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REVIEW ARTICLE

Applications of the Mitsunobu Reaction in Peptide Chemistry

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Abstract: The Mitsunobu reaction – the nucleophilic substitution of an alcoholic hydroxyl group mediated by the redox system trialkylphosphine/dialkyl azodicarobxylate – is widely used in the chemistry of biologically active compounds. The paper deals with applications of the Mitsunobu reaction in amino acid and peptide chemistry. The process provides easy access to many unnatural amino acids and derivatives. Since the reaction occurs with complete inversion of the configuration at the carbinol chiral centre, it can be used for the synthesis of diastereoisomers of hydroxy- and tioprolines. Cyclization of β-hydroxy amino acid containing peptides under Mitsunobu reaction conditions leads to a constrained peptide that mimics the stabilizing reverse turn secondary structure. © 1998 European Peptide Society and John Wiley & Sons, Ltd.

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Keywords: Mitsunobu reaction; *N*-alkyl amino acid synthesis; amino acid derivatives synthesis; β-lactam formation; peptide oxazolines and thiazolines

The Mitsunobu reaction has been known for about three decades. It effectively brings about the nucleophilic substitution of an alcoholic hydroxyl group by the conjugate base of an acidic reactant, with inversion of configuration at the alcohol carbon. It is mediated by the redox combination – triarylphos-phine/dialkyl azodicarboxylate [1–3]. Detailed investigations of the Mitsunobu reaction have led to the commonly accepted two-path mechanism (Figure 1) comprising the following stages: the formation of a triarylphosphine/dialkyl azodicarboxylate zwitterionic adduct, activation of the alcohol by oxyphosphonium salt formation (in the absence of an acid the second route, via dialkoxyphosphorane is favoured), and S_N2-type substitution leading to the product RX and triarylphosphine oxide. For a detailed analysis of the mechanism, see the reviews by Hughes [2, 3].

The efficiency of the reaction depends mainly on the substrate alcohol and the nucleophile; it does not depend on the alkyl group of azodicarboxylate. Thus, ethyl (DEAD) and isopropyl (DIAD) azodicarboxylates work equally well as oxidants and can be used interchangeably. Triphenylphosphine (TPP) is used almost exclusively as the phosphorus component. The sequence of reagent addition has little effect on the yield of the reaction although in some cases the adduct is prepared first to reduce side reactions related to TPP nucleophilicity.

Abbreviations: AA, amino acid residue; Adoc, 1-adamantyloxy-carbonyl; Aloc, allyloxycarbonyl; DEAD, diethyl azodicarboxylate; DIAD, diisopropyl azodicarboxylate; Lac, lactic acid residue; Pht, phthaloyl; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulphonyl; Poc, 4-pyridylmethoxycarbonyl; Ses, 2-[(trimethylsilyl)ethyl]sulphonyl; TCBoc, 2,2,2-trichloro-1,1-dimethylethoxycarbonyl; THP, tetrahydropyranyl; TBDMS, *t*-butyldimethylsilyl; TPP, triphenylphosphine; Troc, 2,2,2-trichloroethoxycarbonyl; WSC, water-soluble carbodiimide; Z(Cl), 4-chlorobenzyloxycarbonyl; Z(*o*-Cl), 2-chlorobenzyloxycarbonyl.

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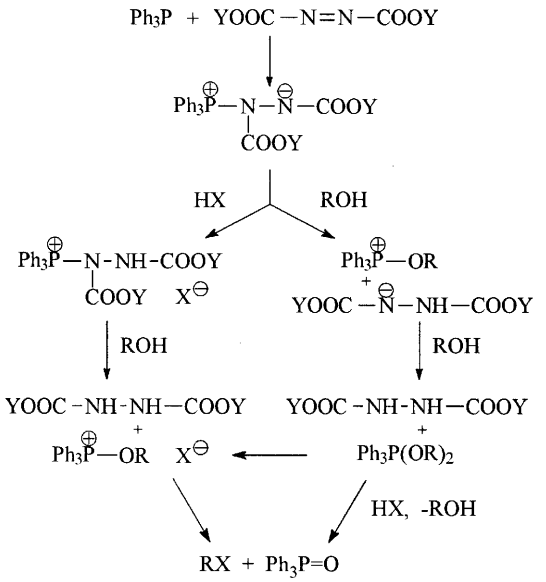


Figure 1.

The Mitsunobu reaction is widely used in organic chemistry owing to its mildness and effectiveness. Several types of natural product, e.g. carbohydrates and alkaloids, have been synthesized or derivatized under Mitsunobu conditions. This paper deals with application of the Mitsunobu reaction in amino acid and peptide chemistry.

Applications of The Mitsunobu Reaction to the Synthesis of Amino Acids and Their Derivatives

The Synthesis of Free and Protected Amino Acids and Their Esters

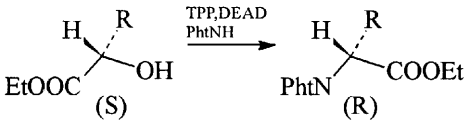
Fabiano *et al.* [4] used the Mitsunobu reaction for the synthesis of amino acids and their esters from corresponding hydroxy acids via an intermediary azide. This one-pot method for converting alcohols into amines was achieved by a combination of three known reactions (Figure 2):

(1) the conversion of an alcohol into an azide with the help of hydrazoic acid in benzene in the presence of TPP and DIAD;
(2) an *in situ* Staudinger reaction [5] producing an iminophosphorane intermediate;
(3) hydrolysis of the iminosphosphorane intermediate by addition of excess of water or dilute hydrochloric acid.

Depending on the hydrolysis conditions, either the free amine or the corresponding hydrochloride was produced. Methyl (*S*)-lactate gave optically pure (*R*)-alanine. Methyl (*S*)-mandelate, however, was converted to the racemic azide and subsequently to the racemic methyl phenylglycinate hydrochloride. Racemization in this case was presumably a consequence of the particular stability of the carbanion intermediate formed by the loss of the α-hydrogen. Z-L-Ser and its methyl ester were similarly converted into the optically active derivatives of diaminopropionic acid.

Viaud and Rollin [6] have developed a novel access to azides using a stable bis-pyridine complex of zinc azide for the one-pot conversion of alcohols into the desired nitrogen compounds via a Mitsunobu-type substitution. The reaction, like the analogous reaction with hydrazoic acid, proceeds with full inversion at the carbinol chiral centre. Thus treatment of ethyl (*S*)-lactate with zinc azide/bis-pyridine complex (1.5 equiv.) and TPP/DIAD (2 equiv.) in toluene smoothly afforded the corresponding pure azide in 83% yield. Complete inversion of the chiral centre was found to occur.

