

Tetrahydroxy 10-Membered Cyclic Eneidyne

Michael Klein,[†] Manfred Zabel,[‡] Günther Bernhardt,[§] and Burkhard König*[†]

Institut für Organische Chemie, Universität Regensburg, D-93040 Regensburg, Germany

burkhard.koenig@chemie.uni-regensburg.de

Received August 27, 2003

The preparation of cyclic 10-membered tetrahydroxy eneidyne is reported. The synthesis starts from tartaric acid and allows the control of the relative stereochemistry. Acetal protection of the 2,3-hydroxy groups stabilizes the eneidyne during synthesis. Removal of the cyclic protecting group with EtSH/TFA transforms the stable compounds into reactive eneidyne, and the rate constants of their cyclization were determined in benzene and water. The cytotoxicity of the activated compounds was assayed against tumor cells in vitro, but the growth inhibitory effect was weak compared to cisplatin.

Introduction

The cytotoxic properties of natural products such as calicheamicin γ_1^I , dynemicin A, or neocarzinostatin¹ have their origin in strained cyclic eneidyne or cumulene-eneidyne structures. Their spontaneous thermal cyclization yields reactive aryl or benzyl diradicals, which abstract hydrogen atoms, leading to DNA strand cleavage and ultimately to cell death.² To fulfill their biological function, the reactivity of the unsaturated macrocycles must be regulated. In the natural products this is achieved either by trigger mechanisms that unlock conformationally constrained precursors³ or by proteins, which tightly bind and stabilize the eneidyne or cumulene-eneidyne chromophore.⁴ To control the reactivity in synthetic eneidyne model compounds, several strategies have been reported: release of a conformational constraint that

prohibits spontaneous cyclization,⁵ isomerization of eneidyne to the more reactive cumulene-eneidyne,⁶ generation of the eneidyne system by retro-Diels–Alder,⁷ S_N2' reaction,⁸ or alkyne deprotection⁹ or the induction of the cyclization by catalytic antibodies,¹⁰ metal cations,¹¹ oxidation,¹² or light.¹³ Hydroxylated eneidyne are well suited to reversibly lock their conformation and therefore modulate their reactivity by cyclic hydroxy protecting

(4) Carrier protein to which the active eneidyne chromophore is tightly noncovalently bound plays an important role in the direction, protection, and regulation of the chromophore, e.g., in the case of the neocarzinostatin chromophore: Chin, D.-H. *Chem. Eur. J.* **1999**, *5*, 1084–1090 and refs cited therein.

(5) (a) Nicolaou, K. C.; Sorensen, E. J.; Discordia, R.; Hwang, C.-K.; Minto, R. E.; Bharucha, K. N.; Bergman, R. G. *Angew. Chem.* **1992**, *104*, 1094–1096; *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1044–1046. (b) Nicolaou, K. C.; Dai, W.-M.; Hong, Y. P.; Tsay, S.-C.; Baldrige, K. K.; Siegel, J. S. *J. Am. Chem. Soc.* **1993**, *115*, 7944–7953. (c) Banfi, L.; Basso, A.; Guanti, G. *Tetrahedron Lett.* **1997**, *53*, 3249–3268. (d) Banfi, L.; Guanti, G.; Basso, A. *Eur. J. Org. Chem.* **2000**, 939–946. (e) Basak, A.; Khamrai, U. K. *Tetrahedron Lett.* **1996**, *37*, 2475–2478. (f) Semmelhack, M. F.; Gu, Y.; Ho, D. M. *Tetrahedron Lett.* **1997**, *38*, 5583–5586.

(6) (a) Wu, M.-J.; Lin, C.-F.; Wu, J.-S.; Chen, H.-T. *Tetrahedron Lett.* **1994**, *35*, 1879–1882. (b) Nicolaou, K. C.; Maligres, P.; Shin, J.; de Leon, E.; Rideout, D. *J. Am. Chem. Soc.* **1990**, *112*, 7825–7826. (c) Suzuki, I.; Bando, N.; Nemoto, H.; Shibuya, M. *Tetrahedron Lett.* **1998**, *39*, 2361–2364.

(7) Bunnage, M. E.; Nicolaou, K. C. *Chem. Eur. J.* **1997**, *3*, 187–192.

(8) Dai, W.-M.; Fong, K. C.; Lau, C. W.; Zhou, L.; Hamaguchi, W.; Nishimoto, S. *J. Org. Chem.* **1999**, *64*, 682–683.

