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Synthetic studies of micropeptin T-20, a novel 3-amino-6-hydroxy-2-piperidone (Ahp)-containing cyclic depsipeptide

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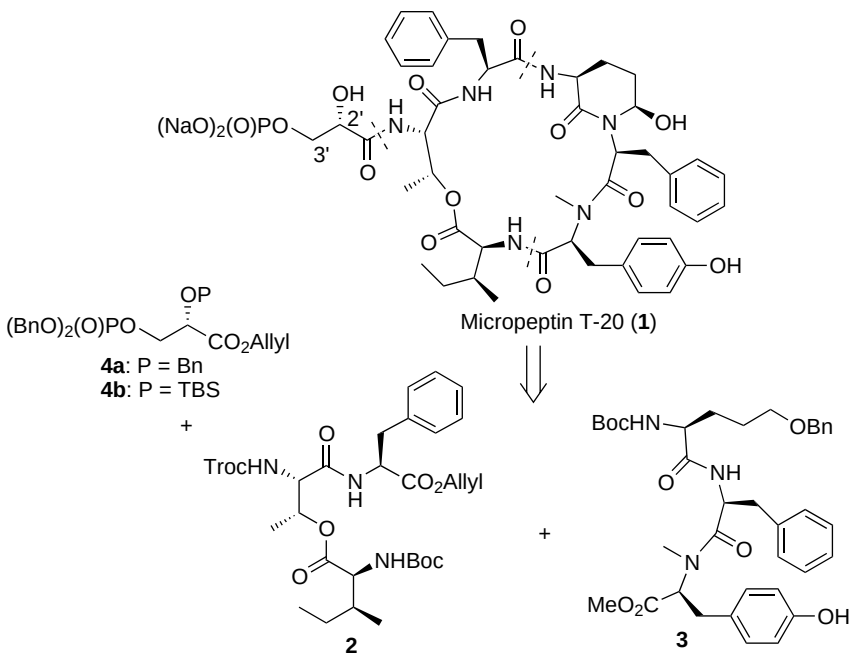
Abstract—The synthetic studies of micropeptin T-20 including the late installation of the Ahp (3-amino-6-hydroxy-2-piperidone) residue through oxidation and cyclization of a homoserine to the requisite hemiaminal are described.

Freshwater cyanobacteria have proven to be an exceptionally rich source of peptidic protease inhibitors containing unusual amino acids.[1] Recently, 3-amino-6-hydroxy-2-piperidone (Ahp) has been found in several cyclic depsipeptides derived from cyanobacteria, and its unprecedented common structure can participate in converting the cyclic peptide into a bioactive conformation due to the conformationally restricted structure and hydrogen bonding with the free hydroxyl group in Ahp.[2,3] Micropeptin T-20 (**1**) was isolated from the cyanobacterium *Microcystis aeruginosa* by Kaya and co-workers,[3a] and its structure was assigned



Scheme 1. Retrosynthetic analysis of micropeptin T-20.

Keywords: micropeptin T-20; 3-amino-6-hydroxy-2-piperidone (Ahp); total synthesis; cyclic depsipeptide.
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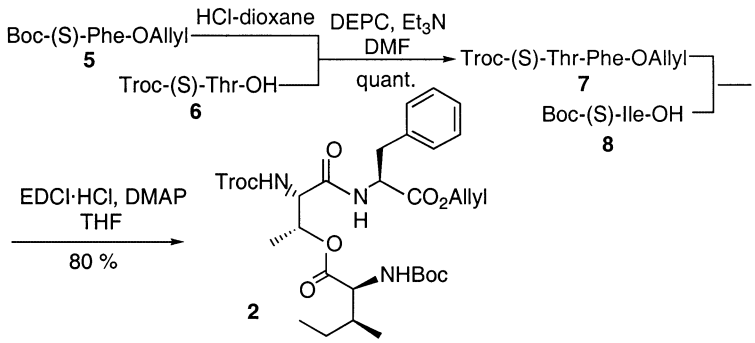
based on extensive NMR and chemical degradation studies. This cyclic depsipeptide contains Ahp together with novel glyceric acid 3′-*O*-phosphate at the N-terminal. Micropeptin T-20 (**1**) strongly inhibits chymotrypsin with an IC_{50} of 2.5 nM, but does not significantly inhibit tyrosinase. Our continued interest in the total synthesis of aquatic natural products having useful biological activities[4] coupled with the novel structure of micropeptin T-20 prompted us to investigate its synthesis. Herein we report our synthetic studies for producing micropeptin T-20, and our finding that the spectroscopic data of our synthetic **1** do not correspond to that of micropeptin T-20.