In their pioneer studies on synthesis of amines under mild conditions Mitsunobu and co-workers [7] described the transformation of ethyl (±)-lactate into racemic ethyl phthaloylalaninate by the use of equimolar amounts of TPP, DEAD and phthalimide, in 58% yield (Figure 3). In the same way optically active N-Pht-Ala and N-Pht-Phe were synthesized from the appropriate hydroxy acid [8]. The poor yield of Pht-Phe obtained initially was significantly improved (46→66%) by the use of a molar excess of TPP and DEAD. Complete or almost complete inversion



R = Me or Bzl

Figure 3.

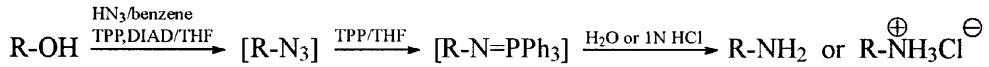
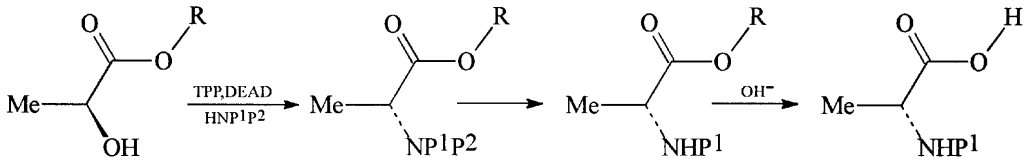


Figure 2.



P1, P2 = Z, Boc, Adoc, Aloc, TCBoc, Troc, Z(OMe), Z(NO2), Z(Cl), Z(o-Cl), Poc, BzlS-CO or Tos
R = Et or Bzl

Figure 4.

of configuration was found to occur during these reactions. Unfortunately, the phthaloyl group is cleaved only under conditions too vigorous to be useful in syntheses involving substrates with sensitive groups.

In model studies aimed at the synthesis of ^{15}N-labelled N-protected chiral amino acids, Degerbeck *et al.* [9] obtained pure, *N,N*-diprotected ethyl (*R*)-alaninates from selected imidodicarbonates or tosyl carbamates and a slight excess of ethyl (*S*)-lactate, under otherwise conventional Mitsunobu conditions (Figure 4). The yield of this conversion was profoundly influenced by the structure of the imidodicarbonate or sulphonylcarbamate used, and varied from $<5\%$ (Boc and Boc) to 93% (Tos and Boc; Tos and Z(NO_2)). The higher yields corresponded to the use of Tos or BzlS-CO protecting groups. The reactivity in the Mitsunobu reaction appears to be dependent on the acidity of the NH function in imidodicarbonates, and steric factors seem to be less important. Being more acidic than amides, sulphonamides were smoothly alkylated in high yield [9]. The degree of racemization was low; in all cases the optical purity of the (*R*)-alanine derivatives produced exceeded 95%. The Fmoc function appeared unstable under the Mitsunobu reaction conditions, and there was no trace of Z(Fmoc)-D-AlaOEt in the mixture resulting from the reaction of Z-NH-Fmoc with ethyl (*S*)-lactate; only decomposition products (Z-NH_2 and dibenzofulvene) were detected. This work based on that of Grehn and Ragnarsson [10] aimed at the design of new synthetic pathways to polyamines related to natural products.

Schmidt *et al.* [11] have described a highly stereoselective synthesis of 2-Fmoc- and 3-Boc-protected (2*S*,3*S*)- or (2*S*,3*R*)-diaminobutyric acid, a component of some antibiotic and immunomodulating peptides found in plant and fungal hydrolysates. (2*S*,3*S*)-3-Azido-*N′*-(Boc)-2-(Fmoc-amino)butyrohydrazide was obtained from (2*S*, 3*R*)-*N′*-(Boc)-2-(Fmoc-amino)-3-hydroxybutyrohydrazide (a threonine derivative) reaction with 10% excess of TPP, HN_3 and DEAD in DMF, and in turn was hydrolyzed to give (2*S*,3*S*)-3-azido-(Fmoc-amino)butyric acid in 81% yield. Subsequent catalytic hydrogenation furnished 3-amino-2-(Fmoc-amino)butyric acid which was converted without isolation into the (2*S*,3*S*)-3-(Boc-amino)-2-(Fmoc-amino)butyric acid. The analogous sequence was accomplished starting from the allo-threonine derivative to yield (2*S*,3*R*)-3-(Boc-amino)-2-(Fmoc-amino)butyric acid. The protocol was also successfully tested for *N*-Troc-threonine hydrazide as starting material [12]; it provides easy access to enantiomerically pure 2,3-diaminobutyric acids bearing compatible protecting groups. The Mitsunobu reaction with Fmoc-Thr-OMe instead of Fmoc-threonine hydrazide was not successful, owing to competitive elimination taking place under these conditions [11].

Campbell and Hart [13] have elaborated a new procedure for the conversion of alcohols into doubly protected amines which could serve as precursors to

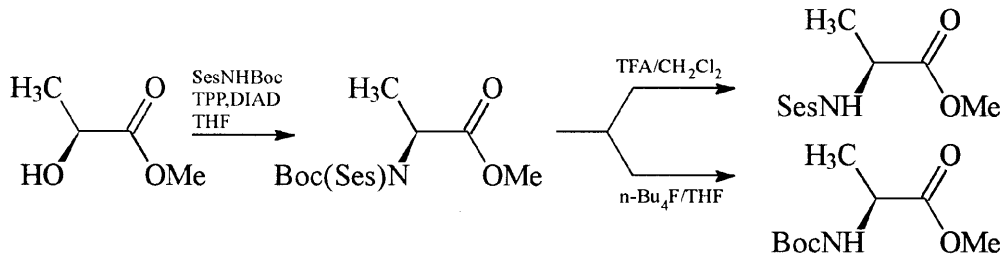


Figure 5.

