esters exhibit good reactivity toward *N*-methylamines, ostensibly through intramolecular general base catalysis (Chart 14),[90] and have generated excellent results in both solution and solid phase synthesis.[94,113] As a representative example, the -OAt active ester method (carbodiimide/HOAt or HATU) gave nearly quantitative yields in the solid phase acylation of the dipeptide MeVal-Ala-resin with Fmoc-MeLeu.[94] Carpino and Rich have independently applied HATU to the first solid phase syntheses of the cyclosporine segments Abu-Sar-MeLeu-Val-MeLeu-Ala and D-Ala-MeLeu-MeLeu-MeVal-Phe-Val.[94,113] HATU out performed BOP, PyBroP, HBTU, and BOP-Cl and also gave better results than the carbodiimide/HOAt methods for these couplings.

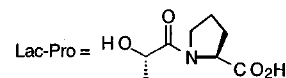
Extremely hindered secondary amines are less amenable to acylations via HATU. Kahn et al. observed low yields for the acylation of a highly hindered *N*-(2-amino-2-methylpropyl)-leucine derivative with excess HATU and Z-Phe (6%, Chart 15).[116] This reaction was more efficiently carried out via the α-azido acid fluoride and the *N*-silyl amine (generated by reaction of the secondary amine with BTSA), although the yields for this difficult transformation were still low.[116] Amino acid fluorides have not been generally applied to *N*-methyl amino acid couplings, and one group suggests that the formation of some *N*-methyl amino acid fluorides, such as Fmoc-MeLeu-F, is difficult and that optimum conditions for these transformations are still needed.[94] The strategy of forming hindered amide bonds from acid fluorides and *N*-silyl amines is discussed further in the next section.

Like cyclosporine, the didemnins (Chart 16) have generated significant pharmaceutical interest due to

Chart 16. Didemnins



didemnin A: R' = Me, R = H
didemnin B: R' = Me, R = Lac-Pro
nor-didemnin B: R' = H, R = Lac-Pro
Lac-Pro =



their immunosuppressive and anticancer activities. The didemnins consist of three to four imino acid residues, including two *N*-methyl residues and one or two prolines, depending on the didemnin subtype (Chart 16). Acylations of proline derivatives are generally not difficult and have been accomplished in good to excellent yield by reagents such as BOP[88] or DEPC.[117] The difficult didemnin linkages Pro-MeTyr and Pro-MeLeu have been formed through a number of different methods by several different groups. As part of Schmidt's synthesis of the didemnin peptolide ring, the phenyltetrazolinethione/*tert*-butylisocyanide reagent gave moderate results (75%) for the Pro-MeTyr linkage,[118] whereas Rinehart et al. obtained higher yields for a similar coupling through a standard carbodiimide acylation (EDC, 89%) en route to didemnin A.[119] The Rinehart group prepared didemnins B (Chart 16) and C (not shown) by acylating the exocyclic D-MeLeu residue of didemnin A with the requisite side chains Lac-Pro and Lac, respectively, via DCC and azide methods, but these yields were considerably lower.[119]

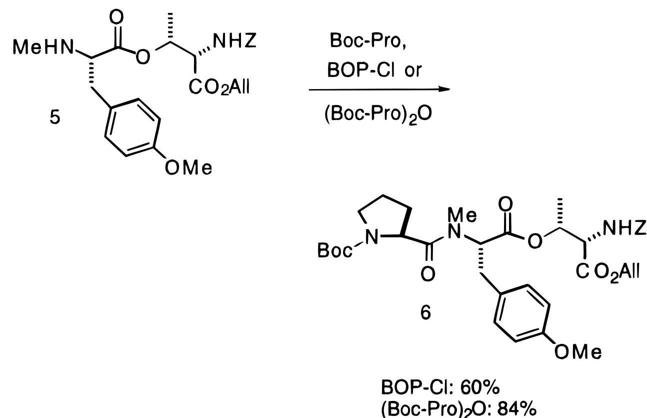
Shioiri's route to didemnin B from didemnin A employed BOP-Cl to append an *O*-benzyl protected Lac-Pro side chain to the exocyclic MeLeu appendage of the didemnin A macrocycle in 68% yield. Although the incorporation of an *N*-methyl residue late in a synthesis is risky, BOP-Cl gave good results here, perhaps because the acylation involved activation of a nonepimerizable proline residue.[117] During Shioiri's synthesis, the Pro-MeTyr linkage was formed during the macrocyclization and will be discussed under that topic.

The Jouin group observed moderate yields for the BOP-Cl-mediated acylation of the MeTyr derivative **5** with Boc-Pro (Chart 17) to give dipeptide **6**, an intermediate in their nordidemnin B (Chart 16) synthesis.[89] The solvent-free symmetrical anhydride method of Goodman gave better results for this coupling (84%, Chart 17), although the 130 °C reaction temperatures may not be compatible with some substrates.[89,120] Rich et al. have also advocated the symmetrical anhydride method for secondary amine acylations.[75]

III. α,α-Disubstituted and Other Hindered Residues

Peptide synthesis with bulky α,α-disubstituted residues, such as Aib and α-ethylalanine (Chart 18),

Chart 17. Symmetrical Anhydride Coupling



is complicated by problems similar to those presented by the *N*-methyl amino acids; however, some unique problems also exist. Diketopiperazine cyclization, attributed to the gem dialkyl effect,[121] can occur readily with certain Aib-containing dipeptides and related fragments during *N*-deprotection.[14,122] For example, hydrogenolytic deprotection of the tripeptide Z-Aib-Pro-Trp-OH gives a mixture of products consisting mainly of the diketopiperazine *cyclo*-[Aib-Pro] and free tryptophan (Chart 19), although, in this case, proline certainly contributes to the propensity for cyclization.[123] This type of cyclization was less problematic in Baldwin's synthesis of chlamydocin: the tripeptide Z-Aib-Phe-Pro-OMe was deprotected (H_2/Pd-C, MeOH) and coupled with *N*-Z-(S)-2-amino-5-chloropentanoic acid (via BOP/DIEA) in 66% yield (Chart 19), although the presence or absence of the diketopiperazine product *cyclo*-[Aib-Phe] was not mentioned in that report. Another interesting problem associated with Aib incorporation is extensive racemization in R-Aib-Xaa-OH sequences during carbodiimide activation, although racemization-suppressing additives can alleviate this problem. Racemization of the penultimate residue of peptides containing *C*-terminal Aib residues during acitvation has also been documented and is discussed further in this paper (see Chart 24). Further complications with Aib couplings can arise from the acid lability of Aib-Pro sites,[124] as well as the usual racemization associated with coupling optically-active amino acid derivatives with amines of low reactivity.[19]

The sterically congested α,α-disubstituted residues inhibit efficient peptide bond formation in all cases involving these amino acid derivatives, but, as expected, these couplings are especially difficult when both the carboxyl and the amino coupling components are α,α-disubstituted. During the activation of urethane protected Aib, oxazolone formation is facilitated by the gem-dimethyl groups.[121] Furthermore, although the Aib derived oxazolone cannot racemize, the *N*-Boc oxazolonium ions decompose readily through loss of the *tert*-butyl cation (this side reaction was also discussed in the *N*-methyl amino acid section—see Chart 5). In one example, the BOP-Cl-mediated coupling of Boc-Aib with Aib-OBn failed due to the formation of the oxazolium ion and its subsequent decomposition to the *N*-carboxy anhydride.[74,87] Other carbamate protecting groups (Cbz, Fmoc, etc.) are much less susceptible to this decomposition and give better results in these cases.

For peptide assembly with Aib residues, the older methods of peptide bond formation (anhydrides, oxazolones,[125] active esters, carbodiimide/HOBt[14]) are inefficient, suffering from low yields, slow reaction rates, and the necessity for large reagent excesses or multiple subjections to the reaction conditions.[126] In spite of these inefficiencies, the DCC-utilizing methods did enable important early syntheses of the fungal antibiotic alamethicin and various analogues (Chart 20).[14,91,127,128]

Other older methods include the use of diethyl phosphobromidate (Chart 21), which has the advan-

Chart 18. Aib Residues and Chlamydocin

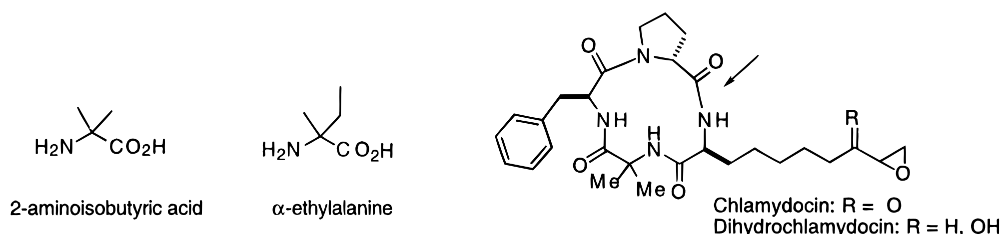


Chart 19. Aib Diketopiperazine Cyclization

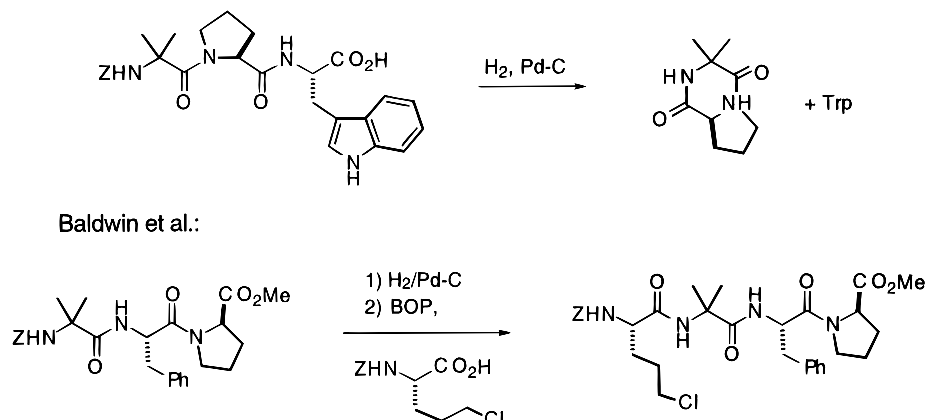


Chart 20

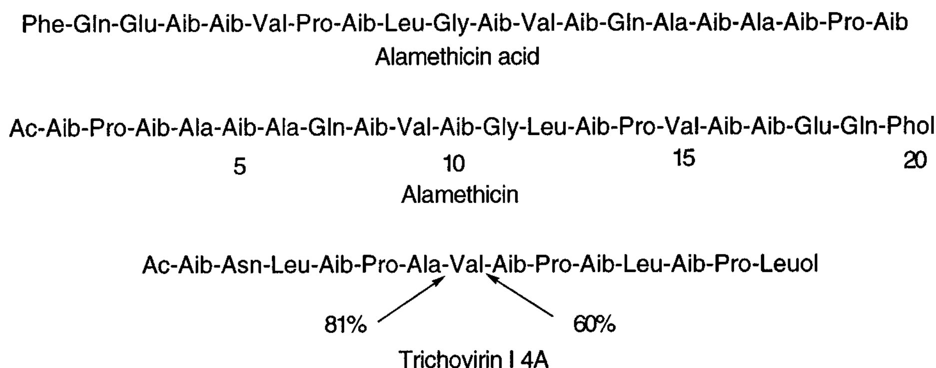
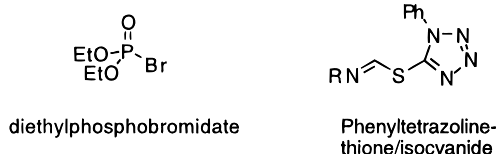


