A Short Preparation of an Advanced Intermediate for Lactacystin Synthesis: The Complete Carbon Skeleton of Clasto-Lactacystin Dihydroxyacid

Philip C. Bulman Page,* David C. Leach, Colin M. Hayman, A. Sazali Hamzah,[1] Steven M. Allin, Vickie McKee

Department of Chemistry, Loughborough University, Loughborough, Leicestershire LE11 3TU, UK
Fax +44(1509)223926; E-mail: p.c.b.page@lboro.ac.uk

Received 26 March 2003

Abstract: An advanced intermediate **22** for lactacystin synthesis, containing the full carbon skeleton of the pyrrolidinone component, has been achieved in four steps from a protected glycine ester.

Key words: lactacystin, clasto-lactacystin, Dieckmann, acylation

(+)-Lactacystin (**1**) is a microbial metabolite isolated by Omura from the culture broth of a *Streptomyces* species.[2] It inhibits cell growth and induces neurite outgrowth in the murine neuroblastoma cell line (Neuro 2a), and this property has prompted study of the utility of the compound in treating neurologically-related diseases, including Alzheimer's.[3] (+)-Lactacystin is a uniquely selective and powerful inhibitor of the 20S proteasome,[4] which is part of a 28-protein complex that is involved in controlling cell growth and metabolism, and which is responsible for the normal turnover of cellular proteins and the removal of damaged proteins.[5] As a result, lactacystin is in demand in many laboratories; a number of synthetic approaches to the compound have been reported,[6,7] and the synthetic work has been reviewed.[8] The β-lactone **2**, known as clasto-lactacystin β-lactone (omuralide), into which lactacystin is converted in vivo prior to hydrolysis to clasto-lactacystin dihydroxyacid, is the active molecule, and also inhibits the 20S proteasome (Figure 1).

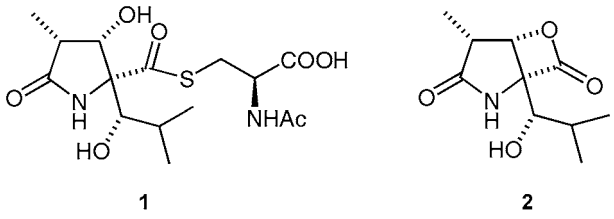
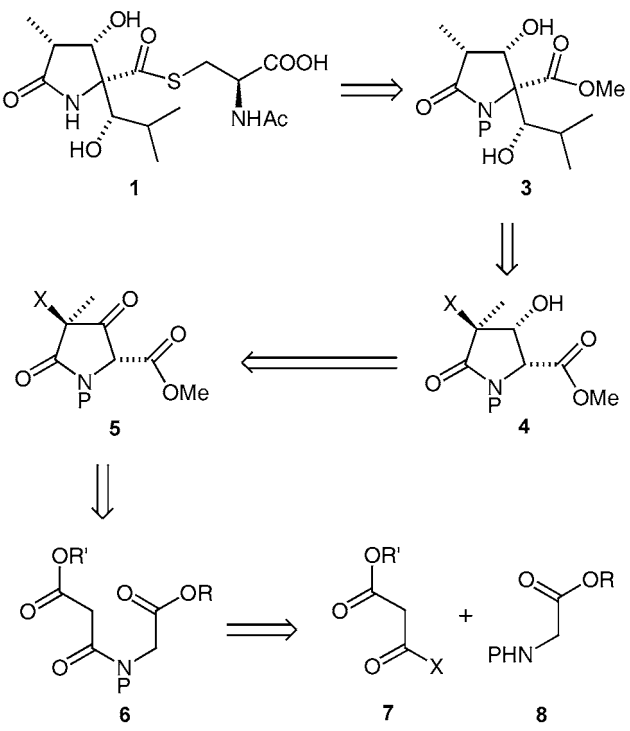