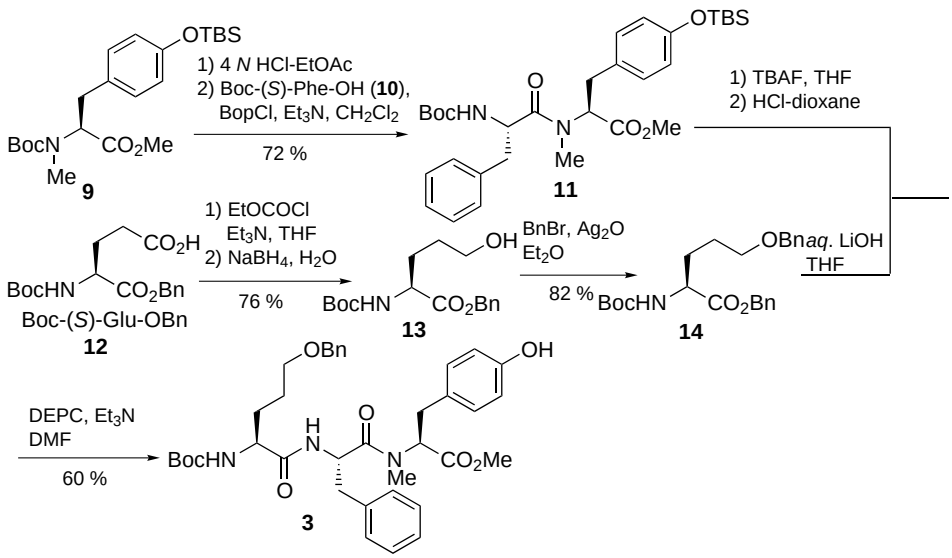
Our convergent synthetic strategy for micropeptin T-20 is shown in Scheme 1. The pentahomoserine derivative was used as a precursor of the Ahp, and the oxidation of its primary alcohol followed by cyclization to Ahp was postponed to the final stages of the synthesis, because the hemiaminal portion of Ahp will be very sensitive to the various reaction conditions. The glyceric acid phosphate **4** was introduced after the macrocyclization of the depsipeptide, which was obtained by a [3+3] convergent strategy.

The depsipeptide **2** was prepared from Boc-(*S*)-Phe-OAllyl (**5**) through (1) acidic removal of the Boc group, (2) coupling with Troc-(*S*)-Thr-OH (**6**) using diethyl phosphorocyanidate (DEPC),[5] and (3) esterification of the threonine hydroxy residue with Boc-(*S*)-Ile-OH (**8**) using *N*-ethyl-*N*′-(3-(dimethylamino)-propyl)carbodiimide hydrochloride (EDCI·HCl) (Scheme 2).

The synthesis of the tripeptide **3** started from Boc-(*S*)-*N*-MeTyr(TBS)-OMe (**9**),[6] from which was deprotected the Boc group, followed by coupling with Boc-(*S*)-Phe-OH (**10**) using bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BopCl)[7] to give the dipeptide **11** in 72% yield. The pentahomoserine derivative **13** was obtained from Boc-(*S*)-Glu-OBn (**12**) by in situ $NaBH_4$ reduction of its mixed anhydride with ethyl chloroformate in 76% yield.[8] Protection of the primary alcohol of **13** as its benzyl ether followed by saponification of the benzyl ester **14** gave the carboxylic acid, which was condensed with the Boc and TBS deprotected derivative of the dipeptide **11** to afford the required tripeptide **3** (Scheme 3).



