A Facile Synthesis of Naturally Occurring Aminopeptidase Inhibitor Tyromycin A†

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Several structurally interesting and bioactive compounds with disubstituted maleic anhydride moieties, such as CP-225,917/CP-263,114,[1] byssochlamic acid,[2] tautomycin,[3] chaetomellic acid A,[4] and tyromycin A[5] have been recently (except byssochlamic acid) isolated as natural products. Recently we completed the synthesis of the 2,3-disubstituted maleic anhydride segment of antibiotic tautomycin[6] and three alternate syntheses of ras farnesyl protein transferase inhibitor chaetomellic acid A.[7−9] A number of FPTase inhibitors are in human clinical trails by several companies.[10] Work on the total synthesis of byssochlamic acid is in progress in our laboratories,[11] and recently we planned for the synthesis of tyromycin A (**1**). It has been isolated from mycelial cultures of the basidiomycete *Tyromyces lacteus* (Fr.) Murr,[5] and its structure was established as 1,16-bis[4-methyl-2,5-dioxo-3-furanyl]hexadecane (**1**) by using spectral and analytical data and by transformation into the corresponding tetramethyl ester and diimide derivatives.[5] Among the enzymes bound to surfaces of mammalian cells, aminopeptidases have been recognized as a potential target for the immunomodulating drugs.[12] Tyromycin A was found to inhibit the leucine and cysteine aminopeptidases bound to the outer surface of HeLa S3 cells, and it also exhibits cytostatic activity.[5] Development of a new facile synthetic route to this bioactive natural product, **1**, is a task of current interest, and its first synthesis has been completed by Samadi et al.[13] by using the well-known decarboxylative Barton−radical coupling reaction. Recently we reported the first coupling reaction of citraconimide **6** and triphenylphosphine (TPP) adduct with aliphatic aldehydes to complete the most efficient synthesis of chaetomellic acid A,[8] and herein we report the extension of this coupling reaction to complete a practical synthesis of tyromycin A via a double Wittig reaction and hydrolysis pathway starting from juniperic acid.[14]

The PCC oxidation[15a] of 1,16-hexadecanediol (**4**) in dichloromethane gave the desired dialdehyde **5**[15b] in 77% yield (Scheme 1). The reaction of dialdehyde **5** with an excess of citraconimide−TPP adduct in refluxing glacial acetic acid followed by removal of acetic acid in vacuo furnished a mixture of bis-condensed exo Wittig products **7** (*E*,*E* major), **8** (*E*,*Z* minor), and **9** (*Z*,*Z* minor) in 70% yield with an 85:15 ratio of *E*:*Z* geometry of the carbon−carbon double bond. In the above reaction, when the acetic acid was distilled off under normal atmospheric pressure and the residue was heated for 30 min at 140−150 °C, the reaction directly furnished the endo bisimide **10** in 72% yield. The mixture of **7** + **8** + **9** in refluxing tetralin underwent a smooth trisubstituted exocyclic to tetrasubstituted endocyclic double bond isomerization to yield the bismaleimide derivative **10** in quantitative yield. The mixture of exo-isomers **7** + **8** + **9** upon treatment with sodium methoxide in methanol followed by acidification gave tyromycin A in 60% yield. The reaction of bisimide **10** upon treatment with KOH in water + THF + CH_3OH (1:1:1) followed by acidification, ethyl acetate extraction, and silica gel column chromatographic purification[16] gave tyromycin A in 98% yield. The spectral and analytical data obtained for tyromycin A (**1**) were in agreement with reported data.[5,13]

In summary, we have demonstrated yet another application of our recently reported citraconimide−TPP adduct coupling reaction with aliphatic aldehydes to complete the practical two-step synthesis of bioactive natural product tyromycin A in 71% overall yield, and our method also offers potentially easy access to tyromycin congeners for structure−activity relationship studies. A one-pot double Wittig reaction has been used previously in the synthesis of β-carotene.[17]

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(16) Samadi et al.[13] believe that the tyromycin A during the silica gel column chromatographic purification undergoes a ring opening to form the corresponding di- and tetracarboxylic acids. We chromatographed 100 mg of pure tyromycin A over silica gel column (60−120 mesh) using a petroleum ether (60−80 fraction)−ethyl acetate mixture (9:1), obtained more than 98 mg of tyromycin A, and proved that it is fairly stable to silica gel column purification conditions. The disubstituted maleic anhydrides under neutral and acidic conditions stay in a ring-closed form, while in basic medium they exist in dianionic form.[4] To our knowledge, the conditions needed to obtain them in the cis-dicarboxylic acid form are still elusive.

