Uncialamycin

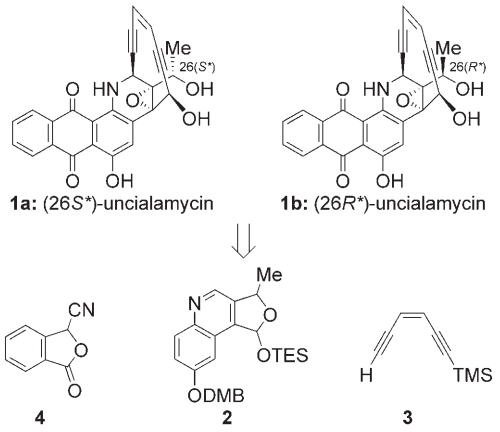
DOI: 10.1002/anie.200700917

Total Synthesis and Stereochemistry of Uncialamycin**

K. C. Nicolaou, Hongjun Zhang, Jason S. Chen, James J. Crawford, and Laxman Pasunoori*

In memory of Yoshihiko Ito

Uncialamycin (**1a** or **1b**, Scheme 1) is a newly discovered enediyne antibiotic that possesses an intriguing molecular architecture and extremely potent biological properties.[1]

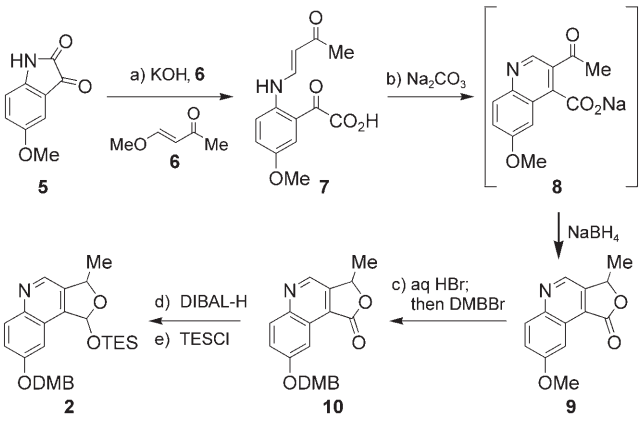


Scheme 1. Structures and retrosynthetic analysis of (26*S**)- and (26*R**)-uncialamycins (**1a** and **1b**). DMB = 3,4-dimethoxybenzyl, TES = triethylsilyl, TMS = trimethylsilyl.

Uncialamycin was isolated from an unreported strain of Streptomycete, related to *Streptomyces cyanogenus*, and exhibits potent plasmid DNA cleavage and phenomenal in vitro activity against *Staphylococcus aureus* (MIC 0.0000064 $\mu g\,mL^{-1}$), as well as activity against *Escherichia coli* (MIC 0.002 $\mu g\,mL^{-1}$) and *Burkholderia cepacia* (MIC 0.001 $\mu g\,mL^{-1}$).[1] The scarcity of uncialamycin (only 300 μg were isolated) limited both the structural elucidation (the stereochemistry of the C26 hydroxy group remained unassigned) and the biological investigation. In view of this state of affairs and our earlier experience with enediyne antitumor antibiotics,[2] we set out to synthesize the two C26 epimers of uncialamycin (**1a** and **1b**) to address the above issues. Herein we report the first total synthesis of both C26 epimers of racemic uncialamycin and assign the 26*R** structure (**1b**) as that of the natural substance through spectroscopic studies and X-ray analysis.

Our strategy for the construction of uncialamycin centered around three key reactions: addition of an acetylide to a pyridinium species, an intramolecular acetylide addition to form the enediyne ring system, and Hauser annulation to form the anthraquinone moiety.[3] Fragments **2**–**4** were thus identified as the key building blocks for the projected synthesis.

