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Efficient Synthesis of Lactate-Containing Depsipeptides by the Mitsunobu Reaction of Lactates

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Abstract: The Mitsunobu reaction has been used as an efficient tool for the synthesis of orthogonally-protected and unprotected depsipeptides such as Boc/Fmoc-L-Lys(Alloc)-D-Ala-D-Lac-OAllyl or L-Lys-D-Ala-D-Lac that are bacterial cell wall precursor analogues found in vancomycin-resistant enterococci.

Keywords: allyl lactate; antibiotic resistance; depsipeptides; Mitsunobu reaction; vancomycin

The synthesis of the small depsipeptide L-Lys-D-Ala-D-Lac (**1**) is relevant to problems associated with the resistance of bacteria to the glycopeptide antibiotic vancomycin (**2**), which is considered to be the last resort for the treatment of infections caused by Gram-positive bacteria.[1] The latter is well known to inhibit cell wall biosynthesis of pathogenic bacteria and effects bacterial cell lysis by binding to the C-terminal L-Lys-D-Ala-D-Ala motif present in cell-wall precursors (Figure 1). A 10^3-fold loss of binding affinity to the resistant cell-wall sequence is due to the bacterial formation of L-Lys-D-Ala-D-Lac. The simple replacement of an amide NH group for an ester oxygen results in the replacement of a stabilizing hydrogen bond with a destabilizing lone pair-lone pair repulsion within the vancomycin-ligand complex.[2]

In the course of an antimicrobial discovery project, we needed a larger amount of the orthogonally protected depsipeptides **3**, **4** or their derivatives (Figure 2). In contrast to the published syntheses by Williams[3] and Boger,[4] we proposed to generate the ester bond *via* the Mitsunobu reaction, the advantages being economical starting material L-lactate and potentially high yields. While Li-D-Lac costs EUR 195.52 per g, L-lactic acid is obtainable for EUR 0.04 per g.[5]

The Mitsunobu reaction, first reported by Oyo Mitsunobu[6] in 1967, is today one of the most important organic transformations. The generation of the esters in high yields from the reaction of alcohols and carboxylic acid in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine (PPh_3) proceeds with complete Walden inversion of the alcohol component.[7]

However, Mitsunobu reactions at sterically and/or electronically disfavoured hydroxy compounds can proceed in part *via* acyloxyphosphonium ions and, thus, they might occur with retention of the configuration.[8]

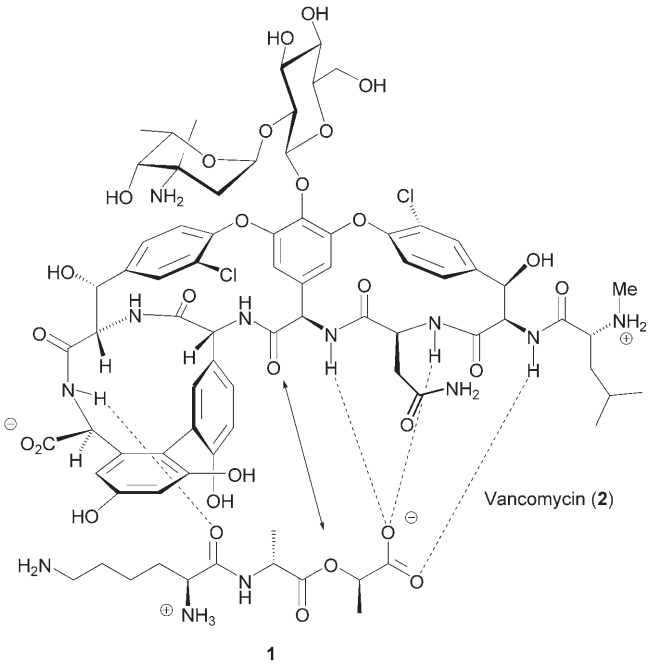


Figure 1. L-Lys-D-Ala-D-Lac (**1**) and vancomycin (**2**).

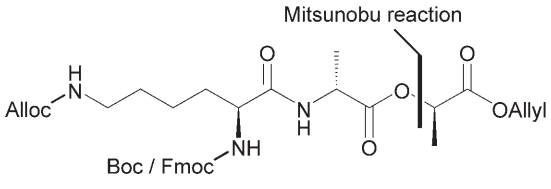


Figure 2. Depsipeptides Boc-L-Lys(Alloc)-D-Ala-D-Lac-OAllyl (**3**) and Fmoc-L-Lys(Alloc)-D-Ala-D-Lac-OAllyl (**4**).

