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# Kahalalide B. Synthesis of a natural cyclodepsipeptide

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**Abstract**

A suitable combination of soluble and polymeric protecting groups and coupling reagents has allowed the first synthesis of the natural cyclodepsipeptide of marine origin Kahalalide B to be carried out.


*Keywords:* cyclization; marine natural products; orthogonal protecting groups; peptides; solid-phase synthesis.

The isolation and structural determination of a family of peptides called kahalalides, from the sacoglossan mollusk *Elysia rufescens* and the green alga *Bryopsis* sp. on which it feeds, have been reported.[1–5] The members of this family range from a $C_{31}$ tetrapeptide to a $C_{75}$ tridecapeptide and besides having several non natural amino acids, most of them are cyclic and have an ester bond in their structure. Furthermore, each peptide contains a fatty acid at the N-terminus. Kahalalide B (**1**) is a cyclic depsipeptide formed by seven different amino acids (Gly, Thr, Pro, D-Leu, Phe, D-Ser, Tyr), and the fatty acid 5-methylhexanoic (5-MeHex), an aliphatic isoacid also present in the structure of other members of the series.

*Abbreviations:* DIEA, *N*,*N*-diisopropylethylamine; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; DMAP, 4-*N*,*N*-dimethylaminopyridine; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HOBt, 1-hydroxybenzotriazole; PyBOP, benzotriazol-1-yl-*N*-oxytris(pyrrolidino)-phosphonium hexafluorophosphate; TFA, trifluoroacetic acid.

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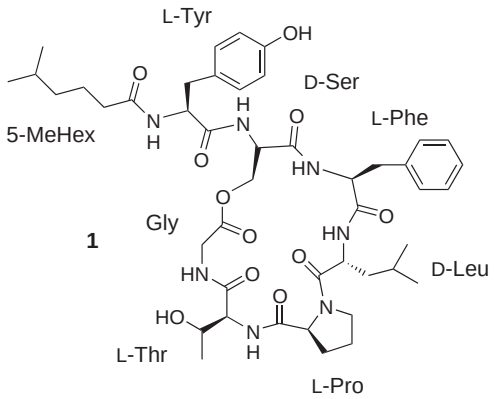
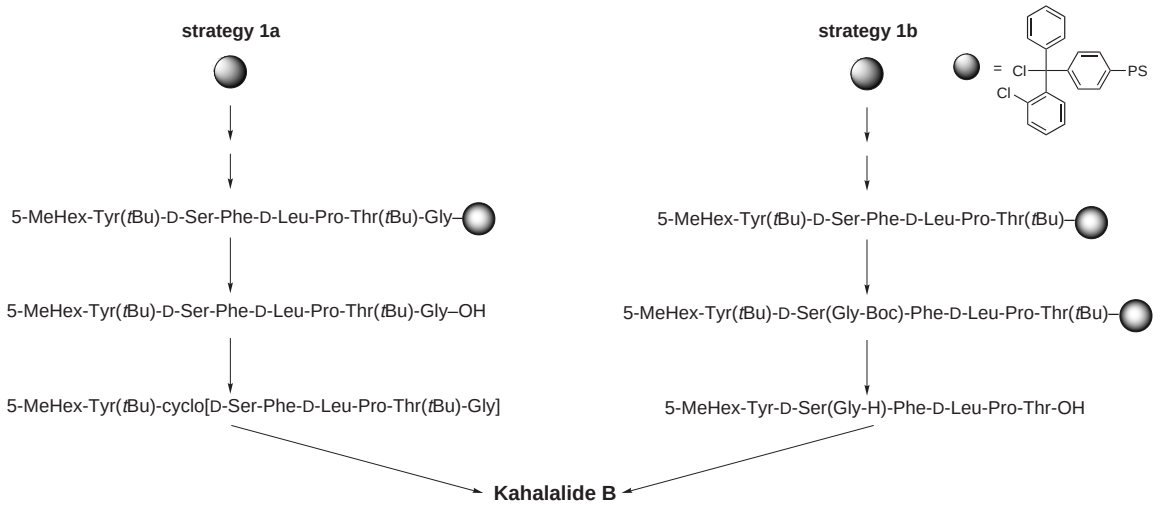