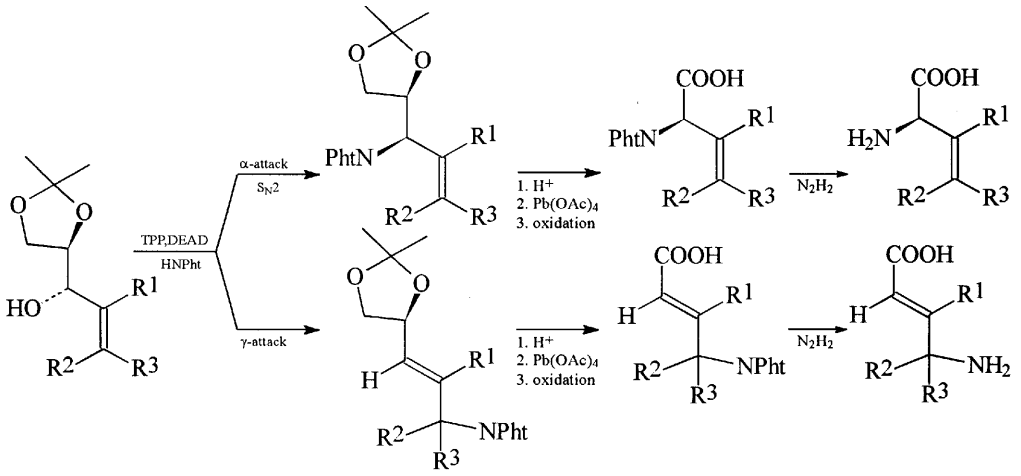


Figure 6.

either carbamates or sulphonamides. They proposed *tert*-butyl-[[2-(trimethylsilyl)ethyl]sulphonyl]-carbamate (Ses-NH-Boc) as an amine synthon which could lead to either Ses- or Boc-protected amines or amino acids (Figure 5). Using methyl (*S*)-lactate (1 equiv.), Ses-NH-Boc (2 equiv.), TTP (3 equiv.) and DEAD (2.4 equiv.) they obtained the methyl ester of *N*-Boc-*N*-Ses-D-alanine as a model compound. Subsequently, the sulphonamide group was removed using tetra-*n*-butylamonium fluoride, giving methyl *N*-*tert*butoxycarbonyl alaninate as a pure D-enantiomer (proving inversion of the configuration).

The amination of allylic alcohols has been employed for efficient syntheses of optically pure β,γ-unsaturated α-amino acids and α,β-unsaturated γ-amino acids, starting from (*R*)-isopropylidene glyceraldehyde and ethyl (*S*)-lactate, respectively (Figure 6) [14]. The key step was the Mitsunobu reaction of chiral secondary allylic alcohols with phthalimide as the nucleophile. Here, α,γ-allylic transpositions were observed for the first time in Mitsunobu chemistry. The α-substitution proceeded with a clean S_N2 inversion, whereas the γ-substitution corresponded to an (*E*)-*anti* (to the -$OPPh_3$ leaving group) attack of the nucleophile, with varying stereoselectivities. The results indicated that the γ-attack in the Mitsunobu reaction proceeded via a partial S_N1 type mechanism in which the leaving group Ph_3PO shields the *syn* face of the allylic cation in a contact ion pair, and directs the attack to the *anti* face (Figure 7).

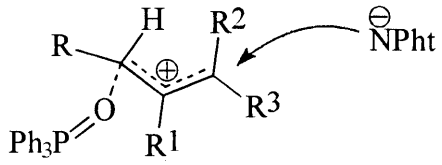


Figure 7.

Kolasa and Miller [15] have elaborated a synthesis of a variety of a substituted chiral α-N-hydroxy amino acids based on chiral α-hydroxy esters as components in the Mitsunobu reaction (Figure 8). This method is especially appealing since the required α-hydroxy carboxylic acids are readily available in optically pure form by deaminative hydroxylation of amino acids. Alkylation of simple N-acyl O-protected hydroxylamines gives mixtures of N-alkylated products (hydroxamates) and O-alkylated products (hydroximates). Alternatively, alkylation of N-alkoxycarbonyl O-substituted hydroxylamines proceeds cleanly on the nitrogen [16].

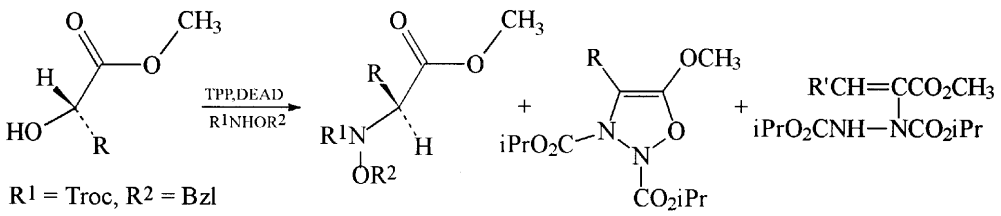


Figure 8.

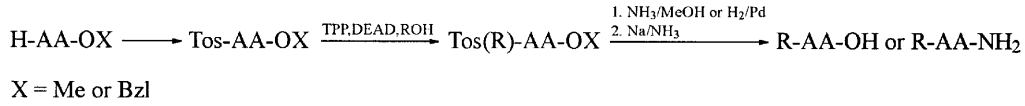


Figure 9.

Thus, as an acidic component of the Mitsunobu reaction *N*-Troc-*O*-Bzl-hydroxylamine was proposed. Reaction of the Troc-NHO-Bzl with a number of α-hydroxy acid methyl esters and TPP/DEAD in THF gave *N*-benzyloxy-*N*-Troc-α-amino acid esters in 20–82% yields. In no case was the desired N-hydroxy amino acid derivative the sole product. The yield of the desired products seemed to depend on the steric effects of the alkyl substituent of the α-hydroxy acid esters.

The Synthesis of *N*-Alkylated Amino Acid Derivatives

The accommodation of alkyl groups of diverse structure and bulk in peptides is still a challenging task. A mild and efficient procedure for the preparation of N-monoalkylated amino acids and peptides would therefore be extremely useful. Papaioannou *et al.* [17] have developed a general approach based on the Mitsunobu reaction (Figure 9). Methyl or benzyl esters of N-Tos protected amino acids in the presence of the TPP/DEAD system underwent N-alkylation with alcohols, such as MeOH, EtOH or *i*-PrOH and the desired products were obtained in high yields (80–95%).

Severe steric congestion could be tolerated in this method. This was shown by the condensation of Tos-Ile-OMe and Tos-Val-OBzl with *i*-PrOH, which cleanly produced the corresponding *N*-isopropylated derivatives. Since deprotection of the methyl ester results in racemization, the benzyl group was an obvious choice for carboxy function protection if the free N-alkyl amino acid was the synthetic target. The mildness of this method allows its application to peptides, as was exemplified by the synthesis of Tos-EtAla-Leu-OMe and Tos-MeAla-Phe-OMe. The concept of application of sulphonamides as acidic components of the Mitsunobu reaction had previously been exploited [18] in the synthesis of the protected amines.