Figure 1

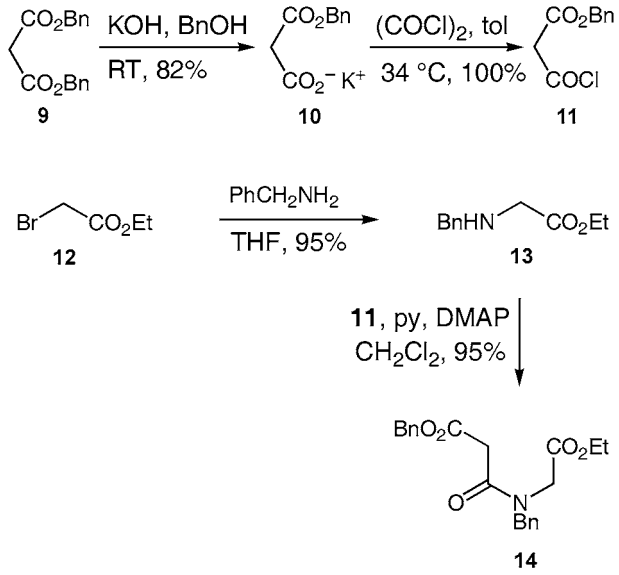
Our approach to the synthesis of lactacystin involves a new route to the advanced intermediate **3**,[9] from which the natural product can be obtained in two steps by a documented procedure.[6] We first envisaged an aldol condensation of **4** to form the C-5 quaternary centre of **3**; reduction of the β-keto ester **5** would provide the β-hydroxyester **4**. The pyrrolidinone ring would be formed by a Dieckmann cyclization of the diester **6**, which would in turn be prepared by condensation of a protected glycine ester **7** with activated malonate **8**. We anticipated that a blocking group X would be required at position C-3 of **5** to avoid problems during the subsequent aldol or other enolate reactions, and an ester moiety was chosen for this purpose as this would aid pyrrolidinone formation and would be potentially removable by subsequent decarboxylation (Scheme 1).



Scheme 1

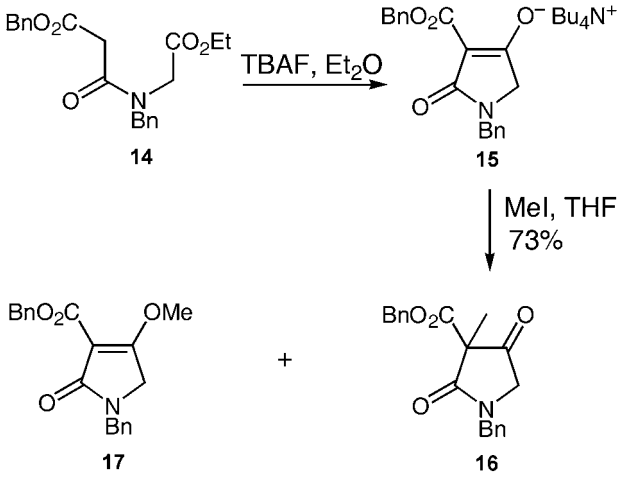
After some experimentation, benzyl groups were chosen for the protecting groups of **7** and **8**. Benzyl malonyl chloride (**11**) was generated by careful partial hydrolysis of commercial dibenzyl malonate (**9**) followed by treatment with oxalyl chloride. *N*-Benzyl glycine ethyl ester (**13**) is commercial but expensive, but can be easily prepared from a condensation of ethyl bromoacetate (**12**) and benzylamine.[10] The precursor **14** to the Dieckmann cyclization (Scheme 2) was prepared by condensation of benzyl malonyl chloride **11** with *N*-benzyl glycine ethyl ester (**13**) in 95% yield.

Synlett 2003, No. 7, Print: 02 06 2003.
Art Id.1437-2096,E;2003,0,07,1025,1027,ftx,en;D07003ST.pdf.