The synthesis of Kahalalide B (**1**) was carried out as a model for the synthesis of other peptides of the same family, because they have several points in common: (i) the cycle is formed by six amino acids, resulting in a 19-membered ring; (ii) the depsipeptide bond is endocyclic, formed by the carboxyl group of an amino acid and the hydroxyl group of the side-chain of another amino acid; (iii) the fatty acid at the N-terminus is the same. The principal challenge associated with the synthesis of Kahalalide B (**1**) was then the formation of the 19-membered cyclic depsipeptide.

First, the synthesis of the non-commercial fatty acid 5-MeHex had to be achieved. It was obtained by a malonic synthesis in a two-step sequence from diethyl malonate and 1-bromo-3-methylbutane in an overall yield of 22%. The synthesis of the natural cyclodepsipeptide was first attempted to be carried out completely on solid-phase[6] using the backbone amide linker (BAL) developed in our laboratory.[7] In this approach, the growing peptide is anchored through a backbone nitrogen, thus allowing the carboxyl at the C-terminus free for the final cyclization step. Unfortunately after different attempts the desired product was not obtained.

A second approach to the synthesis of Kahalalide B (**1**) was attempted by elongating the peptide chain on the solid-phase and carrying out the cyclization in solution after the cleavage of the peptide from the resin. However, there were six different possibilities depending on the cyclization bond chosen and we decided to compare the synthesis of the peptide by two different strategies. These differed in the fact that while in the first one the cyclization was carried out in solution through the ester bond between the carboxyl group of Gly and the side-chain hydroxyl of D-Ser (strategy **1a**), in the second one the ester bond was formed on the solid-phase and the cyclization was through an amide bond between the carboxyl group of Thr and the amine group of Gly (strategy **1b**).

The linear sequence was synthesised on a 2-chlorotrityl chloride-resin (ClTrt–Cl–resin),[8] which allowed the cleavage of the peptide under very mild acid conditions and in the presence of other acid labile protecting groups if necessary. The limited incorporation of the first amino acid of the sequence in both strategies was performed with Fmoc-Gly-OH (strategy **1a**)/Fmoc-Thr($^t$Bu)-OH (strategy **1b**) (0.2 equiv.) in the presence of DIEA (2 equiv.).[9,10] The elongation of the peptide chains was carried out using the Fmoc/$^t$Bu strategy.[11] The Thr and Tyr residues were introduced with protection at the hydroxyl side-chain function ($^t$Bu). DIPCDI–HOBt in DMF was used for all the amide formation (a single coupling for 90 min with 7 equiv. each of Fmoc-amino acid, HOBt, and DIPCDI gave a negative ninhydrin or chloranil (after acylation of Pro) test in all cases except for D-Ser in which recoupling with the Fmoc-amino acid and HATU (7 equiv. each) and DIEA (14 equiv.) in DMF was necessary. In strategy **1b**, the formation of the ester bond took place as the last step on the solid-phase with Boc-Gly-OH (9 equiv.), DIPCDI (9 equiv.) and DMAP (0.9 equiv.)[12] in DMF for 2.5 h and a recoupling under the same conditions but for 1.5 h. The synthetic yields were 83% for strategy **1a** and 77% for strategy **1b**.[13]