Chart 21. Diethyl Phosphobromidate and Phenyltetrazolinethione Isocyanide



tage of being easy to prepare from bromine and triethyl phosphite. This reagent has given good results in Aib couplings and, for example, provided the dipeptide Z-Aib-Aib-OMe in 84% yield.[129] Schmidt's phenyltetrazolinethione/isocyanide reagent (Chart 21) gave moderate to good results in the preparation of hindered peptides such as Z-Pro-Val-OMe (80%), Z-Aib-Ile-OMe (63%), and Z-Ile-Aib-OMe (60%),[130] although its performance in forming Aib-Aib linkages was not reported.

Heimgartner et al.[126,131] devised a unique and efficient route to these peptides, which circumvented carbodiimide acid activation altogether, by treating the Z-protected amino acid **7** with the azirine **8** (Chart 22). The peptide bond is formed by an intramolecular rearrangement of the intermediate **9** to give the carboxamide **10**. The terminal carboxyl group can be liberated under mildly acidic conditions, even in the presence of acid labile Aib-Pro linkages,[124] and treatment of the resultant acid **11** with DCC generates the oxazolone **12**, the acylating agent for the next coupling reaction. This method was applied to the preparation of the 12−20 nonapeptide section of alamethicin (Chart 20), giving high yields and no racemization even for difficult linkages such as Z-Val--Aib (95%) and Z-Val-Aib--Aib-NMePh (99%). Two minor drawbacks to this method are that the azirine **8** must be prepared beforehand, and the method is not applicable to solid phase synthesis.

Recent advances in coupling technology have simplified the synthesis of Aib-rich peptides. In one study, Coste et al. applied the reagents BOP PyBOP, BroP, and PyBroP (Chart 13) to Aib coupling reactions with various levels of success.[132] Coupling efficiencies with these reagents were similar regardless of whether Aib represented the amine or the acid component, but with adjacent Aib residues, BOP and PyBOP out-performed BroP and PyBrop. In one example, the former two reagents gave the dipeptide Boc-Aib-Aib-OMe in 80−89% yield, whereas the latter two reagents gave poor yields (25%) of the same dipeptide. Catalysis by DMAP provided slightly improved yields.[132,133] Reactions with BroP and PyBrop were reportedly more sensitive to steric bulk, with Z-protection giving better yields than the bulkier Boc protection and methyl esters giving better results than benzyl esters.[132] In a subsequent study, PyBrop and Fmoc-NCA methodology were combined in an efficient preparation of the tripeptide Fmoc-Aib-Aib-Aib-OMe.[133]

Heathcock et al. applied PyBrop with good results to the synthesis of (−)-mirabazole C (Chart 28).[134] The difficult coupling of adjacent 2-methylcysteine residues was thus accomplished to yield a dipeptide in 90% yield, although a similar stepwise coupling yielding a tripeptide was slower and lower yielding (60%, see Chart 23). Other attempts to form these types of linkages with BOP, BOP-Cl, DPP, DCC, or acid chlorides were not effective.

With BOP-related reagents, Aib esters can be coupled with activated urethane-protected amino acids without significant racemization of the acid component, and urethane-protected Aib can likewise be coupled with amine components without racemization because it lacks an acidic α-proton. However, sluggish segment couplings with carboxy-terminus

Chart 22. Aib Couplings by the Azirine Method

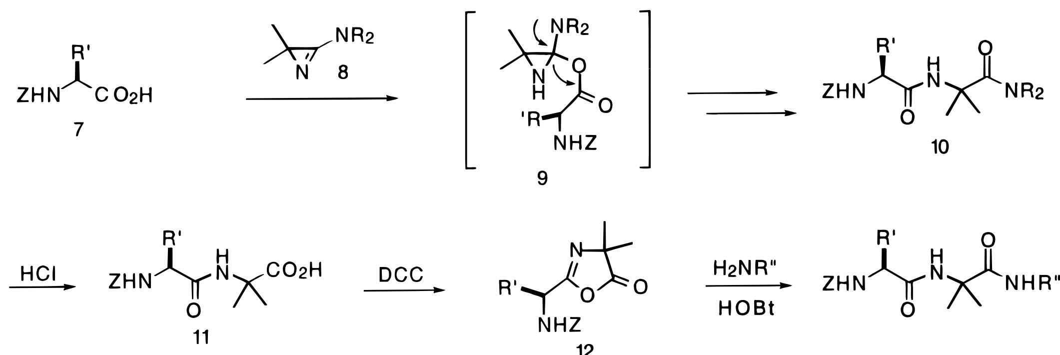


Chart 23

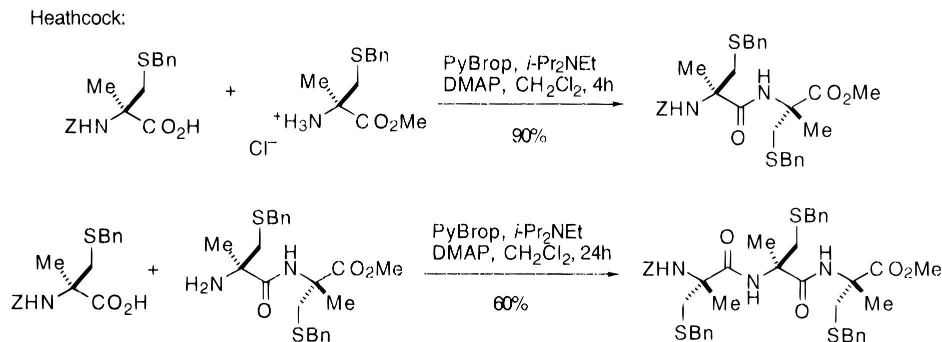


Chart 24. Epimerization during Aib Couplings

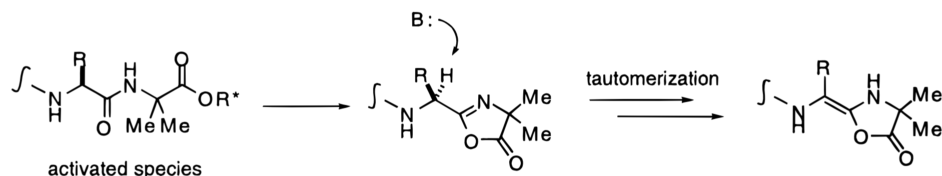
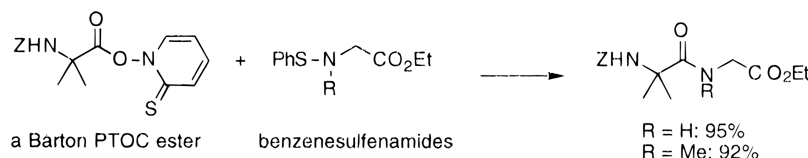
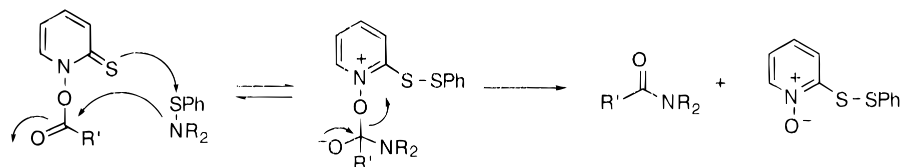


Chart 25. Dipeptides via the Barton PTOC Ester



Proposed mechanism:



Aib residues are not necessarily racemization-free because epimerization can occur at the penultimate *C*-terminal residue by tautomerization of the oxazolone intermediate as shown in Chart 24. This side reaction, which involves an oxazolone intermediate, can be prevented by catalysis with camphor sulfonic acid or zinc chloride.[135] Coste suggests that this type of epimerization is not likely under normal coupling conditions.[132]

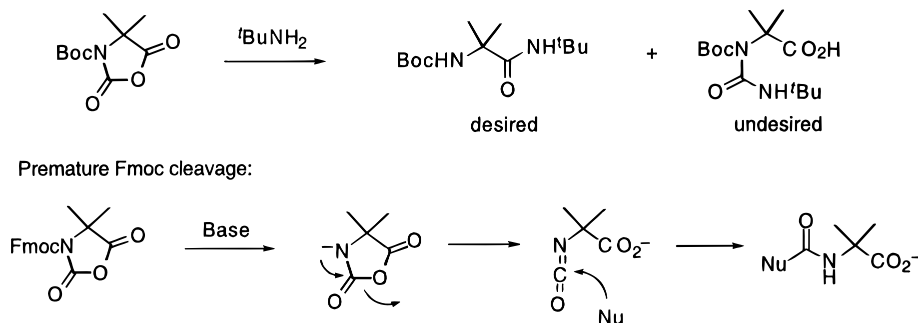
Barton has recently reported on the use of PTOC esters and arylsulfenamides for forming hindered dipeptides such as Z-Aib-Gly-OEt (95%) and Z-Aib-Sar-OEt (92%, Chart 25).[136] Amide bond formation according to this method is thought to proceed according to the mechanism shown in Chart 25. The PTOC ester can be generated in situ and coupled, but higher yields require that the active ester be prepared and isolated beforehand. Reaction of the PTOC ester with the sulfenamide advantageously proceeds under neutral conditions (i.e., without the need for tertiary amine base), which enables racemization free coupling. Free amines can be used in place of the benzene sulfenamides, thus eliminating a synthetic step, but this strategy requires base additives and results in lower coupling rates and slightly more racemization.