(9) (a) Jones, G. B.; Hynd, G.; Wright, J. M.; Purohit, A.; Plourde, G. W., II; Huber, R. S.; Li, A.; Kilgore, M. W.; Buble, G. J.; Yancisin, M.; Brown, M. A. *J. Org. Chem.* **2001**, *66*, 3688–3695. (b) Jones, G. B.; Plourde, G. W., II; Wright, J. M. *Org. Lett.* **2000**, *2*, 811–813.

(10) Jones, L. H.; Harwig, C. W.; Wentworth, P., Jr.; Simeonov, A.; Wentworth, A. D.; Py, S.; Ashley, J. A.; Lerner, R. A.; Janda, K. D. *J. Am. Chem. Soc.* **2001**, *123*, 3607–3608.

(11) (a) König, B. *Eur. J. Org. Chem.* **2000**, 381–385 and refs cited therein. (b) Rawat, D. S.; Zaleski, J. M. *J. Am. Chem. Soc.* **2001**, *123*, 9675–9676. (c) O'Connor, J. M.; Friese, S. J.; Tichenor, M. J. *Am. Chem. Soc.* **2002**, *124*, 3506–3507.

(12) For induction of eneidyne cyclization by oxidation, see: (a) Ramkumar, D.; Kalpana, M.; Varghese, B.; Sankararaman, S. *J. Org. Chem.* **1996**, *61*, 2247–2250. (b) Semmelhack, M. F.; Neu, T.; Foubelo, F. *J. Org. Chem.* **1994**, *59*, 5038–5047. For pH-dependent cyclization at higher temperatures, see: (c) Kim, C.-S.; Diez, C.; Russell, K. C. *Chem. Eur. J.* **2000**, *6*, 1555–1558. For the effect of vinyl substitution, see: (d) Jones, G. B.; Warner, P. M. *J. Am. Chem. Soc.* **2001**, *123*, 2134–2145.

* Fax (int.): +49-941-943-1717.

[†] Institut für Organische Chemie.

[‡] Current address: Zentrale Analytik der NWF IV, Universität Regensburg, 93040 Regensburg, Germany.

[§] Current address: Institut für Pharmazie, Universität Regensburg, D-93040 Regensburg, Germany.

(1) (a) Nicolaou, K. C.; Smith, L. A. In *Modern Acetylene Chemistry*; Stang, P. J., Diederich, F., Eds. VCH: Weinheim, 1995; pp 203–283. (b) Maier, M. E. *Synlett* **1995**, 13–26. (c) Sorensen, E. J.; Nicolaou, K. C.; Winssinger, N. *J. Chem. Edu.* **1998**, *75*, 1225–1258. (d) Groneberg, R. D.; Miyazaki, T.; Stylianides, N. A.; Schulze, T. J.; Stahl, W.; Schreiner, E. P.; Suzuki, T.; Iwabuchi, Y.; Smith, L. A.; Nicolaou, K. C. *J. Am. Chem. Soc.* **1993**, *115*, 7593–7611. (e) Myers, A. G.; Liang, J.; Hammond, M.; Harrington, M. P.; Wu, Y.; Kuo, E. Y. *J. Am. Chem. Soc.* **1998**, *120*, 5319–5320. (f) Clive, D. L. J.; Bo, Y.; Tao, Y.; Daigneault, S.; Wu, Y.-Y.; Meignan, G. *J. Am. Chem. Soc.* **1998**, *120*, 10332–10349.

(2) Initially formed diradical is less reactive than the phenyl radical, which is obtained after one hydrogen abstraction. This fact is of importance for efficient DNA-strand cleavage by eneidyne antibiotics. Schottelius, M. J.; Chen, P. *J. Am. Chem. Soc.* **1996**, *118*, 4896–4903.