A modification of the N-alkylation procedure described above has recently been devised in our group. We have proposed the use of N-Pmc-amino acid esters instead of corresponding tosyl derivatives [19]. The Pmc group is well known in peptide chemistry as an acid-labile group for arginine guanidino function protection [20]. It can be removed from the amino function by means of HBr in acetic acid and is therefore an attractive alternative to the tosyl group. It is noteworthy that methods of N-alkylation based on sulphonamides can be employed for the selective N-alkylation of diamino acid derivatives. It was shown, however, that N^{ω}-alkylation of Orn and Lys derivatives proceeds at a much slower rate than N^{α}-alkylation and that the former cannot be driven to completion even by adding large excess of reagents [17]. Our own experiments with Pmc-derivatives have fully confirmed the literature results with the N-alkylation of tosyl protected diamino acid derivatives [19].

We have also used the Mitsunobu reaction for obtaining the peptide part of peptide nucleic acid (PNA) monomers (Figure 10) [21]. Condensation of *N*-Boc-ethanolamine with either methyl *N*-tosylglycinate or benzyl *N*-tosylglycinate mediated by TPP/DEAD produced the desired compounds in high yield. Reduction (sodium in liquid ammonia) of prehydrolysed methyl or benzyl *N*-(2-Boc-aminoethyl)-*N*-Tos-glycinate furnished appropriate pro-

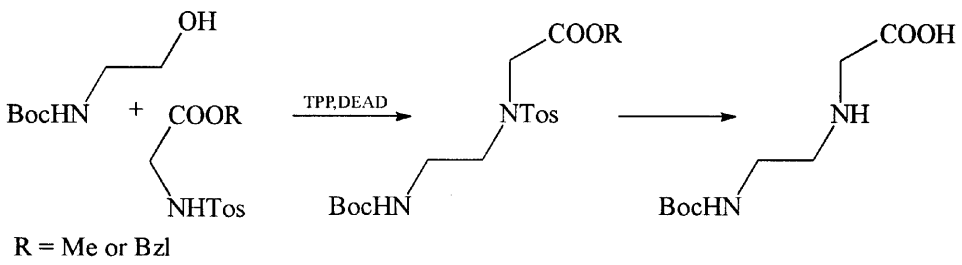


Figure 10.

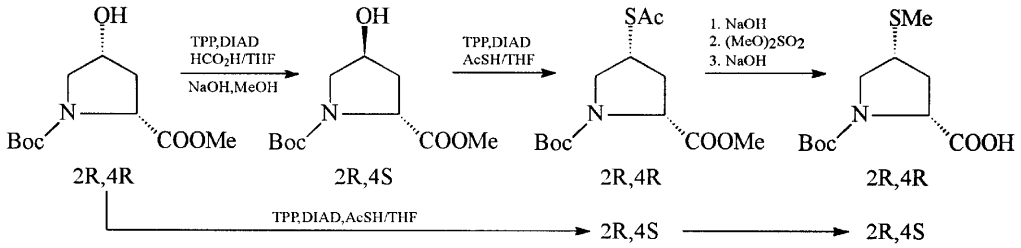


Figure 11.

ducts as substrates for the condensation with thymin-1-yl acetic acid.

Transformations of Hydroxy Amino Acids

The use of hydroxy amino acids as alcohol components in the Mitsunobu reaction and the possibility of the hydroxy group replacement by nucleophiles of different types pointed to routes for the synthesis of diverse amino acid derivatives. The principle has been successfully applied by Kolodziej *et al.* [22] to the synthesis of *N*-Boc-4-*cis*- and *N*-Boc-4-*trans*-(methylthio)prolines, which were used to explore the structural requirements of the Met[31] side chain in the conformation of the C-terminal fragment of cholecystokinin. The synthetic strategy has based upon the reaction of methyl *N*-Boc-(*2R,4R*)-4-hydroxyprolinate or methyl *N*-Boc-(*2R,4S*)-4-hydroxyprolinate with thiolacetic acid under Mitsunobu conditions, to give methyl (*2R,4R*)- or methyl (*2R,4S*)-*N*-Boc-4-(acetylthio)prolinate in 79% or 85% yields, respectively (Figure 11). Selective hydrolysis of thiolacetate, alkylation of the resulting thiol and subsequent hydrolysis of the methyl ester was accomplished in a one-pot sequence. It is worth mentioning that methyl Boc-(*2R,4S*)-4-hydroxyprolinate, which is not easily available, was obtained by epimerization of the C-4 carbon by a Mitsunobu inversion using formic acid, followed by hydrolysis of the resulting formate ester.

Ceulemans *et al.* [23] have used *N*-Boc-protected homoserine for the synthesis of the monomers of peptide nucleic acid consisting of *S*-α-amino-γ-thymid-1-yl butyric acid and L-valine subunits. Reaction of *N*-Boc-L-homoserine benzylester with N^3-benzoylthymine under the Mitsunobu conditions afforded (*S*)-*N*-Boc-α-amino-γ-(N^3-benzoylthymidyl)-butyric acid benzylester (Figure 12). After the removal of *N*-benzoyl and *O*-benzyl protecting group, the product was used in the synthesis of DNA peptide analogues.

Wojciechowska *et al.* [24] have reported the preparation of dehydroamino acids from N-protected (*Z*-, Pht-, Boc-) serine and threonine methyl esters under the intramolecular Mitsunobu dehydration conditions. The reaction of an appropriate hydroxy amino acid derivative was run with the equimolar amounts of TPP and DEAD, and the corresponding

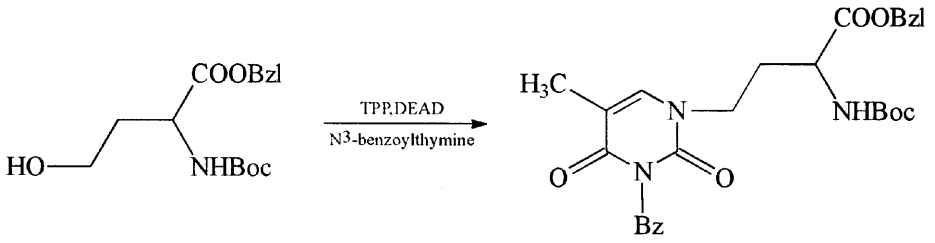


Figure 12.

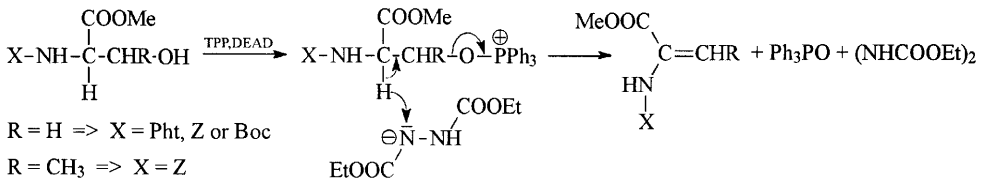


Figure 13.