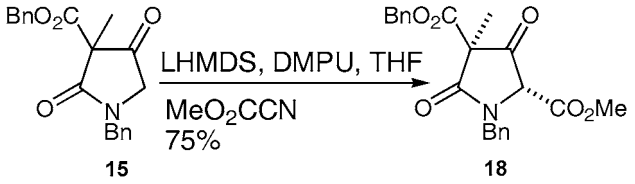
Scheme 2

The Dieckmann cyclization proved problematic under standard conditions, but was found to be successful using TBAF, producing the tetrabutylammonium salt **15**. This compound can be treated in situ with methyl iodide to produce **16** in 73% yield from **14**, together with the regioisomer **17** as an easily separable 3.5:1 mixture (Scheme 3). It is pleasing to note that this is the first stage where purification by column chromatography is required.



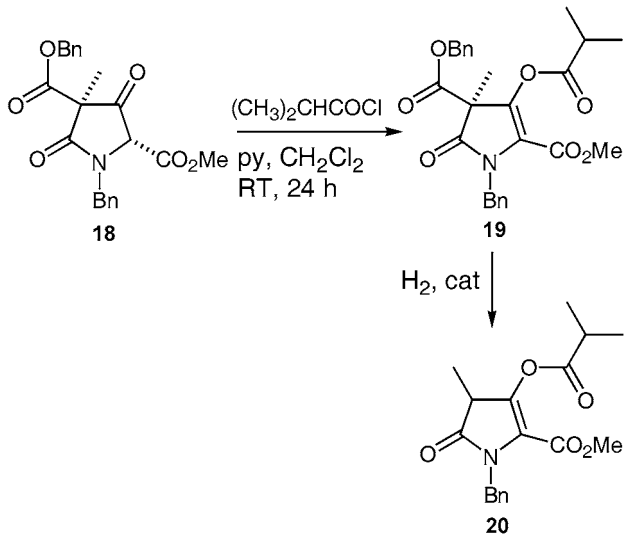
Scheme 3

Addition of the C-5 ester functionality was achieved using Mander's protocol: generation of the enolate from **16** using LHMDS in THF in the presence of DMPU was followed by addition of methyl cyanoformate to give **18** in 70% yield (Scheme 4).[11] This short sequence provides the pyrrolidinone ring, the C-5 ester unit, and the C-3 methyl group along with a blocking group at the same position in the form of a benzyl ester labile towards hydrogenolysis and decarboxylation.



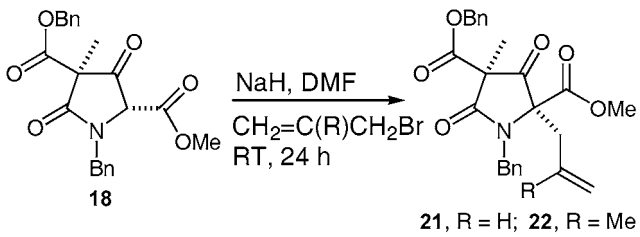
Scheme 4

We were unable to achieve the envisaged aldol condensation with **18** to form the C-5 quaternary centre. Using isobutyraldehyde under various conditions including Mukaiyama's protocol failed in our hands.[12] Having successfully C-alkylated **16** with methyl cyanoformate, we attempted acylation with isobutyryl cyanide,[13] but no reaction was observed. Attempted acylations using Katritzky's benzotriazole procedure also gave no reaction.[14] Acylation of **18** with isobutyryl chloride, however, gave rise exclusively to **19** through O-acylation, in 68% yield. Attempted hydrogenolysis and decarboxylation of the benzyl ester of **19** over palladium with concomitant hydrogenation of the enol ether double bond gave only **20**, in which the enol ether is still intact but the benzyl ester had been removed, in 98% yield (Scheme 5).



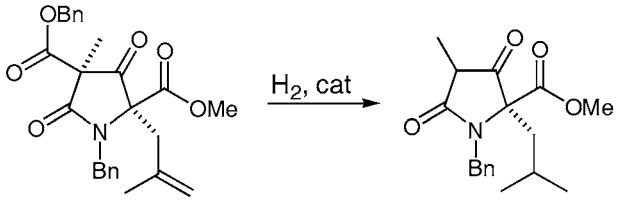
Scheme 5

O-Acylation is therefore feasible. Similar introduction of an allyl substituent could give either O-alkylation or C-alkylated compound **21**.