Scheme 2. Synthesis of the depsipeptide **2**.



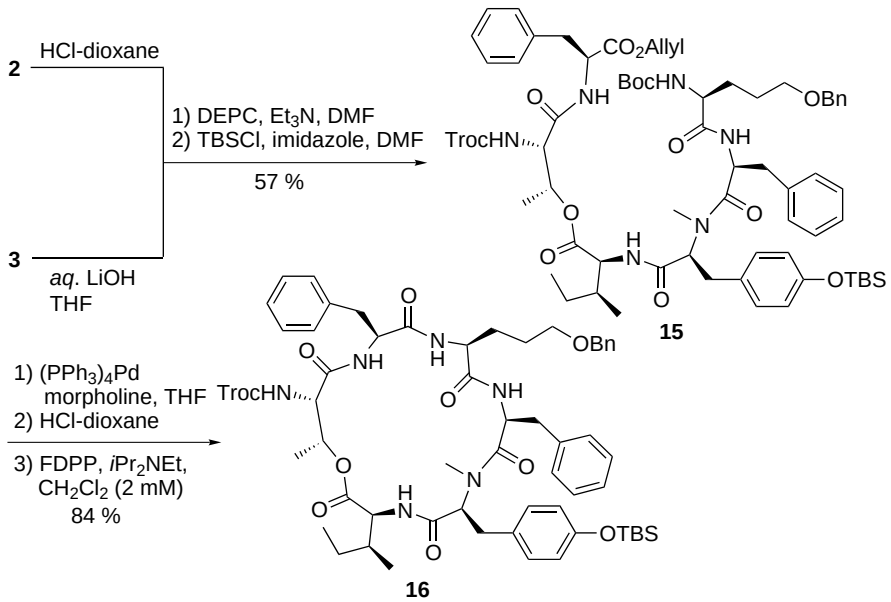
Scheme 3. Synthesis of the tripeptide **3**.

After cleavage of the Boc group from **2** and the methyl ester from **3**, segment condensation of the two deprotected fragments smoothly proceeded with DEPC, followed by reprotection of the phenolyic OH group of tyrosine to give the linear depsipeptide **15** in 57% yield. After removal of the allyl group at the C-terminus with $Pd(Ph_3P)_4$ in the presence of morpholine and then deprotection of the Boc group at the N-terminus with HCl, the macrolactamization was effectively achieved using pentafluorophenyl diphenylphosphinate (FDPP)[9] to afford the macrocyclic depsipeptide **16** in 84% yield (Scheme 4).

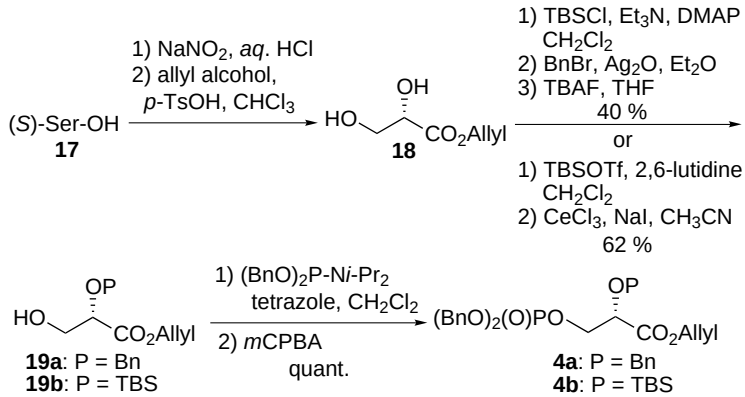
For the preparation of the glyceric acid phosphates **4a** and **4b**, the protected (*S*)-glyceric acid derivatives **19a** and **19b** from (*S*)-serine (**17**) were synthesized through (1) deamination, (2) protection of the carboxylic acid with the allyl group, (3) monosilylation of the primary alcohol or bis-silylation of the diol, and (4) benzylation of the secondary alcohol followed by the deprotection of the primary TBS ether or selective deprotection of the primary TBS ether.[10] Introduction of the phosphate group to the primary alcohols in **19a** and **19b** was achieved using *N*,*N*-diisopropyl dibenzyl phosphoramidite ($(BnO)_2P\text{-}NiPr_2$) in the presence of tetrazole, followed by the oxidation with *m*-chloroperbenzoic acid (MCPBA)[11] to afford the desired phosphates **4a** and **4b** in quantitative yield (Scheme 5).