The cleavage reaction of the peptide from the resin was different for both strategies. In strategy **1a**, it was necessary to obtain the protected peptide so that the hydroxyl groups of Thr and Tyr residues could not react with the carboxyl group of Gly and therefore, it was carried out with TFA–$CH_2Cl_2$ (1:99) (5×0.5 min) with a 67% yield. On the other hand, in strategy **1b** it was not necessary to obtain the protected peptide because the amine function is much more nucleophilic than both hydroxyl groups. The cleavage reaction took place with TFA–$H_2O$ (95:5) for 2 h in 89% yield. The cyclization step was performed at a concentration of $10^{-3}$ M with PyBOP–DIEA (3:6 equiv.) in DMF for 23 h (strategy **1a**) or 1 h (strategy **1b**).[14] The difference in time for the cyclization step is probably due to the lower nucleophilicity of the hydroxyl compared to the amine. Final deprotection in strategy **1a** was carried out with TFA–$H_2O$ (95:5) for 1 h. Crude products were purified by medium pressure chromatography[15] to give the title compound (28% yield for strategy **1a** and 22% yield for strategy **1b**), which in both cases showed high purity by HPLC >98%, a correct MALDI-TOF-MS (calcd for $C_{45}H_{63}N_7O_{11}$, 877.5. Found: $m/z$ 878.7 $[M+H]^+$, 900.6 $[M+Na]^+$, 916.5 [M+K]) and AAA [Gly 1.02 (1), Thr 0.95 (1), Phe 0.99 (1), Ser 1.00 (1), Pro 1.19 (1), Leu 1.00 (1), Tyr 0.87 (1)] and $^1$H-NMR spectra [(500 MHz, $d^6$-DMSO) (see Table 1), ($^1$H, TOCSY (72 ms), ROESY (200 ms)] according to the structure.

In conclusion, an optimum combination of soluble and polymeric protecting groups and coupling reagents (DIPCDI–HOBt, HATU, DIPCDI–DMAP, PyBOP)[16] has allowed the first synthesis of Kahalalide B, to be achieved on a solid support by two different strategies without a significant difference in yield (16% for strategy **1a** versus 15% for strategy **1b**). However, strategy **1b** had some experimental advantages compared to strategy **1a**: (i) cleavage reaction was easier to handle and took place in better yield; (ii) the cyclization reaction was much faster (1 h versus 23 h); (iii) it was not necessary to carry out a final deprotection step of the cyclic depsipeptide because the side-chain protecting groups were removed during the cleavage reaction. This strategy should be of general applicability for the synthesis of other cyclodepsipeptides.

Table 1
$^1$H-NMR (500 MHz, $d^6$-DMSO) data for **1**

| aa | N-H | Hα | Hβ | Side-Chains |
|---|---|---|---|---|
| Gly | 7.34 (t, *J*=6.0 Hz) | 3.89 (1H, dd, $J_1$=17.5 Hz, $J_2$=6.0 Hz) 3.62 (1H, dd, $J_1$=17.5 Hz, $J_2$=6.0 Hz) | - | - |
| Thr | 7.18 | 4.19 (dd, $J_1$=9.0 Hz, $J_2$=2.5 Hz) | 4.41 | 5.42 (d, *J*=9.0 Hz, OH), 1.17 (d, *J*=6.5 Hz, $CH_3$-γ) |
| Pro | - | 4.37 | 2.07 (m), 1.99 (m), 1.79 (m) ($CH_2$-β, $CH_2$-γ), 3.97 (1H, m, $CH_2$-δ), 3.50 (1H, m, $CH_2$-δ) | |
| D-Leu | 8.89 (d, *J*=3.5 Hz) | 4.08 (m) | 1.31 (m), 1.48 (m) ($CH_2$-β, CH-γ), 0.86 (d, *J*=6.5 Hz, $CH_3$-δ), 0.78 (d, *J*= 6.0 Hz, $CH_3$-δ) | |
| Phe | 7.64 (d, *J*=9.0 Hz) | 4.81 (m) | 2.87 (m) | 7.13, 7.17 (5H, m, Ar) |
| D-Ser | 8.01 (d, *J*=10.0 Hz) | 4.40 | 4.40 (1H) 3.37 (1H, dd, $J_1$=11.0 Hz, $J_2$=2.5 Hz) | - |
| Tyr | 8.31 (d, *J*=5.5 Hz) | 4.27 (m) | 2.78 (m) | 9.16 (s, OH), 6.99 (2H, d, *J*=8.5 Hz, Ar), 6.66 (2H, d, *J*=8.0 Hz, Ar) |
| 5-MeHex | - | 2.10 (m) | 1.41 (m, $CH_2$-β, CH-δ), 1.05 (m, $CH_2$-γ), 0.73 (d, J=6.5 Hz, $CH_3$-ε), 0.72 (d, J=6.0 Hz, $CH_3$-ε) | |

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