Scheme 2 summarizes the synthesis of the required building block **2**. Subjecting 5-methoxyisatin (**5**)[6] and



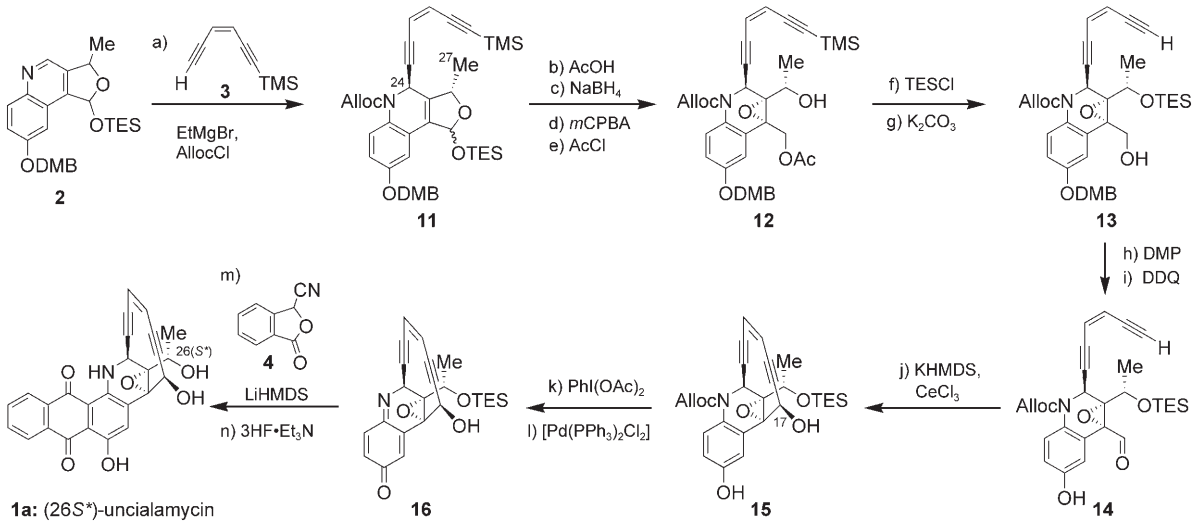
Scheme 2. Synthesis of quinoline system **2**: a) KOH (1.0 equiv), H_2O, 25 °C, 5 min; then **6** (1.5 equiv), 25 °C, 30 min; b) 10% aq Na_2CO_3, 80 °C, 30 min; then $NaBH_4$ (4.0 equiv), 80 °C, 5 min, 86% over 2 steps; c) aq HBr, 120 °C, 18 h, then DMBBr (2.5 equiv), K_2CO_3 (10 equiv), [18]crown-6 (0.1 equiv), DMF, 25 °C, 4 h, 50%; d) DIBAL-H (1.1 equiv), CH_2Cl_2, −78 to 25 °C over 1 h; e) TESCl (1.3 equiv), imidazole (2.6 equiv), DMF, 25 °C, 20 min, 86% over 2 steps, ca. 1:1 mixture of diastereoisomers. DIBAL-H = diisobutylaluminum hydride.

methoxy enone **6** to a two-step Friedlander quinoline synthesis[7] via intermediate **7** afforded the keto carboxylate **8**, which was reduced in situ ($NaBH_4$) to furnish the tricyclic lactone **9** in 86% overall yield. Exchange of the phenolic methyl group for a DMB group led to **10** (50% overall yield for the two steps). The lactone moiety of **10** was reduced (DIBAL-H) and protected as a TES lactol to afford the

[*] Prof. Dr. K. C. Nicolaou, Dr. H. Zhang, J. S. Chen, Dr. J. J. Crawford, L. Pasunoori
Department of Chemistry and
The Skaggs Institute for Chemical Biology
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)
Fax: (+1) 858-784-2469
E-mail: kcn@scripps.edu
and
Department of Chemistry and Biochemistry
University of California, San Diego
9500 Gilman Drive, La Jolla, CA 92093 (USA)

[**] We thank Dr. D. H. Huang and Dr. G. Siuzdak for assistance with NMR spectroscopy and mass spectrometry, respectively, and Dr. R. K. Chadha for X-ray analysis. Financial support for this work was provided by The Skaggs Institute for Chemical Biology and a National Defense Science and Engineering Graduate fellowship (J.S.C.). J.J.C. acknowledges a Fullbright–AstraZeneca Research Fellowship.