Boger[9] reported the first total synthesis of a cyclic heptadepsipeptide *via* a Mitsunobu reaction in 1999. However, the full exploration of this useful method using a range of aliphatic carboxylic acids has not yet been devised. This reaction is known both in liquids[10] and on solid supports.[11] Therefore, we decided to choose this etherification of the depsipeptides with lactates.

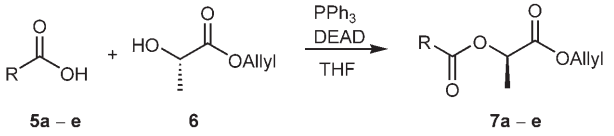
The protecting group strategy is based on the demands of former studies. Thus, the carbonyl function of L-lactate was protected as an allyl ester. For the Mitsunobu reaction, D-alanine was protected by the common amino protective groups Boc and Fmoc. In a first trial, the Mitsunobu reaction was tested with the nucleophiles Boc-D-Ala-OH (**5**), Boc-L-Ala-OH (**5a**), Fmoc-D-Ala-OH (**5b**), benzoic acid (**5c**), 2-naphthoic acid (**5d**), and 2-chloro-4-nitrobenzoic acid (**5e**) with different results (Scheme 1, Table 1).

The starting materials and PPh_3 were dissolved in THF, DEAD was added dropwise at 0 °C and the reaction mixture was warmed to room temperature within 4 h while stirring. To work up the reaction mixtures the solvent was removed, the residue was dissolved in ethyl acetate and washed with saturated aqueous $NaHCO_3$ solution. The crude products were purified by column chromatography (cyclohexane/ethyl acetate).

While Boc-D-Ala-OH (**5**) reacted with allyl L-lactate (**6**) in quite good yields to Boc-D-Ala-D-Lac-OAllyl (**7**) (89% with 2 equivalents of DEAD/PPh_3, 84% with 4 equivalents of DEAD/PPh_3, **5a** to **7a** in 83%), the Mitsunobu reaction with Fmoc-D-Ala-OH (**5b**) resulted in only 14% of the desired product **6b** (2 equivalents of DEAD/PPh_3). Even increasing the amount of DEAD/PPh_3 up to four equivalents did not result in higher yields. By means of ^{31}P, ^{1}H and ^{13}C NMR, it became obvious that the particular cleavage of the Fmoc protecting group already took place at a temperature of 0 °C. The Fmoc protective group is labile under the conditions used. The basic hydrazide anion, formed during the Mitsunobu reaction, induces the deprotection. The nucleophiles benzoic acid (**5c**), 2-naphthoic acid (**5d**) and 2-chloro-4-nitrobenzoic acid (**5e**) reacted in the same way with L-Lac-OAllyl (**6**). In addition to DEAD, the Mitsunobu reaction was also tested with di-*tert*-butyl azodicarboxylate, and dibenzyl azodicarboxylate without significant differences. To prove the stereochemical integrity of this reaction, both enantiomers of alanine, Boc-D-Ala-OH (**5**) and Boc-L-Ala-OH (**5a**) were tested. The optical rotation showed that the stereochemical integrity remained throughout the reaction.

In preparation for the following coupling with L-Lys derivatives, the *N*-terminus of the protected depsipeptides PG-D/L-Ala-D-Lac-OAllyl **7**, **7a**, **7b** were deprotected under classical conditions. The controlled deprotection of Boc and Fmoc shows an interesting feature. The deprotection of Boc (5% TFA/CH_2Cl_2) under classical conditions without any scavenger was successful (90, 92%) and left the labile ester bond intact to give the amine **8** (Scheme 2), removing the Fmoc group (20% piperidine/DMF) in **7b** lead to a diketomorpholine **9** (79%). Obviously, basic conditions induce a nucleophilic attack of the deprotected amine to the lactate carboxylic carbon (Figure 3).

