Enzymatic Preparation of an Optically Active Precursor of the CC-1065/Duocarmycin Pharmacophore

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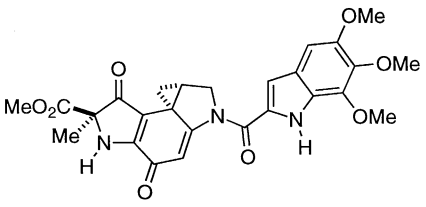
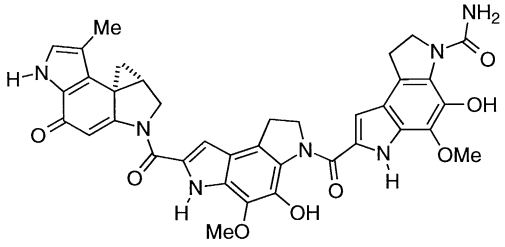
Acetylation of 2-[4-(benzyloxy)-2-nitrophenyl]propane-1,3-diol with vinyl acetate in the presence of porcine pancreatic lipase gave the (R)-mono-acetate (ee = 92%). The (S)-mono-acetate was obtained via acetylation of the diol followed by transesterification in ethanol in the presence of the same enzyme. Incorporation of these optically active mono-acetates into the established synthetic routes provided access to both enantiomers of the common pharmacophore of CC-1065/duocarmycin.

Duocarmycin A (**1**) and CC-1065 (**2**) are exceptionally potent antitumor antibiotics isolated in trace quantities from *Streptomyces* strains.[1] These natural products contain a common pharmacophore, a spirocyclic 1,2,7,7a-tetrahydrocycloprop[1,2-c]indol-4-one subunit. These compounds exert their biological effects by a common or related mechanism. They have been found to bind to double stranded β-DNA within the minor groove with a sequence preference and to N-alkylate purines by the electrophilic activated cyclopropane ring.[2] Despite its remarkable antineoplastic effects, CC-1065 has not been used in clinical trials because it was found that it causes delayed lethality in experimental animals. Subsequent investigation revealed that this delayed toxicity can be removed by changing the side chain attached to the pharmacophore. The synthesis of these natural products and structural analogues as well as their mechanism of action have been extensively investigated in recent years.[2,3] Enantiomers of these compounds usually showed different biological activities. For instance, natural (+)-duocarmycin A was found to be about two orders of magnitude more active against P388 murine leukemia cells than the non-natural enantiomer.[4]



Duocarmycin A (**1**)

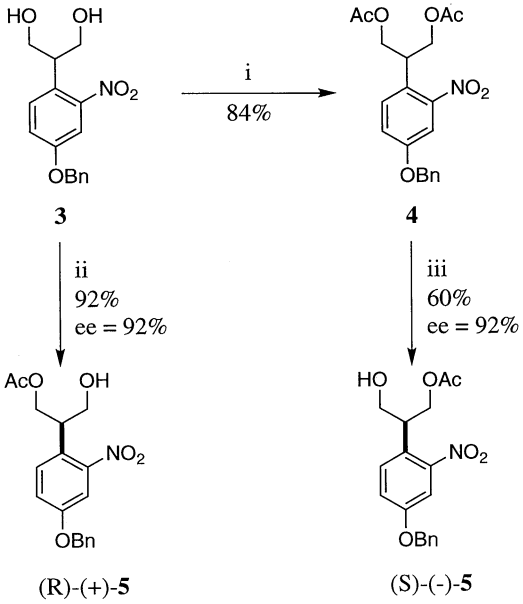


CC-1065 (**2**)

The first syntheses of optically active compounds related to these natural products involved the classical resolution of racemic intermediates by the physical separation (HPLC, crystallization) of diastereomeric derivatives. Recently, enantioselective syntheses of these compounds have been reported.[5] Herein we report the enzymatic desymmetrization of 2-[4-(benzyloxy)-2-nitrophenyl]-propane-1,3-diol, an intermediate in the synthesis of the common pharmacophore of CC-1065/duocarmycin.

Diol **3** was synthesized from commercially available 4-chloro-3-nitrophenol in 3 steps according to the procedure reported by Warpehoski *et al.*[6] Both enantiomers of mono-acetate **5** were prepared via enzymatic reactions that utilize PPL (porcine pancreatic lipase) as catalyst (scheme 1). Acetylation of diol **3** with vinyl acetate in the presence of PPL gave mono-acetate (R)-(+)-**5** in high chemical (92%) and enantiomeric (92%) yields.[7] The other enantiomer, (S)-(−)-**5** was obtained via acetylation of **3** and then transesterification of diester **4** in diisopropyl ether-ethanol in the presence of PPL (yield 60%, 92% ee).[8] The enantiomeric purities were measured by ^{19}F NMR analysis of the corresponding (+)-α-methoxy-α-(trifluoromethyl)-α-phenyl-acetates (MTPA, Mosher's esters). The absolute configuration of **5** could be predicted at this stage using active site models.[9] These configurations were confirmed by conversion of **5** to the title compound **11** of known absolute configuration (*vide infra*).[10]

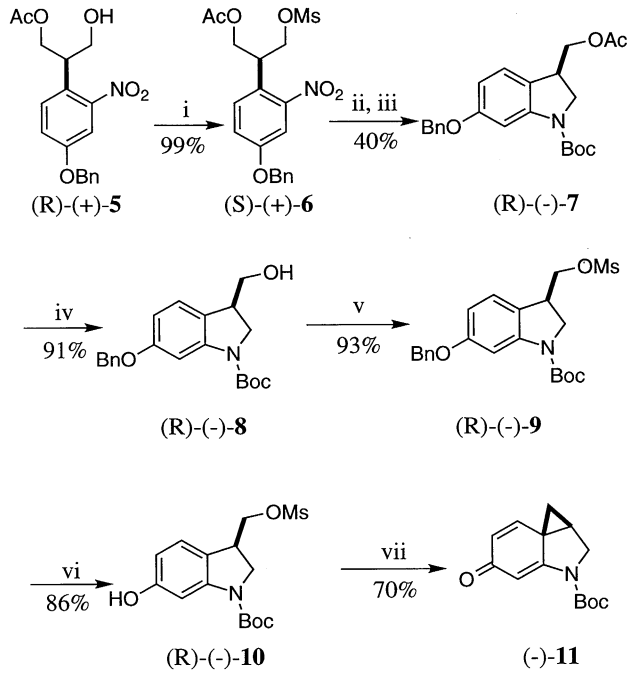
Scheme 1.



i) Ac_2O, pyridine; ii) PPL, vinyl acetate; iii) PPL, EtOH, isopropyl ether.

Mesylation of (R)-**5** provided mesylate (S)-**6** (Scheme 2). Catalytic hydrogenation of the nitro group in the presence of triethylamine also effected cyclization to give the indoline nucleus, and the nucleophilic nitrogen was protected *in situ* by a tert-butoxycarbonyl group. Hydrolysis of acetate (R)-**7** in basic ethanol yielded alcohol (R)-**8**. The enantiomeric purity of **8** was further checked by ^{19}F NMR analysis of the MTPA derivative (ee = 92%). This procedure proved that there is complete retention of configuration in the previous steps. Activation of the primary alcohol through methane sulfonate formation ((R)-**9**) and subsequent deprotection of the benzyl ether by catalytic hydrogenation gave (R)-**10**. $[\alpha]_D^{25}$ – 47.3 (c 1.83, CH_2Cl_2); lit.[10] – 48.9 (c 0.19, CH_2Cl_2). Treatment of **10** with sodium hydride in THF promoted spirocyclization to yield (R,R)-(–)-**11**.[10,11]

Scheme 2.



i) MsCl, Et_3N, CH_2Cl_2; ii) H_2 (3 atm), PtO_2, THF, Et_3N; iii) $(Boc)_2O$, CH_2Cl_2; iv) K_2CO_3, EtOH; v) MsCl, Et_3N, CH_2Cl_2; vi) H_2 (1 atm), Pd/C, THF; vii) NaH, THF.

