A formal synthesis of (+)-lactacystin†

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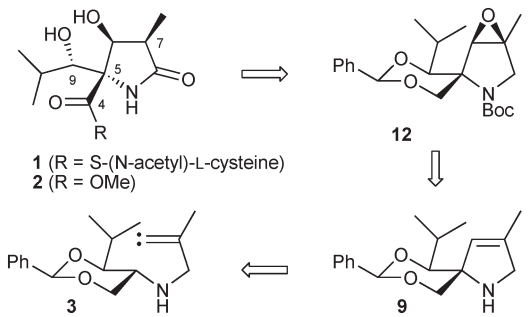
A synthetic route to the neurotrophic agent (+)-lactacystin has been developed utilizing a base-promoted intramolecular alkylidenecarbene C–H insertion as the key transformation.

(+)-Lactacystin (**1**), a secondary metabolite of the bacterium *Streptomyces* sp. OM-6519, was discovered by Omura during a screen for natural product inhibitors of mouse neuronal cell differentiation.[1] Studies by Fenteany and co-workers[2] have revealed that lactacystin covalently modifies and irreversibly-inhibits the 20*S* proteasome, whose function within the cell is to process and eliminate damaged, misfolded and ubiquitinated proteins. The universal presence of this enzyme complex ensures that **1** exhibits a host of biological activities that, in conjunction with its complex structure, have stimulated considerable synthetic interest.[3] Herein we report the asymmetric synthesis of compound **2**, which Smith and co-workers[4] have previously converted to the natural product **1** in two steps (Scheme 1).[5]

While many of the existing routes to **1** utilize aldol chemistry to set the key quaternary C-5 stereocenter, we envisioned that this goal could be achieved through intramolecular C–H insertion of the alkylidenecarbene intermediate **3**. Since alkylidene insertion is known to proceed with retention of configuration,[6] we would parlay the asymmetry of an existing and readily accessible tertiary center into a quaternary carbon center. With regards to the C-6 and C-7 stereocenters, we anticipated that the equatorially-positioned isopropyl group of the 1,3-dioxane ring of **9** would effectively shield the α-face of the 3-pyrroline ring and ensure that dihydroxylation or epoxidation proceeded exclusively from the less hindered face to form **12**, thereby setting the correct stereochemistry at C-6.



Scheme 1 Retrosynthetic analysis of (+)-lactacystin (**1**).

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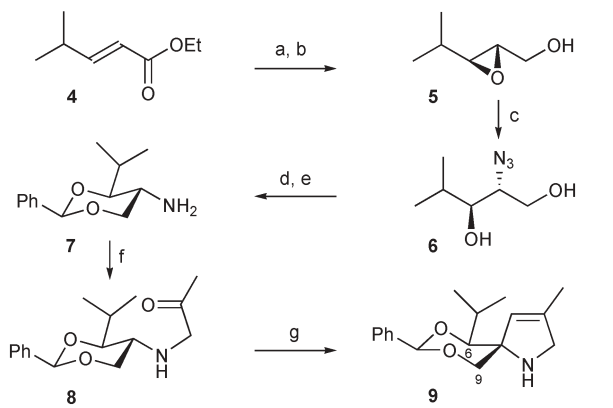
The formation of 3-pyrrolines *via* alkylidenecarbene C–H insertion was first reported in 1970 by Walsh and Bottini, who found that treatment of simple 3-bromopropenyldialkyl amines with potassium *tert*-butoxide afforded 3-pyrrolines in low yield.[7a] While Gilbert,[7b] Shiori[7c] and Baird[7d] have subsequently reported similar cyclizations employing alternative strategies for carbene generation, application of this transformation to natural product synthesis has, until recently, been limited, despite its obvious potential. While the work presented therein was in progress, Hayes and co-workers reported a related route to **1** using alkylidene-carbene C–H insertion.[8]