The Mitsunobu products D-Ala-D-Lac-OAllyl (**8**) and L-Ala-D-Lac-OAllyl (**8a**) were coupled to Fmoc-L-Lys (Alloc)-OH (DMF, HBTU[12], 1 h, room temperature) and the depsipeptides Fmoc-L-Lys(Alloc)-D-Ala-D-Lac-OAllyl (**4**) and Fmoc-L-Lys(Alloc)-L-Ala-D-Lac-OAllyl (**4a**) were generated in 89% (**4**) and 91% (**4a**) yield (Scheme 2).



Scheme 1. Mitsunobu reactions of acids with L-Lac-OAllyl (6).

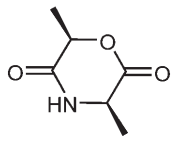
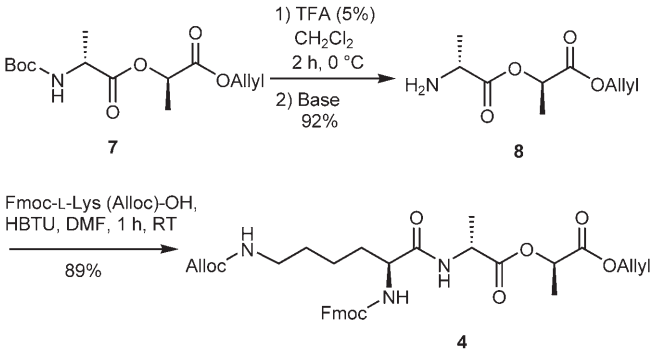


Figure 3. Diketomorpholine 9 after deprotection of Fmoc from 7b.

Table 1.

Carboxylic acid	Conditions	Product	Yield [%][a]
Boc-D-Ala-OH (**5**)	2 equivs. DEAD, PPh_3	**7**	89
Boc-D-Ala-OH (**5**)	4 equivs. DEAD, PPh_3	**7**	84
Boc-L-Ala-OH (**5a**)	2 equivs. DEAD, PPh_3	**7a**	83
Fmoc-D-Ala-OH (**5b**)	2 equivs. DEAD, PPh_3	**7b**	14
benzoic acid (**5c**)	2 equivs. DEAD, PPh_3	**7c**	51
2-naphthoic acid (**5d**)	2 equivs. DEAD, PPh_3	**7d**	43
2-chloro-4-nitrobenzoic acid (**5e**)	2 equivs. DEAD, PPh_3	**7e**	64
Boc-L-Lys(Alloc)-D-Ala-OH (**10**)	4 equivs. DEAD, PPh_3	**3**	40

[a] After column chromatography.



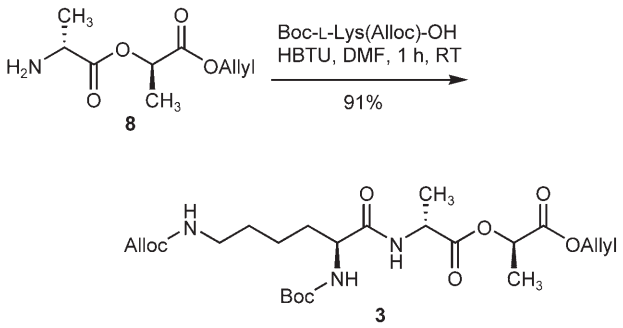
Scheme 2. Synthesis of the depsipeptide **4** (analogue **7a** *via* **8a** to **4a** in 91% yield).

For former studies L-Lys was orthogonally protected in a different manner. Using $CuCO_3$ $Cu(OH)_2$ the ε-position of L-Lys was protected with Alloc (96%).[13] After removing the α-complexing Cu *via* thioacetate, the α-position was protected with Boc[14] (79%). Final coupling with the Mitsunobu product D-Ala-D-Lac-OAllyl (**8**) also succeeded to the depsipeptide Boc-L-Lys(Alloc)-D-Ala-D-Lac-OAllyl (**3**), a starting material for further investigations (Scheme 3).

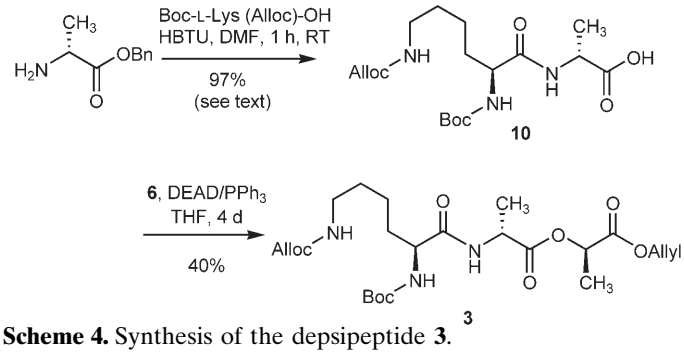
To verify and compare the efficiency of the Mitsunobu reaction for the synthesis of larger depsipeptides, the ester bond was also created as the final assembly step. D-Ala-OBn[15] was coupled with Boc-L-Lys(Alloc)-OH (DMF, HBTU, 1 h, 97%). The benzyl ester was cleaved during the work-up, a controlled deprotection was not necessary. The Mitsunobu coupling with allyl L-lactate (**6**) gave the orthogonally protected depsipeptide **3** (Scheme 4).

These results revealed that an initial Mitsunobu reaction and subsequent peptide coupling led to a much better overall yield (72%) than an inverted strategy (39%).

In comparison to the published syntheses of the cell wall precursor L-Lys-D-Ala-D-Lac,[3,4] this new strategy starting with a Mitsunobu reaction of allyl L-lactate followed by peptide coupling opens an efficient and rational way to this and other important depsipeptides involving the lactate moiety.



Scheme 3. Synthesis of the orthogonally protected depsipeptide 3.



Scheme 4. Synthesis of the depsipeptide **3**.

Experimental Section

Boc-D-Ala-D-Lac-OAllyl (7) *via* Mitsunobu Reaction

A solution of L-Lac-OAllyl (1.30 g, 10 mmol), Boc-D-Ala (1.89 g, 10 mmol) and triphenylphosphine (5.24 g, 20 mmol) in 80 mL absolute THF was cooled to 0 °C under an argon atmosphere. DEAD (3.14 mL, 20 mmol) was added dropwise and the reaction mixture was stirred at room temperature for 4 h. The solvent was removed under reduced pressure. The residue was dissolved in 50 mL EtOAc and washed three times with 50 mL of a saturated solution of $NaHSO_4$. The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure. The crude product was purified by flash chromatography (ethyl acetate/cyclohexane, 3:1) to give the desired product; yield: 2.68 g (89%); R_f = 0.28 (ethyl acetate/cyclohexane 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.44 (d, 3H, ^3J = 7.15 Hz, CH_3-Lac), 1.46 (s, 12H, CH_3-Boc), 1.51 (d, 3H, ^3J = 7.10, CH_3-Ala), 4.15 (q, 1H, ^3J = 7.10 Hz, C*H*-Ala), 4.45 (q, 1H, ^3J = 7.10 Hz, C*H*-Lac), 4,66 (ddd, 2H, ^3J = 5.70, ^4J = 2.40, 1.26 Hz, methylene-CH_2), 5.24 (ddt, 1H, ^3J = 10.20, 2.40, 1.26 Hz, allyl-CH_2), 5.32 (m, 1H, allyl-CH_2), 5.91 (m, 1H, allyl-C*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.1 (*C*H_3-Ala), 17.5 (*C*H_3-Lac), 28.7 (*C*H_3-Boc), 50.3 (*C*H-Ala), 66.8 (*C*H_2-methylene), 70.4 (*C*H-Lac), 80.5 (C_{quart}-Boc), 118.8 (*C*H_2-allyl), 133.0 (*C*H-Allyl), 156.7 (carbamate-Boc), 170.6 (carbonyl-Ala), 174.6 (carbonyl-Lac); IR (KBr): ν = 3382 (s), 2980 (s), 2939 (s), 1747 (m), 1716 (w), 1649 (s), 1513 (m), 1454 (m), 1367 (m), 1297 (m), 1251 (m), 1166 (w), 1132 (s), 1097 (m), 1067 (m), 979 (s), 937 (s), 880 (s), 856 (s), 781 (s), 758 (s) cm^{-1}; FAB-MS: *m/z* (%) = 603 (4) [2 M^+ + H], 302 (16) [M^+ + H], 246 (48), 202 (100) [M^+ − Boc], 189 (8) [M^+ − Lac−OAllyl]; $[\alpha]_D^{20}$: + 5.92 (*c* 0.10 g, THF). [**Boc-L-Ala-D-Lac-OAllyl (7a):** $[\alpha]_D^{20}$: − 0.90 (*c* 0.12 g, THF)].