For the attachment of the side-chain to the cyclic depsipeptide, exchange of the Troc group in **16** with the Boc group and subsequential deprotection of the benzyl ether and Boc group gave the deprotected cyclic depsipeptide, which was condensed with the allyl ester free glyceric acids of **4a** and **4b** to produce the alcohols **21a** and **21b** (Scheme 6).

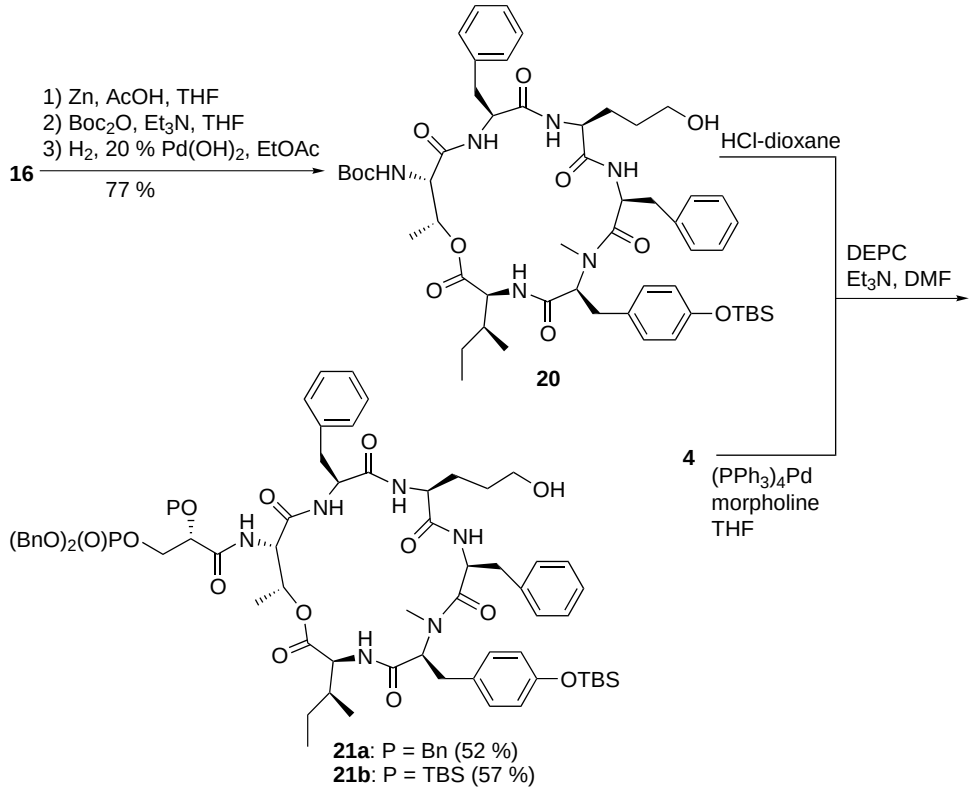
Before the construction of the Ahp derivative, we examined the model cyclization with the aldehyde **22**. We initially treated the aldehyde **22** with several Lewis acids ($ZnCl_2$, $MgBr_2$, $TiCl_4$, $BF_3{\cdot}Et_2O$, $Sc(OTf)_3$), however, no reaction occurred and the aldehyde **22** was only recovered. Treatment with silica gel or *p*-toluenesulfonic acid gave the dehydro derivative **24** as the



Scheme 4. Synthesis of the cyclic depsipeptide **16**.