Scheme 6

Testing the reaction by deprotonation with sodium hydride in DMF at room temperature followed by addition of allyl bromide gave exclusively C-allylated compound **21** in 44% yield. To our delight, repeating the reaction with methallyl bromide gave the analogous compound **22** in 75% yield (Scheme 6),[15] the structure of which was confirmed by single crystal X-ray diffraction.[16] A compound containing all of the carbon atoms present in the carbon skeleton of clasto-lactacystin dihydroxyacid, suitably functionalized for further elaboration, has thus been constructed in only four steps from glycine ester. We have further shown that hydrogenolysis of **22** gives rise as expected to decarboxylation in situ, with concomitant reduction of the double bond, to give **23** in 90% yield (Scheme 7).



22 **23**

Scheme 7

Acknowledgment

This investigation has enjoyed the support of the EPSRC and Charnwood Molecular Ltd.

References

(1) Department of Chemistry, MARA University of Technology, 40450 Shah Alam, Selangor, Malaysia.

(2) Omura, S.; Fujimoto, T.; Otogurro, K.; Matsuzaki, K.; Moriguchi, R.; Tanaka, H.; Sasaki, Y. *J. Antibiot.* **1991**, *44*, 113.

(3) (a) Hefti, F.; Weinen, W. J. *Ann. Neurol.* **1986**, *20*, 275. (b) Dohty, P.; Dickson, J. G.; Flanigan, T. P.; Walsh, F. S. *J. Neurochem.* **1985**, *44*, 1259.

(4) (a) Bogyo, M.; Wang, E. W. *Curr. Top. Microbiol. Immunol.* **2002**, *268*, 185. (b) Lee, D. H.; Goldberg, A. L. *Trends Cell Biol.* **1998**, *8*, 397.

(5) (a) Fenteany, G.; Schreiber, S. L. *J. Biol. Chem.* **1998**, *273*, 8545. (b) Fenteany, G.; Standaert, R. F.; Lane, W. S.; Choi, S.; Corey, E. J.; Schreiber, S. L. *Science* **1995**, *268*, 726. (c) Craiu, A.; Gaczynska, M.; Akopian, T.; Gramm, C. F.; Fenteany, G.; Goldberg, A. L.; Rock, K. L. *J. Biol. Chem.* **1997**, *272*, 13437. (d) Fenteany, G.; Standaert, R. F.; Reichard, G. A.; Corey, E. J.; Schreiber, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3358.

(6) (a) Corey, E. J.; Li, W.-D. Z.; Nagamitsu, T.; Fenteany, G. *Tetrahedron* **1999**, *55*, 3305. (b) Corey, E. J.; Reichard, G. A.; Li, W.-D. Z. *J. Am. Chem. Soc.* **1998**, *120*, 2330. (c) Corey, E. J.; Li, W.-D. Z.; Nagamitsu, T. *Angew. Chem. Int. Ed.* **1998**, *37*, 1676. (d) Corey, E. J.; Li, W.-D. Z. *Tetrahedron Lett.* **1998**, *39*, 7475. (e) Corey, E. J.; Reichard, G. A. *J. Am. Chem. Soc.* **1992**, *114*, 10677.

(7) (a) Chida, N.; Takeoka, J.; Ando, K.; Tsutsumi, N.; Ogawa, S. *Tetrahedron* **1997**, *53*, 16287. (b) Chida, N.; Takeoka, J.; Tsutsumi, N.; Ogawa, S. *J. Chem. Soc., Chem. Commun.* **1995**, 793. (c) Nagamitsu, T.; Sunazuka, T.; Tanaka, H.; Omura, S.; Sprengeler, P. A.; Smith, A. B. *J. Am. Chem. Soc.* **1996**, *118*, 3584. (d) Sunazuka, T.; Nagamitsu, T.; Matsuzaki, K.; Tanaka, H.; Omura, S.; Smith, A. B. *J. Am. Chem. Soc.* **1993**, *115*, 5302. (e) Uno, H.; Baldwin, J. E.; Russell, A. T. *J. Am. Chem. Soc.* **1994**, *116*, 2139. (f) Kang, S. H.; Jun, H.-S. *Chem. Commun.* **1998**, 1929. (g) Kang, S. H.; Jun, H.-S.; Youn, J.-H. *Synlett* **1998**, 1045. (h) Iwama, S.; Gao, W.-G.; Shinada, T.; Ohfune, Y. *Synlett* **2000**, 1631. (i) Panek, J. S.; Masse, C. E. *Angew. Chem. Int. Ed.* **1999**, *38*, 1093.

