Muramyl peptides – synthesis and biological activity

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Muramyl peptides are fragments of peptidoglycan from the cell walls of bacteria. In 1974, elucidated that *N*-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide, MDP) is the minimal structure responsible for the activity exhibited by peptidoglycan of the bacterial cell walls [1,2]. Since 1975, many research group have undertaken synthesis of this highly active glycopeptide and its derivatives. This article concerns muramyl peptides, and new analogues which are considered as prodrugs. Their synthesis and biological activity are also presented. Numerous reviews on muramyl peptides have already been published [3-8], however it may be useful to survey this topic again to recognize the current trends.

Key words: muramyl peptides, immunomodulators, synthesis, biological activity

Introduction

It is a current opinion that the importance of immunotherapy in the general therapy is still growing as it has been proved that immunostimulators are able to strengthen the natural defensive forces of an organism against infection. Very often such therapy is not only effective as prophylaxis and remedy recommended at the beginning of a disease for reduction of disease born complications but also as a help for patients to build up the immune system destroyed by antibiotic or cytostatic treatment.

The whole bacterial cell wall and its components like proteoglycans, lipopolysaccharides, lipoproteins, peptidoglycans and also their fragments possess strong immunostimulating activity. Among them muramyl peptides belong to the strongest, well recognized adjuvants

and immunostimulators and have been already applied in therapy. The immune system is able to recognize muramyl peptides as products of bacteria and to give a proper immunological response similar to a reaction after ordinary infection. One element of the reaction consists in increasing body resistance to infection. This resistance is nonspecific, which means that it is unrelated with species of bacteria, fungi, viruses or even parasites. The main role in the defensive action against microorganisms is played by macrophages. Their activation by muramyl peptides results in increased production of microbicidal oxygen radical substrates such as superoxide and peroxide, and in stimulation of secretion of inflammatory cytokines. Other cells of the immune system are activated by muramyl peptides as well. There is hope that immunostimulators will help to solve the problem of increasing resistance of microbes to antibiotics, and of the increasing number of patients with immunodeficiencies.

N-Acetyl-muramyl-L-alanyl-D-isoglutamine (muramyl dipeptide, MDP 1)

Muramyl dipeptide **1** (Figure 1) was identified in 1974 as the minimal component of bacterial cell wall peptidoglycan retaining immunoactivity similar to whole bacteria [1,2]. Although the larger fragments of bacteria cell wall are obtained by enzymatic hydrolysis of the peptidoglycan, MDP can be prepared only by chemical synthesis. Muramyl peptides, including synthetic muramyl dipeptide (MDP), could replace whole mycobacteria in Freund's adjuvant (killed mycobacteria in a water-in-oil emulsion), which is considered to be the most efficient adjuvant for increasing antibody production and establishing cellular immune response to an antigen.

MDP is usually synthesized by the procedure of Jeanloz and Flowers [9] with later modifications (Scheme 1). The starting material, D-glucosamine hydrochloride **2**, is converted into protected muramic **5** or nor-muramic acid in several steps. In the last step protected muramic or nor-muramic acid is coupled to the dipeptide (L-Ala-D-isoGln) [10,11]. Both

isoglutamine and glutamine are found in natural peptides. D-Isoglutamine is a substantial component of muramyl peptides and glutamine is often present in peptide hormones; for example in vasopressin, oxytocin, growth hormone release factor (GHRF), and gastric peptides inhibitor (GIP). A convenient synthesis of glutamine and isoglutamine derivatives was elaborated in our laboratory [12].

Analogues and derivatives of MDP and their biological activity

Numerous derivatives and analogues of MDP have been synthesized, mainly by researchers of pharmaceutical companies in Europe, Japan and the United States. They endeavor to improve the pharmacological properties of MDP by increasing activity and selectivity. Among the many effects of biological activity of MDP and its derivatives the most promising ones in immunotherapy are: adjuvancity, stimulation of nonspecific resistance against bacterial, viral or parasitic infections, protection against tumors and somnogenicity [3-6,13-15]. MDP also induces other, mostly undesirable or toxic interactions, such as pyrogenicity, induction of autoimmune response, and inflammatory reactions [14-16]. The immunomodulating activity of MDP is based mainly on stimulation of macrophages as well as T and B lymphocyte functions [3-6,13,15,17-20].

The studies of the dependence of the biological activity of MDP on the chemical structure have shown that chemical modifications of the glucopyranose ring and/or of the peptide chain influence the biological properties considerably. Special lipophilic prodrugs of muramyl peptides are listed in Table 1 and are in details described by Baschang [6]. The hydroxy groups at R^1 and R^6 (Table 1) in the pyranose ring are not essential for biological activity of MDP analogues. Lack of the former one or replacement by the -SH group does not change muramyl peptide properties; the latter may be substituted by an amino or acylamido group. The acylamido substituent at C-2 (R^2, Table 1) is required for biological activity. In the side

chain, the methyl groups of the lactyl and alanyl residues are not essential; in fact replacement of the lactyl methyl residue by hydrogen **7** (R^3, nor-MDP) (Figure 2) results in a reduction of some side effects. The nor-MDP is less active than MDP but is also less toxic [3]. L-Alanyl residue may be replaced by an other L-amino acids, e.g. such as L-valine, L-serine, L-leucine, L-proline or L-α-aminobutyric acid. Useful MDP analogues appeared those in which the terminal carboxyl group was transformed into a primary amide and simultaneously the amido group of isoGln by a series of alkyl esters. Most notable in this series Murabutide **8** (R^7, Table 1) (Figure 2) is free of pyrogenicity and equipotent to MDP of antibody production. This substance is currently under clinical evaluation [21]. An attachment of a glucosylamine residue at C-4 of nor-MDP and substitution of the alanyl by α-aminobutyryl residue enhanced the adjuvant activity and reduced pyrogenicity. The FK-156 **9** (Figure 2) has been reported to be a potent stimulant of antibody production and free of pyrogenicity and its analogues FK-565 **10** (Figure 2) a potent anticancer agent. Acylation of MDP at the carbohydrate 6-position (R^6, Table 1) with some mycolic acids or quinonyl group has led to potent adjuvants and analogues presenting considerable antitumor and antibacterial activity [22,23]. Comparable results were obtained for MDP acylated with some branched hydroxy fatty acids [24]. Replacement of the α-carboxamido group of the D-isoglutamine residue of MDP by a carboxyl group left the adjuvant and antiinfection activities almost unchanged; however, the resulting derivatives are faster removed from the organism due to its increased solubility in water [25]. Numerous efforts were undertaken to synthesize analogues of this highly active glycopeptide in order to obtain molecules with improved and more defined pharmacological profiles [3,5]. Studies of the structure-activity relationships for this class of compounds have revealed the importance of the amino acid composition of the peptide moiety for their immunomodulatory activity. It was found that the presence of *N*-acetyl-D-glucosamine residue linked to the muramyl moiety similar to natural sequence is not essential for

immunostimulating properties of such analogues [3,5,25]. Crucial prerequisites for immunostimulatory activity are *S*-configuration of the first amino acid and *R*-configuration of the C-terminal amino acid of the dipeptide unit. Replacement of the *N*-acetylmuramyl moiety with various acyl groups thus represents an important approach to the design and synthesis of new immunologically active MDP analogues – desmuramylpeptides, e.g. FK-156 **9** [26], pimelautide **11** [27] (Figure 2), 7-(oxoacyl)-L-alanyl-D-isoglutamines [28], *N*-[2-(2-amino-alkoxy)propanoyl]-L-alanyl-D-isoglutamine derivatives [29], some carbocyclic MDP analogues [30,31] in which a more lipophilic cyclohexane ring is present instead of the polyhydroxy pyranose ring of D-glucosamine [30,32,33], and the adamantyl-substituted MDP analogue LK 415 [34]. It has been shown that replacement of the polyhydroxy substituted pyranose ring of D-glucosamine by the benzene ring and a partial rigidification of the molecule by incorporation of the lactoyl moiety and acetamido group into a benzo-fused 3-morpholinone ring results in new series of potent immunostimulating compounds [35,36]. The carbocyclic analogues of MDP **12** and **13** (Figure 3) were synthesized and tested by Hasegawa et al. [32]. Compounds in which the carbohydrate moiety of MDP was replaced with cyclohexanol derivatives were inactive as adjuvants for the induction of delayed-type hypersensitivity to azobenzenearsonate *N*-acetyl-L-tyrosine in guinea pigs [33]. This finding led the authors to the speculation that the carbohydrate moiety is essential for the adjuvant activity of MDP and its analogues [33]. Later Barton et al. [30] reported the synthesis of compound **14** (Figure 3), which was also devoid of the sugar part, but some its derivatives were able to stimulate unspecific resistance against bacterial and viral infections, liberation of colony-stimulating factors, induction of interleukin 1 (IL-1) production in macrophages, and antitumor activity. Kikelj et al. [31] described synthesis and biological activity of other carbocyclic MDP analogues of general structure **15** (Scheme 2), obtained by replacement of the *N*-acetyl-muramic acid residue by a *trans*-2-{[2'-(acylamino)-cyclohexyl]oxy}acyl