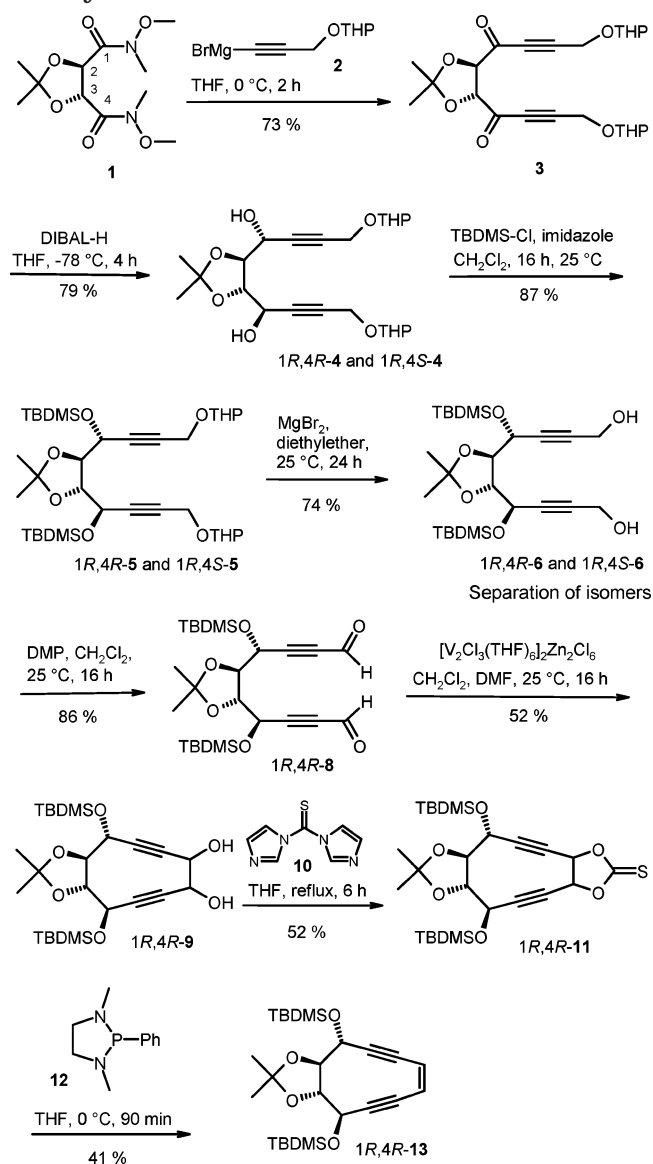
(3) In most cases, a strained, and therefore even at room temperature highly reactive, cyclic eneidyne or cumulene-eneidyne is held in a conformation that kinetically disfavors the cyclization reaction. A small change in conformation, caused, e.g., by a nucleophile that adds to a double bond or an epoxide, releases the constraint, and the cyclization to the arene diradical proceeds instantaneously. (a) Haseltine, J. N.; Cabal, M. P.; Mantlo, N. B.; Iwasawa, N.; Yamashita, D. S.; Coleman, R. S.; Danishefsky, S. J.; Schulte, G. K. *J. Am. Chem. Soc.* **1991**, *113*, 3850–3866. (b) Nicolaou, K. C.; Dai, W.-M. *J. Am. Chem. Soc.* **1992**, *114*, 8908–8921.

groups.¹⁴ Nicolaou et al. reported an enediynes diol and its carbonate as the first example of this kind.^{5a,15} We present here the stereodefined synthesis of enediynes tetraols. The cyclic enediynes become water soluble¹⁶ by the additional hydroxy groups and still can be regulated in their reactivity by cyclic protecting groups.

Results and Discussion

Fallis et al.¹⁷ reported the first synthesis of a protected tetrahydroxy enediyne¹⁸ using Cr(II)/Ni(II)-mediated coupling of an iodoalkyne to an aldehyde for ring closure. In their route, the sensitive enediyne was taken through the synthesis, while we intended to build up the enediyne in the last steps. Starting material **1** was prepared from *L*-tartaric acid. The reaction of (–)-dimethyl-2,3-*O*-isopropylidene-*L*-tartrate¹⁹ with *N,O*-dimethylhydroxylamine hydrochloride and AlMe₃ gave the 2-fold Weinreb amide in 85% yield.²⁰ The reaction of compound **1** with the lithium salt of THP-protected propargylic alcohol gave single substitution as the major product pathway. Using the magnesium salt **2** yielded **3** in 73% yield by clean 2-fold substitution. Initial attempts to continue the enediyne synthesis at the oxidation level of the diketone were not successful.²¹ Therefore, reduction was performed at this stage using DIBAL-H at –78 °C. The diol was obtained in good chemical yield but as an equal mixture of two out of the three possible diastereomers. To improve the diastereoselectivity of the reduction, several alternative reagents and conditions were tested, but the selectivity did not improve and chemical yields decreased. The isomeric mixture was carried through two further steps, TBDMS protection and THP deprotection, to facilitate separation and determination of configuration.²² Selective THP deprotection requires magnesium dibromide in diethyl ether as a reagent. Methanol/acidic ion-exchange resin or methanol/iodine²³ leads to partial TBDMS deprotection. The two isomers of compound **6** were separated by column chromatography on silica gel. ¹H and ¹³C NMR spectra of one isomer reveal a full set of resonances not reduced by symmetry, which corresponds to the nonsymmetric (1*R*,4*S*)-**6** isomer. The NMR spectra of the second

