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TOTAL SYNTHESIS OF CITRAFUNGIN A†

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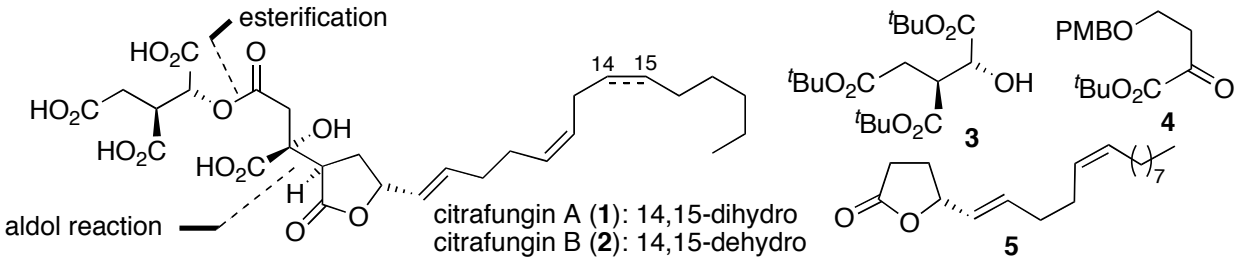
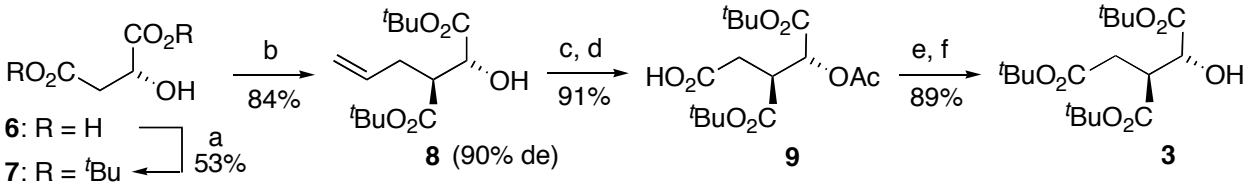
Abstract – Citrafungin A, a member of the alkyl citrate class of natural products, was synthesized for the first time employing a convergent approach involving aldol reaction of (*R*)-4-((1*E*,5*Z*)-tetradeca-1,5-dienyl)-γ-butyrolactone with *tert*-butyl 4-(*p*-methoxybenzyloxy)-2-oxobutanoate and installation of (2*R*,3*S*)-tri-*tert*-butyl isocitrate.

Citrafungins A and B were isolated from the fermentation broth of the fungal sterile mycelium (MF6339) by Singh *et al.*,[1] after screening for substances that exhibit inhibitory activity against geranylgeranyltransferase (GGTase I). It was found that these compounds inhibited GGTase I of various pathogenic fungal species with IC_{50} values of 2.5-15 μM and exhibited potent antifungal activities with MIC values of 0.40-55 μM. Citrafungins A and B have characteristic structures in which a citric acid moiety having the γ-lactone with a C-14 unsaturated long chain connects with isocitric acid. The absolute configurations were determined by chemical degradation and spectroscopic analysis. In connection with our recent synthesis of the alkyl citrate family of natural products,[2] the above-mentioned intriguing biological and structural features prompted us to investigate the synthesis of citrafungin A. We report herein the first total synthesis of citrafungin A via a convergent approach involving aldol reaction of lactone (**5**) with α-keto ester (**4**) and installation of isocitrate (**3**) by esterification.



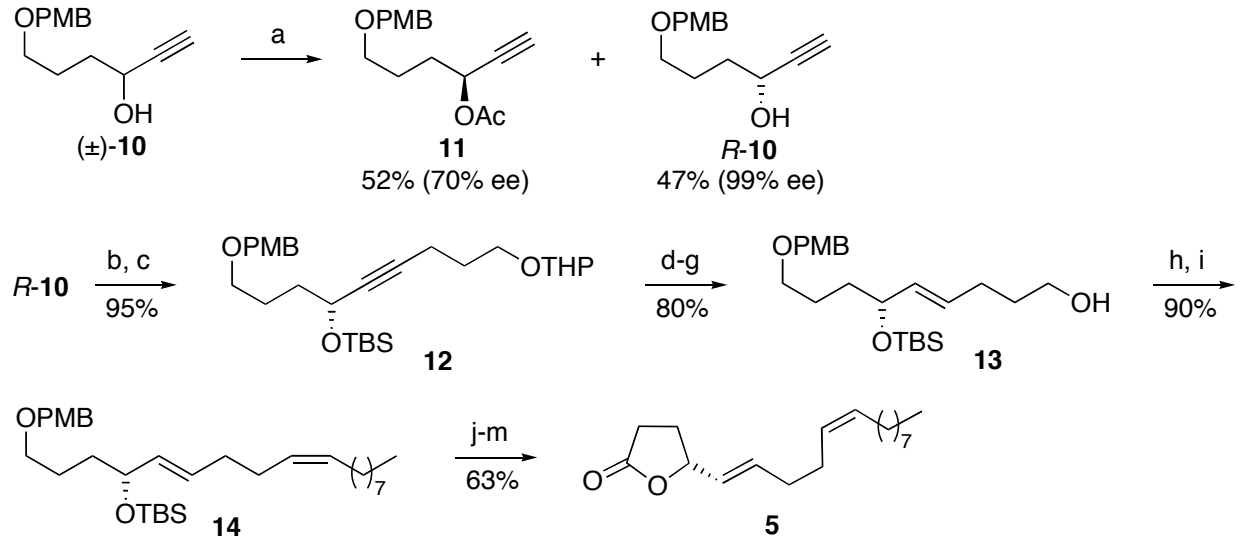
† We would like to dedicate this work to Professor Yoshito Kishi on the occasion of his 70th birthday.