moiety, and compound **19**, resulting from further conformational restriction of the parent carbocyclic analogue - *N*-{*trans*-2-[[2'-(acetylamino)cyclohexyl]oxy]-acetyl}-L-alanyl-D-glutamic acid, **22** and **23** by incorporation of the lactoyl fragment and acetamido group into a cyclohexane-fused 1,4-morpholin-3-one ring. The last analogue protects mice against the immunosuppressive effect of cyclophosphamide treating and increases the nonspecific resistance of mice against fungal infection. It is an immunomodulator which enhances the maturation of lymphocytes B to plasma cells and increases the activity of lymphocytes B and lymphocytes T as well as that of macrophages but does not alter the number of these cells. Tratar et al. [37] described preparation and results of biological assay of two carbocyclic MDP analogues, represented by a general structure **24**, **25** (Figure 4), with a retro-inverso modified peptide bond between the L-alanyl and D-glutamate moieties. Similar modification of the peptide bonds is a frequently applied strategy in the design of peptidomimetics [38]. A series of carbocyclic MDP analogues obtained by Tratar et al. were devoid of immunostimulating activity [37]. The retro-inverso modification of peptide bonds has evolved into one of the most widely used peptidomimetic approaches to the preparation of novel bioactive molecules including immunostimulatory tuftsin analogues [39,40]. The most promising desmuramyl analogues were series of phthalimido desmuramyl dipeptides **26**, **27**, and **28** (Figure 5). In these compounds *N*-acetyl-muramic acid residue was replaced by various *N*-phthaloylated amino acids [29,41] or phthalimido substituted aminoethoxyacetic acid to give immunologically active acyclic MDP analogues like LK 423 **26**. LK 423 has been selected for further studies to develop an anti-inflammatory pharmaceutical agent [42]. Furthermore, this compound is able to stimulate the production of tumor necrosis factor in *in vitro* phorbol-12-myristate-13-acetate (PMA) and ionomycin-stimulated cultures of human peripheral blood mononuclear cells [43]. Gobec and Urleb have synthesized MDP analogues related to LK 423 **26**. They modified the peptide backbone of phthalimido-desmuramyl

dipeptides by introducing various phosphorus-containing molecules [44-46]. Orthogonally protected Abu(P) [benzyl (2*R*, 2*S*)-4-diethyl-phosphonyl-2-phthalimido butanoate] **29** was obtained as a key intermediate for the synthesis of new phthalimido-desmuramyl dipeptide analogues **34** containing diethylphosphonate moiety at the position of ω-carboxylic group of Glu [46] (Scheme 3). The authors also described synthesis of new phosphapeptides, which amide bonds between L-alanyl and D-glutamate moieties were replaced by phosphonamidate and phosphonate groups, respectively **38**, **41** [44] (Scheme 4). Recently attention has been focused on cytokine interfering drugs which suppress or stimulate specific pathways in immune and inflammatory responses. So far, several experimental models have addressed the influence of MDP and some its analogues on the production of cytokines [21,43,47,48]. In 2001 Gobec et al. [34] reported the synthesis of two new adamantyl-desmuramyl dipeptides LK 415 **44** and LK 517 **48** with 1-adamantyl-carboxamido moiety replacing *N*-acetyl-glucosamine fragment in muramyl dipeptides (Scheme 5). Their efficiency in modulating the production of cytokines IL-12, TNFα, IFNγ, IL-4, and IL-10 was measured *in vitro* in ionomycin and phorbol-12-myristate-13-acetate activated cultures of human pheripheral blood mononuclear cells (PBMC), co-incubated with the analogues tested. The results were compared with the activity of MDP. All three substances were strong regulators of IL-12 synthesis and IFN gamma synthesis as well. Introduction of the diethyl phosphonate moiety into LK 517 was of great importance for augmented T-cell cytokine production.

Zemlyakov et al. [49] described a convenient method of synthesis of α-glycosides of methyl *N*-acetyl-muramyl-L-alanyl-D-isoglutaminate **49** (Figure 6). 1-Heptanol, cyclohexanol, and cholesterol were glycosylated with chloride α-glucosaminyl peracetate in the presence of HgI_2 to the corresponding peracetylated α-glycosides of *N*-acetyl-glucosamine. Deacetylation, benzylidenation at positions 4,6 and alkylation with L-2-chloro-propionic acid gave protected D-muramic acid. After coupling with L-Ala-D-Glu(OMe)-NH_2 and deprotection, the target

α-glycosides of methyl *N*-acetyl-muramyl-L-alanyl-D-isoglutaminate **49** were prepared. This method was also used in the synthesis of β-cholesterylglycoside and β-heptylglycoside muramyl dipeptide. The effects of β-heptylglycoside muramyl dipeptide on antibody production and delayed hypersensitivity reaction was studied in mice with weak and strong reactions to sheep erythrocytes. This compound exhibited high immunomodulating activity which depended on the initial genetically determined immunoreactivity of animals and drug dose [50]. Merhi and co-workers [51] have synthesized three lipophilic analogues of muramyl peptide (Scheme 6): a hydrolyzable ester of cholesterol (MTP-Chol) **53**, and two nonhydrolyzable ethers, derivatives of octadecane and heptadecafluorooctadecane (MTP-octadecane **57** and MTP-heptadecafluorooctadecane **63**). Stimulation of the RAW 264.7 cell line by the analogues was studied by measuring nitrite production as an indication of NO-synthase activity. The MTP-Chol incorporated within nanocapsules was as active as free muramyl dipeptide, whereas the lipophilic ether derivatives were inactive. MTP-octadecane in micellar form was not capable of inducing macrophage cytotoxicity either. These results indicate that lipophilic muramyl peptides need to be hydrolyzed inside the cells to yield a hydrosoluble metabolite in order to activate macrophages. Recently Kubasch and Schmidt [52] described synthesis of various 2,6-diaminopimelic acid derivatives containing muramyl- and 1,6-anhydromuramyl di-, tri-, and tetrapeptides. The immunostimulatory properties of these compounds and their comparison with muramyl dipeptide have been not yet reported.

Liu et al. [53] were the first who reported a solid-phase Multipin parallel synthesis of MDP derivatives, a method that can potentially be used to make a diverse MDP derivative library with potential application for drug screening. The macro crowns with a loading capacity of 5-8 μmol/pin from Chiron Mimotopes were used for such synthesis of MDP derivatives **67** (Scheme 7). Furthermore, the synthesis is under way of development by acylation, reductive

alkylation, amine addition, or three-component Ugi reactions based on this solid-phase synthetic method.

Synthesis of conjugates of MDP

In our opinion, the strong immunostimulating activity of MDP and its synergistic effects in combination with other biologically active compounds may not only strengthen the natural biological properties and improve their pharmacological properties, but also increase the self-defense of the infected organism. Titov et al. [54] synthesized conjugates of tuftsin with *N*-acetyl-glucosaminyl muramyl dipeptide (GMDP) **68** in which tuftsin derivatives **69** was attached to the γ-carboxylic group of D-Glu either through the α-amino group of terminal Thr residue **72** or through the ε-amino group of Lys **75** (Scheme 8). A convenient synthetic method for preparation of GMDP from unprotected disaccharide isolated from bacterial media was proposed [55,56]. The conjugates of GMDP and tuftsin were synthesized from unprotected GMDP by a mixed anhydride procedure. It was found that GMDP facilitated the macrophage-stimulating activity of tuftsin. Immunological tests indicate the high adjuvant activity of synthetic GMDP derivatives with tuftsin in stimulation of antibody production against ovalbumin, delayed-type hypersensitivity (DTH) and enhancement of phagocytosis. Mixture of GMDP with tuftsin as well as GMDP coupled covalently bond to this tetrapeptide when administered in saline possessed a high activity comparable with that of incomplete Freund's adjuvant (IFA). We have synthesized two types of conjugates of MDP or nor-MDP with tuftsin derivatives. In the compound **76**, the hydroxyl group at C6 of the sugar moiety was acylated with tuftsin derivatives, whereas the compound **77** was modified at the C-terminal of muramyl peptides by amide bond formation between the isoglutamine carboxyl group and amine group of tuftsin. Main biological effects visible as decreased viability of cancer cells treated with the conjugates were related to generation of free radicals by

monocytes and increased activity of redox enzymes in lymphocytes. The effect was more dependent on monocytes since both changes of the viability and 2',7'-dichlorofluorescein (DCF) fluorescence shift were more tangible in cultures of monocytes [57].

