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Synthesis of natural phaeosphaeride A and semi-natural phaeosphaeride B derivatives

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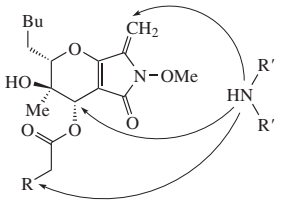
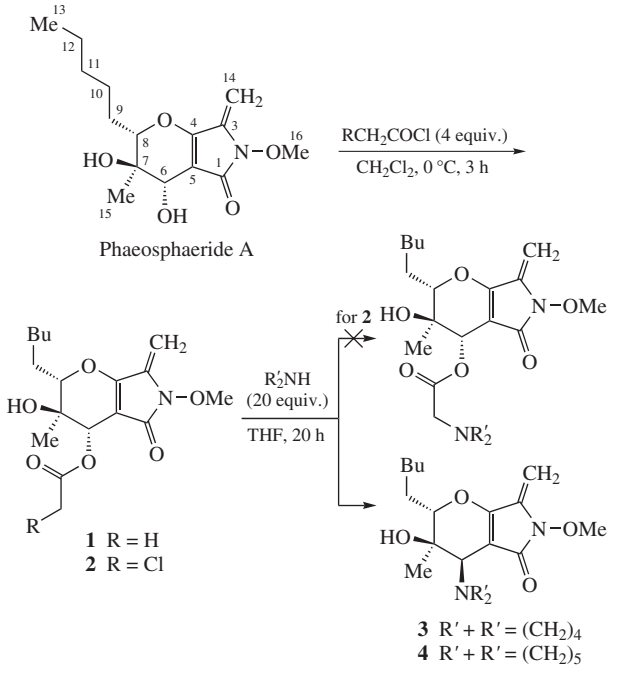
6-O-Acylated phaeosphaeride A and phaeosphaeride B in reaction with secondary cyclic amines undergo stereospecific replacement of acyloxy group by pharmacophoric amino moiety. In case of chloroacetoxy derivative, chlorine displacement by such amines proceeds in the presence of triethylamine.



Phaeosphaeride A (PPA) was originally isolated from the endophytic fungus, FA39 (*Phaeosphaeria avenara*) by Clardy *et al.*,[1] and later its structure was corrected by Kobayashi's[2] and our research groups.[3] It was found to be a potent inhibitor of STAT3/DNA binding with an IC_{50} of 0.61 mM, while exhibiting promising cell growth inhibition in STAT3-dependent U266 multiple myeloma cells with an EC_{50} of 6.7 μM.[1] PPA is a bicyclic C15 substance with three stereocenters including one quaternary center. Previously we have synthesized a series of PPA derivatives and evaluated the influence of the structural fragments in the molecules of PPA and its derivatives on cytotoxic activity.[4,5] Our studies highlighted the C-6 atom in PPA structure as the only possible place for optimizing the biological activity of PPA. The *O*-6-chloroacetyl derivative exhibited more potent cytotoxicity (EC_{50} = 33±7 μM) against A549 cancer cells as compared to natural PPA (EC_{50} = 46±5 μM).

In this paper, the synthesis of new PPA derivatives is described. 6-*O*-Acyl derivatives **1**, **2** were prepared by the reaction of PPA with the corresponding acyl chlorides (Scheme 1).[4] Reactions between chloroacetate **2** and the appropriate amines were expected to give the corresponding products of chlorine displacement.

To our surprise, the substitution afforded the products of replacement of acyloxy group by the amino one with inversion occurring on the C-6 atom.† This reaction course was untrivial since substitution of acyloxy groups by amines usually requires the use of catalysts, *e.g.*, such as $RuCl_3$,[6] $Pd(OAc)_2$,[7] $[Ir(COD)Cl]_2$.[8] The ROESY spectrum of **3** (see Online Supplementary Materials) showed a correlation between the methyl protons at δ 1.06 (H-15)



Scheme 1 The atom numbering is that of Clardy *et al.*[1] and differs from the IUPAC one.

† *General procedure for the synthesis of* **3**, **4**. Pyrrolidine or piperidine (2 mmol, 20 equiv.) was added to a stirred solution of **1** or **2** (0.1 mmol, 1 equiv.) in anhydrous THF (2 ml), and the mixture was stirred at room temperature for 24 h. The mixture was then concentrated under reduced pressure. The crude material was chromatographed on silica gel (CH_2Cl_2–methanol, 100 : 1 v/v).