(2*R*,3*S*)-Tri-*tert*-butyl isocitrate (**3**) was prepared from (*R*)-malic acid (**6**) as depicted in Scheme 1. Thus, after esterification of **6** to di-*tert*-butyl ester (**7**) using *N*,*N*'-diisopropyl-*O*-*tert*-butylisourea,[3] reaction of the lithium enolate derived from **7** with allyl bromide took place with high diastereoselectivity (90% de)[4] to give **8** in 84% yield. Upon acetylation and oxidative cleavage of the alkenic double bond with in situ generated RuO_4,[5] **8** afforded carboxylic acid (**9**) in 91% yield. Esterification of **9** followed by methanolysis allowed us to obtain **3**[6] in 89% yield.



Scheme 1. *Reagents and conditions*: (a) ^{i}PrNHC(O^{t}Bu)=N^{i}Pr, CH_2Cl_2; (b) LDA, THF, –78 °C, then allyl bromide; (c) Ac_2O, Et_3N, DMAP, CH_2Cl_2; (d) $RuCl_3 \cdot nH_2O$ (cat.), $NaIO_4$, MeCN-CCl_4-H_2O (5:5:8); (e) ^{i}PrNHC(O^{t}Bu)=N^{i}Pr, CH_2Cl_2; (f) K_2CO_3, MeOH.

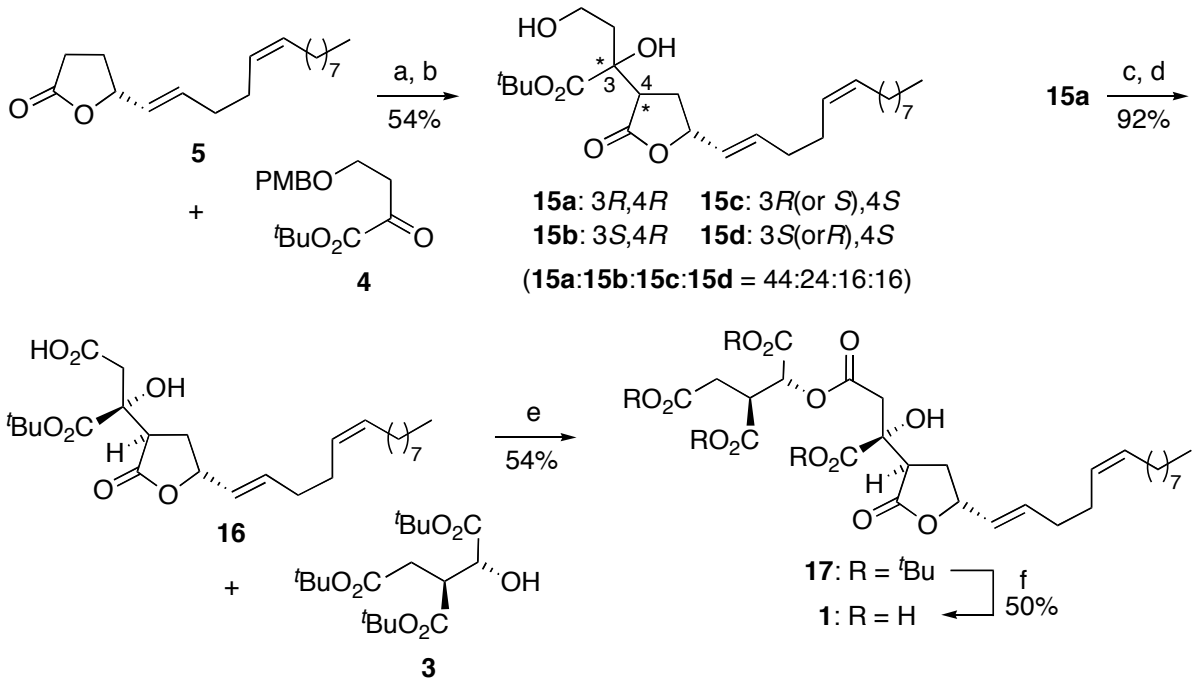
The preparation of lactone (**5**) began with kinetic resolution of racemic propargyl alcohol (**10**) by lipase-mediated acetylation (Scheme 2). According to the procedure[7] which we have previously established, (±)-**10** was reacted with vinyl acetate in the presence of Novozym 435 in di-*i*-propyl ether at room temperature to afford *S*-acetate (**11**) (70% ee) and *R*-**10** (99% ee) in 52% and 47% yields, respectively.