WILEY InterScience DISCOVER SOMETHING GREAT
© 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



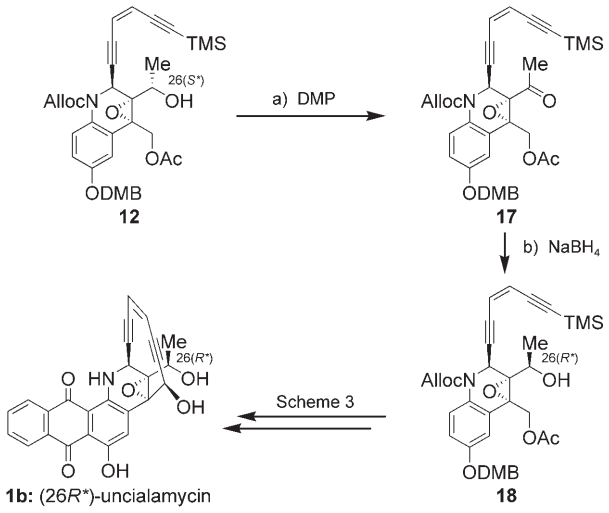
Scheme 3. Synthesis of (26*S**)-uncialamycin (**1a**): a) **2** (1.0 equiv), **3** (1.8 equiv), EtMgBr (1.9 equiv), 25 °C, 30 min; then AllocCl (1.6 equiv), 25 °C, 5 min, 92% (based on 80% conversion); b) MeCN/H_2O/AcOH (4:1:2), 25 °C, 5 h, 91%; c) $NaBH_4$ (1.0 equiv), MeOH, 25 °C, 10 min; d) *m*CPBA (1.4 equiv), CH_2Cl_2, 0 °C, 3 h, 80% over 2 steps; e) AcCl (1.1 equiv), collidine (3.0 equiv), 0 °C, 30 min, then 25 °C, 12 h, 82%; f) TESCl (1.5 equiv), imidazole (3.0 equiv), DMF, 0 °C, 10 min; g) K_2CO_3 (2.0 equiv), THF/MeOH (2:1), 0 °C, 20 min, 78% over 2 steps; h) DMP (2.0 equiv), CH_2Cl_2, 25 °C, 3 h, 87%; i) DDQ (4.0 equiv), CH_2Cl_2/H_2O (10:1), 25 °C, 12 h, 87%; j) $CeCl_3$ (4.0 equiv), 25 °C, THF, 30 min; then KHMDS (5.0 equiv), −78 to −40 °C over 1 h, 61% (based on 80% conversion), plus 30% C17 epimer; k) $PhI(OAc)_2$ (1.1 equiv), MeOH, 25 °C, 10 min, 80%; l) nBu_3SnH (1.1 equiv), H_2O (4.0 equiv), $[Pd(PPh_3)_2Cl_2]$ (0.1 equiv), CH_2Cl_2, 25 °C, 20 min, 74% (based on 70% conversion); m) **4** (3.0 equiv), LiHMDS (3.0 equiv), THF, −78 °C, 20 min; then **16** (1.0 equiv), −78 to 25 °C over 1 h, 63%; n) 3 HF·Et_3N (100 equiv), THF, 25 °C, 1 h, 92%. DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DMP = Dess-Martin periodinane; HMDS = hexamethyldisilazide, *m*CPBA = *meta*-chloroperoxybenzoic acid.

quinoline system **2** (86% overall yield for the two steps of approximately a 1:1 inconsequential mixture of diastereoisomers).