Scheme 5. Synthesis of the glyceric acid phosphates **4a** and **4b**.



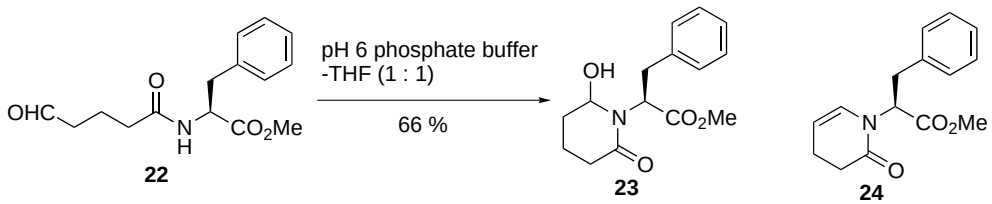
Scheme 6. Synthesis of the alcohols **21a** and **21b**.

major product. Fortunately, the cyclization proceeded by treatment of the aldehyde **22** with pH 6 phosphate buffer to provide the desired hydroxy piperidone **23** in 66% yield (Scheme 7).

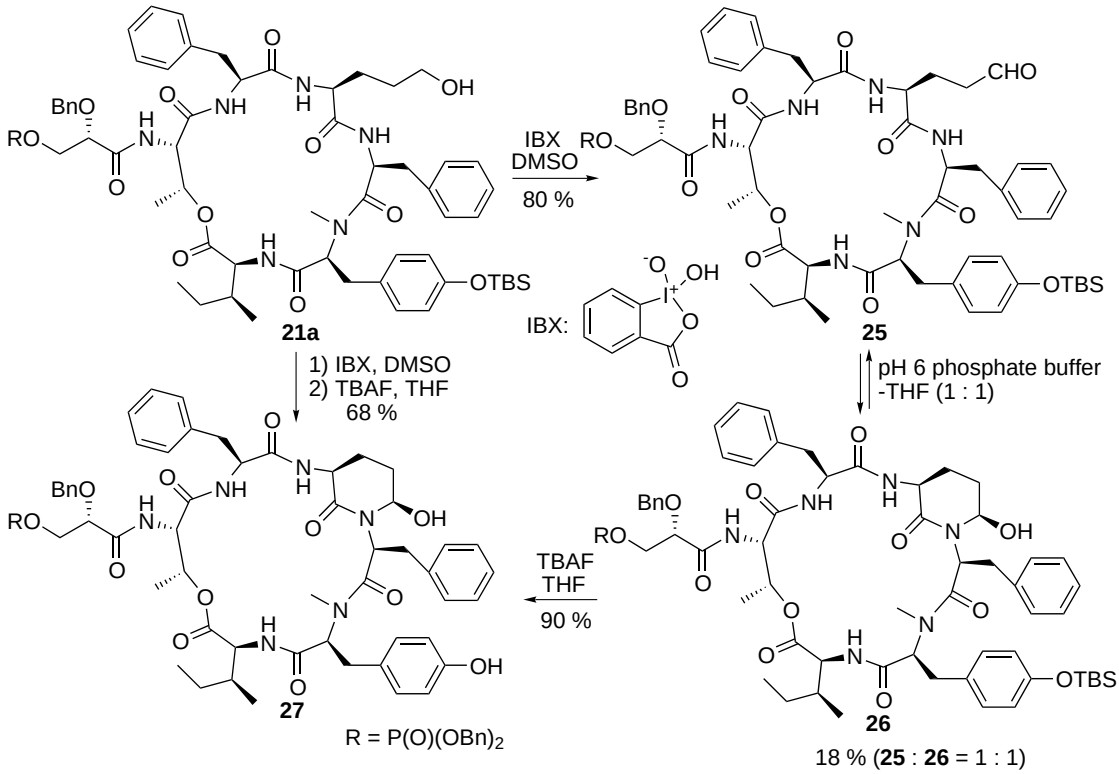
Next, we applied the above optimized condition to the construction of the Ahp moiety in micropeptin T-20. The oxidation of the primary alcohol **21a** was performed under mild, non-acidic conditions using 1-hydroxy-1,2-benziodoxol-3(1H)-one 1-oxide (IBX) in DMSO[12] to afford the aldehyde **25** in 80% yield. Unfortunately, treatment of the aldehyde **25** with pH 6 phosphate buffer afforded the Ahp derivative **26** and the recovered aldehyde **25** as a 1:1 mixture in 18% yield. Interestingly, this mixture treated with tetra *n*-butylammonium fluoride (TBAF) gave only the phenolic OH free Ahp derivative **27** in 90% yield. Therefore, we performed the IBX oxidation followed by deprotection of the TBS ether in the phenolic OH group to directly obtain the protected micropeptin T-20 (**27**) in 68% yield. However, deprotection of the secondary benzyl ether in the glyceric acid fragment was sluggish, and we had to change the protective group at the alcohol from the benzyl group to the more labile TBS group (Scheme 8).