Scheme 2. *Reagents and conditions*: (a) Novozyme 435, CH_2=CHOAc, $^{i}Pr_2O$; (b) $^{t}BuMe_2SiCl$, imidazole, DMF; (c) ^{n}BuLi, $Br(CH_2)_3OTHP$, THF-HMPA, –20 °C to rt; (d) $^{n}Bu_4NF$, THF; (e) $NaH_2Al(MeOCH_2CH_2O)_2$ (Red-Al), THF, 50 °C; (f) $^{t}BuMe_2SiCl$, imidazole, DMF; (g) PPTS, ^{i}PrOH, 60 °C; (h) $(COCl)_2$, DMSO, CH_2Cl_2, –78 °C, then Et_3N, 0 °C; (i) $[Me(CH_2)_8]Ph_3P^+I^-$, ^{n}BuLi, THF, 0 °C; (j) DDQ, CH_2Cl_2-H_2O; (k) PCC, CH_2Cl_2; (l) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, ^{t}BuOH-H_2O (5:1); (m) 47% HF, MeCN-THF.

Protection of *R*-**10** as its *tert*-butyldimethylsilyl ether, followed by alkylation of the terminal acetylene with 2-(3-bromopropoxy)tetrahydro-2*H*-pyran using *n*-butyllithium gave alkyne (**12**) in 95% yield. By successive desilylation, Red-Al reduction, silylation and selective removal of the tetrahydropyranyl ether protecting group, **12** was converted to *E*-alkene (**13**) in 80% yield. After Swern oxidation of **13**, the resulting aldehyde was directly subjected to Wittig reaction using the ylide generated from (*n*-nonyl)triphenylphosphonium iodide and *n*-butyllithium to give *E*,*Z*-diene (**14**) selectively in 90% yield. Upon oxidative removal of *p*-methoxybenzyl ether protecting group, PCC oxidation, Pinnick oxidation[8] and treatment with aqueous HF, lactone (**5**)[9] was obtained in 63% yield.

With the required lactone (**5**) in hand, we then investigated its aldol reaction with α-keto ester (**4**), prepared from *tert*-butyl 4-(*p*-methoxybenzyloxy)butanoate by the method[2b] we have previously established (Scheme 3). Reaction of the lithium enolate generated from **5** with **4**, followed by silica gel column chromatography gave two fractions, each of which contained two aldol products. After removal of the *p*-methoxybenzyl ether protecting group, these aldol products became separable to give 3*R*,4*R*-isomer (**15a**), 3*S*,4*R*-isomer (**15b**), 3*R*(or *S*),4*S*-isomer (**15c**), and 3*S*(or *R*),4*S*-isomer (**15d**) in a ratio of 44:24:16:16 in 54% yield. The stereochemistry of the C4 position of each isomer was unambiguously determined by NOE experiments. As for **15a** and **15b**, the stereochemistry of the C3 quaternary center also became clear by the close similarity of the ^{1}H NMR spectrum of **15a** to that of citrafungin A as well



Scheme 3. *Reagents and conditions*: (a) LDA, HMPA-THF (1:4), –78 °C, then **4**; (b) DDQ, CH_2Cl_2-H_2O (10:1); (c) PCC, CH_2Cl_2; (d) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, ^{t}BuOH-H_2O (5:1); (e) **3**, DCC, DMAP·HCl, 4A molecular sieves, CH_2Cl_2; (f) TFA, CH_2Cl_2.

as the fact that **15a** was transformed into citrafungin A as described below. The C3 stereochemistry of **15c** and **15d** was not determined. Before we examined this aldol reaction, we had expected the higher stereoselectivity[10] at least at the C4 position arising from the bulkier γ-substituent on the lactone ring on the basis of the fact that the reaction of 4-(*tert*-butyldiphenylsilyloxy)methyl-γ-butyrolactone with **4** brought about excellent stereoselection at the C4 position (>90% de) in a model study. This result suggests the poorer shielding effect of a linear γ-substituent on diastereofacial selection of the enolate.