Activation of the pyridine moiety in **2** with allyloxycarbonyl chloride (AllocCl) and trapping of the generated pyridinium species with the acetylide derived from enediyne **3**[8] and EtMgBr furnished the intermediate **11** in 92% yield (based on 80% conversion, Scheme 3).[2d,e,5,9] Removal of the TES group from **11** (AcOH, 91% yield) followed by reduction ($NaBH_4$) of the resulting lactol yielded a diol, which underwent selective epoxidation with *m*CPBA to give, after monoacetylation (AcCl, collidine), the hydroxy epoxide **12** (66% overall yield for the three steps). Protection of the free hydroxy group of **12** as a TES ether followed by K_2CO_3-induced cleavage of both the acetate and TMS groups from the product led to the hydroxy enediyne **13** (78% overall yield for two steps). Oxidation of the hydroxy group in **13** (DMP, 87% yield) followed by removal of the DMB group (DDQ, 87% yield) afforded the cyclization precursor, acetylenic aldehyde **14**.

The crucial cyclization of **14** to afford the desired 10-membered ring enediyne **15**[2d,e,5a,b] was achieved in 61% yield (based on 80% conversion) by exposure to KHMDS in the presence of $CeCl_3$. The undesired C17 epimer of **15** was also formed in 30% yield.[10] Oxidation of **15** to the corresponding methoxy hemiquinone system ($PhI(OAc)_2$, MeOH, 80% yield) and subsequent removal of the Alloc group from the resulting product (cat. $[Pd(PPh_3)_2Cl_2]$, 74% yield based on 70% conversion) furnished the rather labile iminoquinone **16**.[5] Finally, Hauser annulation[11] of **16** with nitrile **4** (LiHMDS, 63% yield) followed by desilylation (3 HF·Et_3N, 92% yield) furnished (26*S**)-uncialamycin (**1a**), whose ^1H and ^{13}C NMR spectroscopic data (Table 1) were consistent with its structure but differed from those reported for natural uncialamycin.[1]

Having proven that the structure of uncialamycin was not **1a**, we then set out to synthesize the 26*R** epimer **1b** to confirm the expectation that the latter was the true structure of the natural product. The task would prove rather simple, for it was soon discovered that an oxidation/reduction sequence using the hydroxy acetate **12** afforded clean inversion of the stereochemistry at the C26 position (Scheme 4). Thus, oxidation of **12** to the ketone **17** through



Scheme 4. Synthesis of (26*R**)-uncialamycin (**1b**): a) DMP (2.0 equiv), CH_2Cl_2, 25 °C, 90 min, 92%; b) $NaBH_4$ (1.0 equiv), MeOH, 25 °C, 10 min, 98%, >96% stereoselectivity.

 © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

the use of DMP, followed by reduction ($NaBH_4$) furnished the hydroxy acetate **18**, which was epimeric at C26, in 90% overall yield and greater than 96% stereoselectivity. The previously developed sequence that produced (26*S**)-uncialamycin (**1a**) from intermediate **12** also served well[12] to deliver, from intermediate **18**, (26*R**)-uncialamycin (**1b**), whose ^1H and ^{13}C NMR spectroscopic data (Table 1) were consistent with those reported[1] for the naturally occurring substance. Synthetic **1b** formed deep-purple crystals (175 °C decomp) from ethyl acetate/hexanes that yielded to X-ray analysis (Figure 1).[13] These results provided unambiguous proof of the structure of uncialamycin as being that of **1b**.