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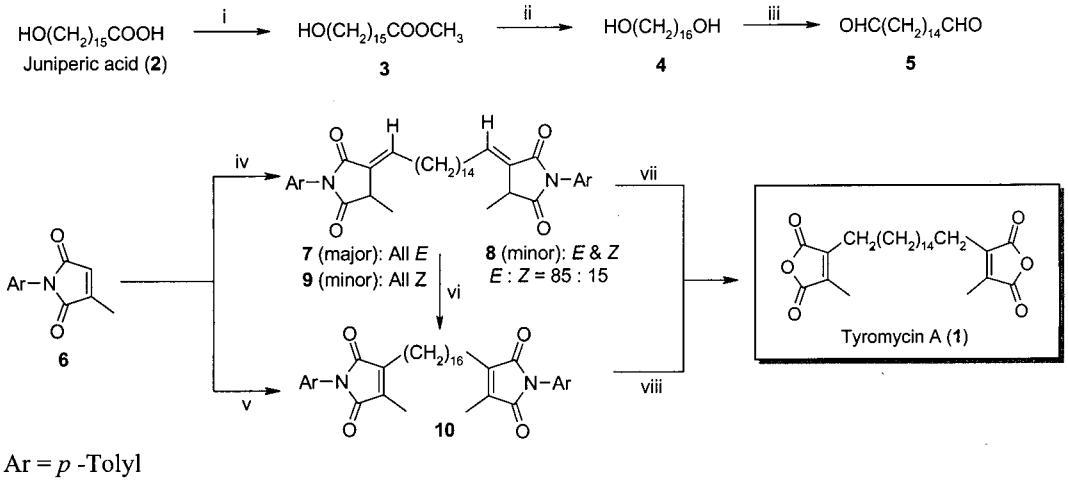
Scheme 1[a]



[a] (i) CH_2N_2, Et_2O, 0 °C, 2 h (95%); (ii) LAH, Et_2O, rt, 2 h (98%); (iii) PCC, CH_2Cl_2, rt, 10 h (77%); (iv) TPP, AcOH, **5**, reflux, 10 h (70%); (v) (a) TPP, AcOH, **5**, reflux, 10 h, (b) Δ, 140−150 °C, 30 min (72%); (vi) Tetralin, reflux, 1 h (98−100%); (vii) (a) CH_3ONa, CH_3OH, reflux, 2 h, (b) H^+/HCl (60%); (viii) (a) KOH, H_2O, THF, CH_3OH, reflux, 2 h, (b) H^+/HCl (98%).

Experimental Section

Melting points are uncorrected. Column chromatographic separations were carried out on ACME silica gel (60−120 mesh). Triphenylphosphine, citraconic anhydride, and juniperic acid were obtained from Aldrich Chemical Co. 1,16-Hexadecanediol (**4**) is commercially available and is a more suitable starting material for the synthesis of tyromycin A (**1**). We began our synthesis with juniperic acid (**2**) as it was immediately available to us from our polymer chemistry division. The citraconimide **6** was obtained in quantitative yield from citraconic anhydride via dehydration of a mixture of the corresponding regioisomers of maleanilic acids.[18]

Methyl Ester of Juniperic Acid (3). A solution of acid **2** (2.72 g, 10 mmol) in ether (30 mL) was treated with a solution of diazomethane in ether at 0 °C until the starting material was completely consumed (2 h). Excess diazomethane was quenched with acetic acid, and the reaction mixture was concentrated in vacuo. Silica gel column purification of the residue using a petroleum ether and ethyl acetate mixture (8:2) gave pure **3**: 2.71 g (95% yield); mp 60−62 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.28 (bs, 22H), 1.45−1.75 (bm, 5H), 2.35 (t, J = 8 Hz, 2H), 3.65 (t, J = 8 Hz, 2H), 3.68 (s, 3H); MS (*m*/*e*) 286, 268, 256, 236, 199, 143, 112, 98, 87, 74, 69, 55; IR ($CHCl_3$) ν_{max} 3400, 1712 cm^{-1}.