SCHEME 1. Synthesis of Protected Tetrahydroxy Enediyne **13**



isomer show a symmetry reduced half set of resonances, which agrees with (1*R*,4*R*)-**6** and (1*S*,4*S*)-**6** but allows no distinction. An X-ray structure analysis (see Supporting Information) of the compound confirmed its structure to be (1*R*,4*R*)-**6**. The pure isomers were taken separately through the following synthetic steps to complete the synthesis (only one isomer reaction is shown in Scheme 1). Oxidation of (1*R*,4*R*)-**6** using the Dess–Martin reagent gave dialdehyde (1*R*,4*R*)-**8** in good yield. A pinacol-type reaction was used in the next step for ring closure. The Pederson vanadium(II) reagent is suitable to mediate the reaction as shown in earlier reports,²⁴ because it favors the formation of the desired *cis*-diol,²⁵ presumably by a cyclic chelate transition state.²⁶ Diol (1*R*,4*R*)-**9** was

(24) Myers, A. G.; Dragovich, P. S. *J. Am. Chem. Soc.* **1992**, *114*, 5859–5860.

(25) Thiocarbonyl reagent reacts preferably with *cis*-diols. *trans*-Diols are converted slowly. Banfi, L.; Guanti, G.; Basso, A. *Eur. J. Org. Chem.* **2000**, 939–946.

(26) Konradi, A. W.; Pedersen, S. F. *J. Org. Chem.* **1992**, *57*, 28–32.

(13) (a) Evenzahav, A.; Turro, N. J. *J. Am. Chem. Soc.* **1998**, *120*, 1835–1841. (b) Jones, G. B.; Wright, J. M.; Plourde, G., II; Purohit, A. D.; Wyatt, K. J.; Hynd, G.; Fouad, F. *J. Am. Chem. Soc.* **2000**, *122*, 9872–9873.

(14) Pitsch, W.; Klein, M.; Zabel, M.; König, B. *J. Org. Chem.* **2002**, *67*, 6805–6807.

(15) For a recent report on the synthesis of β -lactam fused cyclic enediynes, see: Basak, A.; Mandal, S. *Tetrahedron Lett.* **2002**, *43*, 4241–4243.

(16) Unno, R.; Michishita, H.; Inagaki, H.; Suzuki, Y.; Baba, Y.; Jomori, T.; Nishikawa, T.; Isobe, M. *Bioorg. Med. Chem.* **1997**, *5*, 987–999.

(17) Comanita, B. M.; Heuft, M. A.; Rietveld, T.; Fallis, A. G. *Isr. J. Chem.* **2000**, *40*, 241–254.

(18) For the synthesis of a tetrahydroxy dienediyne, see: Toshima, K.; Yanagawa, K.; Ohta, K.; Kano, T.; Nakata, M. *Tetrahedron Lett.* **1994**, *10*, 1573–1576.

(19) Mash, E. A.; Nelson, K. A.; Van Deusen, S.; Hemperly, S. B. *Org. Synth.* **1990**, *68*, 92–103.

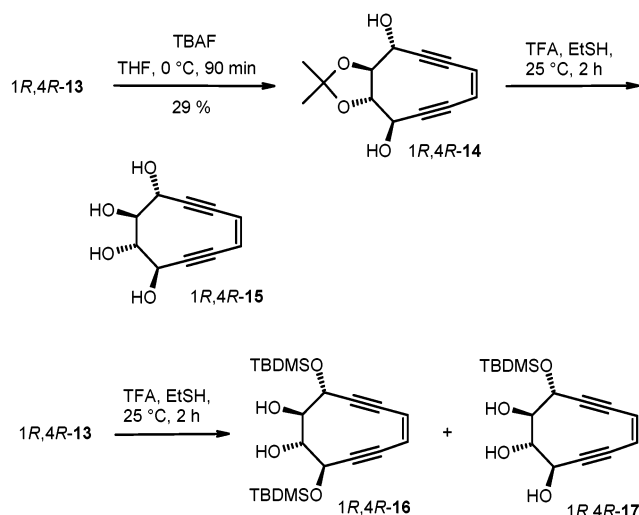
(20) Nugiel, D. A.; Jacobs, K.; Worley, T.; Patel, M.; Kaltenbach, R. F., III; Meyer, D. T.; Jadhav, P. K.; De Lucca, G. V.; Smyser, T. E.; Klabe, R. M.; Bacheler, L. T.; Rayner, M. M.; Seitz, S. P. *J. Med. Chem.* **1996**, *39*, 2156–2169.

(21) Diketodialdehyde analogous to compound **8** is not stable.

(22) Additional racemic stereocenters of the THP groups lead to a complex mixture of possible stereoisomers.

(23) Ramasamy, K. S.; Bandaru, R.; Averett, D. *Synth. Commun.* **1999**, *29*, 2881–2894.

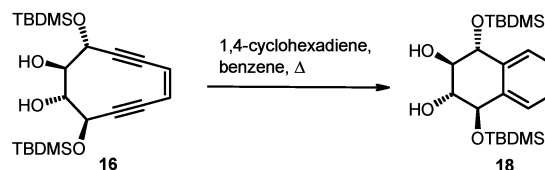
SCHEME 2. Deprotection of Eneidyne 13



obtained in 52% yield as a mixture of diastereomers, which were not separated but reacted with *N,N*-thiocarbonylbisimidazole (**10**) to give thiocarbonate (1*R,4R*)-**11**. The Corey–Hopkins elimination²⁷ is completed by treatment of the thiocarbonate with 1,3-dimethyl-2-phenyl-[1,3,2]diazaphospholidine (**12**) to give enediyne (1*R,4R*)-**13** in 41%. Analogously, compound (1*R,4S*)-**6** was converted into (1*R,4S*)-**13** in 15% overall yield (see Supporting Information for yields of the individual reactions).

To investigate the properties of enediyne **13**, the protecting groups have to be removed under mild conditions. Selective TBDMS deprotection is possible using standard conditions, but the high polarity of the product makes its isolation difficult and low yielding. The enediyne will become reactive after cleavage of the isopropylidene diol protecting group, so heating must be strictly avoided. Removal of the isopropylidene protecting group at room temperature requires treatment with EtSH and concentrated TFA. Under these conditions, a partial loss of the TBDMS groups cannot be avoided. The thermal reactivity of enediynes (1*R,4R*)-**13**, (1*R,4R*)-**16**, and (1*R,4R*)-**15** was investigated by thermolysis and monitored by HPLC analysis²⁸ to derive kinetic data. The cyclization of (1*R,4R*)-**16** was performed in benzene/cyclohexadiene at 69 °C, and the formation of the expected Bergman cyclization product was confirmed by HPLC-MS. Data analysis revealed pseudo-first-order kinetics with a reaction rate constant of $k = 6.4 \times 10^{-4} \text{ s}^{-1}$ and a half-life $t_{1/2} = 18 \text{ min}$. The isopropylidene-protected compound (1*R,4R*)-**13** showed no reaction under these conditions. Tetrahydroxy enediyne (1*R,4R*)-**15** is water soluble. Therefore, the thermal cyclization of this compound was investigated in aqueous solution. Again the data fit to pseudo-first-order kinetics with a rate constant of $k = 4.3 \times 10^{-3} \text{ s}^{-1}$ and a half-life $t_{1/2} = 3 \text{ min}$ at 60 °C.²⁹ The thermolysis data confirm that removal of

SCHEME 3. Thermal Cyclization of Eneidyne 16



the isopropylidene group transforms the stabilized enediynes into thermally reactive compounds. To investigate if the concept of enediyne stabilization and activation holds in vitro, the cytotoxicity of compounds (1*R,4R*)-**13**, (1*R,4R*)-**14**, (1*R,4R*)-**15**, and (1*R,4R*)-**16** was tested against human breast cancer cells MDA-MB-231 in the crystal violet assay³⁰ (see Supporting Information for data). Compounds (1*R,4R*)-**13** and (1*R,4R*)-**14**, stabilized by the isopropylidene protecting group, had no effect on cell growth, while for (1*R,4R*)-**15** and (1*R,4R*)-**16** a weak growth inhibition was observed. However, in comparison to cisplatin, a clinically widely used cytostatic, the inhibitory concentrations of enediynes **15** and **16** are high.

Conclusions

Cyclic tetrahydroxy enediynes were synthesized from tartaric acid in enantiomerically pure form. Each sp^3 -hybridized carbon of the compounds is functionalized. The hydroxy substituents make the compounds water soluble and at the same time lock the ring conformation and control enediyne reactivity by cyclic diol protecting groups. The preparation of enediynes, which can be activated by enzymes, light, or acid in vitro, can be envisaged, choosing a suitable protecting method.

Experimental Section

2,3-*O*-Isopropylidene-*L*-tartaric acid,¹⁷ 2,2-dimethyl-[1,3]-dioxolane-4,5-bis(methoxymethyl-carboxamide) **1**,¹⁸ vanadium tris(tetrahydrofuran) trichloride,³¹ and magnesiumbromide Et_2O^{32} were prepared according to reported procedures.

1-(2,2-Dimethyl-5-[4-(tetrahydropyran-2-yloxy)-but-2-ynyl]-[1,3]dioxolane-4-yl)-4-(tetrahydropyran-2-yloxy)-but-2-in-1-one (3). A mixture of 2-prop-2-ynyloxy-tetrahydropyran (3.22 g, 23.0 mmol) and EtMgBr , prepared from Mg turnings (0.61 g, 25.0 mmol) and bromoethane (2.51 g, 23.0 mmol) in 10 mL of dry THF, was refluxed for 1 h. After cooling to room temperature, the reaction mixture was added dropwise to a solution of 2,2-dimethyl-[1,3]-dioxolane-4,5-bis-(methoxymethylcarboxamide) **1** (1.00 g, 3.62 mmol) in 40 mL of THF, refluxed for 4 h, poured into aqueous KH_2PO_4 (100 mL), and extracted with EtOAc ($3 \times 50 \text{ mL}$). The combined organic phases were washed twice with brine, dried over Na_2SO_4 , and evaporated to dryness in vacuo. Column chromatography (petroleum ether/ EtOAc , 3:1) gave 1.15 g (73%) of **3** ($R_f = 0.32$) as a yellow oil. IR (neat): $\tilde{\nu} = 2944, 2872, 2216, 1688, 1386, 1345, 1241, 1122, 1033, 968, 941, 902, 871, 816 \text{ cm}^{-1}$. UV (CH_2 -

(27) Corey, E. J.; Hopkins, P. B. *Tetrahedron Lett.* **1982**, *23*, 1979–1982.

(28) Analytical method corresponds to previously described procedures: (a) Choy, N.; Kim, C.-S.; Ballester, C.; Artigas, L.; Diez, C.; Lichtenberger, F.; Shapiro, J.; Russell, K. C. *Tetrahedron Lett.* **2000**, *41*, 6955–6958. (b) Wisniewski Grissom, J.; Calkins, T. L.; McMillen, H. A.; Jiang, Y. *J. Org. Chem.* **1994**, *59*, 5833–5835. (c) Kim, C.-S.; Russell, K. C. *J. Org. Chem.* **1998**, *63*, 8229–8234.

(29) Janda et al. have observed the formation of quinones from thermal enediyne cyclization in oxygen-enriched water–DMSO mixtures. Jones, L. H.; Harwig, C. W.; Wentworth, P.; Simeonov, A.; Wentworth, A. D.; Py, S.; Ashley, J. A.; Lerner, R. A.; Janda, K. D. *J. Am. Chem. Soc.* **2001**, *123*, 3607–3608. We could observe neither quinone nor Bergman product formation. The poor hydrogen donor ability of water leads to a large extent of polymer formation.

(30) Bernhardt, G.; Reile, H.; Birnböck, H.; Spruss, T.; Schönenberger, H. *J. Cancer Res. Clin. Oncol.* **1992**, *118*, 35–43.

(31) Manzer, E. L. *Inorg. Synth.* **1982**, *21*, 135–137.

(32) Ashby, E. C.; Arnott, R. C. *J. Organomet. Chem.* **1968**, *14*, 1–11.