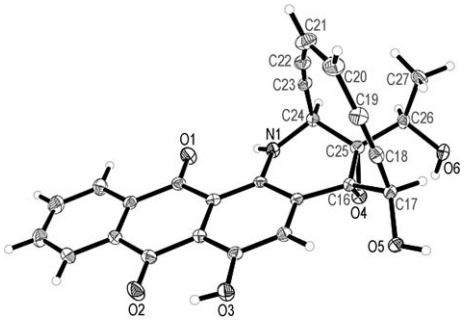
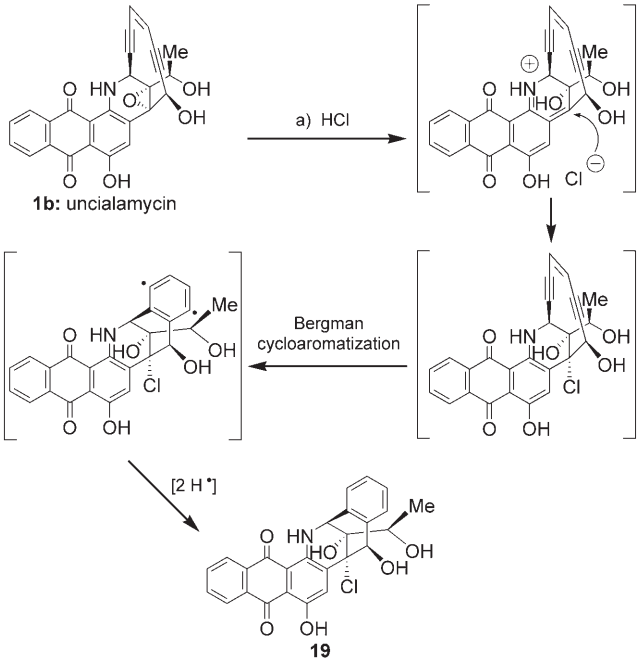


Figure 1. ORTEP drawing of uncialamycin (**1b**). Thermal ellipsoids are set at the 30% probability level.

Uncialamycin (**1b**) proved to be quite stable in the solid phase and in a variety of solvents. In the presence of dry HCl in CH_2Cl_2 at ambient temperature, however, it rapidly converts into the blue hexacyclic compound **19** (90% yield, Table 1), presumably as a consequence of a cascade that involves a Bergman cycloaromatization reaction[14] as shown



Scheme 5. Bergman cycloaromatization of uncialamycin (**1b**): a) 0.005 M HCl in CH_2Cl_2, 25 °C, 5 min, 90%.

Table 1: Selected data for compounds **1a**, **1b**, and **19**.

1a: $R_f = 0.13$ (silica gel, EtOAc/hexanes 2:3); ^1H NMR (600 MHz, [D$_6$]DMSO): $\delta = 13.18$ (s, 1H), 10.01 (d, $J = 4.4$ Hz, 1H), 8.56 (s, 1H), 8.25 (overlapping doublets, 2H), 7.95 (t, $J = 7.6$ Hz, 1H), 7.90 (t, $J = 7.3$ Hz, 1H), 6.68 (d, $J = 5.0$ Hz, 1H), 6.10 (d, $J = 9.9$ Hz, 1H), 5.99 (d, $J = 9.8$ Hz, 1H), 5.62 (d, $J = 5.6$ Hz, 1H), 5.51 (d, $J = 4.9$ Hz, 1H), 5.03 (d, $J = 3.4$ Hz, 1H), 4.20 (quint, $J = 6.4$ Hz, 1H), 1.34 ppm (d, $J = 6.7$ Hz, 3H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta = 186.9$, 182.2, 154.7, 143.5, 135.7, 134.8, 134.3, 133.5, 132.1, 129.9, 126.5, 126.0, 124.0, 123.3, 112.6, 110.4, 101.2, 98.2, 89.7, 88.5, 76.2, 66.0, 65.2, 62.7, 42.3, 21.8 ppm; HRMS (ES): calcd for $C_{25}H_{18}NO_6^+$: 440.1129 [*M*+H]$^+$, found 440.1133.