Rapoport et al. have developed the reagent CBMIT, which mediates peptide coupling under neutral, racemization-free conditions. CBMIT, obtained through the bismethylation of carbonyldiimidazole with methyl triflate, is a highly reactive acylating agent that appears to fare well with hindered substrates.[137] The hindered dipeptide Z-Aib-Aib-OMe was formed in 81% yield with this reagent, although it is noteworthy that the acylation of the *N*-methyl amino ester MeLeu-OMe with Z-Phe was somewhat less efficient (70% yield).

UNCA's and PyBroP are also effective methods in couplings of hindered Aib residues.[138] In one case, an extremely hindered MeAib was reportedly acylated by Boc-Phe-NCA in quantitative yield. The best results were obtained at elevated temperatures (50 °C) with excess UNCA over extended reaction times, and the results provided by UNCA were superior to those provided by PyBrop.[138]

The UNCA method is unfortunately plagued by a number of possible side reactions.[139] For example, when treated with *tert*-butyl amine, Boc-Aib-NCA yielded the expected amide as well as the urea derived from undesired attack at the less hindered carbonyl group (Chart 26). With Fmoc-Aib-NCA, premature base-catalyzed deprotection of the Fmoc group by DIEA occurs more readily than with fluoride (vide infra) or mixed anhydride activation and can generate undesired products arising from nucleophilic attack on the intermediate isourea (Chart 26).

Chart 26. Coupling via UNCA's and Potential Side Reactions



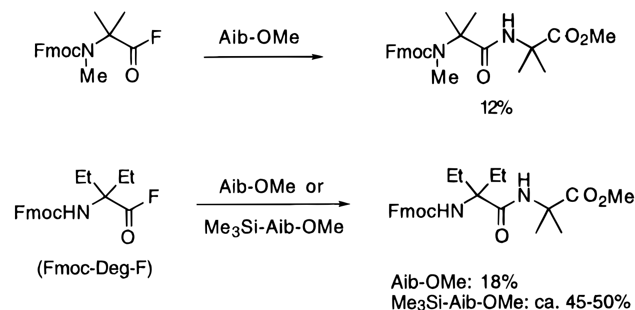
Similar side reactions are known for unprotected Aib *N*-carboxy anhydrides as well.[140]

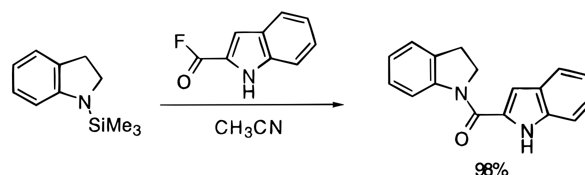
The side reactions observed for UNCA and other reagents can render them less than ideal for solution phase synthesis and incompatible with the solid phase coupling of Aib residues, since incomplete coupling reactions give rise to deletion sequences and impure products. In response to this deficiency, Wenschuh and Carpino investigated Fmoc amino acid fluorides as mediators of these difficult couplings.[141−143] Although a previous report had characterized the amino acid fluorides as sluggish agents for these couplings,[138] the work of Wenschuh and Carpino has elevated them to a position of choice for the solution and solid phase synthesis of Aib containing peptides. Three factors are responsible for the remarkable success of this method: (*i*) the small size of the fluoride ion makes it an ideal activating group for hindered couplings, (*ii*) the acid-stable Fmoc group resists S_N1 or S_N2 side reactions, which can plague peptide synthesis utilizing Boc- or Z-protection schemes,[141] and (*iii*) the amino acid fluorides are more stable and less likely to form oxazolones[143] than the previously investigated amino acid chlorides.[105,108] Furthermore, Carpino has recently shown that amino acid fluoride coupling reactions proceed well under neutral conditions. This enables efficient coupling reactions in the absence of tertiary base, thus minimizing the extent of side reactions such as oxazolone formation, epimerization, or racemization and premature Fmoc cleavage.[144]

Once-difficult couplings involving Aib residues have recently been described as "easy" using the acid fluoride method.[19,139] For example, the 41-residue peptide h-CRF (which contains four contiguous Aib residues) was prepared efficiently,[19,139] whereas the previously recommended UNCA's,[138] PyBrop, and other methods failed completely. The acid fluoride method also permitted the first solid phase synthesis of alamethicin-acid (Chart 20), a 20-residue peptide containing eight Aib and two proline residues.

Extremely hindered sequences, such as Fmoc-MeAib-Aib-OMe or those containing Fmoc-Deg-Aib-OMe linkages, are very difficult to assemble. Standard Fmoc amino acid fluoride conditions gave poor coupling yields for these dipeptides (18% and 12%, respectively, Chart 27), with products arising from competing Fmoc deprotection predominating.[141] These hindered linkages are more efficiently prepared by treatment of the Fmoc amino acid fluorides with the *N*-trimethylsilylamine generated by prior treatment of the free amine with BTSA. This method enabled

Chart 27

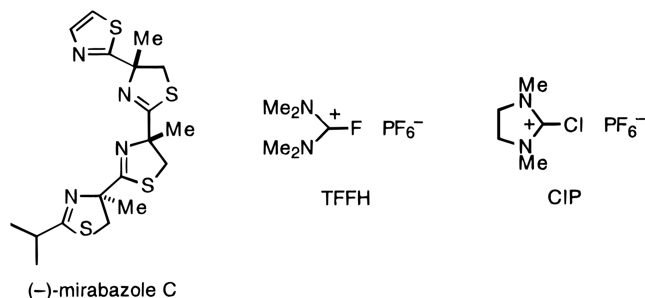




the preparation of the dipeptide Fmoc-Deg-Aib-OMe in yields approaching 50% (Chart 27), and premature Fmoc deprotection was not observed.[141] This method is based on previous work by Cava et al., who showed that *N*-silylation enhanced the reactivity of poorly nucleophilic aromatic amines for acylations with acid fluorides (Chart 27).[145,146]

Racemization of standard amino acid fluorides during couplings to Aib derivatives in standard systems is generally negligible,[19,147] although extreme cases naturally require carefully chosen conditions to minimize racemization. For example, when the racemization-sensitive Z-Phg-F was coupled with the hindered Aib-OMe (CH_2Cl_2/DIEA), up to 15% racemization was observed. Racemization could be reduced in this case by conducting the reaction under two phase conditions (CH_2Cl_2/aqueous $NaHCO_3$);[142] however, the neutral reaction conditions recently described by Carpino should prove even more useful for reducing racemization in sensitive substrates.[148] The urethane-protected amino acid fluorides can be prepared in advance with cyanuric fluoride[149] in pyridine, or with (diethylamino)sulfur trifluoride in CH_2Cl_2,[150] and are generally crystalline solids that are stable for several months. Alternatively, Carpino has reported the use of TFFH (Chart 28), which permits high yield couplings after in situ preparation of the amino acid fluorides. The hindered peptide H-Tyr-Aib-Aib-Phe-Leu-NH_2 was prepared in 88% yield by the solid phase method using this reagent.[151]

Chart 28



Carpino's HATU reagent (Chart 14) also gives excellent results for Aib couplings and has the advantage over the amino acid fluorides of being commercially available and enabling one-pot coupling protocols. HATU permits the linkage of adjacent Aib residues in quantitative yield, whereas HOBt protocols give less than satisfactory results.[113,152] Another similarly based method is CIP (Chart 28) in the presence of additive HOAt, which mediates solution phase Aib couplings in good to excellent yield (82−90% for Z-Aib-Aib-OMe).[152,153] The yields for these couplings in the absence of HOAt were comparable with those obtained from PyBrop (10−60%), but the additive HOAt improved them dramatically.[154] For Aib activation, the CIP-mediated reaction proceeds first through the oxazolone and then through the HOAt active ester. Fmoc and Z-nitrogen protection gives the best yields, while Boc protection is slightly less efficient due to the deleterious *N*-carboxy anhydride formation mentioned previously (Chart 5).[74] CIP/HOAt provided the key activation for the difficult coupling of adjacent 2-methylcysteine residues in Kiso's synthesis of (−)-mirabazole C (Chart 28).[153]

Other types of steric hindrance can also inhibit peptide bond formation. Acylations of Val and Ile are more difficult than the other common amino acids, but this is generally not a major concern unless the other coupling partner is also unusually hindered (these cases were discussed throughout the previous two sections). The hindered nature of the didemnin isostatine nitrogen is reflected by the low yields obtained by Shioiri and Schmidt for its acylation (DEPC, 31%; Pfp-OH, 33%; pivaloyl chloride, 21%; DPPA, 45%; dimethyl-2-thiopyridone-3-carbonitrile, 55%; Chart 29).[117,118]

During the course of our synthesis of microcystin-LA, we encountered another type of hindered residue that was difficult to couple. Our initial attempts to efficiently activate the benzyl/phenylfluorenyl protected aspartate derivative **13** (Chart 30) and couple it with typical nonhindered primary amines proved unsuccessful. Standard methods of acid activation such as DCC/HOBt, DCC/DMAP, or acid chloride activation gave poor results, providing respectively the corresponding HOBt ester, the *N*-acylurea derived from rearrangement of the initial DCC adduct, and decomposition products resulting from overactivation. Structural features of **13** that might contribute to this coupling failure include the steric bulk conferred by α-substitution of the acid component, the bulky protection of the amine group, and a diminished electron withdrawing effect of the β-amine group relative to the typical α-amino acid couplings. The problem was solved by employing HATU with catalytic DMAP, which provided the desired dipeptide **14** in quantitative yield (Chart 30).