We reported the synthesis and both *in vitro* and *in vivo* (hollow fiber assay) biological evaluation of MDP and nor-MDP analogues modified at position 6 of the carbohydrate moiety with acridine/acridone *N*-substituted ω–aminoalkano-carboxylic acids **80** (Scheme 9) and at the C-terminal of the peptide residues by the formation of an amide bond between the isoglutamine carboxylic group and the amine function of acridine/acridone derivatives **83**. These conjugates **80** were synthesized from protected MDP **78**. Heating of **78** in aqueous acetic acid caused deprotection of the C4 and C6 hydroxyl group. The partially protected **79** was acylated acridine/acridone derivatives in a mixture of pyridine/DMF by means of EEDQ or DCC and HOBt as coupling reagents. These conjugates **83** were synthesized from partially protected MDP or nor-MDP **81** by mixed anhydride or DPPA procedures. The synthesis of three analogues of desmuramylpeptides **84**, **85** (Figure 8) modified with an amino-acridine/acridone residue was also reported [58]. These analogues were synthesized by classical mixed anhydride method. The screening data indicate that the analogues **80**, **84** and **85** exhibit low cytotoxic activity, whereas several analogues **83** are potent *in vitro* cytotoxic agents against a panel of human cell lines. Two analogues were active in the *in vivo* hollow fiber assay. Three compounds of this group were selected by the NCI Biological Evaluation Committee for evaluations in subcutaneous human tumor xenograft assays. Benzyl 1-*O*-benzyl-6-*O*-[*N*-(1-nitro-9-acridinyl)-β-alanyl]-*N*-acetyl-muramyl-L-alanyl-D-isoglutaminate **86** (Figure 8) shows an immunostimulating effect on the cytotoxic activity of the NK cells obtained from the spleen of healthy and Ab melanoma bearing animals [59]. We also synthesized MDP and nor-MDP conjugates modified at the peptide part with batracylin or batracylin derivatives [60]. These conjugates **87** were synthesized from partially protected

MDP or nor-MDP **81** by DPPA procedure (Scheme 10). During tests performed at the Medical University of Gdansk two analogues reduced the proliferation of Ab melanoma cells *in vitro* as compared with batracylin alone [60]. The conjugates, however, are better soluble in water and show lower toxicity then batracylin.

Interesting immunostimulant activities might be expected in case of conjugating MDP to macromolecules [61]. Intracellular *N*-acetylglucosaminylmuramyl peptide-binding proteins of murine macrophages and myelomonocytic WEHI-3 cells were characterized. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting revealed proteins with molecular masses of 18, 32 and 34 kDa retaining the ability to specifically bind glucosaminylmuramyl dipeptide. The inhibition analysis demonstrated that only biologically active muramyl peptides could inhibit glucosaminylmuramyl dipeptide-binding to these proteins. Purification of these proteins and sequencing of peptides obtained after in-gel trypsin digestion enabled identification of the above metioned proteins as histones H1 and H3. These findings suggest that nuclear histones might be target molecules for muramyl peptides [62].

Clinical studies

Muramyl peptides and some of their synthetic analogues are now under intensive clinical trials and the obtained results are promising. The lipophilic MDP derivative N^2-[*N*-(acetyl-muramyl)-L-alanyl-D-isoglutaminyl]-N^6-stearoyl-L-lysine (MDP-Lys(L-18), Muroctasin, Romurtide) **88** (Figure 9) was introduced for treatment of patients after radiotherapy-induced leukopenia [63,64]. Muramyl tripeptide phosphatidylethanolamine (MTP-PE) **89**, a synthetic lipophilic analogue of MDP, stimulates monocytes/macrophages to kill a variety of tumor cells *in vitro* and *in vivo*. MTP-PE encapsulated into multilamellar liposomes (L-MTP-PE) is clinically tested in patients with recurrent osteosarcoma and melanoma. L-MTP-PE combined

with other anticancer agents may thus improve long-term cure rates of patients with these diseases [65]. Azuma and Seya [66] reported the usefulness of synthetic immunoadjuvants such as muramyldipeptide (MDP) derivatives, trehalose-dimycolates (TDM) and DNA fraction in treatment of cancer and infectious diseases in experimental systems and cancer patients.

It is generally acknowledged that synthetic muramyl peptides are a very interesting group of immunological adjuvants which are safe and free of contaminants of biological origin. An immunological adjuvant is defined as any substance that accelerates, prolongs, or enhances the specific immune responses to antigens including vaccine. In the mid-1930s J. Freund developed a powerful immunological adjuvant composed of a water-in-mineral oil emulsion containing killed mycobacteria (Freund's complete adjuvant, FCA). Although FCA is one of the most effective known adjuvants, it is highly reactogenic and cannot be used in human vaccines. However, Freund's incomplete adjuvants (a water-in-mineral oil emulsion without mycobacteria, FIA) was employed in an influenza vaccine licensed in the United Kingdom and is used in several anti-HIV vaccines under clinical evaluation. At present, one of the most promising adjuvants seems to be the "Syntex adjuvant formulation" (SAF) – an emulsion containing MDP[Thr] **90** (Figure 9) in a pluronic polyol with squalane and Tween 80 [67]. A veterinary vaccine against the feline leukemia virus based on SAF is available in the United States, and successful experiments with the following infections have been reported: simian acquired immunodeficiency syndrome oncovirus (SAIDS), influenza virus, hepatitis B virus (HBV), Epstein-Barr virus (EBV), herpes simplex virus (HSV), and nonviral antigens of malignant cells, especially B lymphomas [6,68]. Clinical experiments with SAF have already started. The availability of synthetic vaccines is expected as one of the major advances in clinical immunology. Antigenic peptide determinants of various pathogens, capable of vaccinating experimental animals have been defined and synthesized or produced by

recombinant DNA technology. Their coupling to carriers leads to immunogens capable of eliciting an immune response that can be increased by simultaneous administration of MDP and of *N*-acetyl-muramyl-L-alanyl-D-glutamine n-butyl ester (Murabutide) or, better, by chemical combination of MDP or of Murabutide to the hapten-carrier molecule [69]. nor-MDP was used as adjuvant in antifertility vaccine. In the future, an alternative for traditional vaccines could be provided by polyvalent vaccines obtained by polymerization of one or several antigenic determinants with adjuvant [70]. High adjuvant activity of MTP-PE derivative in antivirus type I (HIV-1) and in anti-influenza vaccines has been described [71]. There is a suggestion that the application of the 6-*O*-(2-tetradecylhexa-decanoyl)-*N*-acetyl-muramyl-L-alanyl-D-isoglutamine (B30-MDP) **91** (Figure 9) to liposomal vaccines will aid in the development of improved high immunogenicity of vaccines. In consideration to the effectiveness of B30-MDP as a liposomal vaccine, it is important to evaluate the effect of cholesterol, dimyristoylphosphatidylcholine (DMPC), distearoylphosphatidylcholine (DSPC), dipalmitoylphosphatidylcholine (DPPC) or dipalmitoylphosphatidylglycerol (DPPG) incorporation on the chemical stability of B30-MDP and physicochemical properties of B30-MDP/lipid mixed vesicles from the pharmaceutical point of view [72,73]. The use of immunological adjuvants to enhance and gain direct immune responses to subunit vaccines is a critical stage of rational vaccine design.

Conclusions

Many derivatives of MDP have been synthesized for further studies by *in vitro* and *in vivo* assays. The solution-phase synthesis makes the preparation of a large number of MDP analogues much easier. Several of them, including GMDP [74] **68** (Scheme 8), MDP[Thr]' **90** [75,76], MTP-PE **89** [77,78], Romurtide **88** [79], B30-MDP **91** [80] (Figure 8), Murabutide **8** [21], and lipopeptides, like FK-156 **9**, pimelautide **11** (Figure 2) are now in clinical trials.

During the past 10 years attention was directed towards the synthesis of desmuramylpeptide derivatives and the synthesis of MDP conjugates with different bioactive compounds.

Safe, improved galenicals of muramyl peptides will have to be elaborated – using pluronic polyols and biodegradable microcapsules - instead liposomes for introduction into human and veterinary medicine. The synergism of MDP with other immunomodulators (trehalose dimycolate, cytokines, lipopolysaccharide, lipid A) and with endogenous interleukin will have to be studied. This might lead to improved effects of vaccines and the stimulation of nonspecific immunity. Finally, the combined use of muramyl peptides with other chemotherapeutics is also promising in the therapy of many infectious and autoimmunological diseases.

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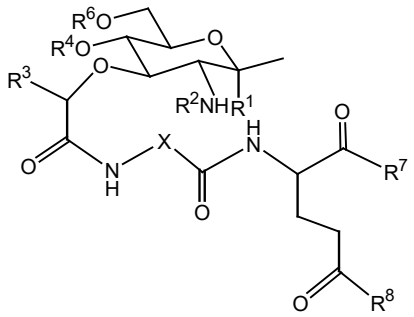
Table 1. The dependence of the biological activity of MDP on the chemical structure.



Group	Substituent	Activity				
		A	T	B	V	P
R^1	α or β-*O*-alkyl (i.e. Me, Bn)	+-				+
	β-SH, β-S-acyl	+				
	α or β-*O*-acyl	+		+		
	glucosamine	+	+			
R^2	H, acyl (MDP)	+				
R^3	H (nor-MDP), CH_3, C_2H_5, C_4H_9	+				-
	$=CH_2$, $=C_3H_7$, $=CHC_6H_5$	+				
R^4	glucosamine	+	+			-
	acyl (i.e. COC_5H_{11})	+	+		+	
R^6	*O*-acyl (i.e. mycolic acid, fatty acid, quinonyl group)	+	+	+		
	NH_2, *N*-acyl	+		+		
	S-acyl	-				
X	L-amino acid (i.e. Ala, Ser, Val, Thr, α-Abu)	+				+-
	D-amino acid (i.e. Ala)	immunosuppression				
R^7	alkoxyl (i.e. *O*-n-Bu-, Murabutide)	+			+	-
	aminoalkyl	-				
	α-amino acid residue	-				
	α-amino acylamide residue	+				
R^8	alkoxyl	+	+			
	aminoalkyl	-				
	acylated lipophilic residue (i.e. MTP-PE)	+	+	+	+	-
FK-156	desmuramylpeptides	+	+	+	+	-
FK-565			+			

A – adjuvant activity; T – anticancer activity; B – antibacterial activity;

V – antiviral activity; P – pyrogenic

Figure 1.