1b: $R_f = 0.14$ (silica gel, EtOAc/hexanes 2:3); ^1H NMR (600 MHz, [D$_6$]DMSO): $\delta = 13.19$ (s, 1H), 10.01 (d, $J = 4.5$ Hz, 1H), 8.53 (s, 1H), 8.24 (overlapping doublets, 2H), 7.94 (td, $J = 7.4$, 1.1 Hz, 1H), 7.89 (td, $J = 7.4$, 1.1 Hz, 1H), 6.68 (d, $J = 5.1$ Hz, 1H), 6.06 (d, $J = 9.8$ Hz, 1H), 5.98 (d, $J = 10.0$ Hz, 1H), 5.39 (d, $J = 5.7$ Hz, 1H), 5.16 (d, $J = 5.1$ Hz, 1H), 5.07 (d, $J = 4.6$ Hz, 1H), 4.33 (quint, $J = 6.2$ Hz, 1H), 1.31 ppm (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (150 MHz, [D$_6$]DMSO): $\delta = 186.8$, 182.1, 154.7, 143.5, 135.5, 134.8, 134.3, 133.5, 132.1, 129.8, 126.5, 126.0, 123.9, 123.2, 112.6, 110.3, 100.3, 98.8, 89.6, 87.3, 75.9, 63.5, 62.9, 59.7, 43.1, 21.9 ppm; HRMS (ES): calcd for $C_{25}H_{18}NO_6^+$: 440.1129 [*M*+H]$^+$, found 440.1123.

19: $R_f = 0.13$ (silica gel, EtOAc/hexanes 2:3); ^1H NMR (600 MHz, CD_3CN): $\delta = 13.26$ (s, 1H), 10.80 (d, $J = 4.7$ Hz, 1H), 8.27 (d, $J = 7.0$ Hz, 1H), 8.24 (d, $J = 7.1$ Hz, 1H), 7.83 (t, $J = 7.0$ Hz, 1H), 7.77 (t, $J = 7.3$ Hz, 1H), 7.77 (s, 1H), 7.45 (d, $J = 8.3$ Hz, 1H), 7.43 (d, $J = 7.6$ Hz, 1H), 7.29 (t, $J = 6.9$ Hz, 1H), 7.25 (t, $J = 7.0$ Hz, 1H), 5.42 (d, $J = 7.6$ Hz, 1H), 5.14 (d, $J = 5.2$ Hz, 1H), 4.25 (d, $J = 7.9$ Hz, 1H), 4.02 (quint, $J = 6.5$ Hz, 1H), 3.70 (s, 1H), 2.92 (d, $J = 7.1$ Hz, 1H), 1.46 ppm (d, $J = 6.4$ Hz, 3H); ^{13}C NMR (150 MHz, CD_3CN): $\delta = 188.3$, 182.8, 156.1, 143.2, 137.6, 136.9, 136.6, 136.1, 135.4, 133.8, 133.6, 133.3, 129.7, 129.1, 128.8, 128.0, 127.5, 126.9, 114.5, 109.1, 80.0, 78.0, 73.5, 69.3, 56.8, 20.4 ppm; HRMS (ES): calcd for $C_{25}H_{21}ClNO_6^+$: 478.1057 [*M*+H]$^+$, found 478.1074.

in Scheme 5. It is assumed that uncialamycin damages DNA and kills cells through a mechanism that involves such a cascade sequence initiated by bioreduction in a similar manner as dynemicin A.[1–5]

While the described study proves the structure of uncialamycin and renders its racemic form readily available, it leaves the absolute stereochemistry unverified, although its structural similarity to dynemicin A[4,5a,b] and its DNA cleavage activity[1] are highly suggestive of the shown enantiomeric form. An asymmetric synthesis of uncialamycin, currently in progress in these laboratories, should prove this hypothesis and provide ample quantities of its natural form and related analogues for biological investigations.