Fmoc-L-Lys(Alloc)-D-Ala-D-Lac-OAllyl (4) *via* Peptide Coupling

A solution of D-Ala-D-Lac-OAllyl [(generated *via* Mitsunobu reaction, 89% yield, and Boc-deprotection, 90% yield); 1.00 g, 5.0 mmol] and DIPEA (646 mg, 5.0 mmol) was prepared in 4 mL of absolute DMF. Fmoc-L-Lys(Alloc)-OH

(2.34 g, 5 mmol) and HBTU (2.09 g, 5.5 mmol) were also dissolved in 4 mL of DMF. Under argon atmosphere both mixtures were added in parallel and dropwise into a vial. The reaction mixture was stirred at room temperature for 1 h and then poured on 250 mL of cold water. The crude product was filtered, washed with water and pentane and purified by flash chromatography (ethyl acetate, methanol) to give a white solid; yield: 2.34 g (91%); R_f = 0.75 (dichloromethane/methanol); ^{1}H NMR (500 MHz, DMSO): δ = 1.25–1.67 (m, 14H, CH_2-Lys, CH_3-Lac, CH_3-Ala), 4.14 (m, 1H, C*H*-Fmoc), 4.20 (q, 1H, ^3J = 7.10 Hz, C*H*-Ala), 4.59 (q, 1H, ^3J = 7.10 Hz, C*H*-Lac), 4.80 (m, 4H, O-CH_2), 5.21 (d, 2H, ^3J = 2.21 Hz, O-CH_2-Fmoc), 5.89 (m, 6H, allyl/Alloc), 7.36–7.74 (m, 8H, Fmoc); ^{13}C NMR (125 MHz, DMSO): δ = 17.1, 17.4, 23.4, 29.5, 30.8, 31.1, 31.2, 32.3, 36.2, 39.5, 39.6, 40.2, 40.4, 47.1, 54.2, 54.7, 64.5, 65.6, 66.0, 117.3, 118.6, 120.5, 125.8, 127.5, 128.1, 132.4, 134.3, 141.1, 144.2, 144.3, 156.3, 162.7, 174.4; IR (KBr): ν = 3302 (s), 3068 (w), 2968 (m), 1956 (w), 1916 (w), 1693 (s), 1651 (m), 1536 (s), 1451 (m), 1410 (w), 1335 (w), 1249 (m), 1150 (w), 1105 (w), 1045 (m) 992 (m), 938 (m), 859 (m), 777 (w), 759 (m), 740 (s), 660 (m), 621 (w) cm^{-1}; FAB-MS: *m*/*z* (%) = 622 (12) [M$^+$ + H], 506 (8), 414 (15), 356 (10), 202 (20) [M$^+$ − Fmoc−Lys(Alloc)], 179 (100), 136 (15); $[\alpha]_D^{20}$: +11.6 (*c* 0.10, THF).

Fmoc-L-Lys(Alloc)-L-Ala-D-Lac-OAllyl (4a): ^{1}H NMR (500 MHz, DMSO): δ = 1.28–1.69 (m, 14H, CH_2-Lys, CH_3-Lac, CH_3-Ala), 4.14 (m, 1H, C*H*-Fmoc), 4.23 (q, 1H, ^3J = 7.10 Hz, C*H*-Ala), 4.61 (q, 1H, ^3J = 7.10 Hz, C*H*-Lac), 4.82 (m, 4H, O-CH_2), 5.21 (d, 2H, ^3J = 2.21 Hz, O−CH_2−Fmoc), 5.88 (m, 6H, allyl/Alloc), 7.36–7.74 (m, 8H, Fmoc); ^{13}C-NMR (125 MHz, DMSO): δ = 17.2, 17.3, 23.3, 29.4, 29.6, 30.8, 31.2, 32.1, 36.2, 39.4, 39.6, 40.3, 40.4, 47.1, 54.2, 54.6, 64.5, 65.6, 66.0, 117.3, 118.5, 120.5, 125.7, 127.5, 128.1, 132.5, 134.3, 141.1, 144.2, 144.3, 156.6, 162.7, 174.4; $[\alpha]_D^{20}$: −0.20 (*c* 0.15, THF).

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