Our route to **2** commenced (Scheme 2) with the reduction of α,β-unsaturated ester **4** using alane (AlH_3). Sharpless asymmetric epoxidation of the resulting allylic alcohol then provided epoxide **5**.[9] Acid-catalyzed ring-opening of **5** with sodium azide in aqueous 2-methoxyethanol gave an inseparable 2 : 1 mixture of **6** and the 1,2-diol arising from C-3 ring opening.[10] Treatment of this mixture with aqueous sodium periodate selectively cleaved the 1,2-diol to the corresponding α-azido aldehyde, which was then separated by chromatography on silica gel. Protection of the 1,3-diol moiety of **6** as a benzylidene acetal and chemoselective catalytic hydrogenation of the azide over $Pd(CaCO_3)$ then provided amine **7** as a single diastereomer. Alkylation of **7** with bromoacetone under standard conditions gave **8**, which was found to be unstable, decomposing within a matter of hours at room temperature (Scheme 2). Nonetheless, when this α-amino ketone was immediately treated with lithium (trimethylsilyl)diazomethane[11] at −78 °C, the desired



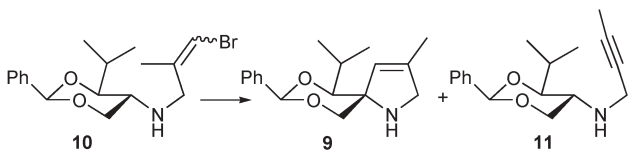
Scheme 2 Reagents and conditions. a: AlH_3, Et_2O, 0 °C, 1 h (85%); b: (+)-DIPT, $Ti(O^iPr)_4$, CH_2Cl_2, 4 Å sieves, −20 °C, 7 h (76%, 96% ee); c: (i) NaN_3, NH_4Cl, $MeOCH_2CH_2OH$, H_2O, reflux, 14 h, (ii) $NaIO_4$, THF, H_2O, rt, 24 h (47%); d: PhCHO, *p*-$TsOH{\cdot}H_2O$, $PhCH_3$, reflux, 1 h (78%); e: H_2 (1 atm), $Pd/CaCO_3$, EtOH, rt, 3 h (98%); f: bromoacetone, K_2CO_3, acetone, reflux, 7 h (56%); g: $Me_3SiCLiN_2$, THF, −78 °C, 1 h (35%). h: 1,3-dibromo-2-methyl-propene (*E* : *Z* = 3 : 2), K_2CO_3, CH_3CN, reflux, 16 h (76%).

3-pyrroline **9** was isolated, albeit in a rather modest yield. Analysis of the 2D-NOESY spectrum of **9** revealed correlations between H_{ax}-6, H_{ax}-9 and the amine proton, thus confirming that insertion proceeded with retention of configuration.

Encouraged by this preliminary result but suspecting that further pursuit of α-amino ketone substrates would be fraught with difficulties, we now decided to examine α-elimination as an alternate method for alkylidenecarbene generation. Thus, heating an equimolar mixture of **7** and 1,3-dibromo-2-methyl-propene ($E : Z = 3 : 2$)[12] in acetonitrile, generated the corresponding mixture of alkenyl bromides **10**, which proved to be stable (Table 1). Upon treatment of an ethereal solution of **10** with potassium hexamethyldisilazide (KHMDS), cyclization proceeded rapidly at room temperature to provide 3-pyrroline **9** and propargyl amine **11**, which arise through a competitive 1,2-rearrangement of the intermediate carbene (Table 1, entry 1). In an effort to ascertain the effect of alkenyl bromide geometry upon insertion, the individual isomers of **10** were separated by careful flash chromatography and submitted to the same cyclization conditions as before (Table 1, entries 2 and 3). Intriguingly, there appears to be a correlation between the efficiency of the insertion and the geometry of the alkenyl bromide precursor, with *E*-**10** giving **9** in 67% yield. This observation is rather surprising, given that Taber has previously demonstrated, as least for carbocyclic systems, that the geometry of the alkenyl halide precursor does not influence the efficiency of C–H insertion.[13]