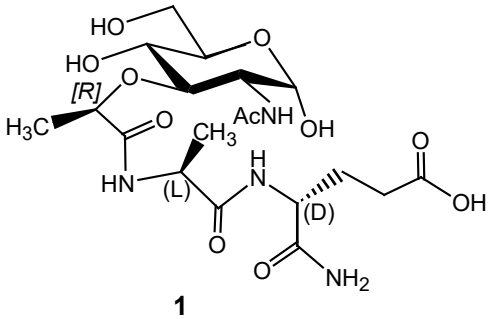


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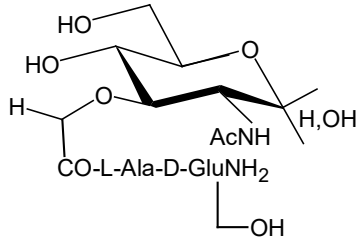
Figure 2.



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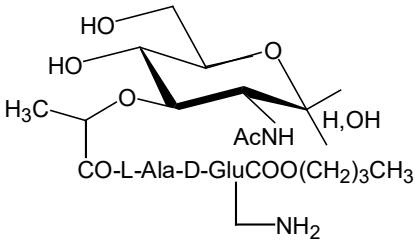

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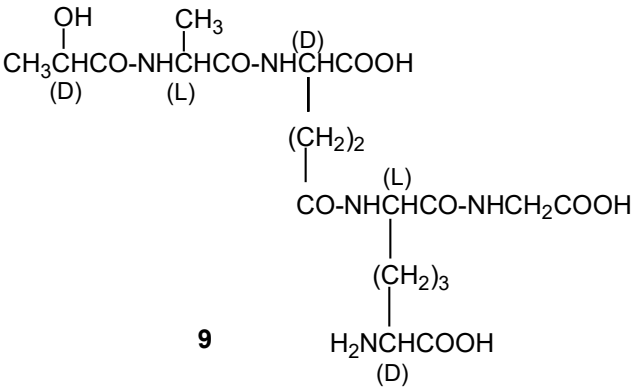

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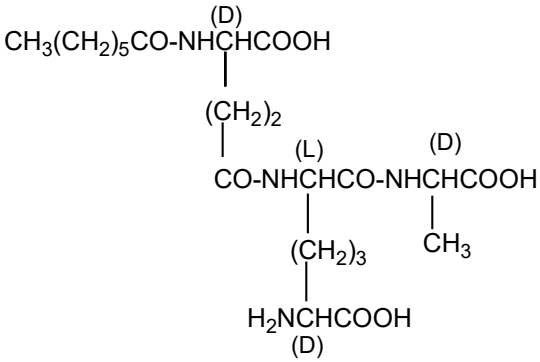

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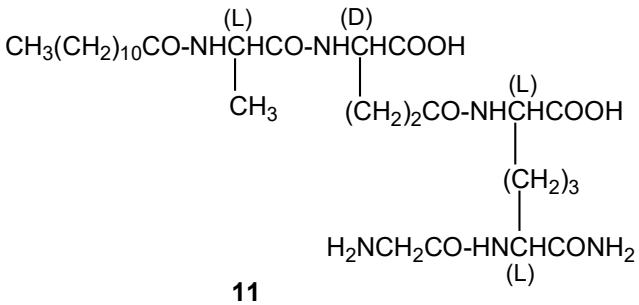

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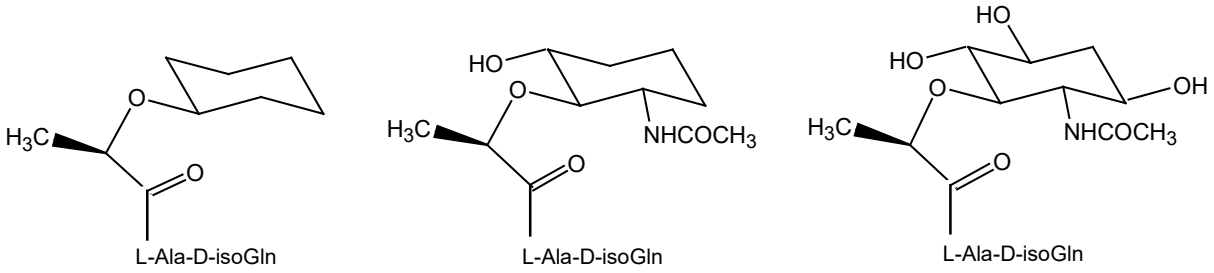
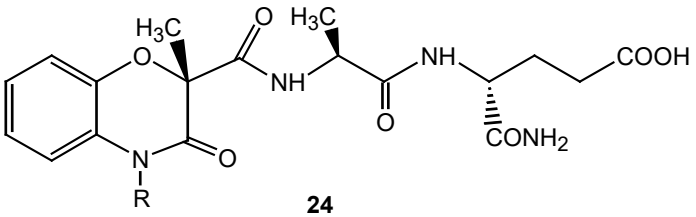
Figure 3.



12 **13** **14**

Figure 4.



24

R = H, CH_3

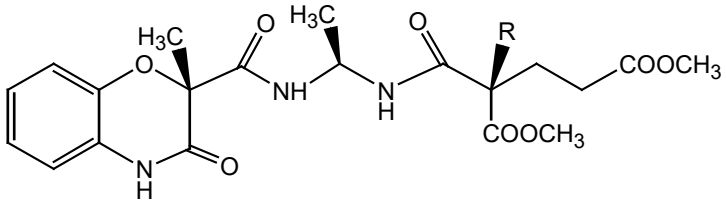


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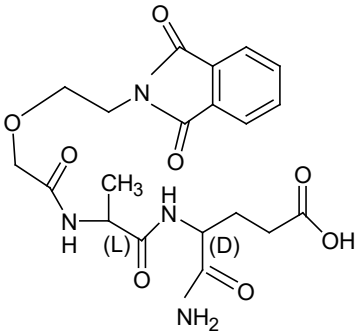
Figure 5.



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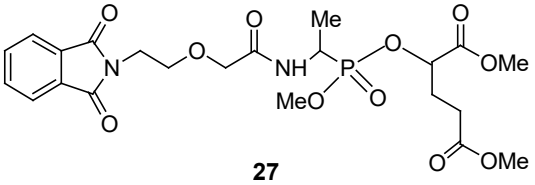
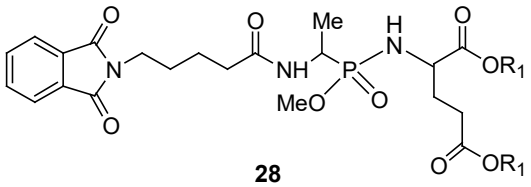


27

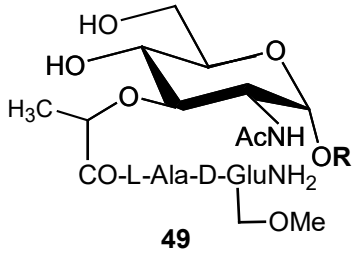
Me = methyl
Et = ethyl
Bn = benzyl



28

R_1 = Me, Et, t-Bu

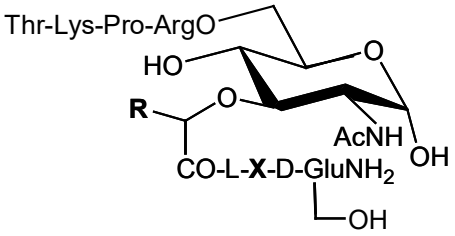
Figure 6.



49

R = 1-heptyl; cyclohexyl; cholesteryl

Figure 7.



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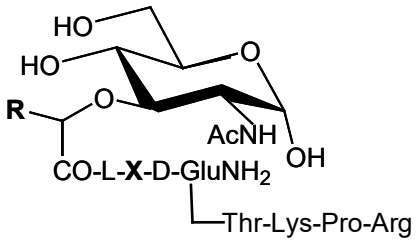
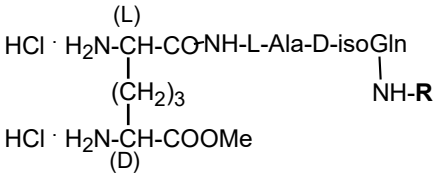


77

R = CH_3, H
X = Ala, Val, Pro

Figure 8.



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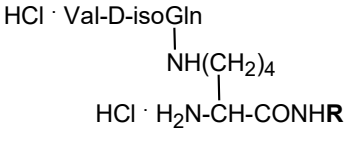
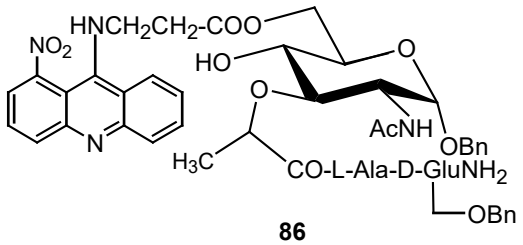


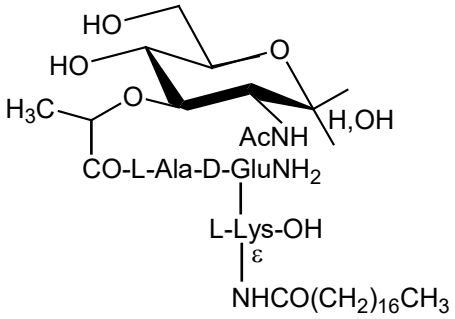
85

R = amino-acridine/acridone derivatives



86

Figure 9.



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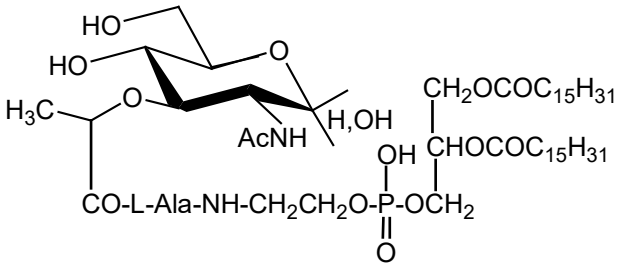

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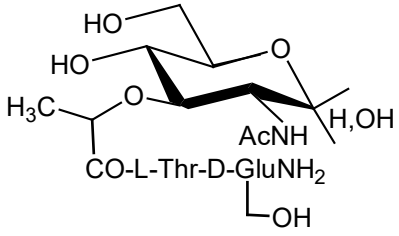

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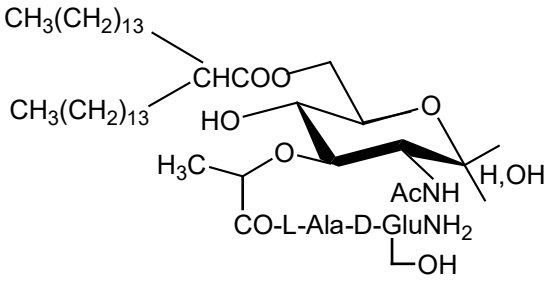
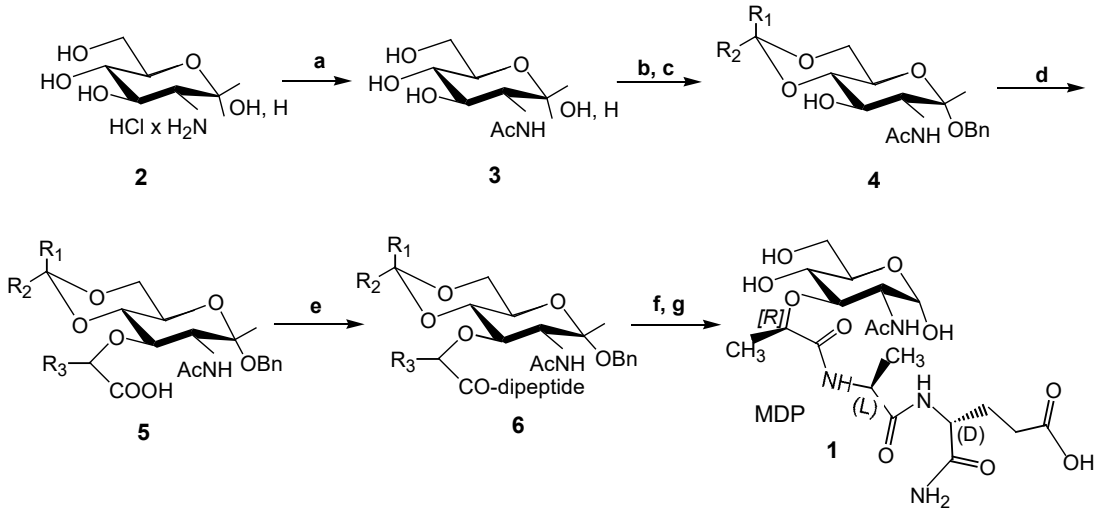


91

Scheme 1. Synthesis of muramyl dipeptide (MDP).

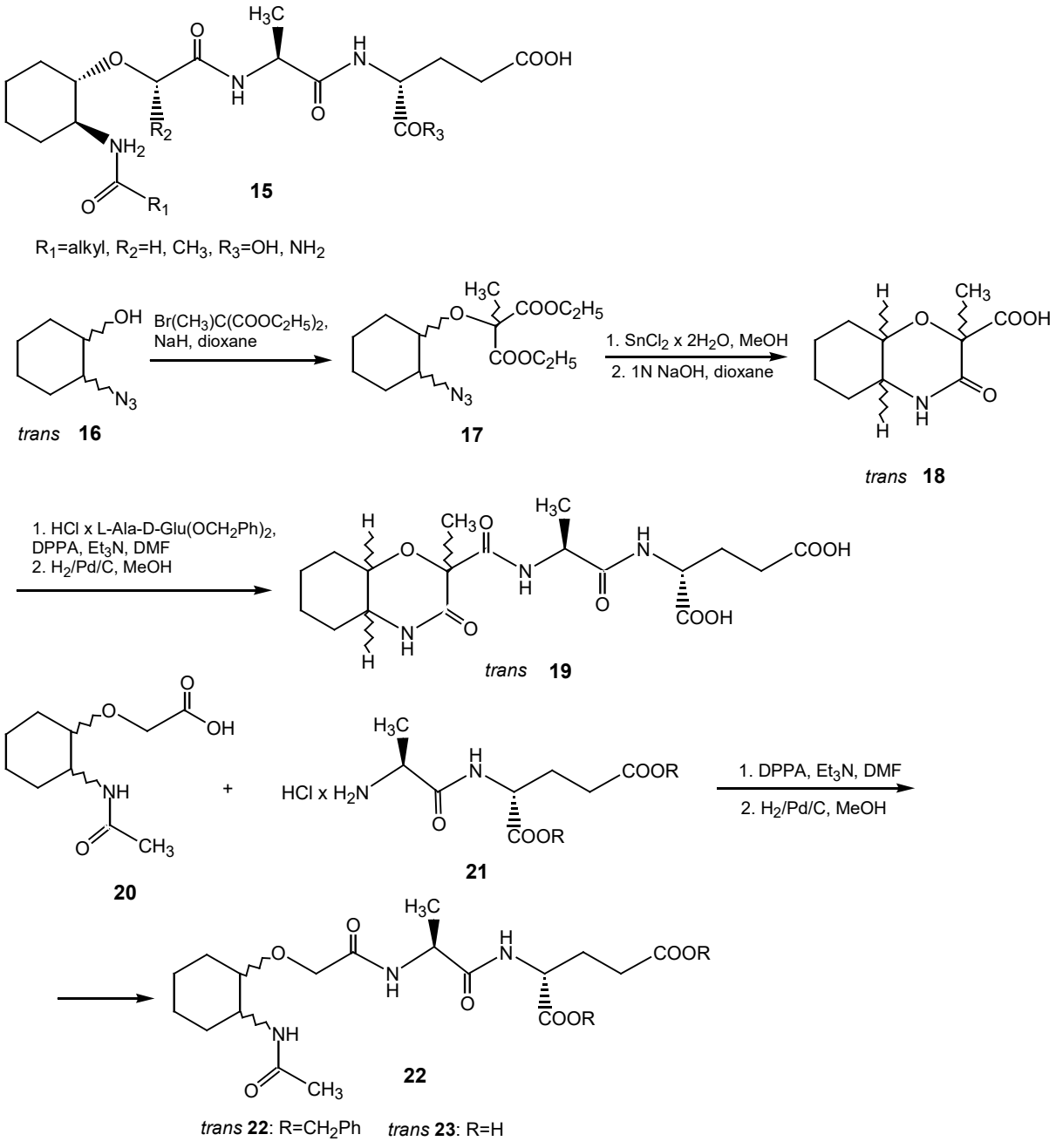


2 **3** **4** **5** **6**

a: acetic anhydride, sodium alcoholate; **b:** benzyl alcohol, H^+; **c:** PhCHO, H^+ or 2,2-dimethoxypropane, H^+; **d:** NaH, DL-α-chloropropionic acid or chloroacetic acid or chlorobutyric acid or lactic acid tosylate; **e:** dipeptide ester and EEDQ or DCC,HOBt or Woodwards Reagent K or isobutyl chlorocarbonate; **f:** acetic acid aq.; **g:** H_2, Pd/C, H^+; R_1,R_2=CH_3 or R_1=H, R_2=C_6H_5; R_3=H,CH_3,C_2H_5; dipeptide = L-Ala-D-isoGln.