IV. Dehydro Amino Acid Containing Peptides

The Dhaa's are fairly reactive Michael acceptors that react readily with thiols and amines but are less reactive toward oxygen nucleophiles (i.e., hydroxide),

Chart 29. Hindered Isostatine Couplings

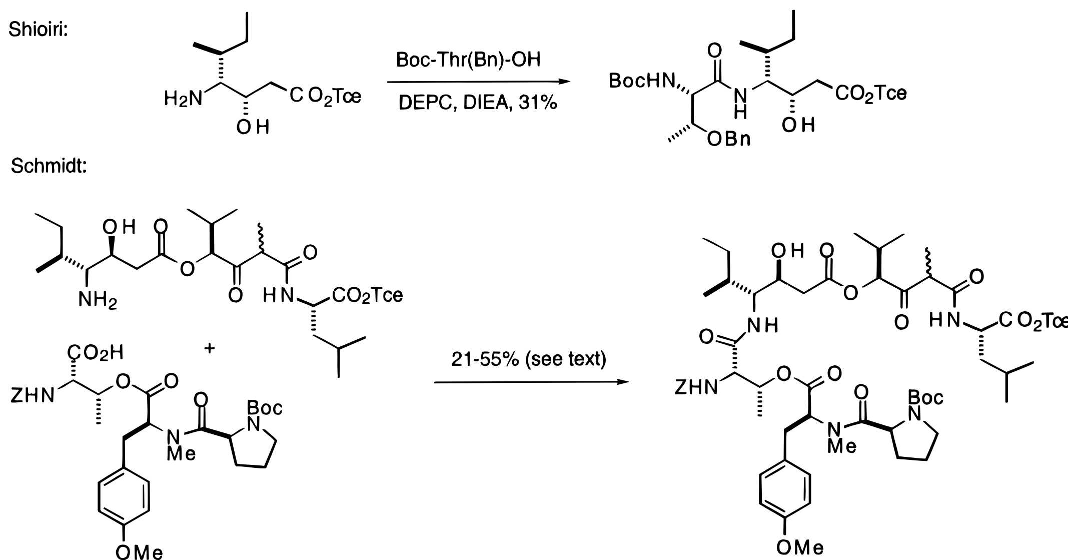


Chart 30. Steric Hindrance Caused by *N*-Phenylfluorenyl Protection

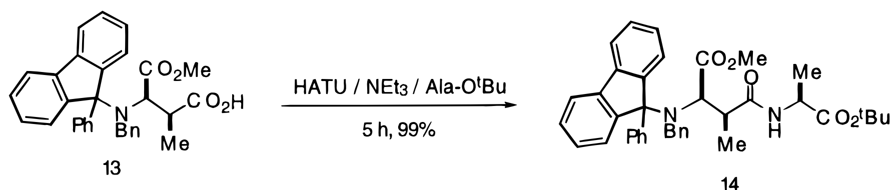
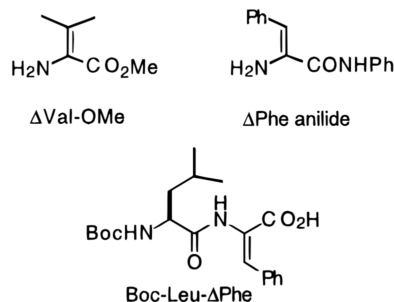


Chart 31. Isolable Dehydroamino Acid Derivatives



although water can add under acidic conditions.[59,75] Substitution at the β-position induces some measure of resistance to these Michael additions.[75] Dehydro residues also engage in electrophilic addition of hydrogen halides to yield α-halo derivatives that equilibrate to the thermodynamically favored β-halo species.[155] Dhaa's undergo facile catalytic hydrogenation, cycloadditions, electrocyclic reactions, ene reactions, and *E/Z*-isomerizations.[59] These residues are fairly stable toward acids with weakly nucleophilic counterions, and although aromatic dehydro residues are reportedly more acid stable (i.e., 1 N HCl) than aliphatic ones, ΔMeAla residues exhibit excellent stability to trifluoroacetic acid. Tripeptides containing dehydro residues appear less prone to diketopiperazine formation than the previously discussed *N*-methyl and Aib containing peptides. For example, the tripeptide Leu-ΔMePhe-Gly-OMe could be acylated (DCC/HOBt/Boc-MeAla) without formation of *cyclo*-[Leu-ΔMePhe] (cf. Chart 6).

The nonacylated α,β-Dhaa derivatives ΔVal-OMe and ΔPhe anilide (Chart 31) are isolable,[26,118] but these amino acids are poor nucleophiles in peptide coupling reactions due to delocalization of the nitrogen lone pairs.[156] This effect is accentuated when the dehydro residue is conjugated with aromatic rings, such as in ΔPhe, and therefore synthetic routes that require extension in the amino direction even from a relatively stable *N*-terminal dehydro residue should be avoided.[26,75] In addition, however, Rich et al. found that the *C*-terminal carboxyl group of Boc-Leu-ΔPhe (Chart 31) was difficult to activate: the DCC method failed to result in coupling with Gly-OMe, and the acid chloride method gave only low yields of Boc-Leu-ΔPhe-Gly-OMe.[75] However, other reports indicate that this can be a viable strategy.[59] These arguments suggest that the smallest synthetically sensible dehydropeptide containing fragment is a trimer wherein the dehydro residue occupies the internal position.

The free amino forms of most Dhaa's and *N*-terminal Dhp's are unstable, readily hydrolyzing in mildly acidic aqueous solution to liberate ammonia (or methylamine with *N*-methyl Dhaa's) and an α-keto acid.[157] In contrast, their *N*-acylated counterparts are stable, isolable compounds that hydrolyze in a similar fashion only under more rigorous conditions (e.g., HCl/HOAc/H_2O). This feature was exploited by Noda et al. in a solid phase synthesis of oxytocin, both to attach the carboxyl terminus to the resin and as a means of introducing Gln and Asn residues, which were initially incorporated as Glu and Asp dehydroalanine ethylamide amides (Chart 32).[118,158] The ΔAla functionality was introduced by *S*-methylating Boc-Asp[L-Cys(Me)-NHEt]-OBn with methyl fluorosulfonate and eliminating dimethyl sulfide with 2 M sodium hydroxide at 0 °C (which also hydrolyzed the ester—see Chart 32). This example demonstrates the stability of internal ΔAla functionality to the basic conditions of ester hydrolysis as well as to multiple standard peptide coupling steps.

In contrast to the above example, urethane-protected peptides with *N*-terminal *N*-methyl dehydro groups are subject to hydantoin formation under the conditions of basic ester hydrolysis or amide

Chart 32. Oxytocin Synthesis: Dehydro-Residues as Synthesis Intermediates

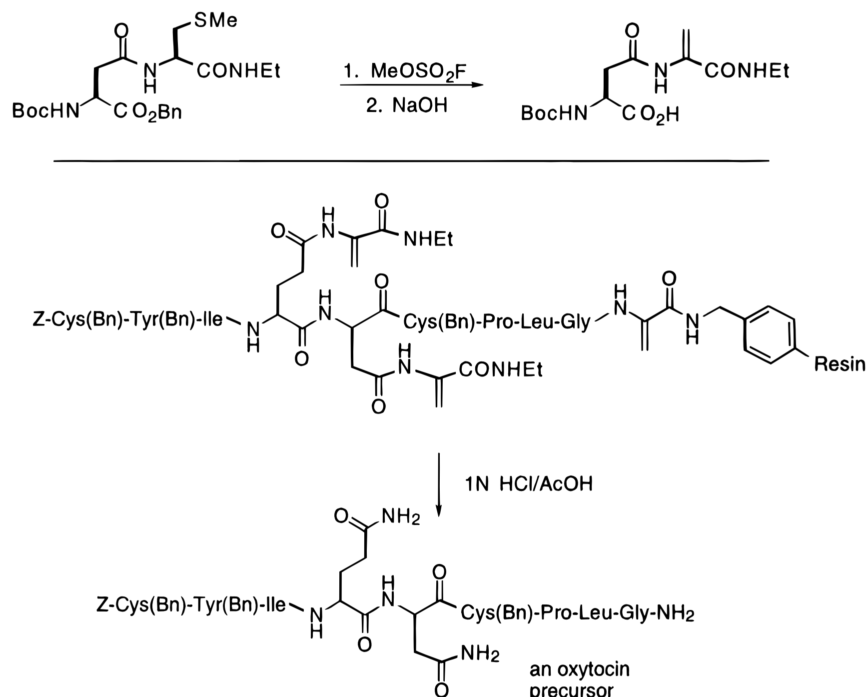


Chart 33. Hydantoin Formation

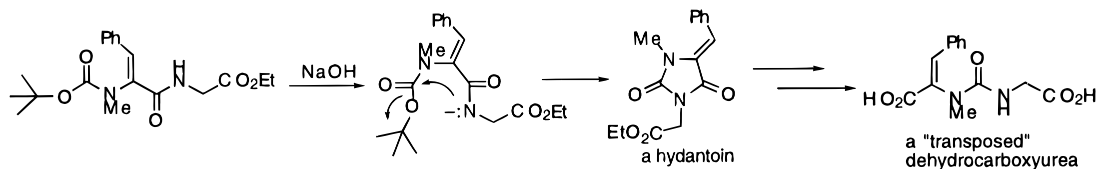
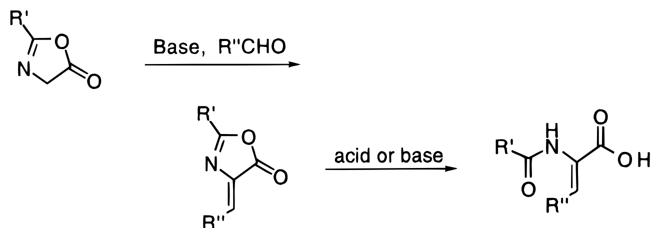


Chart 34. Erlenmeyer Condensation



N-alkylation.[159-161] With standard (non-dehydro) residues, this side reaction is common with Cbz protection, but is suppressed under usual conditions by the more hindered Boc group.[75] However, Boc-protected terminal *N*-alkyl dehydro residues are still prone to hydantoin formation, which results in a "transposed" dehydrocarboxy urea after hydrolysis (Chart 33).[75] Mechanistically, five adjacent trigonal centers and a reduced *trans*-amide preference orients the urethane carbonyl group and the nitrogen of the adjacent residue in close proximity, thereby favoring hydantoin formation.

Of the many known routes to dehydroamino acids and peptides, relatively few are directly amenable to the preparation of *N*-alkylated derivatives.[26,59,60] For example, the classic Erlenmeyer condensation reaction is useful for introducing aromatic dehydroamino acid residues at a *C*-terminal glycine in a growing peptide chain (Chart 34), but incorporation of the *N*-methyl group would require an additional alkylation step.