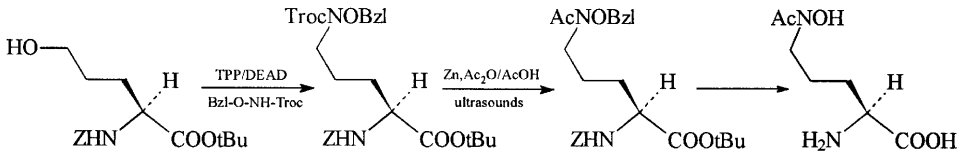


Figure 14.

dehydroamino acid was in 55–69% yield isolated (Figure 13). In the case of methyl 2-Z-aminocrotonate, an equimolar mixture of *Z*- and *E*-isomers was obtained.

N^5-Acetyl-N^5-hydroxy-L-ornithine is the key constituent of several siderophores, which are low molecular weight iron(III)-transport compounds excreted by number of microorganisms. Dolence *et al.* [25] have reported the transformation of a 5-hydroxy-L-norvaline derivative to an L-ornithine derivative under Mitsunobu reaction conditions. Starting from N-Z-5-hydroxy-L-norvaline *tert*-butyl ester and *N*-Troc-*O*-benzyl-hydroxylamine, they obtained N^2-Z-N^5-benzyloxy-N^5-Troc-L-ornithine *tert*-butyl ester. The Troc group in the protected product was then easily replaced by an acetyl group. Removal of other protecting groups gave the siderophore component containing both amino acid and hydroxamic acid functionalities (Figure 14).

The Mitsunobu reaction has also been explored as a method of the synthesis of phosphonates from primary alcohols [26]. Phosphonamidate and phosphonate analogues of γ-glutamyl peptides have been synthesized as a key intermediates in syntheses of precursors of γ-glutamyl hydrolase and folylpoly-γ-glutamate synthetase inhibitors. It was shown that, contrary to experience with phosphonamidates, phosphonates are not accessible in the reaction of phosphonochlorinate with the secondary alcohol nucleophiles (Figure 15) [27]. Malachowski and Coward [27] therefore, used the TPP/DIAD system to prepare the phosphonate from diethyl 2-hydroxyglutarate and monomethyl phosphonic derivative.

The Mitsunobu reaction occurs with the inversion of configuration at the carbinol chiral centre, yielding a product of high optical purity. It is an excellent procedure for transforming hydroxy acids, hydroxy amino acids or peptides into esters whose subsequent hydrolysis leads to a stereoisomer of the initial compound with inverted configuration at the carbinol centre. The reaction has been used very often in this way. Golubev *et al.* [28] have described an efficient synthesis of *cis*- and *trans*-4-hydroxy-L-pipecolic acid from L-aspartic acid. The *trans* isomer

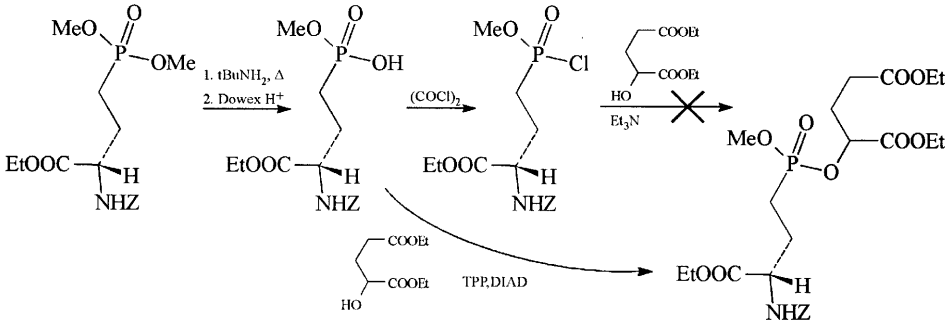


Figure 15.

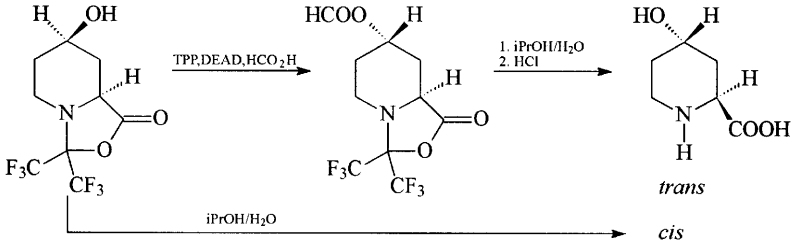


Figure 16.

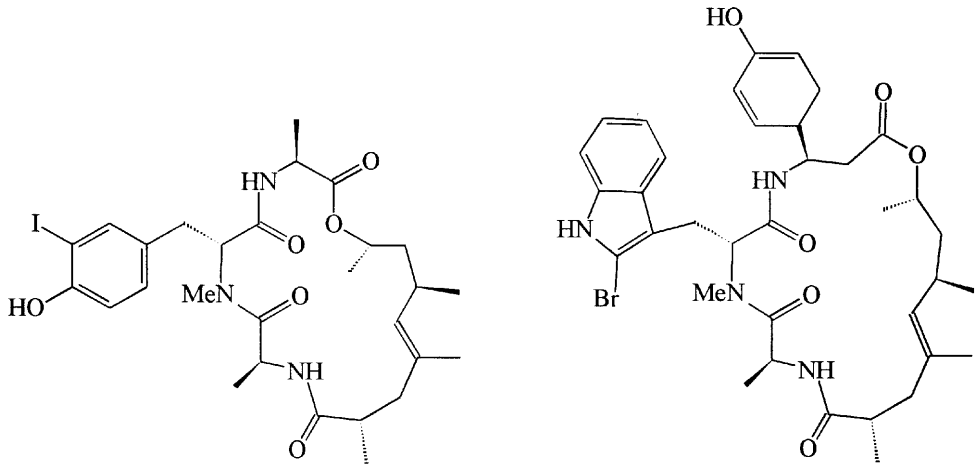


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Figure 17.

was obtained in a good yield *via* inversion of hexafluoroisopropylidene-protected *cis*-4-hydroxy-L-pipecolic acid through the formation of *O*-formyl derivative and subsequent hydrolysis of the intermediary ester (Figure 16). An identical sequence of reactions was employed for the conversion of methyl *N*-Boc (*2R,4R*)-4-hydroxyprolinate into methyl *N*-Boc (*2R,4S*)-4-hydroxyprolinate [21] (Figure 11).