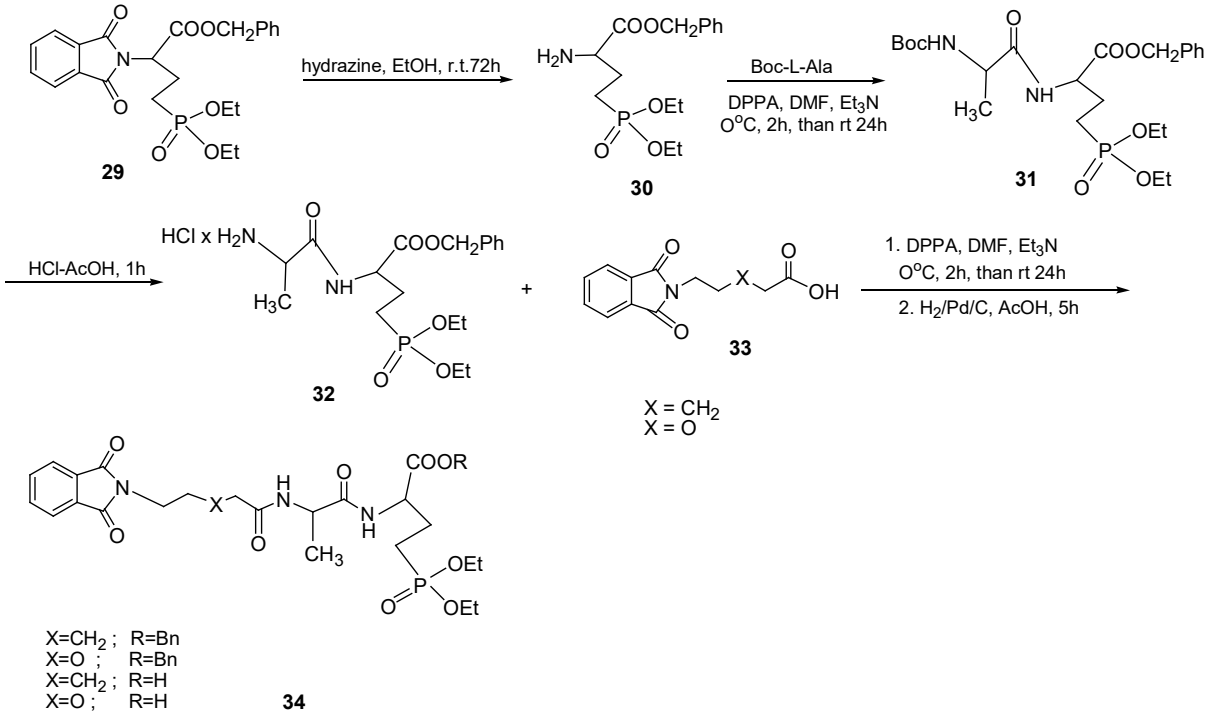
EEDQ = *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline,coupling reagent
DCC = *N,N'*-dicyclohexylcarbodiimide, coupling reagent
HOBt = 1-hydroxybenzotriazole hydrate, antiracemizating additive
Woodwards Reagent K = *N*-ethyl-5-phenylisoxazolium-3'-sulfonate, coupling reagent

Scheme 2. Synthesis of non-pyrogenic carbocyclic muramyl dipeptide analogue [31].



DPPA = diphenyl azidopghosphate, coupling reagent
DMF = dimethylformamide

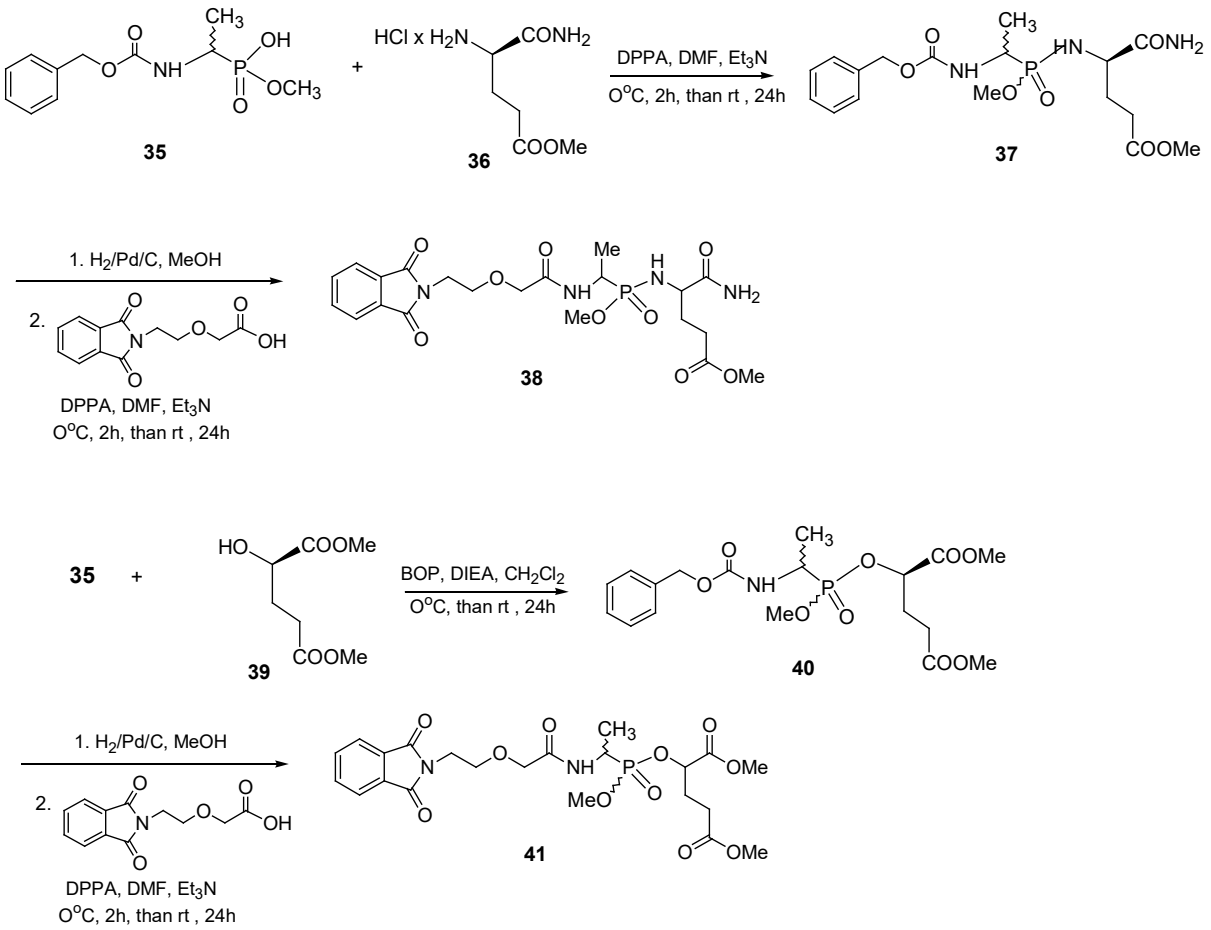
Scheme 3. Synthesis of phosphono phthalimido-desmuramyldipeptide analogues [46].



29 **30** **31** **32** **33** **34**

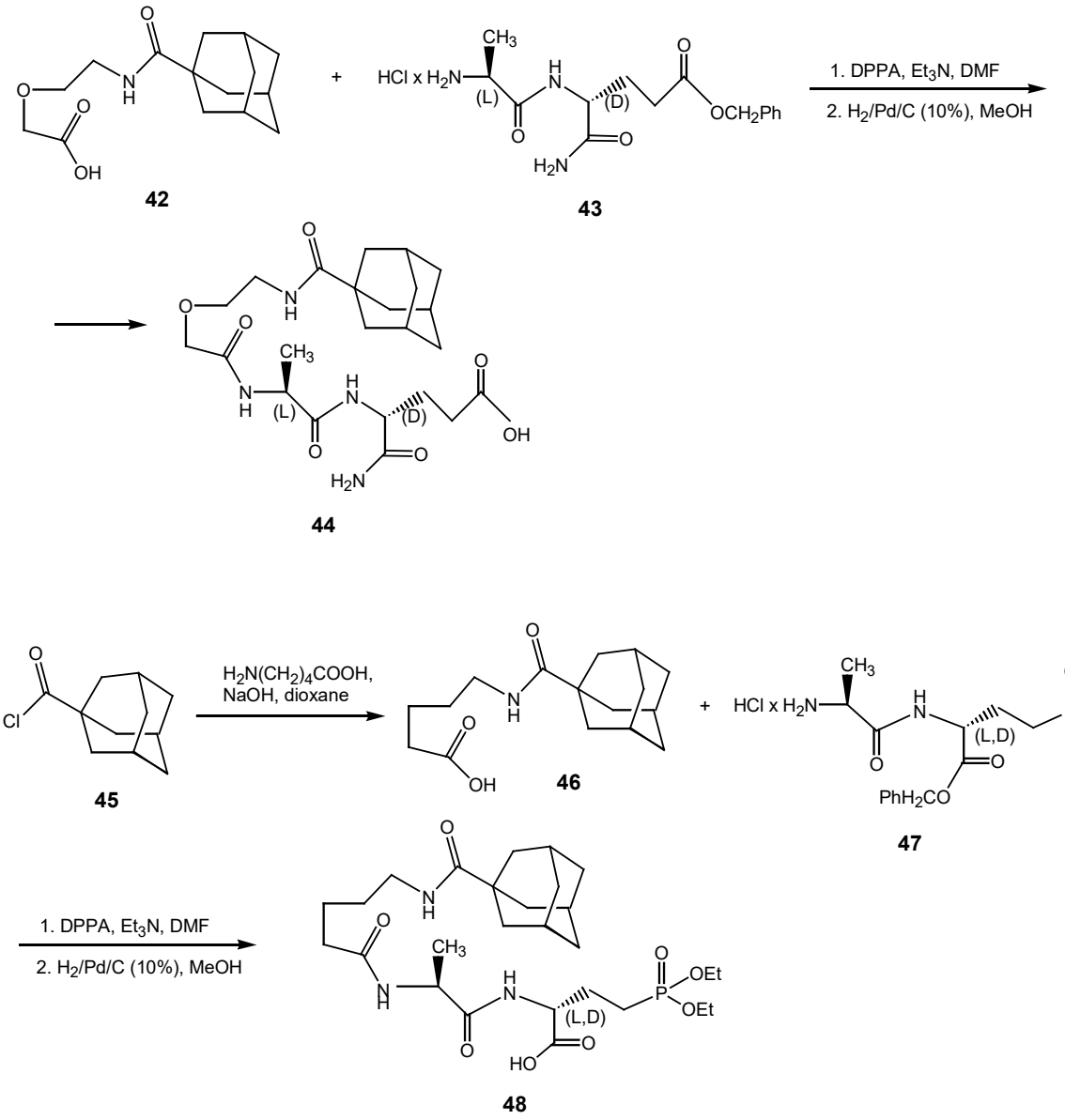
DPPA = diphenyl azidophosphate, coupling reagent
DMF = dimethylformamide
Boc = *tert*-butoxycarbonyl group