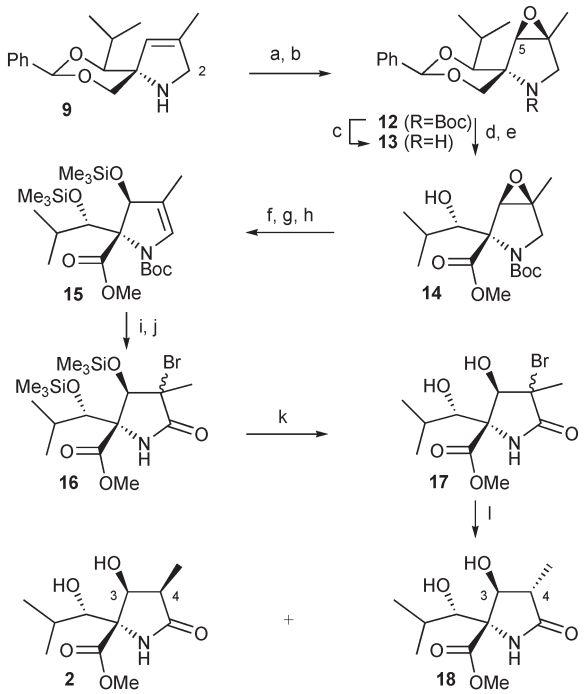
Having established the C-5 center, we now turned our attention to installing the remaining stereocenters and adjusting the oxidation state at C-4 (Scheme 3). In view of the sensitivity of **9** to aerial oxidation, we opted now to acylate the nitrogen atom. However, introduction of *N*-Boc protection proved to be rather troublesome, primarily because of the highly hindered nature of this substrate. The most effective conditions identified involved treating **9** with *tert*-butyl aminocarbonate, generated *in situ* from $(Boc)_2O$ and hydroxylamine.[14] Under these conditions, *N*-acylation was slow but ultimately proceeded in excellent yield. Epoxidation with *m*-CPBA in the presence of 2,6-di-*tert*-butyl-4-methylphenol (BHT)[15] then proceeded smoothly to furnish **12**. Attempts to accomplish this transformation without a radical inhibitor failed to provide any epoxide, but instead generated the α,β-unsaturated lactam resulting from allylic oxidation at C-2. That epoxidation of the pyrroline ring had proceeded from the less hindered face could not be immediately determined because of the

Table 1 2-Pyrrolines *via* alkylidenecarbene C–H insertion[a]



Substrate	Yield of **9** (%)[b]	Yield of **11** (%)[b]
(*E*/*Z*)-**10**	50	36
(*E*)-**10**	67	25
(*Z*)-**10**	14	42

[a] A solution of **10** in Et_2O was added to 1.5 equiv. of KHMDS in Et_2O and the resulting mixture stirred at ambient temperature for 15 min. [b] Yield of isolated product after flash chromatography.



Scheme 3 Reagents and conditions. a: $(Boc)_2O$, $NH_2OH{\cdot}HCl$, Et_3N, CH_2Cl_2, rt, 96 h (93%); b: *m*-CPBA, BHT, CH_2Cl_2, rt, 18 h (90%); c: $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt, 15 min (90%); d: H_2 (2300 psi), $Pd(OH)_2$, EtOAc, rt, 24 h (94%); e: (i) TEMPO, trichloroisocyanuric acid, CH_2Cl_2, rt, 2 h, (ii) $NaClO_2$, ^{t}BuOH, 2-methyl-2-butene, 0 °C, 1 h, (iii) CH_2N_2, Et_2O, rt, 10 min (96%); f: Me_3SiCl, imidazole, DMF, rt, 4 h (94%); g: LDA, THF, 0 °C, 2 h (93%); h: Me_3SiCl, imidazole, DMF, rt, 1 h (85%); i: NBS, H_2O, 1,4-dioxane, rt, 3 h (95%); j: PDC, DMF, rt, 4 h, then: $Mg(ClO_4)_2$, CH_3CN, 40 °C, 1 h (67%); k: NH_4F, MeOH, reflux, 1 h (95%); l: SmI_2, THF, rt, 15 min (80%, 1 : 2).

presence of amide rotamers in the ^{1}H NMR spectrum. Accordingly, the *N*-Boc group was removed under mild conditions ($BF_3{\cdot}Et_2O$)[16] to provide the corresponding amine **13** as a single diastereomer. Analysis of this material by a 2D-NOESY experiment revealed correlations between H-5 and the methyl protons of the isopropyl group, thereby confirming that epoxidation had indeed proceeded from the less hindered face of **12**.