Received: February 28, 2007
Published online: May 11, 2007

Keywords: antibiotics · enediynes · natural products · structure elucidation · total synthesis

[1] J. Davies, H. Wang, T. Taylor, K. Warabi, X.-H. Huang, R. J. Andersen, *Org. Lett.* **2005**, *7*, 5233–5236.
[2] a) K. C. Nicolaou, W.-M. Dai, *Angew. Chem.* **1991**, *103*, 1453–1481; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1387–1416; b) K. C. Nicolaou, W.-M. Dai, S.-C. Tsay, V. A. Estevez, W. Wrasidlo,

 © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Science **1992**, *256*, 1172-1178; c) K. C. Nicolaou, A. L. Smith, E. W. Yue, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 5881 – 5888; d) K. C. Nicolaou, C.-K. Hwang, A. L. Smith, S. V. Wendeborn, *J. Am. Chem. Soc.* **1990**, *112*, 7416 – 7418; e) K. C. Nicolaou, P. Maligres, T. Suzuki, S. V. Wendeborn, W.-M. Dai, R. K. Chadha, *J. Am. Chem. Soc.* **1992**, *114*, 8890 – 8907.

[3] For related studies that culminated in elegant total syntheses of dynemicin A,[4] which is a related enediyne antitumor antibiotic, see reference [5].

[4] a) M. Konishi, H. Ohkuma, T. Tsuno, T. Oki, G. D. VanDuyne, J. Clardy, *J. Am. Chem. Soc.* **1990**, *112*, 3715 – 3716; b) M. Konishi, H. Ohkuma, K. Matsumoto, H. Kamei, T. Miyaki, T. Oki, H. Kawaguchi, G. D. VanDuyne, J. Clardy, *J. Antibiot.* **1989**, *42*, 1449 – 1452.

[5] a) A. G. Myers, M. E. Fraley, N. J. Tom, S. B. Cohen, D. J. Madar, *Chem. Biol.* **1995**, *2*, 33 – 43; b) A. G. Myers, N. J. Tom, M. E. Fraley, S. B. Cohen, D. J. Madar, *J. Am. Chem. Soc.* **1997**, *119*, 6072 – 6094; c) M. D. Shair, T. Y. Yoon, S. D. Danishefsky, *Angew. Chem.* **1995**, *107*, 1883 – 1885; *Angew. Chem. Int. Ed. Eng.* **1995**, *34*, 1721 – 1723; d) M. D. Shair, T. Y. Yoon, K. K. Mosny, T. C. Chou, S. J. Danishefsky, *J. Am. Chem. Soc.* **1996**, *118*, 9509 – 9525.

[6] S. E. V. Bell, R. F. C. Brown, F. W. Eastwood, J. M. Horvath, *Aust. J. Chem.* **2000**, *53*, 183 – 190. Purchased from TCI America.

[7] H. Bretschneider, K. Hohenlohe-Oehringen, A. Rhomberg, US patent 3,311,632, **1967**.

[8] A. Ernst, L. Gobbi, A. Vasella, *Tetrahedron Lett.* **1996**, *37*, 7959 – 7962.

[9] The lactol ring led to the rigid positioning of the methyl group which effectively blocked the formation of the alternative diastereoisomer in this reaction. Furthermore, this rigid ring system allowed the definitive assignment of the relative stereochemistry of **11** on the basis of HMQC NMR analysis (to unambiguously identify the H24 position) and ROESY NMR analysis, which indicated a correlation between the H24 and H27 atoms.

[10] A tentative stereochemical assignment of C17 was made on the basis of ROESY analysis of both C17 epimers of **15**. Definitive assignment was afforded by ROESY analysis of **1a**, derived from the major epimer **15**, which indicated a correlation between the H17 and H26 atoms.

[11] a) F. M. Hauser, R. P. Rhee, *J. Am. Chem. Soc.* **1979**, *101*, 1628 – 1629; b) G. A. Kraus, H. Cho, S. Crowley, B. Roth, H. Sugimoto, S. Prugh, *J. Org. Chem.* **1983**, *48*, 3439 – 3444.

[12] The key cyclization to form the desired 10-membered enediyne ring system now occurred with a 5:1 diastereomeric ratio (80% total yield).

[13] CCDC-638436 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[14] a) R. R. Jones, R. G. Bergman, *J. Am. Chem. Soc.* **1972**, *94*, 660 – 661; b) R. G. Bergman, *Acc. Chem. Res.* **1973**, *6*, 25 – 31.

 © 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim