Facile Synthesis of (+)-Brefeldin A Utilizing Two Optically Active Synthons Prepared by Different Enzyme-catalysed Reactions

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The lactone **4a** and the alcohol **6** (both available in optically active form from biocatalytic processes) have been used as synthons in the preparation of (+)-brefeldin A.

Brefeldin A **1** was first isolated in 1958 from *Penicillium decumbens* and was subsequently found as a secondary metabolite in other cultures.[1] The structure and stereochemistry was confirmed by X-ray crystallography in 1971.[2] Several partial, formal and total syntheses of brefeldin A have been reported[3,4] while biological testing has shown that the compound exhibits a wide range of biological activities including antibiotic, antiviral, cytostatic and antimitotic effects.[5]

We envisaged that this natural product could be synthesised, in single-enantiomer form, from the *exo*-hydroxylactone **4a**, a compound which we have been able to obtain in an optically pure state by a simple biotransformation.[6] Fig. 1 shows our retrosynthetic approach. Disconnection of the lactone group and the vinyl side chain of brefeldin A gives a cyclopentenone **2**, bearing a four carbon side chain, that could ostensibly be prepared from the *exo*-hydroxylactone **4a**. The partner in the coupling reaction *i.e.* organometallic reagent **3** is derived from the (*S*)-hept-6-yn-2-ol **6**.

Addition of glyoxylic acid to cyclopentadiene in water produces a mixture of *exo*-**4a** and *endo*-hydroxy bicyclic lactone **5** in a ratio of 1 : 4.[6] We have previously reported the enzymatic resolution of both these hydroxylactones by enzyme action at their hydroxyl functions using *Pseudomonas fluorescens* or *Candida cylindracea* lipase, and showed that the *endo*-hydroxylactone **5** and its enantiomer are convenient synthons for the preparation of intermediates for hypocholestemic agents and the anti-HIV agent (−)-carbovir respectively.[6]

For the synthesis of brefeldin A the minor isomer from the above preparation (*i.e.* the *exo*-hydroxylactone **4a**) is the requisite starting material. In order to have suitable amounts of this synthon, the *endo*-isomer **5** was epimerized in two steps (Scheme 1). Thus Mitsunobu inversion of the *endo*-isomer **5** (available as a single enantiomer in kilogram quantities) using chloroacetic acid as the nucleophile provided the ester **4b**, which was treated with thiourea and sodium bicarbonate in refluxing ethanol[7] (in order to chemoselectively hydrolyse the chloroacetyl group) to yield the *exo*-hydroxylactone **4a** in 86% overall yield.

The protected hydroxylactones **4c** and **4d** were prepared by

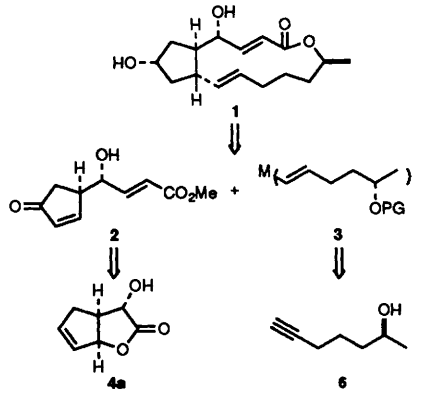
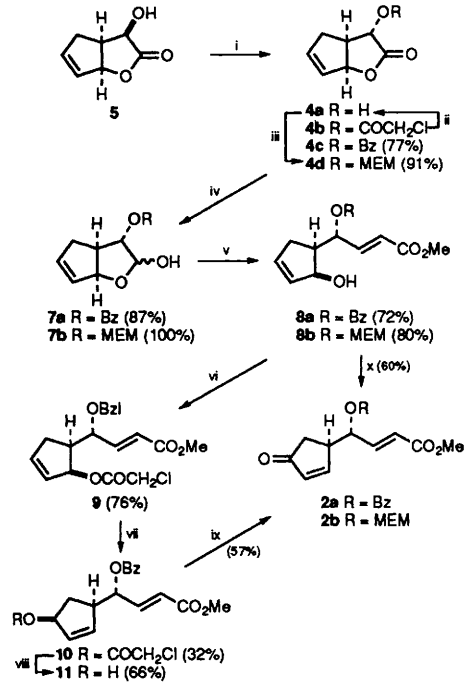


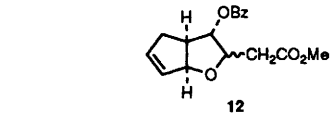
Fig. 1

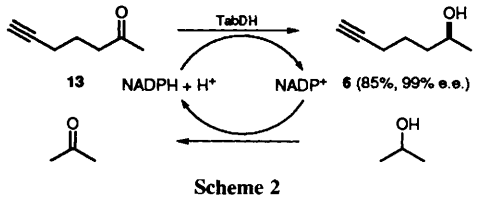


Scheme 1 *Reagents and conditions*: i, Ph_3P, DEAD, THF, benzoic acid (for **4c**) or chloroacetic acid (for **4b**); ii, H_2NCSNH_2, $NaHCO_3$, EtOH, heat [86% from **5**]; iii, MEM-Cl, DIPEA, CH_2Cl_2; iv Diisobutylaluminium hydride (Dibal-H), THF, −78 °C; v, methyl (triphenyl phosphoranylidene) acetate, toluene; vi, chloroacetic acid anhydride, pyridine; vii, $PdCl_2[MeCN]_2$, 1,4-benzoquinone; viii, thiourea, $NaHCO_3$, EtOH; ix, PCC, CH_2Cl_2; x, PCC, *p*-TsOH, CH_2Cl_2 (60%)