The same reaction sequence can be used to transform (S)-**5** to the corresponding (+)-**11**.[10] Using this methodology, both enantiomers of the product **11** are available from the same intermediate **3** and by the use of a single enzyme. Also, Terashima *et al.*[3f] reported the synthesis of duocarmycin from compound **7**.

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References and Notes

1 a) I. Takahashi, K. Takahashi, M. Ichimura, M. Morimoto, K. Asano, I. Kawamoto, F. Tomita, and H. Nakano, *J. Antibiot.*, **41**, 1915 (1988). b) L.J. Hanka, A. Dietz, S.A. Gerpheide, S.L. Kuentzel, and D.G. Martin, *J. Antibiot.*, **31**, 1211 (1978). c) T. Yasuzawa, K. Muroi, M. Ichimura, I. Takahashi, T. Ogawa, K. Takahashi, H. Sano, and Y. Saitoh, *Chem. Pharm. Bull.*, **43**, 378 (1995).

2 D.L. Boger, *Acc. Chem. Res.*, **28**, 20 (1995).

3 a) L.F. Tietze and T. Grote, *J. Org. Chem.*, **59**, 192 (1994). b) D.L. Boger and P. Mesini, *J. Am. Chem. Soc.*, **116**, 11335 (1994). c) H. Muratake, I. Abe, and M. Natsume, *Tetrahedron Lett.*, **35**, 2573 (1994). d) Y. Wang and J.W. Lown, *Heterocycles*, **36**, 1399 (1993). e) J.H. Tidwell and S.L. Buchwald, *J. Org. Chem.*, **57**, 6380 (1992). f) Y. Fukuda, K. Nakatani, Y. Ito, and S. Terashima, *Tetrahedron Lett.*, **31**, 6699 (1990).

4 Y. Fukuda, K. Nakatani, and S. Terashima, *Tetrahedron*, **50**, 2809 (1994).

5 a) D.L. Boger, J.A. McKie, T. Nishi, and T. Ogiku, *J. Am. Chem. Soc.*, **118**, 2301 (1996). b) L. Ling and J. W. Lown, *J. Chem. Soc., Chem. Commun.*, **1996**, 1559.

6 M.A. Warpehoski, I. Gebhard, R.C. Kelly, W.C. Krueger, L.H. Li, J.P. McGovren, M.D. Prairie, N. Wicnienski, and W. Wierenga, *J. Med. Chem.*, **31**, 590 (1988).

7 Enzyme-catalyzed acylation of diol 3. To a stirred solution of diol **3** (525.5 mg, 1.733 mmol) in vinyl acetate (9 mL) were added powdered molecular sieves (300 mg) and PPL supported on Celite (4000 units) and the reaction was monitored by TLC (~ 3h). The mixture was filtered, the solvent was evaporated and the crude product was purified by flash chromatography on silica gel (ethyl acetate/hexane, 3/3, as eluent) to give (2R)-3-acetoxy-2-[4-(benzyloxy)-2-nitrophenyl]propane-1-ol (**5**) as an oil (551 mg, 92%). $[\alpha]_D^{25}$ + 30.5 (c 1.95, CH_2Cl_2).

8 Enzyme-catalyzed transesterification of diacetate 4. Diacetate **4** (200 mg, 0,516 mmol) and ethanol (0.6 mL, 10.2 mmol) were dissolved in diisopropyl ether (50 mL), PPL on Celite (4000 units) was added and the mixture was stirred at room temperature. The reaction was monitored by TLC (4 days). The mixture was filtered, the solvent was evaporated and the crude product was purified by flash chromatography (ethyl acetate/hexane), 2/3 as eluent) to give (S)-**5** (110 mg, 61%). $[\alpha]_D^{25}$ – 27.3 (c 5.05, CH_2Cl_2).

9 P.G. Hultin and J.B. Jones. *Tetrahedron Lett.*, **33**, 1399 (1992).

10 D.L. Boger, R.J. Wysocki, and T. Ishizaki, *J. Am. Chem. Soc.*, **112**, 5230 (1990).

11 The spectral and physical data agreed with those reported. The specific rotation of (R,R)-(–)-**11** was $[\alpha]_D^{25}$ – 138 (c 0.52, CH_2Cl_2); the previous report did not indicate the $[\alpha]_D$.