Dehydro peptides can be prepared through the base-catalyzed dehydration of MeThr or MeSer tosylates or similar residues, but the potential side reactions are numerous.[162] In their recent synthesis of the (*N*-methylamino)dehydrobutyrate-containing cyclic peptide motuporin, Schreiber and Valentekovich showed that activation of the free hydroxyl group of these residues was not necessary to effect β-elimination: barium hydroxide-mediated dehydration of the MeThr residue of an advanced intermediate accompanied bis methyl ester hydrolysis to give motuporin in 52% yield (Chart 35).[98]

Dehydro residues can also be prepared from cysteine derivatives by oxidation to the sulfoxide followed by the thermolytic elimination of sulfenic acid. Rich et al.[28,75,162] applied this method to a synthesis of tentoxin (Chart 36): the 3-benzylthio-D,L-phenylalanine containing tetrapeptide was oxidized to the sulfoxide, and the elimination was induced by heating in refluxing xylene to give the *E*- and *Z*-ΔPhe peptides in 60% yield.[162] This method is obviously limited to substrates that can withstand periodate oxidations and prolonged heating, although new, milder protocols allow the elimination to proceed at somewhat lower temperatures.[162] Methods for obtaining the *Z*-isomer selectively were also described.[75] The *N*-methyl group was subsequently introduced in 89% yield by treatment with MeI and K_2CO_3 in DMF over several days. These conditions selectively alkylate dehydro residues over standard ones, although the method has its limitations. Anhydrous reaction conditions and lengthy reaction periods are required for good results, and some dehydro residues are unreactive.[159]

The versatile chemistry of Schmidt et al. has proven to be a valuable and practical method of preparing dehydroamino acids. The precursor phosphonoglycine (Chart 37) is extended on either side with standard coupling procedures to give a phosphonopeptide, which is then smoothly converted into a dehydropeptide in excellent yield via the Horner Emmons reaction (Chart 37). A variety of aromatic and aliphatic aldehydes have been employed, and the utility of this method was demonstrated in the synthesis of natural products such as chlamydocin (Chart 18) and hexaacetylcelenamide A (Chart 38).[35,36] This method was recently adapted to the preparation of *N*-methyl Dhaa's in our synthesis of microcystin LA (Charts 37 and 48).[163]

V. Segment Coupling Strategies

Peptide synthesis is normally carried out by coupling an activated *N*-protected amino acid to the *N*-terminal residue of the growing chain. On occasion,

Chart 35. Threonine Dehydration Leading to Motuporin

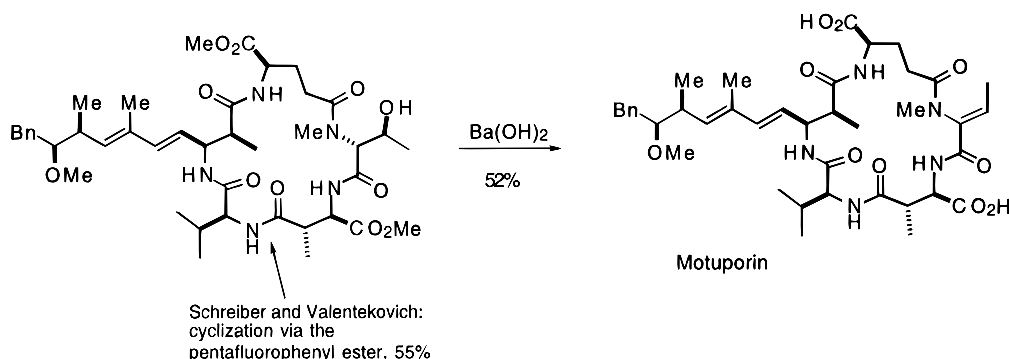
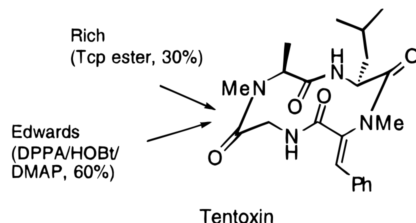


Chart 36. Tentoxin



it is necessary—or desirable for convergence—to proceed in the other direction, i.e., to couple the *N*-terminal amine of a peptide (or a simple amino acid) to the activated carboxyl group of another peptide (e.g., Chart 4). Such "segment couplings" are, however, prone to epimerization via oxazolones that form readily during the activation of *N*-acyl amino acids.[161,164] Due to low coupling rates, the most difficult segment couplings are those involving hindered peptide components; furthermore, problems are compounded with larger peptides because the greater size of the activated peptide fragment can result in a further reduction in the coupling rate.[165] In addition to epimerization, segment couplings can lead to premature cleavage of Fmoc groups. For example, Fmoc-Leu-Aib--Pro-Val-Aib-Aib-NMePh was obtained (DCC/HOBt) in only 19% yield because of competing deprotection of the Fmoc group by the basic proline residue.[126] Fmoc protection of the *N*-terminal amino group is therefore not ideal for segment couplings involving secondary amines or other hindered units.

In spite of these problems, segment couplings offer opportunities for increasing convergence,[166] and furthermore, the synthesis of cyclic peptides usually requires at least one segment coupling (i.e., macrocyclization). Racemization can be reduced or avoided altogether in some cases through the judicious choice of peptide bond disconnections. For example, Kiso et al. found that CIP/HOAt segment coupling at the Ala-Val site of Trichovirin I 4A (Chart 20) proceeded in 81% yield without epimerization, whereas segment coupling at the more hindered Val-Aib site produced a lower yield (60%) of extensively epimerized product.[167] Segment couplings involving the activation of nonepimerizable acid terminal residues such as Gly, Pro, or in some cases Aib can give good results, although, as mentioned in the section on Aib couplings, racemization of the penultimate residue of *C*-terminal Aib containing peptides is a potential side reaction (Chart 24). In Wenger's synthesis of cyclosporine (Chart 10),[168] the 2 + 4 segment coupling between Boc-Abu-Sar and MeLeu-Val-MeLeu-Ala-OBn (via the mixed pivalic anhydride) was free from racemization because the activation occurred at a nonepimerizable sarcosine (MeGly) residue. Rich et al. also benefitted from this feature, employing BOP-Cl for the same 2 + 4 segment coupling in 90% yield.[95] Proline activation gives good results as well: Heimgartner et al. prepared Z-Leu-Aib-Pro--Val-Aib-Aib-NMePh via the isobutyl chloroformate-derived mixed anhydride in 74% yield.[126] This peptide was further extended with another segment coupling, this time via carbodiimide/HOBt activation of a nonepimerizable Aib residue to give the nonapeptide Z-Leu-Aib-Pro-Val-Aib-Aib--Glu(OBn)-Gln-Phol in 65% yield.[126] Shioiri's preparation of didemnin B from didemnin A also involved a segment coupling at proline: in this case, a high yielding BOP-Cl-mediated linkage of the exocyclic MeLeu residue with the nonepimerizable proline segment shown in Chart 39. However, BOP-Cl-mediated segment couplings involving unusually hindered sites remain difficult and racemization-prone: Z-MeVal-MeVal--MeVal-MeVal-O^{t}Bu was prepared in only 52% yield, with 5% epimerization by this method.[88] Other segment couplings involving an *N*-methyl acid terminal proceed in good yield (70−90%) but with severe epimerization.[88]

Non-α-amido peptide residues such as *iso*-Glu or *iso*-Asp make good segment coupling sites because epimerization through the oxazolone route is impossible. Schreiber and Valentekovich took advantage of this feature in preparing the pentapeptide motuporin precursor **17** via pentafluorophenyl ester activation of the *β*-amido Adda dipeptide **15** and treatment with the amine **16** (Chart 40).

Castro's BOP reagent has been applied to segment couplings with moderate success. Wenger's acylation of the *N*-methyl heptapeptide MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn with the hindered valine terminal of Boc-D-Ala-MeLeu-MeLeu-MeVal was mediated by BOP in 73% yield.[168] The moderate yield of this coupling was acceptable considering the hindered nature of the linkage and might even be considered surprisingly high for such an HOBt-mediated *N*-methyl amino acid acylation. In other cases, the BOP reagent performed poorly: the most troublesome reaction during Rich's synthesis of D-lysine^8-cyclosporine analogue (Chart 10) was a BOP-mediated 4 + 7 segment coupling between the hindered MeVal and MeBmt residues. This method gave the resultant undecapeptide precursor to D-Lys^8 cyclosporine in 64% yield but only after recovery and recycling of the starting material.[95] During Jouin's synthesis of nordidemnin B (Chart 16), the Lac-Pro-D-MeLeu fragment was appended to the macrocycle by using BOP in only 57% yield.[89] This segment coupling was chosen over the alternative nonconvergent strategy, which would have required carrying the valuable macrocycle through a number of step-

Chart 37. Dehydropeptides by the Horner Emmons Reaction

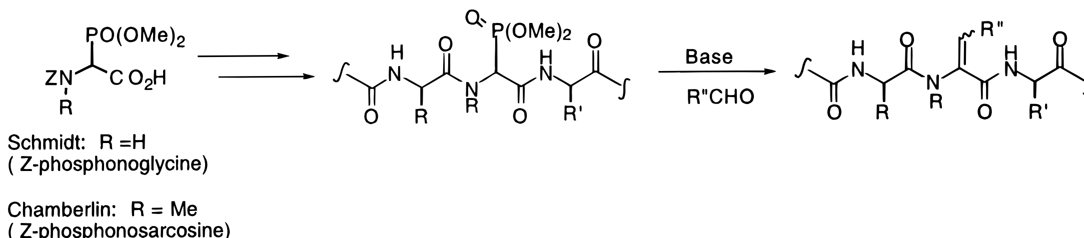
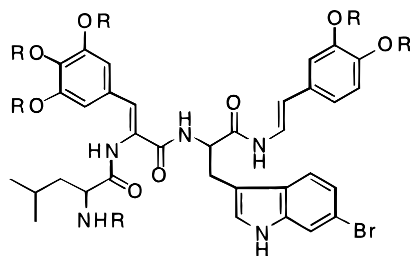


Chart 38. Celenamides



wise couplings, including a potentially difficult Me-Leu acylation. Shioiri's route to the Lac-Pro-MeLeu side chain of didemnin B (Chart 16) involved BOP-Cl-mediated formation of the Pro-D-MeLeu linkage and furnished the higher yields.[89] Segment coupling protocols involving secondary amine acylations should not involve HOBt catalysis for reasons mentioned in the section on *N*-methyl amino acid couplings (vide supra). Grieco et al. attempted one such DCC/HOBt-mediated segment coupling in their synthesis of jasplakinolide (Chart 50) and obtained a 50% yield for this reaction (Chart 41).[169]

Carpino has recently shown that HATU in the presence of collidine (rather than the more commonly used DIEA) affords many segment couplings in high yield and without the complication of epimerization.[113,151,170] For example, the particularly epimerization-prone coupling of Z-Phe-Val-OH and Pro-O*t*Bu was mediated by HATU/DIEA/DMF in 80% yield with 6.5% epimerization, while HATU/collidine/DMF gave 86% yield and only 1.7% epimerization.[170] The reagent HAPyU (Chart 14) gave the best results for this segment coupling, providing only 0.1% epimerization with collidine as the base, or 6.3% with DIEA. The extent of epimerization in these segment couplings involving proline acylations should generate concern for similar couplings with other hindered or otherwise unreactive nucleophiles, such as the *N*-methyl amino acid derivatives. Faster segment couplings, such as that between Z-Gly-Gly-Val-OH and the less hindered Ala-Gly-Gly-OMe, can generally be accomplished with HAPyU/collidine/DMF in >80% yield with less than 0.1% racemization.[170] Carpino's HATU/collidine conditions provided good results for a 4 + 2 segment coupling to give a microcystin precursor in 80% yield and with no detectable epimerization (Chart 42).[163] Methods giving poor segment coupling results can be improved with additive HOAt. For example, the coupling of Z-Phe-Val and Ala-OMe via TFFH/collidine gave 6% of the epimer, whereas epimerization was reduced to <0.1% in the presence of HOAt.[151]