IBX oxidation of the alcohol **21b** followed by TBAF treatment simultaneously led to the deprotection of both TBS groups in tyrosine and glyceric acid and cyclization to the Ahp derivative to give the requisite Ahp ring **28** as a single isomer, which was speculated to have the same configuration as the natural product.[13] Finally, deprotection of the benzyl phosphate was achieved by hydrogenolysis, followed by treatment with phosphate buffer and purification by ODS silica-gel chromatography to afford the putative structure of micropeptin T-20 (**1**) (Scheme 9).[14]

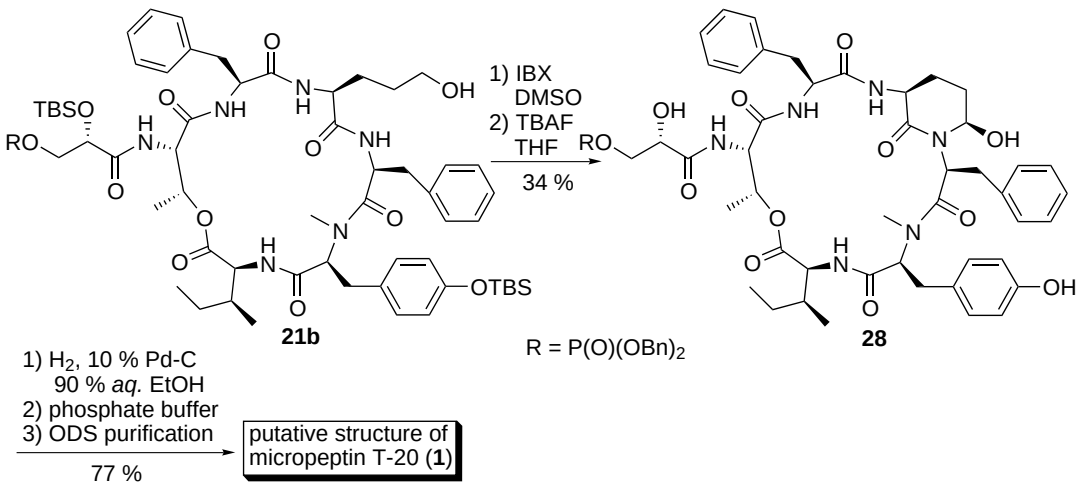
While all the spectroscopic data for synthetic **1** were consistent with the structural assignment, there were some differences between the ^{1}H and ^{13}C NMR spectra for **1** and the corresponding data reported for the natural product. Their conspicuous differences are found in the chemical shift of the glyceric acid frag-



Scheme 7. Preliminary model studies for the construction of hydroxy piperidone.