The benzylidene acetal of **12** was now removed by hydrogenolysis over Adam's catalyst [$Pd(OH)_2$] at high pressure and the resulting 1,3-diol converted to ester **14**. Thus, selective oxidation of the primary alcohol using TEMPO and trichloroisocyanuric acid[17] provided the corresponding β-hydroxy aldehyde in excellent yield. This compound proved to be quite unstable and accordingly was immediately converted to the corresponding ester through Pinnick oxidation and methylation with diazomethane. The secondary hydroxyl group was then protected as a trimethylsilyl ether and the epoxide ring opened using LDA to afford the corresponding hydroxy enamide, which was then silylated to furnish **15**. That the exocyclic olefin product arising from deprotonation at the methyl group was not observed in the ring opening reaction is likely a reflection of the chelational and electronic directing effects of the carbamate group.[18] Hydrobromination of **15** with *N*-bromosuccinamide (NBS) in aqueous 1,4-dioxane now proceeded with complete regioselectivity to form a mixture of α-bromo carbinolamine diastereomers. After screening a number

of oxidizing reagents, pyridinium dichromate was found to most successfully mediate the transformation to the corresponding α-bromolactam. Removal of the *N*-Boc group, to form **16**, was then accomplished under mild conditions, using magnesium perchlorate.[19] Removal of the silyl ethers was effected by NH_4F in methanol to give diol **17**. Finally, reduction of the α-bromo ester with samarium diiodide gave a mixture of C-4 epimers **2** and **18** (1 : 2) which were separated to give **2**. The spectral and physical properties of **2**[20] were in accordance with those previously reported.[4] This compound has been previously been converted to **1** by Smith[4], and hence the route described above represents a formal, total synthesis of (+)-lactacystin.

In summary, we have developed a novel synthetic route to (+)-lactacystin, based on the [1,5]-C–H insertion of alkylidenecarbenes.

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Notes and references

1 S. Omura, K. Matsuzaki, T. Fujimoto, K. Kosuge, T. Furuya, S. Fujita and A. Nakagawa, *J. Antibiot.*, 1991, **44**, 117.

2 G. Fenteany, R. F. Standaert, W. S. Lane, S. Choi, E. J. Corey and S. L. Schreiber, *Science*, 1995, **268**, 726.

3 (*a*) For an excellent review, see: C. E. Masse, A. J. Morgan, J. Adams and J. S. Panek, *Eur. J. Org. Chem.*, 2000, 2513; (*b*) P. C. B. Page, A. S. Hamzah, D. C. Leach, S. M. Allin, D. M. Andrews and G. A. Rassias, *Org. Lett.*, 2003, **5**, 353; (*c*) C. J. Brennan, G. Pattenden and G. Rescourio, *Tetrahedron Lett.*, 2003, **44**, 8757.

4 T. Nagamitsu, T. Sunazuka, H. Tanaka, S. Omura, P. A. Sprengeler and A. B. Smith, *J. Am. Chem. Soc.*, 1996, **118**, 3584.

5 Presented in part at the 222nd National Meeting of the American Chemical Society: D. J. Wardrop and E. G. Bowen, *Abstracts of Papers*, 200th National Meeting of the American Chemical Society, Division of Organic Chemistry, Chicago, IL, August 26–30, 2001, abstract no. 200.