Table 1 Enzymatic acylation of **6** (using vinyl acetate as solvent and acylating agent)

Lipase	*t*/h	Conversion (%, ±5)	% e.e. Acetate	% e.e. **6**
PS	72	50	83	80
P30	41	54	90	90
AK	25	50	93	83
AY30	50	49	0	0





Scheme 2

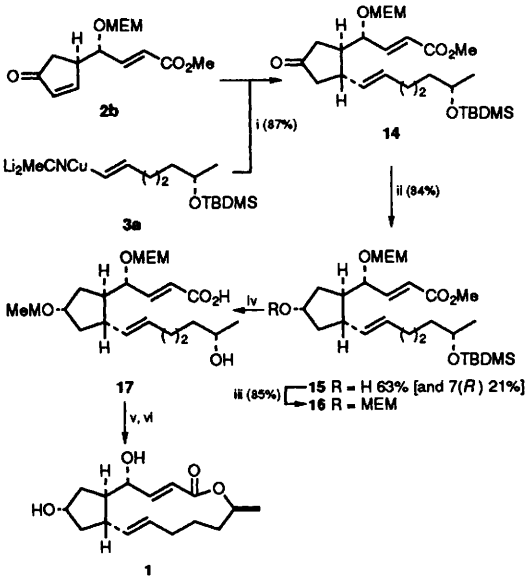
standard methods, and reduced to the corresponding lactols **7a** and **b** using diisobutylaluminium hydride[8] (Scheme 1). Addition of the Wittig reagent Ph_3PCHCO_2Me to these lactols gave the alcohols **8a** and **b**. As expected, on prolonged reaction an intramolecular conjugate addition of the newly formed hydroxy group to the unsaturated ester took place, forming a bicyclic ether (*e.g.* **12**).

The cyclopentenol **8a** was acylated to provide the allylic chloroacetate **9**. A (3,3)-sigmatropic rearrangement to a less hindered alcohol was promoted by catalytic amounts of Pd^{II}.[9] Selective hydrolysis of the chloroacetate with thiourea[7] and oxidation with PCC gave the desired intermediate **2a**. Notably, the rearrangement and oxidation of cyclopentenols **8a** and **b** to the corresponding cyclopentanones **2a** and **b** was also achieved in one-pot using Baekstrom's conditions[10] in 60% yield.

The lower side chain of brefeldin A was prepared by a second enzyme catalysed process. Thus hept-6-yn-2-one **13** was enantioselectively reduced using the alcohol dehydrogenase from *Thermoanaerobium brockii* (TabDH) in high optical purity (99% e.e.) and excellent yield in a process that is much cleaner and efficient than the prescribed biotransformation using bakers' yeast (Scheme 2).[11] The resolution of (±)-hept-6-yn-2-ol using lipases was also not as effective as the dehydrogenase-catalysed reaction for the production of optically active material (Table 1). The alcohol **6** was protected as the *tert*-butyldimethylsilyl ether before being converted into the cuprate reagent **3a**, which was contaminated with 15–20% of the isomeric (*Z*)-alkene.

Conjugate addition[12] of cuprate **3a** to the cyclopentenone **2a** gave complex mixtures. However addition of **3a** to the compound **2b** occurred smoothly at the more reactive cyclopentenone unit from the unhindered *alpha* face (Scheme 3).

The disubstituted cyclopentanone **14** so formed was reduced with some selectivity using K. Selectride, and the major product **15** was purified by chromatography before being further protected with a second MEM group. This diprotected diol **16** was identical to Taber's intermediate,[4] *e.g.*



Scheme 3 *Reagents and conditions*: i, Compound **3a**, THF, −78 °C; ii, K. Selectride, THF, −78 °C; iii, MEM-Cl, DIPEA, CH_2Cl_2; iv, HCl (1 mol dm^{-3}) then LiOH; v, 2,4,6-trichlorobenzoyl chloride, THF then DMAP, toluene, heat (80%); vi, $TiCl_4$, CH_2Cl_2, 0 °C (80%)

$[\alpha]_D$ = −34 ° (*c* 1.0, $CHCl_3$), lit.[4] $[\alpha]_D$ = −27.7 ° (*c* 1.44, $CHCl_3$), ^{13}C NMR: δ($CDCl_3$) −4.71, −4.42, 18.08, 23.73, 25.58, 25.87, 32.43, 32.89, 39.21, 40.21, 42.98, 48.30, 51.40, 58.88, 58.93, 66.83, 67.52, 68.44, 71.71, 71.81, 75.73, 76.81, 94.19, 94.34, 121.29, 131.05, 133.30, 148.14, 166.50.