Scheme 8. Synthesis of the protected micropeptin T-20 (**27**).



Scheme 9. Synthesis of the putative structure of micropeptin T-20 (**1**).

ment, whereas the similarly synthesized 2′(*R*)-glyceric acid containing micropeptin T-20 is also not identical to the natural product. Although these NMR spectral differences might be affected by the pH values or concentrations in solution, the detailed conditions of the NMR spectra of natural product are not clear. Unfortunately, since no natural micropeptin T-20 is presently available, direct comparison of synthetic **1** with natural product under the same conditions will require re-isolation.

In summary, we have developed a novel strategy for the synthesis of the Ahp-containing cyclic depsipeptide and demonstrated its application to the synthesis of the putative structure of micropeptin T-20. Further applications of our strategy to other Ahp-containing natural products are currently underway.

Acknowledgements

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spectra of micropeptin T-20. The high resolution FAB mass spectrum was measured by Dr. Akito Nagatsu (Nagoya City University), to whom the authors' would like to give thanks.

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14. Synthetic **1**; a pale yellow solid: $[\alpha]_D^{23}$ −24.6 (c 0.15, CH_3OH); ^1H NMR (500 MHz, CD_3OD): δ (ppm) 0.87 (3H, t, $J=7.4$ Hz, Ile-5-**H**), 0.91 (3H, d, $J=6.7$ Hz, Ile-6-**H**), 1.07–1.15 (1H, m, Ile-4-**H**), 1.27 (3H, d, $J=6.4$ Hz, Thr-4-**H**), 1.28–1.35 (1H, m, Ile-4-**H**), 1.60–1.66, 1.68–1.75 (2H, m, Ahp-5-**H**), 1.77–1.89 (2H, m, Ahp-4-**H**, Ile-3-**H**), 1.98–2.04 (1H, m, Tyr-3-**H**), 2.56–2.66 (1H, m, Ahp-4-**H**), 2.66–2.80 (2H, m, Phe(1)-3-**H**, Phe(2)-3-**H**), 2.85 (3H, s, N**Me**), 2.92–2.98 (1H, m, Tyr-3-**H**), 3.36–3.45 (2H, m, Phe(1)-3-**H**, Phe(2)-3-**H**), 3.83 (1H, ddd, $J=6.7$, 9.4, 12.2 Hz, Ahp-3-**H**), 4.00–4.07 (1H, m, Ga-3-**H**), 4.13-4.18 (2H, m, Ga-2,3-**H**), 4.53 (1H, br, Thr-2-**H**), 4.65–4.71 (2H, m, Ile-2-**H**, Phe(1)-2-**H**), 4.90–4.96 (1H, m, Tyr-2-**H**), 5.03–5.06 (1H, m, Phe(2)-2-**H**), 5.16 (1H, br, Ahp-6-**H**), 5.46–5.52 (1H, m, Thr-3-**H**), 6.82 (2H, d, $J=8.5$ Hz, Tyr-6,8-**H**), 6.89 (2H, d, $J=7.1$ Hz, Tyr-5,9-**H**), 7.08–7.21 (10H, m, $C_6\mathbf{H_5}\times2$); ^{13}C NMR (500 MHz, CD_3OD): δ (ppm) 11.6, 16.7, 18.2, 22.4, 26.2, 30.6, 31.6, 34.3, 36.4, 37.4, 39.1, 50.8, 52.5, 55.8, 56.1, 57.4, 63.2, 68.3, 73.5, 73.7, 75.8, 116.7, 127.5, 127.7, 129.1, 129.4, 129.5, 130.1, 130.7, 131.8, 137.7, 139.0, 157.7, 190.9, 171.2, 171.5, 172.9, 173.3, 174.3, 174.6; HRFABMS (*m*-nitrobenzyl alcohol) calcd for $C_{46}H_{57}N_6Na_2O_{15}P$ $[M+H]^+$: 1011.3492. Found: 1011.3533.