3*R*,4*R*-Isomer (**15a**) thus obtained was subjected to PCC oxidation followed by Pinnick oxidation to give carboxylic acid (**16**) in 92% yield. Esterification of **16** with **3** was achieved by Keck's method[11] using dicyclohexylcarbodiimide and 4-dimethylaminopyridine hydrochloride to afford ester (**17**)[12] in 54% yield. Finally, exposure of **17** to trifluoroacetic acid at room temperature furnished citrafungin A. The spectral data were identical with those reported for natural citrafungin A.[1]

In conclusion, we have achieved the first total synthesis of citrafungin A although the stereoselectivity of the key aldol reaction was disappointing. Efforts to improve the synthetic route are now underway.

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6. $[\alpha]^{27}_{D}$ +7.3 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 18H), 1.45 (s, 9H), 1.49 (s, 9H), 2.48 (dd, *J* = 6.3, 16.6 Hz, 1H), 2.73 (dd, J = 8.8, 16.6 Hz, 1H), 3.14 (s, 1H), 3.28 (ddd, *J* = 2 .4, 6.3, 8.8 Hz, 1H), 4.12 (d, *J* = 2.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.1, 33.9, 45.8, 70.9, 81.0,

81.7, 83.1, 170.2, 171.0, 172.3; FTIR (neat) 3493, 1731 cm^{-1}; HRMS (EI) calcd for $C_{18}H_{32}O_7$ [$(M+1)^+$] 361.2226, found 361.2218.

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9. $[\alpha]^{24}_D$ –17.1 (*c* 1.00, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 8.0 Hz, 3H), 1.26 (brs, 12H), 1.93-2.03 (m,3H), 2.13 (brs, 4H), 2.37 (m, 1H), 2.51-2.56 (m, 2H), 4.89 (q, *J* = 7.2 Hz, 1H), 5.33-5.43 (m, 2H), 5.52 (dd, *J* = 6.8, 14.8 Hz, 3H), 5.79-5.83 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.8, 26.6, 27.3, 28.7, 28.9, 29.4, 29.6, 29.7, 31.9, 32.2, 81.0, 127.7, 128.1, 130.8, 134.8, 163.8; FTIR (neat) 1776, 1172 cm^{-1}; HRMS(EI) calcd for $C_{18}H_{30}O_2$ (M^+) 278.2246, found 278.2234.
10. For diastereofacial selectivity of the lithium enolates derived from 4-alkoxymethyl-γ-butyrolactones, see: (a) S. Takano and K. Ogasawara, *J. Synth. Org. Chem. Jpn.*, 1982, **40**, 1037. (b) S. Takano, *Pure. Appl. Chem.*, 1987, **59**, 353.
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12. $[\alpha]^{24}_D$ +2.0 (*c* 1.00, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.26 (s, 12H), 1.46 (m, 36 H), 1.91-2.02 (m, 2H), 2.11 (bs, 3H), 2.34 (m, 1H), 2.44 (dd, *J* = 4.4 Hz, 16.6 Hz, 1H), 2.62 (dd, *J* = 9.8 Hz, 16.6 Hz, 1H), 2.83 (q, *J* = 4.9 Hz, J = 10.2 Hz, 1H), 3.04 (d, *J* = 17.6 Hz, 1H), 3.30 (td, *J* = 3.9, 5.9 Hz, 1H), 3.72 (d, *J* = 17.6 Hz, 1H), 4.06 (s,1H), 4.99 (q, *J* = 7.3 Hz, 1H), 5.17 (d, *J* = 2.9 Hz, 1H), 5.31-5.46 (m, 3H), 5.77-5.80 (m,1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 26.5, 27.3, 27.7, 27.8, 28.0, 29.2, 29.3, 29.3, 29.4, 29.5, 29.5, 29.6, 30.5, 31.9, 32.2, 32.5, 33.0, 40.4, 43.6, 46.5, 72.3, 75.2, 77.2, 79.8, 80.9, 81.9, 83.0, 83.9, 128.0, 128.2, 130.9, 135.1, 166.4, 168.9, 169.6, 170.4, 171.9, 174.9; FTIR (neat) 3483, 1776, 1737, 1154 cm^{-1}; HRMS (EI) calcd for $C_{44}H_{72}O_{13}$ (M^+) 808.4973 found 808.4917.