Scheme 4. Synthesis of phosphonamidate- and phosphonate-desmuramyldipeptide analogues [44].



DPPA = diphenyl azidophosphate, coupling reagent
DMF = dimethylformamide
BOP = benzotriazolyloxy-tris-(dimethylamino)phosphonium hexafluorophosphate
DIEA = diethylamine

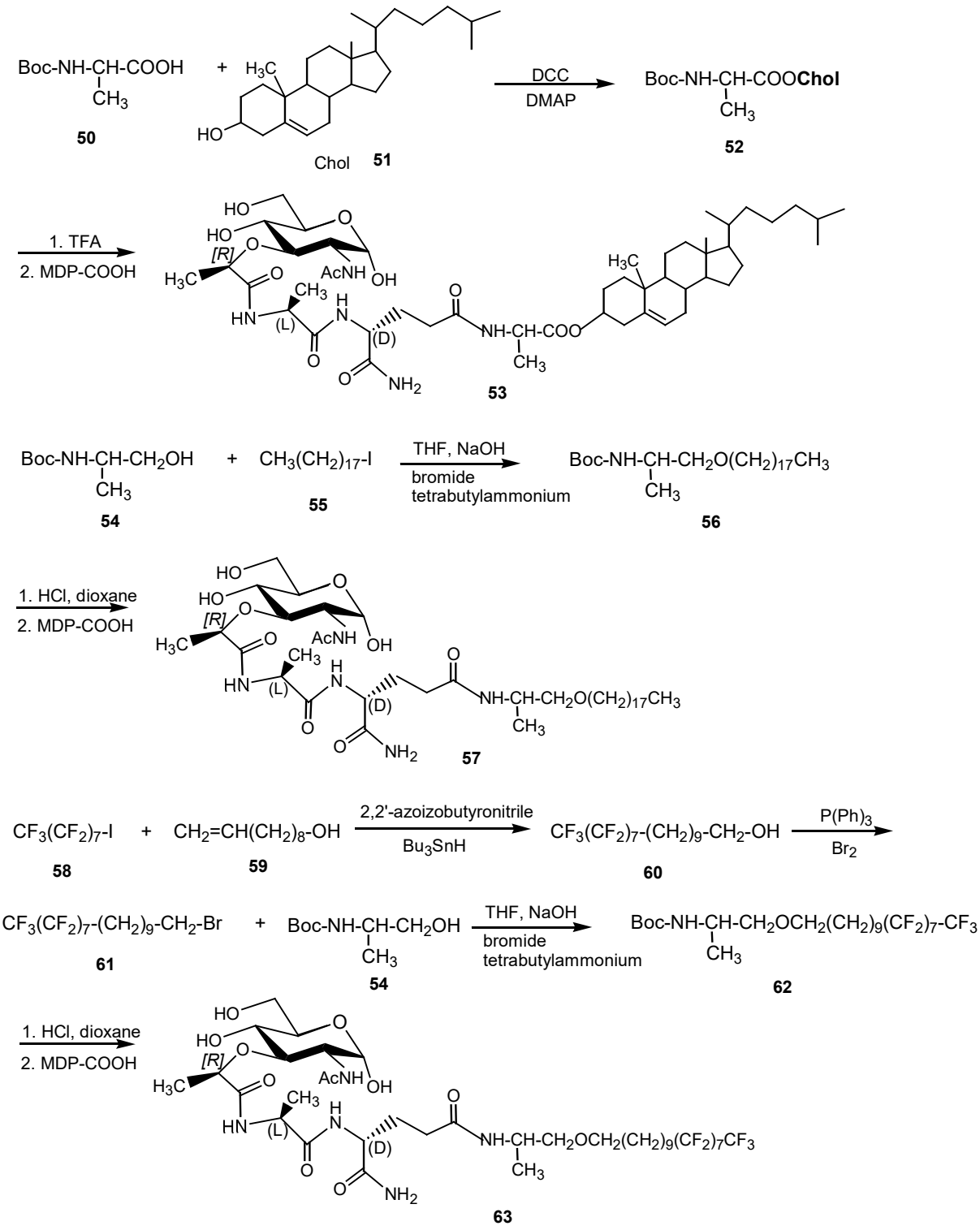
Scheme 5. Synthesis of adamantyl-desmuramyldipeptides LK 415 **44** and LK 517 **48** [34].



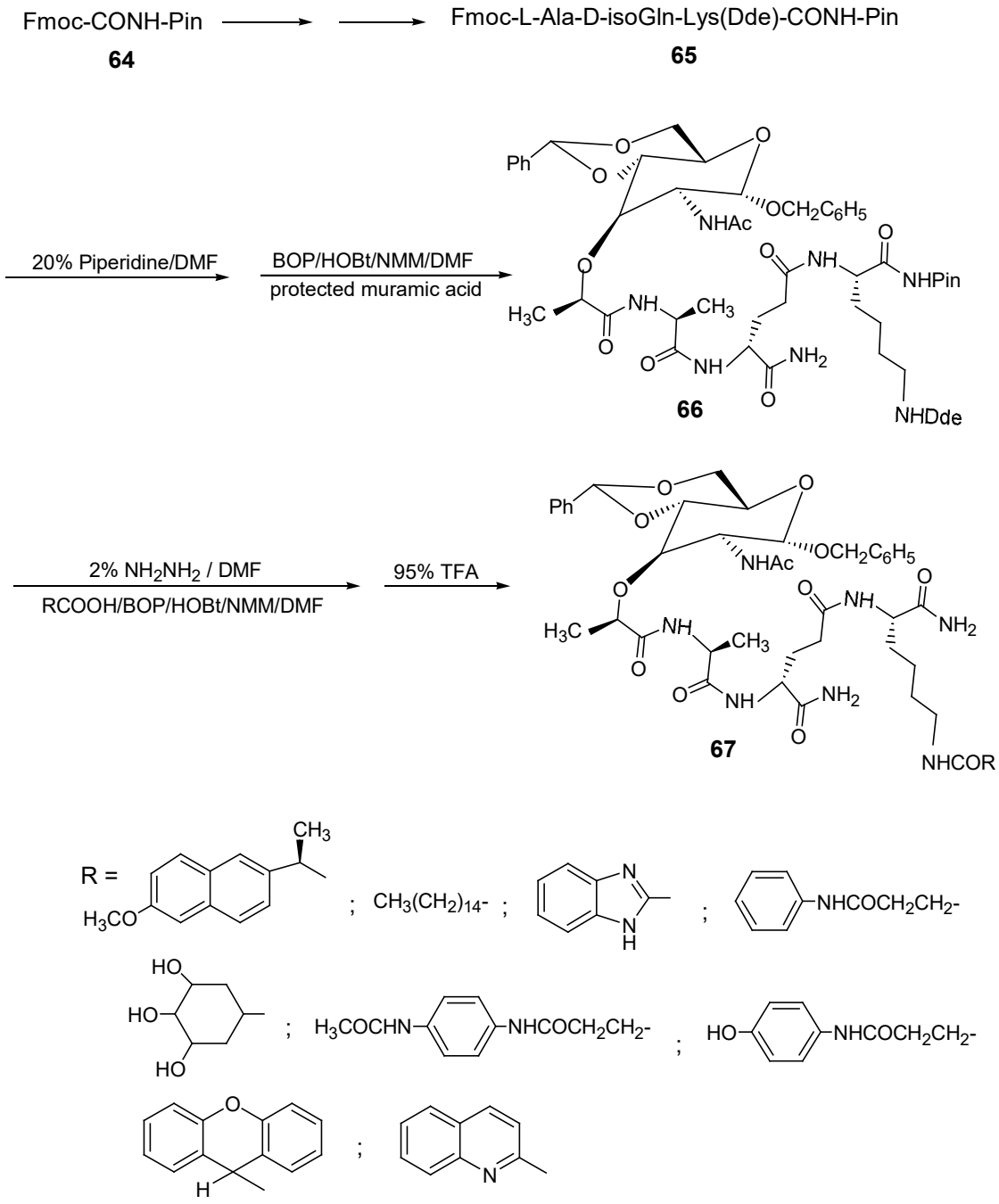
42 **43** **44** **45** **46** **47** **48**

DCC = *N,N'*-dicyklohexylcarbodiimide, coupling reagent
DMAP = 4-dimethylamino-pyridine
THF = tetrahydrofuran
Boc = *tert*-butoxycarbonyl group

Scheme 6. Synthesis of MTP-Chol **53**, MTP-octadecane **57**, and MTP-heptadecafluoro-octadecane **63** [51].