The Synthesis of Carboxylic Acid Active Esters

Grochowski and Jurczak [29] have performed the synthesis of *N*-acyloxyphthalimides and *N*-acyloxysuccinimides – derivatives of carboxylic acids (so-called active esters, useful as reactive acylating agents in peptide synthesis) – under Mitsunobu conditions in a neutral reaction medium. Both *N*-hydroxyphthalimide and *N*-hydroxysuccinimide, in the presence of carboxylic acid with a 10% excess of DEAD and TPP underwent facile esterification. The O-acyl derivatives of *N*-hydroxyphthalimide or *N*-hydroxysuccinimide were obtained in high yields (72–96%). The method may be especially useful for the preparation of active esters of N-protected amino acids.

Esterification/Lactonization in Peptolides

The Mitsunobu reaction has been used in the synthesis of semisynthetic derivatives of a newly isolated cyclopeptolide comprising nine *S*-amino acids and one *R*-lactic acid [30]. Transformation of the *R*-lactic acid residue into its *S*-counterpart in the cyclopeptolide c-[Pip-MeVal-Val-MeAsp-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Lac] was achieved by opening of the lactone ring of the protected cyclopeptolide and recyclization of the linear peptide obtained – H-*R*-Lac-Pip-MeVal-Val-MeAsp(*t*-Bu)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-OH – under Mitsunobu conditions. Re-macrolactonization was performed at high dilution (approx. 1 mM) with three equivalents of both TPP and DEAD over a period of 24 h to give the cyclic product in 67% yield, with inverted configuration at the lactic acid residue.

Imaeda *et al.* [31] employed the Mitsunobu reaction in the first efficient and stereoselective syntheses of geodiamolide A (Figure 17(A)) and jaspamide (Figure 17(B)). These are cyclic peptolides isolated from sponge species and showing interesting cytotoxic and antifungal activities. The key step of the synthesis, i.e. the coupling of the tripeptide unit with the polypropionate unit was found to be exceptionally difficult to achieve, and at first was realized (in 74% yield) by high-pressure esterfication of the activated as the imidazolide tripeptide substrate. The same reaction was later easily performed in high yield (95%) using *N*-protected tripeptide, the hydroxy ester and typical Mitsunobu reaction components. It is noteworthy that the inversion of the configuration at the carbinol centre of the hydroxy component needed for the Mitsunobu reaction was also carried out under Mitsunobu reaction conditions, using formic acid, TPP and DEAD.

Preparation of Peptide Oxazolines, Thiazolines and Aziridines

Oxazolines and thiazolines rings are the building blocks of many biologically active natural products. The thiazoline ring is a characteristic structural segment of cyclopeptide alkaloids such as lissoclinamide [32]. The presence of these heterocyclic units stabilizes the reverse turn secondary structure [33], and the conformational constraint introduced by these 'peptide-mimics' is widely used in the design of peptide analogues of pharmacological interest. Chiral oxazolines are also a powerful tool in asymmetric syntheses [34]. Some oxazolines which were not attainable by standard methods (e.g. 2,5-diphenyl-1,3-oxazolines or 2-trifluoromethyl-1,3-oxazolines) were successfully obtained under the mild, neutral conditions of the Mitsunobu reaction [35]. The method employs the intramolecular reaction of *N*-(*β*-hydroxyalkyl)amides (Figure 18).

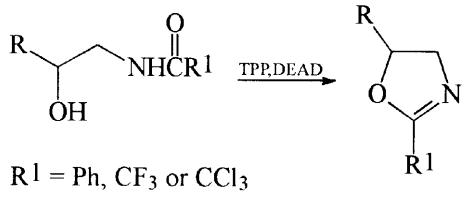


Figure 18.

The same reaction has been utilized to form oxazoline and thiazoline units in *β*-hydroxy-*α*-amino acid-containing peptides (Figure 19) [36]. Since the oxazoline ring is acid-sensitive, and the thiazolidine ring is easily epimerized and opened under basic conditions, these heterocyclic systems are preferably introduced at the final stage of peptide synthesis. Cyclization to the five-membered ring proceeded smoothly in case of serine derivatives, whereas in the threonine derivatives 1,2-dehydration was observed. The dehydration was not detected in thioamide derivatives, probably because of the higher nucleophilicity of the thioamide function [36].

Surprisingly, Wipf and Miller found that the Mitsunobu-type cyclization of Z-Gly-allo-Thr-NHMe led to the desired oxazoline derivative whereas the analogous reaction with Z-Gly-Thr-NMe proceeded to the formation of *N*-acyl aziridine (Figure 20) [37].

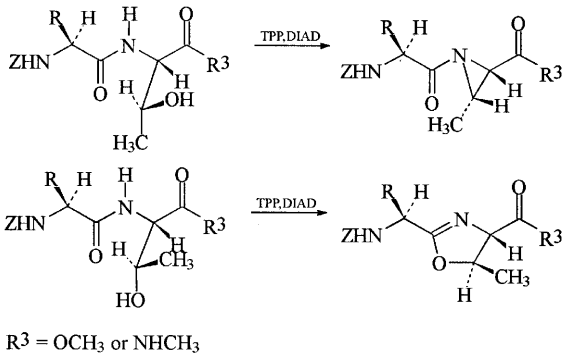


Figure 20.

Later results [38] confirmed that, in general, treatment of threonine peptides with TPP/DEAD redox system results in aziridine formation, whereas allo-threonine derivatives and thiopeptides give the oxazolines and thiazolines, respectively. The significant difference in the reaction pathway of Thr and allo-Thr derivatives is unique to Mitsunobu conditions and is not observed in the analogous cyclization with the Burgess reagent [37]. Presumably, partly responsible for this effect is the destabilizing *gauche* interaction of the threonine *α*-carboxyl and *β*-methyl groups which leads to the *cis*-oxazoline. Additionally, deprotonation of the amide N–H bond by the reduced DIAD anion generates a small amount of anions which are far more reactive toward E_i cyclization, and with amide anions an intramolecular N-alkylation leading to aziridine formation is expected. Thus, the presence of a sufficiently strong

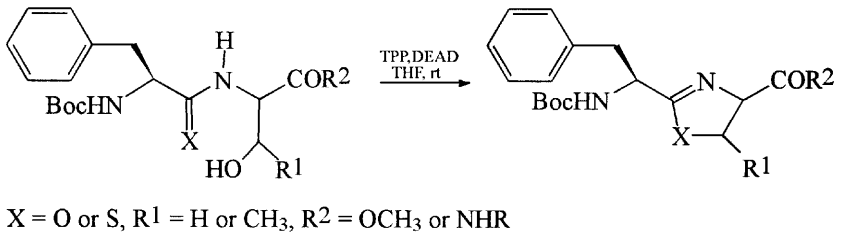


Figure 19.

base induces amide deprotonation and aziridine formation [38] and in some measure determines the course of these reactions. The relatively easy formation of thiazolidine derivatives by the standard TPP/DIAD mediated system is accompanied, however, by significant side-chain epimerization [39].