Chart 39. Didemnin B via BOP-Cl Segment Coupling

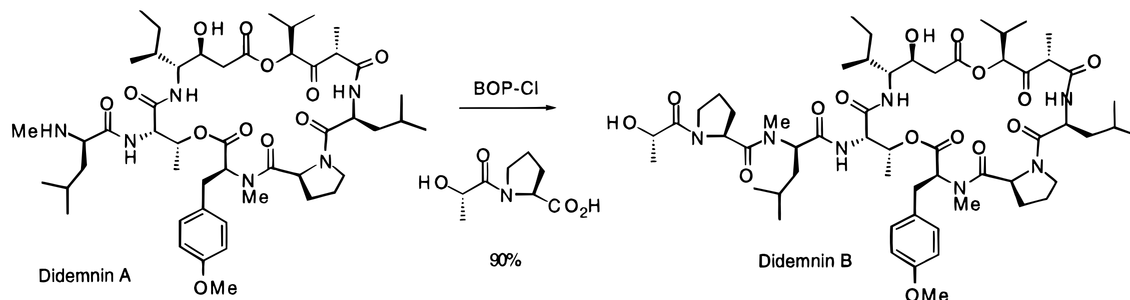


Chart 40. Segment Couplings at Non-α-Amido Acid Residues

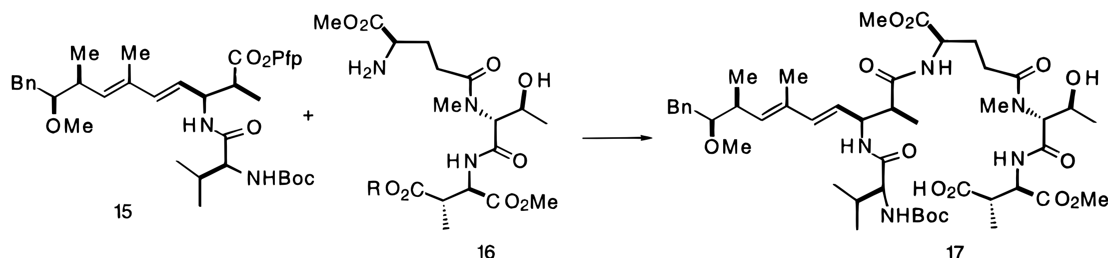


Chart 41

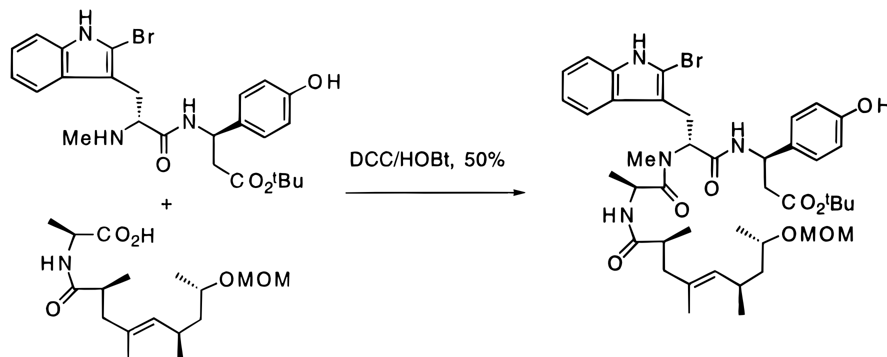


Chart 42

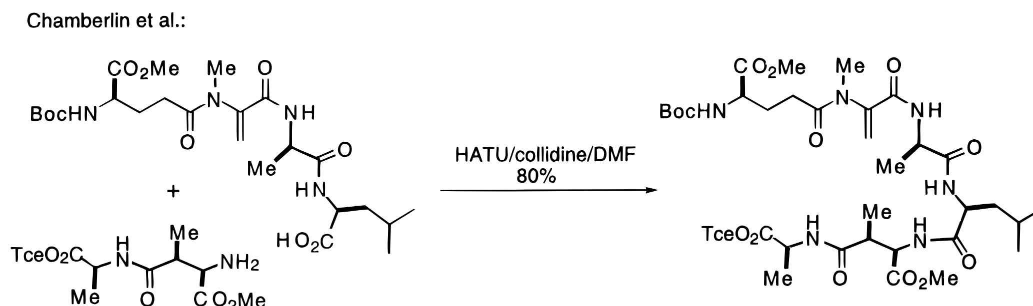
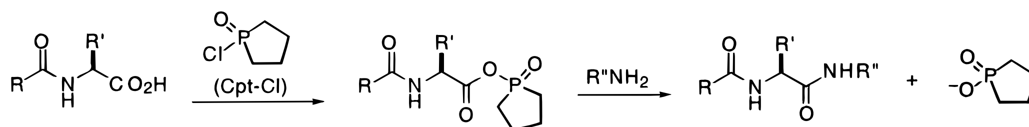


Chart 43



Ho et al. have recently described a two-phase coupling method that also reduces epimerization during segment couplings. Good results were obtained in CH_2Cl_2/water or isopropyl acetate/water with the water soluble carbodiimide EDC and an additive. This method proved especially useful for couplings of racemization-prone *R*-Val or *R*-phenylglycine. In this system, 2-hydroxypyridine *N*-oxide was slightly better at reducing epimerization than HOBt and HOAt. The dipeptide Ac-Val-Val-OBn was obtained in 93% yield with negligible racemization, and the coupling giving rise to Z-Phg-Val-OBn proceeded in 99% yield with no detectable racemization.[171]

The carboxylic–phospholanic mixed anhydrides reported by Poulos et al. also mediate segment couplings with little epimerization (Chart 43).[165] This is attributed to the resistance of the phospholanic mixed anhydrides to form epimerization-prone oxazolones below 0 °C. The mixed anhydrides, prepared by reaction of the free acid with Cpt-Cl, decompose at room temperature.

VI. Peptide Macrocyclization

Peptide macrocyclization reactions have been reviewed extensively,[3,93,168,172–177] so this section will limited to a brief survey of this topic as it relates to the synthetic examples already mentioned or referenced to above, with emphasis on structural features that either promote or hinder the cyclization process. For macrocyclic peptide synthesis, the ring disconnection carries significant strategic importance and can dictate the level of success of the synthesis. Poor disconnections can lead to slow cyclization rates, in turn facilitating side reactions such as dimerization, oligomerization, and/or epimerization of the *C*-terminal residue. The cyclization position should therefore be carefully chosen according to a number of simple guidelines:[93,173] (*i*) The cyclization site should not be sterically encumbered by *N*-alkyl, α,α-disubstituted, or *β*-branched amino acids such as Val or Ile, although one possible exception to this general rule is cyclization at proline, which has given good results (vide infra). (*ii*) If possible, the cyclization should occur between a D- and an L-residue, since this reaction tends to proceed faster than cyclization between residues of the same α-center configuration. It has been further stated by Veber et al. that the best cyclization precursor contains a D-residue amino terminus.[178] Interestingly, peptides consisting of only L-residues and no other turn-inducing structures will often not cyclize until the carboxyl terminal epimerizes to the D-configuration.[177] (*iii*) Intrachain hydrogen bonds can facilitate peptide cyclization. Potentially useful hydrogen bond interactions might be identified by X-ray crystallography[168] or molecular modeling. (*iv*) Turn-inducing structures such as Gly or secondary amide linkages facilitate cyclization,[173] and the position of these residues within the cyclization precursor may be important. It has been suggested that the turn-inducing structure should reside midway along the cyclization precursor for the best results, although in other cases the location of the turn-structure does not significantly affect the success of the cyclization.[3,175]

Wenger designed his cyclosporine synthesis based on these guidelines.[168] The cyclization coupling was chosen between L-Ala (position 7) and D-Ala (position 8), the only adjacent non-*N*-methylated residues in the molecule (Chart 10). Furthermore, the opposite configurations and small side chains of these Ala residues were expected to facilitate cyclization, and intramolecular H-bonds and a *cis*-amide linkage between the MeLeu residues at positions 9 and 10 were likewise predicted to aid cyclization. For the macrolactamization, the azide method was attempted and then abandoned due to low yields attributed to acid catalyzed epimerization of the *N*-methyl residues during the diazotization reaction, but three other cyclization methods, BOP, propylphosphonic anhydride, and DCC/pentafluorophenol, each gave good yields (62–65%). Rich et al. cyclized their D-Lys[8] cyclosporine analogue (Chart 10) with a coupling reaction between D-Lys and L-Ala according to similar arguments and achieved 68% yield via propylphosphonic anhydride/DMAP activation.[95]

Galpin's ring disconnection[110] between the hindered cyclosporine 1 and 11 positions (Chart 44) was designed to enable the facile preparation of cyclosporine analogues differing from the natural compound at position 1, which had been implicated as being central to the immunosuppressant activity. That this

Chart 44. MeThr¹-Cyclosporine

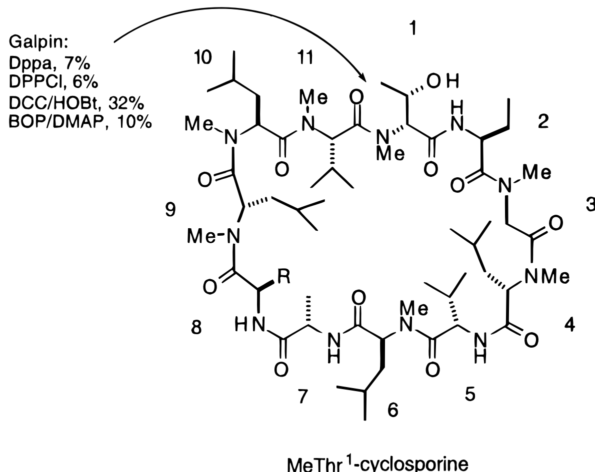
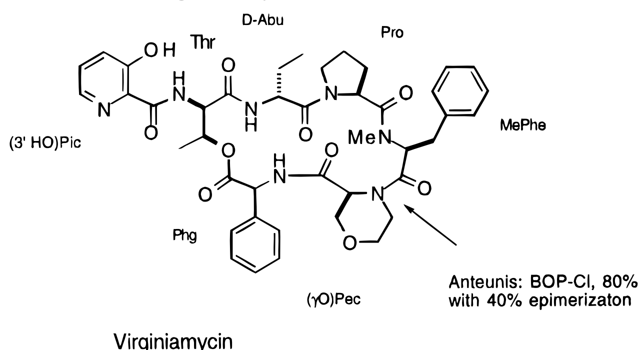


Chart 45. Virginiamycin



strategy was aimed at the rapid preparation of analogues rather than at maximizing cyclization efficiency was reflected in the cyclization yields, which were generally low. For the cyclization of a MeThr¹ analogue (Chart 44), a variety of methods including DPPA, DPPCl, DCC/HOBt, and BOP/DMAP generally gave very poor results after lengthy reaction periods (several days), with yields based on final products that appear to be mixtures. An analogue with hydroxyproline at position 1 (Chart 10) gave the best cyclization results, 65% via BOP/DMAP, but only low yields by other methods. Application of the BOP reagent for *N*-methyl couplings and cyclization at this hindered position were found to be not generally conducive to high yields.