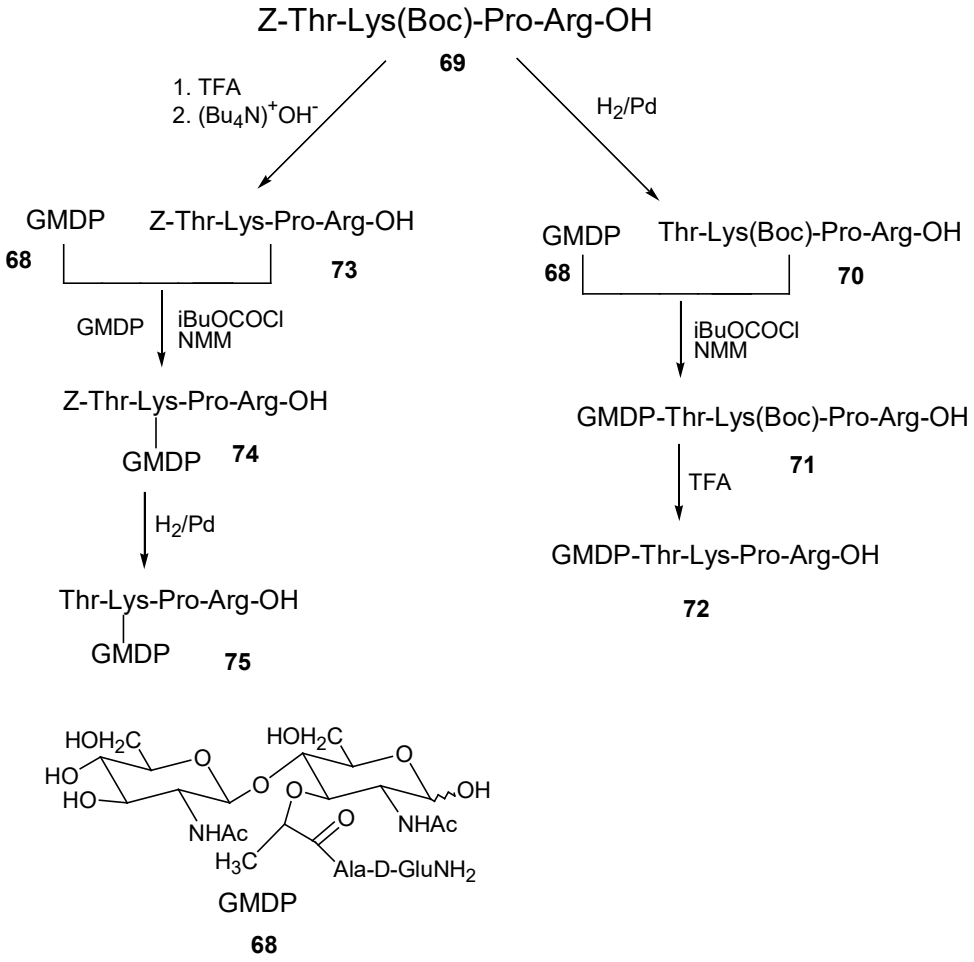


Scheme 7. Synthesis of muramyl dipeptide derivatives on the crown [53].



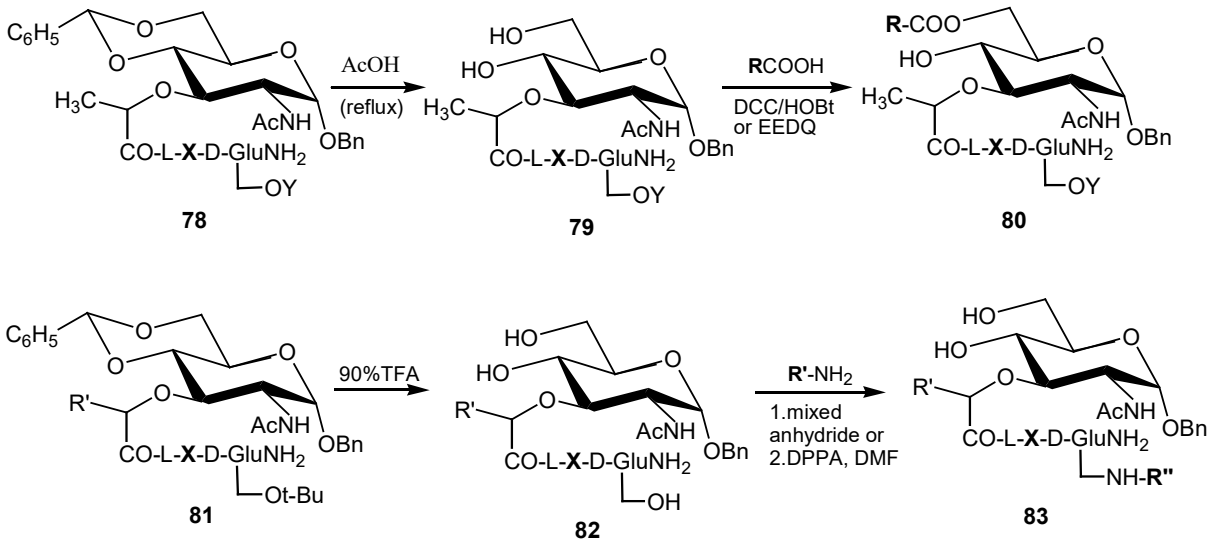
BOP = benzotriazolyloxy-tris-(dimethylamino) phosphonium hexafluorophosphate
Dde = 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl
DMF = dimethylformamide
Fmoc = 9-fluorenylmethoxycarbonyl
HOBt = 1-hydroxybenzotriazole hydrate
NMM = *N*-methylmorpholine
Pin = macro crown with a loading capacity of 5-8μmol/pin from Chiron Mimotopes
TFA = trifluoroacetic acid

Scheme 8. Synthesis of GMDP-tuftsin conjugates [54].



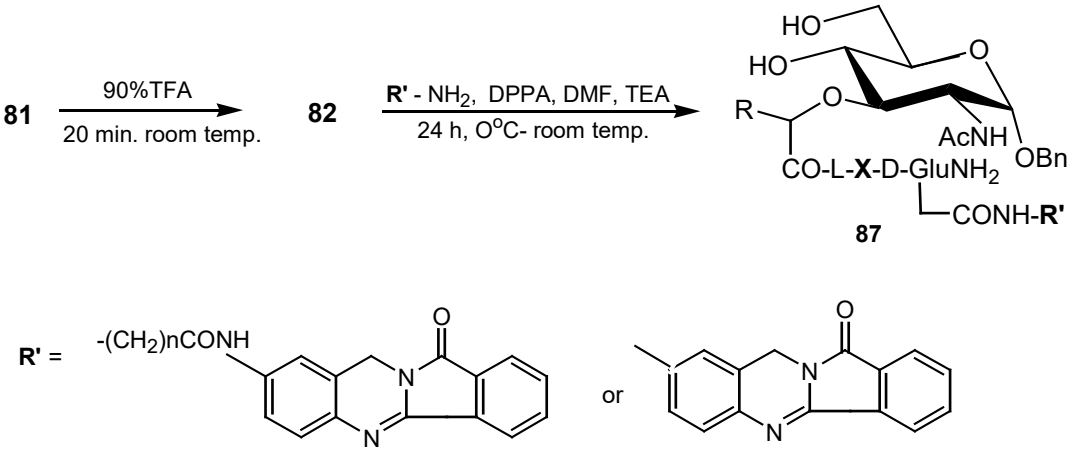
Boc = *tert*-butoxycarbonyl group
iBuOCOCl = isobutyl chloroformate
Z = benzyloxycarbonyl group

Scheme 9. Synthesis of MDP or nor-MDP conjugates of acridine/acridone derivatives [58].



X = Ala, Val
Y = Bn, Me
R = carboxy-acridine/acridone derivatives
R' = H, CH_3
R'' = amino-acridine/acridone derivatives
DCC =*N*,*N'*-dicyklohexylcarbodiimide, coupling reagent
DMF = dimethylformamide
DPPA =diphenyl azidophosphate, coupling reagent
HOBt = 1-hydroxybenzotriazole hydrate
EEDQ =*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, coupling reagent
TFA = trifluoroacetic acid

Scheme 10. Synthesis of MDP or nor-MDP conjugates of batracylin [60].



X = Ala, Val,Pro
n = 2,3,5
R = H, CH_3
DMF = dimethylformamide
DPPA = diphenyl azidophosphate, coupling reagent
TEA = triethylamine
t-Bu = *tert*-butyl
TFA = trifluoroacetic acid