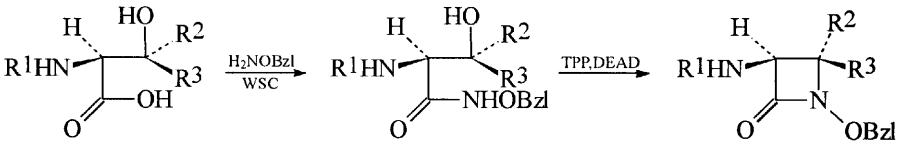
Both, serine and threonine N-protected esters are reported to give α,β-dehydroamino acids under Mitsunobu conditions [24, 37].

Formation of β-lactams

Miller and co-workers have devised an excellent method of the synthesis of 2-azetidinones, the basic structural units of β-lactam antibiotics, based upon the fact that the NH bond of *O*-acyl and *O*-alkylhydroxamic acids have p*K* values in the range of 6–10 and therefore undergo the Mitsunobu reaction [40, 41]. The method, resembling a biogenetic type of β-lactam synthesis, relies on the direct ring closure of *O*-alkyl-β-hydroxyhydroxamates derived from N-protected serine or threonine under the Mitsunobu conditions (Figure 21).

Contrary to intermolecular reactions between hydroxamic acid derivatives and Z-Ser-OBzl, where the dehydroalanine derivative was the predominant product, during the intramolecular cyclization of substituted β-hydroxyhydroxamic acids neither β-elimination, nor racemization was detected and the cyclic product of N-alkylation was the only isolated compound. It was demonstrated that the method allows complete control of stereochemistry on the β-lactam (Thr → *trans* β-lactam, allo-Thr → *cis* β-lactam) while remaining compatible with the incorporation of sensitive peripheral functionality [40]. The procedure is an efficient entry to antibiotics of nocardicine type [42]. This ring closure has also been employed for the preparation of a new class of β-lactam antibiotics – monobactams, containing an azetidinone ring sulphonated at position 1 [43]. Floyd and co-workers used Boc-serine *N*-methoxy amide as a starting material for their preparation [44]. After the ring formation, the *N*-methoxy group was removed by Birch reduction, and the azetidinone ring was then N-sulphonated and Boc-deprotected. The zwitterionic products serve as precursors to a wide variety of synthetic monobactams which are potent antimicrobial agents. The cyclization of hydroxamates in Mitsunobu conditions is an extremely facile and elegant reaction; the yields, however, were only 65–70% due to the necessity of chromatographic separation of the by-products. Therefore, for the large-scale production of monobactams derived from Ser, Thr and allo-Thr the cyclization *via* mesylate derivatives was chosen.

Aziridine and azetidinone rings were formed when arylamides of N-protected β-hydroxy amino acids were subjected to the Mitsunobu reaction conditions [45]. β-Lactam formation occurred in the case of serine derivatives, whereas aziridine was produced from threonine derivatives (Figure 22). The stereochemistry of the cyclic products proved that both β-lactam and aziridine formation involve inversion at the carbinal carbon. The latter reaction could be circumvented by employing a N-phthaloyl protecting group, as proposed by Bose and coworkers [46], who studied in detail the influence of various factors on the selectivity of the process.



R^1 = Z or Boc, R^2, R^3 = H or CH_3

Figure 21.

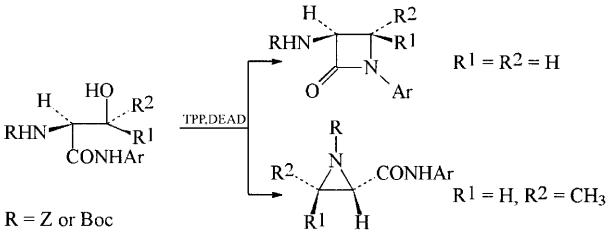


Figure 22.

Miller and Mattingly have explored the β-lactam synthesis from a variety of serylpeptides [47]. Using full protection of serine nitrogen to avoid the side reaction mentioned above, they subjected several peptides to Mitsunobu reaction conditions. The results indicated that a β-lactam ring formation strongly depends on Cα'–H bond acidity (C-H[1],

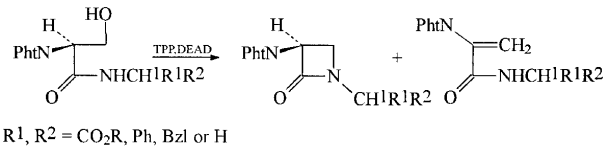


Figure 23.

Figure 23). Thus, serylglycine derivative was converted to a mixture of dehydropeptide and β-lactam whereas serylaminomalonate was converted mainly to the β-lactam. Moreover, the analogous compound lacking H^1, *N*-phthaloyl-L-seryl-amino-(2-methyl)-malonate, was converted under the same conditions almost exclusively to the dehydropeptide. The facilitation of β-lactam formation by the acidity of the α′–CH bond was rationalized by the hypothesis of a cyclization intermediate in which the α′-carbon is sp^2-hybridized. The idea is consistent with the fact that in some cases ($R^1 = COOR$, $R^2 = C_6H_4OBzl$) racemization at C α′ was observed [47].

Farouz-Grant and Miller showed that the Mitsunobu reaction may be also employed to the cyclization of 1-aminocyclopropane-1-carboxylic acid (ACC)-containing seryl dipeptides (Figure 24) [48].

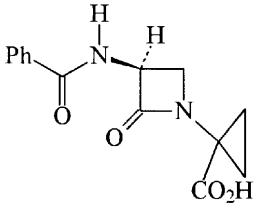


Figure 24.

To prevent aziridine or oxazoline formation, the seryl nitrogen was temporarily doubly protected with a 4,5-diphenyloxazoline-2-one (Ox) group [49]. The Mitsunobu cyclization of more complex β-hydroxyamino acid-containing ACC peptides failed.

The formation of a four-membered ring of the β-lactam type was the key step in a synthesis of statine (4(*S*)-amino-3(*S*)-hydroxy-6-methylheptanoic acid) derivatives. This amino acid is a building block of pepstatin, a naturally occurring pentapeptide with aspartyl protease inhibitory activity [50]. In the search for renin inhibitors some analogues of statine were prepared. Among them 3(*S*),4(*S*)-diamino-6-methylheptanoic acid [51] and its 2,2-difluoro derivative [52] are of particular interest. To displace the statine β-hydroxy group with a nitrogen nucleophile the intramolecular inversion strategy was chosen (Figure 25), in order to avoid difficulties anticipated in the intermolecular reaction. Thus, 2,2-difluoro statine derivative was converted to its *p*-methoxyphenyl amide and a Mitsunobu-type cyclization was performed; the resulted β-lactam was hydrolysed to the desired N-protected derivative.

Synthesis of β-lactones and other Cyclic Compounds

The efficient closure of β-lactone rings can be achieved under Mitsunobu conditions [53]. The treatment of *N*-protected serine derivatives with the preformed TPP/DMAD adduct at 78 °C gave the expected products in reasonable yield (60–72%). These results are in contrast to very poor results of the cyclization obtained by Parker *et al.* [54]. β-Lactones are valuable intermediates for the synthesis of β-substituted alanines, which are common constituents of microbial antibiotic or antitumour peptides. Stereochemically pure N-protected β-substituted L- or D-alanines were easily obtained by Mitsunobu-type cyclization of readily available L- or D-serine derivatives, followed by nucleophilic opening of the resulting β-lactones (Figure 26). Only relatively hard nucleophiles like ammonia and methoxide attack the carbonyl to give acyl-oxygen instead of C^{β}-O cleavage. Nevertheless, the amide/amine ratio may be inverted simply by altering the solvent [53].

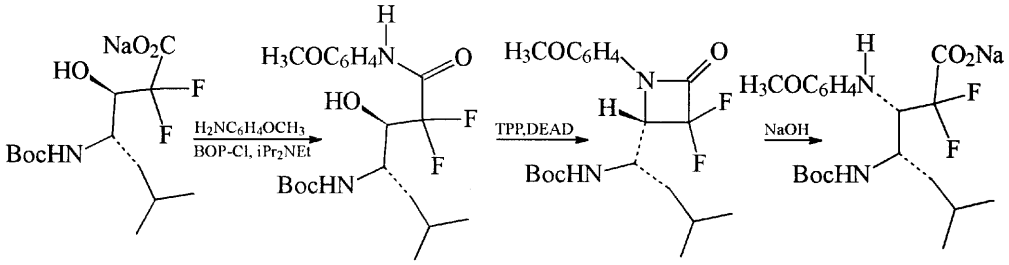
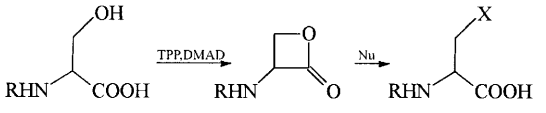


Figure 25.



R = Z or Boc, Nu = AcONa, $(NH_2)_2CS$, $N(CH_3)_3$, $PhCH_2S^-Na^+$, etc.

Figure 26.

Papaioannou *et al.* [55] have used the intramolecular Mitsunobu reaction for the conversion of *trans* -4-hydroxy-*N*-trityl-L-proline to *cis*-hydroxyproline derivatives *via* 5-triphenylmethyl-2-oxa-5-aza-bicyclo[2.2.1]heptan-3-one formation. If methanol was present in the mixture, the transesterification of the bicyclic lactone to the *cis*-4 hydroxy-*N*-trityl-L-proline methyl ester catalysed by an excess of the TPP/DEAD was observed (Figure 27).

Alternatively, ammonolysis of the intermediary lactone in isopropyl alcohol provided *cis*-4 hydroxy-

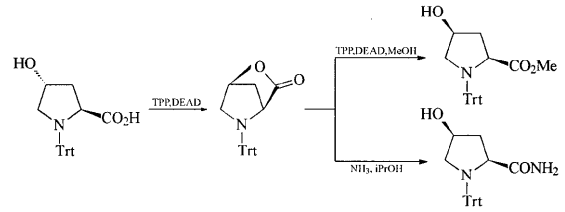


Figure 27.

N-trityl-L-proline amide; its saponification with 2 M KOH in DMSO-MeOH gave the *cis*-4-hydroxy-*N*-trityl-L-proline, which was isolated as the corresponding diethylammonium salt in 68% yield. The lactone was thus proved to be a key intermediate in the synthesis of *cis*-4-hydroxy-L-proline and derivatives thereof. The formation of a C-terminal 4-hydroxyproline lactone was a key step in the synthesis of TRH analogues containing *cis*-hydroxyproline residue [56, 57]. The closure of a seven-membered ring *via* a Mitsunobu reaction has been reported by Maurer and Miller [58]. In the course of the synthesis of mycobactins, the most structurally complex of the known siderophores [59], they converted *N*-Boc-ε-hydroxynorleucine *O*-benzyl hydroxamate into a product of intramolecular alkylation. The reaction with the TPP/DEAD system gave a seven-membered *N*-benzyloxy lactam – an important unit of the siderophores and the hydroximate side-products (Figure 28). In the same synthesis, a Mitsunobu reaction was also utilized to form an ester bond which could not be achieved by standard carboxyl group activation [58].

Summary

Because of its mildness and effectiveness, the Mitsunobu reaction is very useful not only in amino acid but also in peptide chemistry. It provides easy access to many unnatural amino acids, their derivatives and amino acid-related cyclic compounds. By reversing the activation process it allows formation of ester bonds which are otherwise difficult to achieve. The stereochemical course of the reaction has been used many times to make hydroxy amino acid derivatives of inverted chirality at a carbinol centre, and in peptolide modifications.

Acknowledgements

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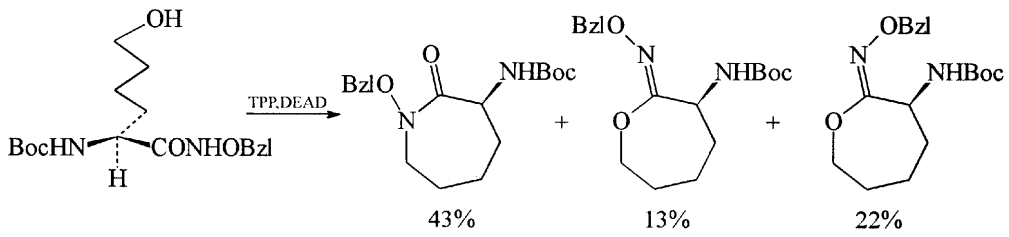

Figure 28.

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