Ring closures at *N*-terminal *N*-methyl residues are not recommended, even with nonbulky acid components such as glycine. Rich attempted this type of cyclization between Gly and MeAla of tentoxin (Chart 36), obtaining only 30% of the desired product.[75] Interestingly, the reaction proceeded at high dilution via the trichlorophenyl ester in refluxing pyridine, but no desired product was obtained at ambient temperatures. Tentoxin analogues containing D-MeAla at position 1 gave yield improvements of up to 20% for the cyclization reaction.[75] Edwards et al. employed Dppa/HOBt/DMAP for a similar tentoxin cyclization and obtained a much improved yield of 60% (Chart 36).[179] Anteunis' decision to close virginiamycin S at the hindered MePhe-(γO)Pec position via BOP-Cl (Chart 45) was met with 40% epimerization at the MePhe residue, although the combined yield of the epimers was 80%. The level of epimerization was doubled in the presence of HOBt, which evidently inhibited the cyclization reaction.

During Rinehart's synthesis of didemnin A, the hindered nature of the isostatine-Thr linkage was reflected in the low cyclization yield (18%) obtained via EDC/HOBt (Chart 46). On the other hand, Jouin et al. took clever advantage of the opportunity for racemization-suppressing urethane protection in his nordidemnin B macrocyclization, achieving an improved yield of 54% by cyclizing a Z-Thr derivative in "stepwise coupling" fashion.

Shioiri et al. avoided the hindered isostatine residue altogether and instead chose to cyclize at the seemingly more hindered MeTyr-Pro site. This risky strategy paid off, supplying the cyclized product in 68% yield after 3 days reaction with BOP-Cl (Chart 16).[117] Schmidt's route to the didemnin macrocycle was the most attractive of the four in terms of macrocyclization yields. In choosing the Pro-Leu ring disconnection, he bypassed problems associated with the hindered isostatine and MeTyr groups, and cyclization via the pentafluorophenyl ester proceeded in 70% yield (Chart 16).[118]

That the position of ring closure can have a dramatic effect on the cyclization yield is further illustrated by Schmidt's synthesis of the cancerostatic peptide WF-3161.[180] Model studies on a simplified WF-3161 analogue, which contained the substitution of Ala for Aoe, showed that cyclization was most efficient between D-Phe and L-Ala (cf. 70% vs 0−16% for the other three positions—see Chart 47). Subsequent Pfp ester induced cyclization at the analogous position of an Aoe-containing WF-3161 precursor furnished the macrocycle in 74% yield.

Chart 46. Didemnin Cyclizations

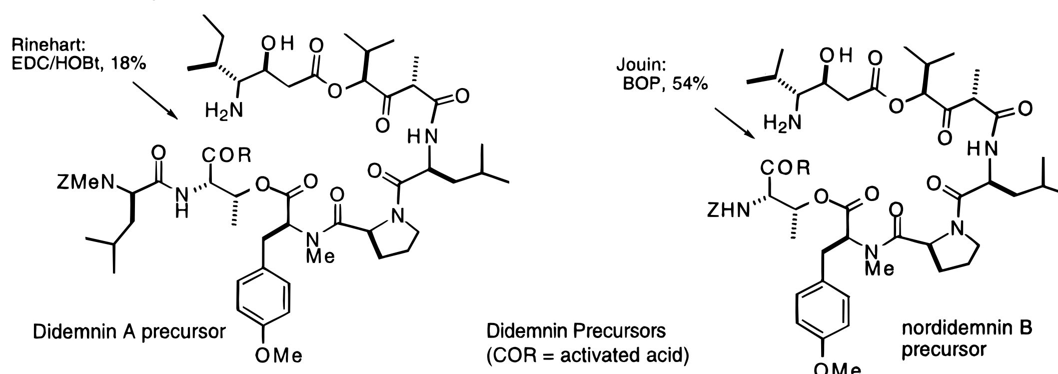
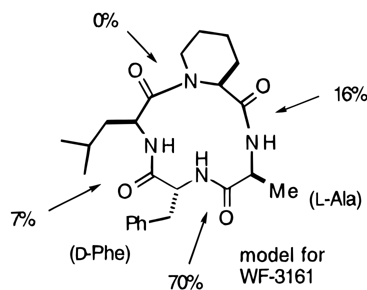


Chart 47. WF-3161 Cyclizations via the Pfp Ester

Schmidt & Beutler:



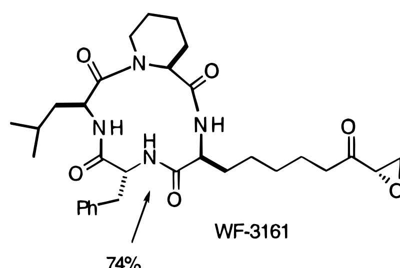
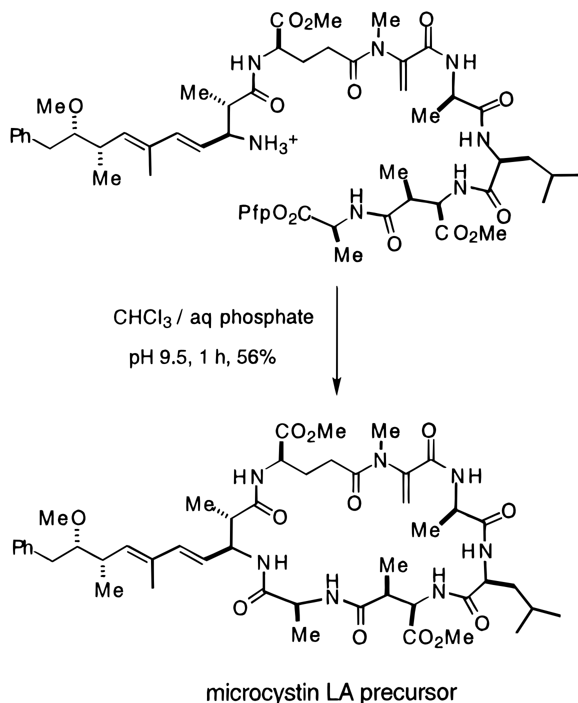


Chart 48. Pfp Ester-Induced Cyclization Leading to Microcystin-LA



The pentafluorophenyl ester method often gives better results than other active ester methods and has been applied to many cyclopeptide syntheses.[23,46,48,50,51,54,98,181] We employed this method of activation for the cyclization of a microcystin-LA precursor and achieved rapid ring closure in a two-phase $CHCl_3$/pH 9.5 phosphate buffer system to give the macrocycle in 56% yield within 1 h (Chart 48).[163] The positioning of the turn-inducing MeΔAla residue midway along the microcystin cyclization precursor may have contributed significantly to the rapid cyclization observed in this case. The pentafluorophenyl ester method was also chosen by Schreiber and Valentekovich for their motuporin ring closure between Val and β-methyl D-Asp (Chart 35).[98] Cyclization in this case required several days at high dilution and supplied the cyclized product in 55% yield. The hindered *N*-terminal Val residue is a suboptimal nucleophile in peptide coupling reactions, but cyclization at this site was nevertheless attractive because it enabled activation of a β-amido residue, which is less prone to epimerization than the usual α-amido residues. The activation of other nonepimerizable *C*-termial residues such as Gly or Pro present similar cyclization advantages.[173]

For sluggish cyclizations, the Pfp ester may not supply sufficient activation. Schmidt and Baldwin had each reported fast and efficient Pfp ester-induced ring closures en route to syntheses of chlamydocin (Chart 49),[182,183] but Schreiber found that a similar cyclization leading to the structurally related cyclopeptide trapoxin B was difficult and required more powerful activation.[184] The major structural difference between these peptides is the absence of an Aib residue in trapoxin, suggesting that Aib positively influences this type of cyclization reaction. The trapoxin cyclization required treatment with BOP/DMAP in DMF over 3 days.

For depsipeptides such as jasplakinolide (Chart 50), macrolactonization at nonepimerizable sites is often preferred over macrolactamization at epimerizable sites, and for these types of cyclizations, the Boden–Keck conditions[185] generally give the best results. For example, Grieco et al.[169] cyclized jasplakinolide according to the Boden–Keck protocol (DCC/DMAP-TFA) in high yield (79%), while Konopelski et al.[96] obtained a much lower yield for this transformation under the more traditional conditions (DCC, 36%). The DMAP-TFA salt employed in the Boden–Keck protocol presumably raises the effective concentration of proton sources, which mediate the proton transfer steps required for this high dilution intramolecular esterification reaction. The result is an enhanced cyclization rate and a reduced occurrence of side reactions. However, macrolactonization is not always the best choice for depsipeptide cyclizations. White and Grieco independently obtained poor yields for the macrolactonization of geodiamolide A (20%)[99] and B (15%),[97,186] respectively (Chart 50), using the Boden–Keck cyclization method. In a related case, Yamada's Boden–Keck-mediated macrolactonization of a doliculide analogue resulted in complete epimerization at the MeTyr moiety during cyclization (Chart 51). Better results were obtained via BOP-Cl mediated macrolactamization at the nonepimerizable MeTyr-Gly site, which gave the doliculide macrocycle in ~80% yield.[186]

The new HOAt reagents HAPyU and TAPipU (Chart 52) excel in peptide macrocyclizations.[172] These reagents offer an attractive alternative to the traditional azide methods, which have long been known to reduce racemization but may be incompatible with *N*-methyl peptides,[168] or to the HOBt reagents, which improve cyclization rates in some cases but can also give more racemization. For the difficult cyclization of the hexapeptide H-Val-Arg-Lys(Ac)-Ala-Val-Tyr-OH, which contains no turn