6 (*a*) D. F. Taber, R. P. Meagley and D. D. Doren, *J. Org. Chem.*, 1996, **61**, 5723; (*b*) Reviews covering the 1,5-C–H insertion of alkylidenecarbenes: P. J. Stang, *Chem. Rev.*, 1978, **78**, 383; P. J. Stang, *Angew. Chem., Int. Ed. Engl.*, 1992, **31**, 274; D. F. Taber, in *Methods of Organic Chemistry*, ed. G. Helmchen, Thieme, Stuttgart, 1995, vol. E21, pp. 1127; W. Kirmse, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 1164.

7 (*a*) R. A. Walsh and A. T. Bottini, *J. Org. Chem.*, 1970, **35**, 1086; (*b*) J. C. Gilbert and B. K. Blackburn, *J. Org. Chem.*, 1986, **51**, 3656; (*c*) H. Ogawa, T. Aoyama and T. Shioiri, *Heterocycles*, 1996, **42**, 75; (*d*) M. S. Baird, A. G. W. Baxter, A. Hoorfar and I. Jefferies, *J. Chem. Soc., Perkin Trans. 1*, 1991, 2575.

8 (*a*) M. P. Green, J. C. Prodger, A. E. Sherlock and C. J. Hayes, *Org. Lett.*, 2001, **3**, 3377; (*b*) M. P. Green, J. C. Prodger and C. J. Hayes, *Tetrahedron Lett.*, 2002, **43**, 6609.

9 Y. Gao, J. M. Klunder, R. M. Hanson, H. Masamune, S. Y. Ko and K. B. Sharpless, *J. Am. Chem. Soc.*, 1987, **109**, 5765.

10 M. Caron, P. R. Carlier and K. B. Sharpless, *J. Org. Chem.*, 1988, **53**, 5185.

11 S. Ohira, K. Okai and T. Moritani, *J. Chem. Soc., Chem. Commun.*, 1992, 721.

12 W. Fischetti, K. T. Mak, F. G. Stakem, J. Kim, A. L. Rheingold and R. Heck, *J. Org. Chem.*, 1983, **48**, 948.

13 D. F. Taber and T. D. Neubert, *J. Org. Chem.*, 2001, **66**, 143.

14 R. B. Harris and I. B. Wilson, *Tetrahedron Lett.*, 1983, **24**, 231.

15 Y. Kishi, M. Aratani, H. Tanino, T. Fukuyama, T. Goto, S. Inoue, S. Sugiua and H. Kakoi, *J. Chem. Soc., Chem. Commun.*, 1972, 64.

16 E. F. Evans, N. J. Lewis, I. Kapfer, G. Macdonald and R. J. K. Taylor, *Synth. Commun.*, 1997, **27**, 1819.

17 L. De Luca, G. Giacomelli and A. Porcheddu, *Org. Lett.*, 2001, **3**, 3041.

18 P. Beak, A. Basu, Y. S. P. Gallagher and S. Thayumanavan, *Acc. Chem. Res.*, 1996, **29**, 552.

19 J. A. Stafford, M. F. Brackeen, D. S. Karanewsky and N. L. Valvano, *Tetrahedron Lett.*, 1993, **34**, 7873.

20 Selected physical and spectroscopic data for **2**: $[\alpha]_D^{25}$ = +60.0 (*c* 0.2 in CH_3OH), lit.[4] $[\alpha]_D^{24}$ = +65.0 (*c* 0.3 in CH_3OH). δ_H (400 MHz, CD_3OD): 4.43 (1 H, d, *J* = 6.2 Hz), 3.90 (1 H, d, *J* = 7.0 Hz), 3.73 (3 H, s), 3.00–2.90 (1 H, m), 1.69–1.61 (1 H, m), 1.06 (3 H, d, *J* = 7.6 Hz), 0.97 (3 H, d, *J* = 7.0 Hz) and 0.83 (3 H, d, *J* = 7.0 Hz). δ_C (100 MHz, CD_3OD): 182.3, 172.9, 80.1, 76.9, 76.8, 52.5, 42.5, 32.3, 20.1, 19.5 and 8.8. HRMS-EI: Found 245.12350, $C_{11}H_{19}NO_5$ $[M]^+$ requires 245.12363.