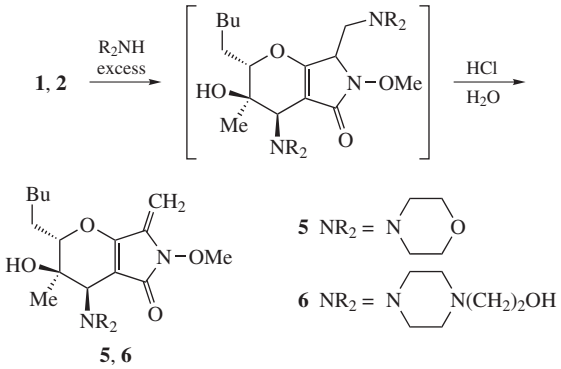
(2S,3R,4R)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-2-pentyl-4-(pyrrolidin-1-yl)-3,4,6,7-tetrahydropyrano[2,3-c]pyrrol-5(2H)-one **3**: 0.112 mmol scale, 11 mg, yellow oil, 28% yield. R_f 0.65 (CH_2Cl_2–methanol, 10 : 1 v/v). $[\alpha]_D^{20}$ = –178.91 (*c* 0.37, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.40 (s, 1H), 5.00 (dd, 2H, *J* 17.8, 1.4 Hz), 3.92 (s, 3H), 3.64 (d, 1H, *J* 9.9 Hz), 3.31 (s, 1H), 3.10–2.55 (m, 4H), 2.03–1.89 (m, 1H), 1.84–1.70 (m, 4H), 1.69–1.52 (m, 2H), 1.45–1.29 (m, 5H), 1.05 (s, 3H), 0.91 (t, 3H, *J* 6.7 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.11 (s), 158.44 (s), 137.00 (s), 101.65 (s), 91.38 (s), 83.60 (s), 68.00 (s), 64.41 (s), 59.55 (s), 31.73 (s), 28.21 (s), 26.43 (s), 23.91 (s), 22.55 (s), 19.66 (s), 14.04 (s). IR (KBr, ν/cm^{-1}): 3430, 2957, 2930, 2860, 1722, 1633, 1438, 1190, 1144. HRMS, *m/z*: 351.22745 $[M+H]^+$ (calc. for $C_{19}H_{31}N_2O_4$, *m/z*: 351.227834).

For characteristics of compounds **4**, see Online Supplementary Materials.

and the proton at δ 3.31 (H-6), confirming the inversion of configuration of the C-6 atom, which was not previously observed for starting compounds **1**, **2**.

To check whether chlorine does effect the reaction, the experiment was repeated using *O*-acetyl PPA derivative **1**. The results were analogous with the whole ester groups being substituted.

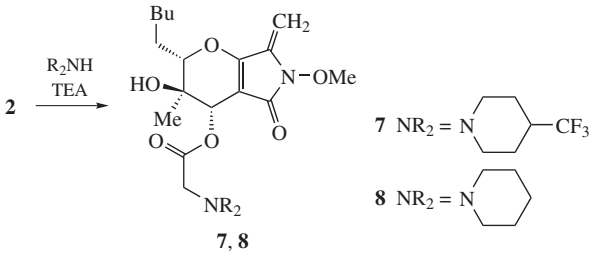
When morpholine or 1-(2-hydroxyethyl)piperazine were used as the amines, one more reaction took place: along with the substitution of the acyloxy group, addition of the amine at the exocyclic double bond occurred (Scheme 2).‡ MS revealed an $[M+H]^+$ ion with exact mass 454.29, corresponding to the molecular formula $C_{23}H_{39}N_3O_6$ + H (calc., *m/z* 454.2912, for a morpholine derivative). These 'addition–substitution' products were prone to partial decomposition during work up and purification by column chromatography. Therefore, they were used in the next step without purification. Upon treatment with HCl in water and THF compounds **5** and **6** were formed by a Cope elimination reaction[9–11] (see Scheme 2).§



Scheme 2

Since initially our goal was to obtain chlorine substitution products for various amines, we succeded to solve the problem using triethylamine as a base. In this way, products **7** and **8** were obtained (Scheme 3).¶

Recently Kobayashi and his research group have accomplished the first total synthesis of phaeosphaeride A and its inactive stereoisomer phaeosphaeride B.[12] To obtain products similar



Scheme 3

to compounds **3**–**6** but with opposite configuration at C-6, we have prepared phaeosphaeride B using Kobayashi's method and synthesized its chloroacetyl derivative **9** (Scheme 4). The ROESY spectrum of **9** showed a correlation between the methyl protons at δ 1.19 (H-7) and the proton at δ 5.52 (H-6), confirming the configuration of the C-6 atom (see Online Supplementary Materials). We have tried to replace the ester group in compound **9** with piperidine in an attempt to synthesize a compound where both the tertiary hydroxy group (C-7) and H-6 proton would be on the

‡ Similar products of addition–substitution judging by mass spectra were obtained in trace amounts during the synthesis of products **3**, **4**.