Chart 49. Chlamydocin and Trapoxin B Cyclizations

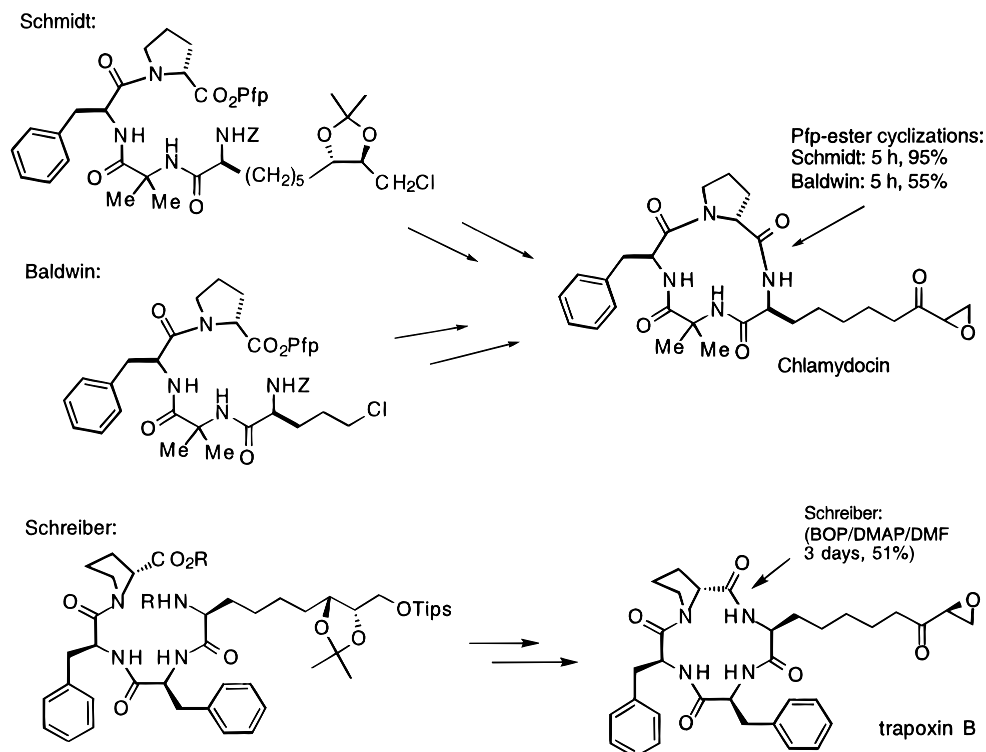


Chart 50. Jasplakinolide and Geodiamolide

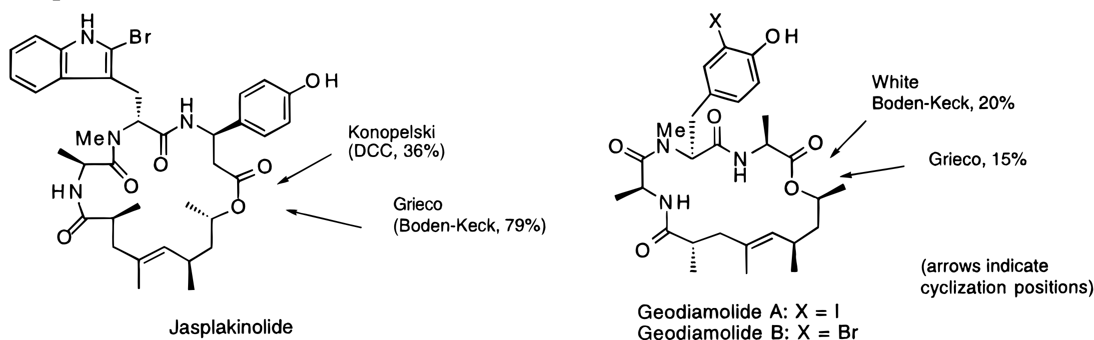
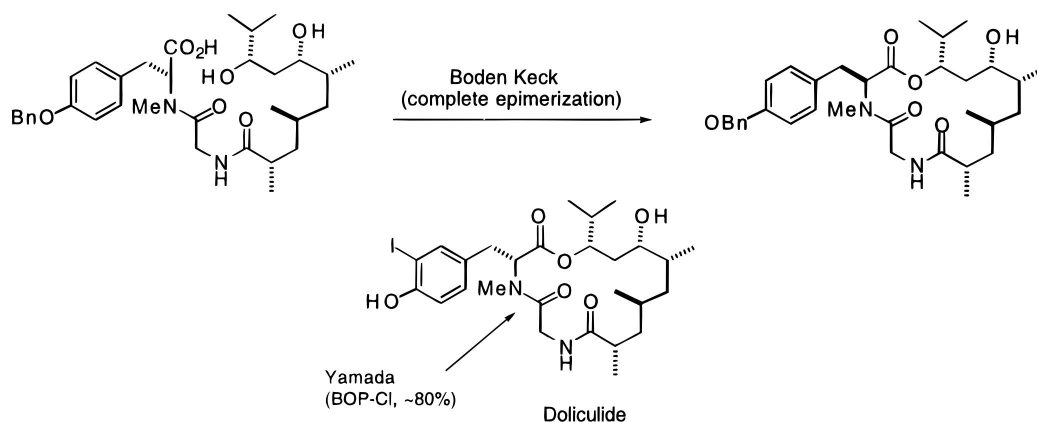
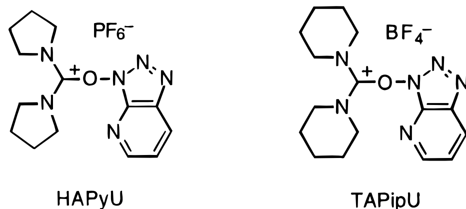


Chart 51. Doliculide Cyclizations



structures, the HOBt reagents BOP and TBPipU gave low yields (5−10%) and extensive racemization (24 and 7%, respectively). In marked contrast, HAPyU gave 55% yield and <0.5% epimerization. The HOAt reagents mediate rapid, high yielding cyclizations for typical decapeptides, generally requiring only 30 min for complete cyclization without the necessity of high dilution protocols. On the other hand, severely unfavorable peptide conformational preferences still cannot be overcome. Attempted HAPyU cyclizations of the pentapeptide H-Arg-Lys(Ac)-Ala-Val-Tyr-OH, which contains only L-amino acids, led only to dimerization, even at high dilution. That the peptide must epimerize in order to cyclize is evidenced by the HOBt reagents giving small amounts of the epimerized macrocycle and

Chart 52. HAPyU and TAPipU



HAPyU giving only a trace due to its ability to suppress racemization. Coupling with HATU (Chart 14) was shown to be effective at reducing epimerization in medium ring cyclizations during solid phase synthesis.[34]

VII. Conclusion

In recent years, new reagents and methods have significantly advanced the field of nonribosomal peptide synthesis. Once-troublesome *N*-substituted amino acids can now be coupled smoothly with a number of reagents, of which Carpino's HATU generally gives the best results. Synthetic methods for traditionally difficult Aib-containing peptides have also improved dramatically in recent years, to the extent that even solid phase synthesis of these compounds is now relatively simple via the Fmoc amino acid fluorides, which are currently the best reagents for these couplings. Many routes are available to dehydropeptides, with Schmidt's phosphonoglycine method proving among the most versatile, and this method has recently been extended to include the preparation of *N*-methyl dehydroamino acid residues. Other work includes improvements in segment coupling protocols and peptide macrocyclizations, which should facilitate natural product synthesis in this field.

VIII. Abbreviations

Abu	aminobutyric acid
Aib	2-aminoisobutyric acid
Aoe	(2*S*,9*S*)-2-amino-9,10-epoxy-8-oxodecanoic acid
AOP	7-azabenzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate
Ala-OMe	alanine methyl ester
Gly-OMe	glycine methyl ester, etc.
Bn	benzyl
Boc	*tert*-butoxycarbonyl
BOP	benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate
BOP-Cl	bis(2-oxo-3-oxazolidinyl)phosphinic chloride
BroP	bromotris(dimethylamino)phosphonium hexafluorophosphate
BTSA	*N*,*O*-bis(trimethylsilyl)acetamide
CBMIT	1,1′-carbonylbis(3-methylimidazolium) triflate
Cbz	carbobenzyloxycarbonyl
CIP	2-chloro-1,3-dimethylimidazolidium hexafluorophosphate
collidine	2,4,6-trimethylpyridine
Cpt-Cl	1-oxochlorophospholane
DEPC	diethylphosphorocyanidate
Deg	diethylglycine
Dhaa	α,β-dehydroamino acid
Dhp	dehydropeptide
DIEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
Dppa	diphenylphosphoryl azide
Dpp-Cl	diphenylphosphinic chloride
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
Fmoc	9-fluorenylmethoxycarbonyl
HAMDU	*O*-(7-azabenzotriazol-1-yl)-1,3-dimethyl-1,3-dimethyleneuronium hexafluorophosphate
HAPipU	*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-pentamethyleneuronium hexafluorophosphate
HAMTU	*O*-(7-azabenzotriazol-1-yl)-1,3-dimethyl-1,3-trimethyleneuronium hexafluorophosphate
HAPyU	*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyleneuronium hexafluorophosphate
HATU	*N*-[(dimethylamino)-1*H*-1,2,3-triazole[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate, previously known as *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate[177]
HBTU	*O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
HOSu	*N*-hydroxysuccinimide
h-CRF	human corticotropin releasing factor
Lac-OH	lactic acid
MeAla	*N*-methyl Ala
MeIle	*N*-methyl-Ile, etc.
Meaa	*N*-methyl amino acid
ΔMeAla	*N*-methyldehydroalanine
ΔPhe	dehydrophenylalanine, etc.
NCA	*N*-carboxyanhydride
(γO)Pec	γ-oxopipecolinic acid
Phg	phenylglycine
Phol	phenylalinol
Pic	picolinic acid
PTOC	pyridine-2-thione-*N*-oxycarbonyl
PyAOP	7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate
PyBroP	bromotripyrrolidinophosphonium hexafluorophosphate
PyCloP	chlorotripyrrolidinophosphonium hexafluorophosphate
PyClU	1,1,3,3-bis(tetramethylene)chlorouronium hexafluorophosphate
SPip	γ-thiopipecolinic acid
SPPS	solid phase peptide synthesis
TAPipU	*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-pentamethyleneuronium tetrafluoroborate
TBPipU	*O*-(7-benzotriazol-1-yl)-1,1,3,3-pentamethyleneuronium tetrafluoroborate
Tce	trichloroethyl
TFFH	tetramethylfluoroformamidium hexaflourophosphate
UNCA	urethane protected *N*-carboxy anhydride
Z	benzyloxycarbonyl

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X. References

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