Removal of the silyl protecting group and hydrolysis of the methyl ester gave the corresponding hydroxy acid **17**. $[\alpha]_D$ = −9.33 ° (*c* 0.4, $CHCl_3$), ^1H NMR: δ($CDCl_3$) 6.86 (dd, *J* 15.8, 6.3 Hz, 1H), 5.95 (dd, *J* 15.8, 1.2 Hz, 1H), 5.33 (m, 2H), 4.68 (m, 4H), 4.15 (m, 2H), 3.78 (m, 2H), 3.66 (m, 4H), 3.55 (m, 5H), 3.38 (s, 3H), 3.37 (s, 3H), 2.31 (m, 1H), 2.15 (m, 1H), 2.0–1.2 (m, 10H), 1.18 (d, *J* 6 Hz, 3H). Lactone formation and removal of the MEM protecting groups yields brefeldin A **1**.[4] This new synthesis of (+)-brefeldin A is comprised of only 11 steps starting from the readily available bicyclic lactone **4a**.

We thank the SERC, DTI and Chiroscience for support under the Biotransformations LINK Scheme (Fellowships to G. G. and H. F. O.) and Wiley for a post-doctoral Fellowship (to G. C.). The skilled assistance of Rosemary MacKeith and Claire Morgan-Smith is greatly appreciated.

Received, 31st January 1994; Com. 4/00595C

References

1 V. L. Singleton and N. Bohonos, *Nature*, 1958, **181**, 1072.
2 H. P. Weber, D. Hauser and H. P. Sigg, *Helv. Chim. Acta*, 1971, **54**, 2763.
3 Partial Synthesis: *e.g.* T. Livinghouse and R. V. Stevens, *J. Chem. Soc., Chem. Commun.*, 1978, 754; Formal Synthesis: *e.g.* K. Ueno, H. Suemune, S. Saeki and K. Sakai, *Chem. Pharm. Bull.*, 1985, **33**, 4021; total Synthesis: *e.g.* E. J. Corey and R. H. Wollenberg, *Tetrahedron Lett.*, 1976, **51**, 4705; E. J. Corey, R. H. Wollenberg and D. R. Williams, *Tetrahedron Lett.*, 1977, **26**, 2243.
4 D. F. Taber, L. J. Silverberg and E. D. Robinson, *J. Am. Chem. Soc.*, 1991, **113**, 6639.
5 For example, V. Betina, L. Drobnica, P. Nemec and M. J. Zenanova, *J. Antibiot. Ser. A*, 1964, **17**, 93; V. Betina, M. Betinova and M. Kutkova, *Arch. Mikrobiol.*, 1966, **55**, 1; G. Tamura, K. Ando, S. Susuki, A. Takatsuki and K. Arina, *J. Antibiot.*, 1968, **21**, 160.
6 R. A. MacKeith, R. McCague, H. F. Olivo, C. F. Palmer and S. M. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 1993, 313; R. McCague, H. F. Olivo and S. M. Roberts, *Tetrahedron Lett.*, 1993, **34**, 3785; R. A. MacKeith, R. McCague, H. F. Olivo, S. M. Roberts, S. J. C. Taylor and H. Xiong, *Bioorg. Med. Chem.*, 1994 in the press.
7 M. Naruto, K. Ohno, N. Naruse and H. Takeuchi, *Tetrahedron Lett.*, 1979, **3**, 251.
8 L. Van Hijte, R. D. Little, J. L. Petersen and K. D. Moeller, *J. Org. Chem.*, 1987, **52**, 4647.
9 L. E. Overman, *Angew. Chem., Int. Ed. Engl.*, 1984, **23**, 579; P. A. Grieco, T. Takigawa, S. L. Bongers and H. Tanaka, *J. Am. Chem. Soc.*, 1980, **102**, 7587; S. J. Danishefsky, M. Paz-Cabal and K. Chow, *J. Am. Chem. Soc.*, 1989, **111**, 3456.
10 P. Baekstrom, S. Okecha, N. De Silva, D. Wijekoon and T. Norin, *Acta Chem. Scand. B*, 1982, **36**, 31.
11 G. Casy and S. M. Roberts, in *Preparative Biotransformations*, ed. S. M. Roberts, Wiley, 1993, and references therein.
12 O. W. Gooding, *J. Org. Chem.*, 1990, **55**, 4209; O. W. Gooding, C. C. Beard, G. F. Cooper and D. Y. Jackson, *J. Org. Chem.*, 1993, **58**, 4209; J. R. Behling, K. A. Babiak, J. S. Ng, A. L. Campbell, R. Moretti, M. Koerner and B. H. Lipshutz, *J. Am. Chem. Soc.*, 1988, **110**, 2641.