§ *General procedure for the synthesis of compounds* **5**, **6**. Mopholine or 1-(2-hydroxyethyl)piperazine (2 mmol, 20 equiv.) was added to a stirred solution of **1** or **2** (0.1 mmol, 1 equiv.) in anhydrous THF (2 ml), and the mixture was stirred at room temperature for 24 h. The mixture was then concentrated under reduced pressure. The residue was dissolved in THF (4 ml), diluted with H_2O. The resulting solution was acidified with concentrated HCl to pH 3 and stirred for 24 h. The mixture was then again concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (10 ml), treated with saturated $NaHCO_3$ to pH 8 and extracted with CH_2Cl_2 (2×20 ml). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was chromatographed on silica gel (CH_2Cl_2–methanol, 50 : 1 v/v).

(2S,3R,4R)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-4-(morpholin-4-yl)-2-pentyl-3,4,6,7-tetrahydropyrano[2,3-c]pyrrol-5(2H)-one **5**: 0.158 mmol scale, 10 mg, yellow oil, 17% yield. R_f 0.56 (CH_2Cl_2–methanol, 10 : 1 v/v). $[\alpha]_D^{20} = -188.10$ (*c* 0.33, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.04–5.00 (m, 3H), 3.93 (s, 3H), 3.71 (m, 4H), 3.60–3.52 (m, 1H), 2.98 (s, 1H), 2.89 (m, 2H), 2.65–2.58 (m, 2H), 2.03–1.90 (m, 1H), 1.68–1.54 (m, 2H), 1.42–1.31 (m, 5H), 1.06 (s, 3H), 0.91 (t, 3H, *J* 6.8 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.64 (s), 158.42 (s), 136.79 (s), 100.74 (s), 91.68 (s), 83.65 (s), 68.50 (s), 67.43 (s), 64.46 (s), 63.18 (s), 31.75 (s), 28.27 (s), 26.45 (s), 22.54 (s), 19.95 (s), 14.04 (s). IR (KBr, ν/cm^{-1}): 3427, 2955, 2925, 2854, 1723, 1636, 1438, 1190, 1120. HRMS, *m/z*: 367.22192 $[M+H]^+$ (calc. for $C_{19}H_{31}N_2O_5$, *m/z*: 367.222749).

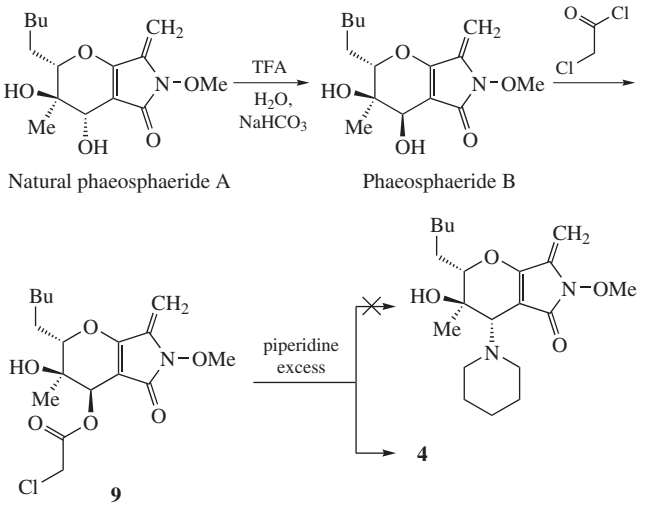
(2S,3R,4R)-3-Hydroxy-4-[4-(2-hydroxyethyl)piperazin-1-yl]-6-methoxy-3-methyl-7-methylidene-2-pentyl-3,4,6,7-tetrahydropyrano[2,3-c]pyrrol-5(2H)-one **6**: 0.160 mmol scale, 23 mg, yellow oil, 35% yield. R_f 0.75 (CH_2Cl_2–methanol, 5 : 1 v/v). $[\alpha]_D^{20} = -185.79$ (*c* 0.47, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.07–4.95 (m, 2H), 3.93 (s, 3H), 3.58 (m, 3H), 3.12–2.17 (m, 13H), 1.98–1.92 (m, 1H), 1.69–1.54 (m, 2H), 1.40–1.30 (m, 5H), 1.05 (s, 3H), 0.91 (t, 3H, *J* 6.6 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.81 (s), 158.28 (s), 136.84 (s), 101.03 (s), 91.70 (s), 83.50 (s), 68.42 (s), 64.50 (s), 62.44 (s), 59.04 (s), 57.62 (s), 53.37 (s), 31.75 (s), 29.70 (s), 28.29 (s), 26.46 (s), 22.55 (s), 19.92 (s), 14.04 (s). IR (KBr, ν/cm^{-1}): 3484, 3297, 2930, 2856, 1721, 1635, 1438, 1192, 1002. HRMS, *m/z*: 410.26492 $[M+H]^+$ (calc. for $C_{21}H_{36}N_3O_5$, *m/z*: 410.26495).