(8) (a) Masse, C. E.; Morgan, A. J.; Adams, J.; Panek, J. S. *Eur. J. Org. Chem.* **2000**, 2513. (b) Corey, E. J.; Li, W.-D. *Chem. Pharm. Bull.* **1999**, *47*, 1.

(9) Page, P. C. B.; Hamzah, A. S.; Leach, D. C.; Allin, S. M.; Andrews, D. M.; Rassias, G. A. *Org. Lett.* **2003**, 353.

(10) Kruijtzer, J. A. W.; Hofmeyer, L. J. F.; Heerma, W.; Versluis, C.; Liskamp, R. M. J. *Chem.–Eur. J.* **1998**, *4*, 1570.

(11) Mander, L. N.; Sethi, S. P. *Tetrahedron Lett.* **1983**, *24*, 5425.

(12) Mukaiyama, T.; Bonno, K.; Naraska, K. *J. Am. Chem. Soc.* **1974**, *96*, 7503.

(13) Howard, A. S.; Meerholz, C. A.; Michael, J. P. *Tetrahedron Lett.* **1979**, *20*, 1339.

(14) Katritzky, A. R.; Pastor, A. *J. Org. Chem.* **2000**, *65*, 3679.

(15) Analytical data of compound **22**: Colorless powder, 1:1 mixture of diastereoisomers, mp 109 °C. (Found: C, 69.26%; H, 5.98%; N, 3.10%. $C_{26}H_{27}NO_6$ requires C, 69.47%; H, 6.05%; N, 3.12%). IR(nujol): ν_{max} = 1778, 1748, 1698 cm^{-1}. [Found (M^+): 449.18307. $C_{26}H_{27}NO_6$ requires 449.18384]. MS: *m/z* (%) = 449 (2) [M^+], 394 (8), 314 (3), 91 (100), 65 (3). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.48 (3 H, s), 1.59 (3 H, s), 1.69 (3 H, s, major), 1.72 (3 H, s, minor), 2.67 (3 H, s, major), 2.77–2.96 (4 H, m, major + minor), 3.11 (3 H, s, minor), 4.03 (1 H, d, *J* = 15.2 Hz, major), 4.18 (1 H, d, *J* = 15.2 Hz, minor), 4.51 (1 H, bs, minor), 4.56–4.57 (1 H, m, minor), 4.67 (1 H, br s, major), 4.97–4.98 (1 H, m, major), 5.06 (1 H, d, *J* = 12.4 Hz, minor), 5.14 (1 H, d, *J* = 12.4 Hz, major), 5.12–5.25 (1 H, m, minor), 5.12–5.25 (1 H, m, minor), 5.24 (1 H, d, *J* = 12.4 Hz, major), 5.36 (1 H, d, *J* = 15.2 Hz, major), 7.17–7.35. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.35 (major), 19.19 (minor), 23.73 (minor), 24.45 (major), 36.92 (major), 37.81 (minor), 44.47, 44.55, 52.19, 52.84 (OCH_3, minor), 57.59, 57.97, 68.11, 68.28, 76.11, 75.85, 118.21(minor), 118.78(major), 127.68, 127.94, 128.26, 128.29, 128.31, 128.48, 128.51, 128.53, 128.58, 128.63, 128.75, 129.08, 134.82, 135.15, 135.39, 137.83, 138.15, 164.84, 165.09, 166.72, 167.15, 170.62, 170.89, 199.95, 200.22.

(16) Crystallographic data for **22** are available on request from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44(1223)336033, deposit@ccdc.cam.ac.uk, quoting the deposition number CCDC 206650.