1,16-Hexadecanediol (4). A solution of **3** (2.29 g, 8 mmol) in ether (25 mL) was added dropwise to a suspension of lithium aluminum hydride (379 mg, 10 mmol) in ether (20 mL) at room temperature over a period of 10 min. The reaction mixture was stirred at room temperature for 2 h, and excess reagent was decomposed by cautious addition of moist ether (30 mL) and stirring for 30 min. To this mixture was added dilute HCl (20 mL), and the reaction mixture was extracted with ether (3 × 25 mL). The organic layer was washed with brine, dried over Na_2SO_4, concentrated, and dried in vacuo. Silica gel column chromatographic purification of the residue using petroleum ether−ethyl acetate (1:1) gave pure **4**: 2.02 g (98% yield); mp 91−92 °C (lit.[19] mp 89−90 °C); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.30 (bs, 24H), 1.58 (q, J = 6 Hz, 4H), 3.65 (t, J = 6 Hz, 4H); MS (*m*/*e*) 258, 240, 228, 222, 210, 194, 180, 166, 152, 137, 123, 109, 95, 82, 67, 54; IR (Nujol) ν_{max} 3415, 3356, 1462 cm^{-1}.

1,16-Hexadecanedial (5). To a stirred suspension of PCC (3.24 g, 15 mmol) in CH_2Cl_2 (35 mL) at 0 °C was added a solution of 1,16-hexadecanediol (1.29 g, 5 mmol) in CH_2Cl_2 (15 mL) over a period of 20 min, and the reaction mixture was further stirred for 10 h at room temperature. The reaction mixture was diluted with anhydrous ether (40 mL) and then stirred vigorously for 30 min. The supernatant of the reaction mixture was filtered through a small pad of silica gel and concentrated in vacuo. The silica gel column purification of the residue using a petroleum ether and ethyl acetate mixture (9:1) gave pure **5**: 978 mg (77% yield); mp 52−53 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.26 (bs, 20H), 1.63 (quintet, J = 6 Hz, 4H), 2.43 (dt, J = 6 and 2 Hz, 4H), 9.77 (s, 2H); MS (*m*/*e*) 254, 236, 192, 136, 108, 95, 81, 68, 57; IR (Nujol) ν_{max} 2748, 1713 cm^{-1}.

(*E*/*Z*)-1,16-Bis[4-methyl-2,5-dioxo-1-*p*-tolylpyrrolidin-3-yl]-1,15-hexadecadiene (7/8/9). A mixture of citraconimide **6** (3.01 g, 15 mmol), triphenylphosphine (3.93 g, 15 mmol), and 1,16-hexadecanedial (762 mg, 3 mmol) in glacial acetic acid (40 mL) was refluxed with stirring for 10 h. Acetic acid was distilled off in vacuo at 45−50 °C, and the residue was dissolved in ethyl acetate (50 mL). The organic layer was washed with water and brine and dried over Na_2SO_4. Concentration of the organic layer followed by silica gel column chromatographic purification of the residue using a mixture of petroleum ether and ethyl acetate (85:15) gave a mixture of **7**, **8**, and **9** (*E*:*Z* = 85:15 by ^{1}H NMR): 1.31 g (70% yield); mp 96−100 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.29 (bs, 20H), 1.48 (d, J = 8 Hz, 0.9H) (*Z*-isomer), 1.53 (d, J = 8 Hz, 5.1H), 1.40−1.60 (m, 4H), 2.00−2.40 (m, 3.4H), 2.39 (S, 6H), 2.70−2.90 (m, 0.6H) (*Z*-isomer), 3.35 (q, J = 8 Hz, 0.3H) (*Z*-isomer), 3.46 (q, J = 8 Hz, 1.7 H), 6.23 (dt, J = 8 and 2 Hz, 0.3 H) (*Z*-isomer), 6.93 (dt, J = 8 and 2 Hz, 1.7 H), 7.21 (d, J = 8 Hz, 4H), 7.29 (d, J = 8 Hz, 4H); MS (*m*/*e*) 624, 518, 423, 396, 312, 242, 228, 215, 202, 186, 133, 118, 107, 95, 81, 68, 55; IR (Nujol) ν_{max} 1770, 1710, 1672 cm^{-1}. Anal. Calcd for $C_{40}H_{52}N_2O_4$: C, 76.88; H, 8.38; N, 4.48. Found: C, 77.11; H, 8.12; N, 4.32.

1,16-Bis[4-methyl-2,5-dioxo-1-*p*-tolylpyrrol-3-yl]hexadecane (10). The imide **10** was prepared using the same procedure as described for the preparation of **7** + **8** + **9** except that the acetic acid was distilled off slowly over a period of 15 min at 140−150 °C bath temperature and the oily residue was further heated with stirring for 30 min at same temperature. **10**: 1.34 g (72% yield); mp 96−98 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.29 (bs, 20H), 1.35 (bs, 4H), 1.60 (quintet, J = 4 Hz, 4H), 2.07 (s, 6H), 2.40 (s, 6H), 2.48 (t, J = 6 Hz, 4H), 7.24 (d, J = 6 Hz, 4H), 7.28 (d, J = 6 Hz, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 8.2, 20.5, 23.2, 27.6, 28.7, 29.1 (5 × CH_2), 125.1 (2-carbons) 129.0 (2-carbons), 136.5, 140.7, 170.2, 170.5; MS (*m*/*e*) 624, 438, 242, 228, 215, 202, 154, 136, 120, 106, 91, 81, 55; IR (Nujol) ν_{max} 1770, 1713 cm^{-1}. Anal. Calcd for $C_{40}H_{52}N_2O_4$: C, 76.88; H, 8.38; N, 4.48. Found: C, 77.01; H, 8.22; N, 4.57.

Isomerization of Exo Isomers (7 + 8 + 9) to Endo Isomer (10). A solution of the **7**, **8**, and **9** mixture (500 mg) in tetralin (10 mL) was refluxed for 1 h with stirring. The reaction mixture was then cooled to room temperature, and silica gel column purification of the reaction mixture using a petroleum ether and ethyl acetate mixture (9:1) gave pure **10**: 490 mg (98% yield).

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1,16-Bis[4-methyl-2,5-dioxo-3-furanyl]hexadecane (Tyromycin A, 1). (a) A mixture of **7** + **8** + **9** (500 mg) in a solution of sodium methoxide (500 mg) in methanol (20 mL) was refluxed for 2 h with stirring and then the methanol was distilled off in vacuo. The residue was acidified with dilute HCl and extracted with ether (30 mL × 2), and the organic layer washed with water and brine and dried over Na_2SO_4. Concentration of the organic layer in vacuo followed by silica gel column chromatographic purification of the residue furnished pure **1**: 214 mg (60% yield). (b) To a solution of imide **10** (500 mg) in a THF−methanol mixture (12 mL, THF:MeOH = 1:1) was added a solution of KOH (1.2 g) in water (6 mL), and the reaction mixture was refluxed for 2 h with stirring. The solvent mixture was removed in vacuo, and the residue was acidified with dilute HCl. Repetition of the above workup procedure followed by silica gel column chromatographic purification furnished pure **1**: 350 mg (98% yield); mp 60 °C (lit.[5] mp 59−60 °C); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.23 (bs, 24H), 1.55 (quintet, J = 8 Hz, 4H), 2.05 (s, 6H), 2.43 (t, J = 8 Hz, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 9.5, 24.4, 27.5, 29.2, 29.4, 29.6 (4 × CH_2), 140.4, 144.7, 165.8, 166.2; MS (*m*/*e*) 446, 428, 400, 373, 210, 196, 149, 126, 98, 55; IR (Nujol) ν_{max} 1855, 1767, 1670 cm^{-1}. Anal. Calcd for $C_{26}H_{38}O_6$: C, 69.95; H, 8.52. Found: C, 70.08; H, 8.28.

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Supporting Information Available: ^{1}H NMR spectra of **1**, **3**, **4**, **5**, **7** + **8** + **9** mixture, and **10** and ^{13}C NMR spectra of **1** and **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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