¶ *General procedure for the synthesis of* **7**, **8**. The appropriate amine (0.6 mmol, 3 equiv.) was added to a stirred solution of **2** (0.2 mmol, 1 equiv.) and TEA (0.8 mmol, 4 equiv.) in anhydrous THF (2 ml), and the mixture was stirred for 24 h. Then, the mixture was filtered and concentrated *in vacuo*. The residue was diluted with CH_2Cl_2 (20 ml), washed with saturated $NaHCO_3$ and brine. After extraction, the organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was chromatographed on silica gel (CH_2Cl_2–ethyl acetate, 1 : 1 v/v).

(2S,3S,4S)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-c]pyrrol-4-yl [4-(trifluoromethyl)piperidin-1-yl]acetate **7**: 0.201 mmol scale, 15 mg, colorless oil, 15% yield. R_f 0.50 (CH_2Cl_2–ethyl acetate, 1:1). $[\alpha]_D^{20} = -25.91$ (*c* 0.73, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.67 (s, 1H), 5.06 (dd, 2H, *J* 18.0, 1.5 Hz), 4.10 (d, 1H, *J* 12.1 Hz), 3.92 (s, 3H), 3.36 (br. s, 1H), 3.07 (dd, 2H, *J* 36.3, 11.2 Hz), 2.38–2.23 (m, 2H), 2.10–1.97 (m, 1H), 1.94–1.56 (m, 8H), 1.49–1.29 (m, 6H), 1.18 (s, 3H), 0.93 (t, 3H, *J* 6.8 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 171.40 (s), 164.40 (s), 158.03 (s), 136.12 (s), 101.07 (s), 92.29 (s), 86.18 (s), 70.77 (s), 64.56 (s), 59.01 (s), 52.04 (s), 51.74 (s), 40.03–39.76 (m), 31.49 (s), 27.36 (s), 26.02 (s), 24.56 (s), 22.50 (s), 13.98 (s). IR (KBr, ν/cm^{-1}): 3428, 2957, 2928, 2857, 1722, 1638, 1452, 1279, 1256, 1133, 1085, 1005. HRMS, *m/z*: 491.23591 $[M+H]^+$ (calc. for $C_{23}H_{34}F_3N_2O_6$, *m/z*: 491.236348).

(2S,3S,4S)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-c]pyrrol-4-yl piperidin-1-ylacetate **8**: 0.155 mmol scale, 12 mg, yellow oil, 18% yield. R_f 0.2 (CH_2Cl_2–methanol, 20 : 1 v/v). $[\alpha]_D^{20} = -31.25$ (*c* 0.4, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.63 (s, 1H), 5.02 (dd, 2H, *J* 17.8, 1.3 Hz), 4.08 (m, 1H), 3.90 (s, 3H), 3.51 (br. s, 1H), 3.35–3.26 (m, 2H), 2.61–2.48 (m, 4H), 1.90–1.70 (m, 1H), 1.72–1.58 (m, 5H), 1.47–1.27 (m, 8H), 1.15 (s, 3H), 0.90 (t, 3H, *J* 6.6 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 171.86 (s), 164.39 (s), 158.05 (s), 136.19 (s), 101.17 (s), 92.08 (s), 86.18 (s), 70.80 (s), 64.53 (s), 59.89 (s), 54.23 (s), 31.49 (s), 27.38 (s), 26.04 (s), 25.80 (s), 23.83 (s), 22.50 (s), 14.00 (s). IR (KBr, ν/cm^{-1}): 3438, 2932, 2857, 1723, 1638, 1450, 1167, 1130. HRMS, *m/z*: 423.24795 $[M+H]^+$ (calc. for $C_{22}H_{35}N_2O_6$, *m/z*: 423.248963).

For synthesis and characteristics of compound **9**, see Online Supplementary Materals.



Scheme 4

same face. However, reaction of **9** with piperidine afforded the same product **4**, probably, due to the rigidly fixed methyl group at the C-7 position. Its ^{1}H and ^{13}C spectra were identical to those of the sample **4** synthesized *vide supra*, and the ROESY spectrum showed a correlation between the methyl protons at δ 1.04 (H-7) and the proton at δ 2.95 (H-6). This result confirms the assumption made by Kobayashi[12] that a nucleophile would preferentially attack the C-6 center from the more accessible β-face.

Thus, depending on the reaction conditions, new products can be obtained as a result of the substitution of the chlorine atom, or of the acyloxy group, and in some cases, of the simultaneous replacement of the acyloxy group and the addition of the N-nucleophile to an exocyclic double C=C bond. A new family of chemical substances was synthesized, utilizing a method of introducing secondary amines as pharmacophores into the structure of phaeosphaeride A. Further research involving the synthesis of natural phaeosphaeride A derivatives and the testing of their biological activity is currently being carried out in our laboratory.

This paper is dedicated to the memory of V. M. Berestovitskaya.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2017.09.020.

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