CN): λ_{\max} (lg ϵ) = 224 nm (4.114). $^1\text{H NMR}$: δ 1.40–1.88 (m, 18 H), 3.50–3.61 (m, 2 H), 3.76–3.90 (s, 2 H), 4.48 (m, 4 H), 4.84 (m, 4 H). $^{13}\text{C NMR}$: δ 18.8 (–), 25.2 (–), 26.8 (+), 30.0 (–), 53.7 (–), 62.0 (–), 82.5 (C_{quat}), 82.7 (+), 94.6 (C_{quat}), 97.2 (+), 114.6 (C_{quat}), 183.8 (C_{quat}). MS (CI), m/z (%): 85 (12) [DHP], 102 (70), 118 (100), 368 (7) [$\text{M} + \text{NH}_4 - \text{DHP}^+$], 453 (40) [$\text{M} + \text{NH}_4^+$]. Anal. Calcd for $\text{C}_{23}\text{H}_{30}\text{O}_8$ (434.49): C, 63.58; H, 6.96. Found: C, 63.18; H, 7.00.

1-[5-[1-Hydroxy-4-(tetrahydropyran-2-yloxy)but-2-ynyl]-2,2-dimethyl-[1,3]dioxolan-4-yl]-4-(tetrahydropyran-2-yloxy)but-2-in-1-ole ((1*R*,4*S*)-4, (1*R*,4*R*)-4). To a solution of **3** (5.40 g, 12.4 mmol) in THF (130 mL) was added DIBAL-H (50 mL, 50 mmol, 1 M solution in THF) at -78°C over 1 h. The reaction mixture was stirred for 24 h; methanol (25 mL) was added, and the solvent was removed in vacuo. The residue was redissolved in saturated K/Na tartrate solution (340 mL) and EtOAc (310 mL) and stirred for 30 min; the organic phase was separated, and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with brine and dried over Na_2SO_4 , and the solvent was removed in vacuo; column chromatography (petroleum ether/EtOAc, 1:1) yielded 4.31 g (79%) of **4** as a stereoisomeric mixture ($R_f = 0.45$), yellow oil. IR (neat): $\tilde{\nu} = 3415, 2942, 2871, 1738, 1455, 1373, 1243, 1119, 1025, 903, 872, 815\text{ cm}^{-1}$. $^1\text{H NMR}$: δ 1.34–1.92 (m, 18 H), 3.04 (s, 2 H), 3.45–3.61 (m, 2 H), 3.71–3.91 (m, 2 H), 4.10–4.43 (m, 6 H), 4.53–5.07 (m, 4 H). MS (CI), m/z (%): 85 (100) [DHP $^+$], 102 (49), 118 (85), 288 (10) [$\text{M} + \text{NH}_4 - 2\text{DHP}^+$], 372 (10) [$\text{M} + \text{NH}_4 - \text{DHP}^+$], 456 (17) [$\text{M} + \text{NH}_4^+$]. Anal. Calcd for $\text{C}_{23}\text{H}_{34}\text{O}_8$ (438.52): C, 63.00; H, 7.81. Found: C, 62.68; H, 7.65.

4,5-Bis-[1-(tert-butyl dimethylsilanyloxy)-4-(tetrahydropyran-2-yloxy)but-2-ynyl]-2,2-dimethyl-[1,3]dioxolane ((1*R*,4*S*)-5, (1*R*,4*R*)-5). Diol **4** (745 mg, 1.70 mmol), imidazole (748 mg, 11.0 mmol), and *tert*-butylchloro dimethylsilane (TBDMS-Cl, 640 mg, 4.25 mmol) were dissolved in 20 mL of CH_2Cl_2 and stirred for 16 h at room temperature. The reaction mixture was washed with aqueous NaHCO_3 and brine and dried over Na_2SO_4 , and the solvent was removed in vacuo. Column chromatography (petroleum ether/EtOAc, 4:1) gave 980 mg (87%) of **5** as a mixture of stereoisomers ($R_f = 0.52$), colorless oil. IR (neat): $\tilde{\nu} = 2931, 2857, 1742, 1464, 1370, 1253, 1217, 1121, 1078, 1027, 945, 903, 838, 779, 669\text{ cm}^{-1}$. $^1\text{H NMR}$: δ 0.06–0.19 (m, 12 H), 0.80–0.98 (m, 18 H), 1.36–1.96 (m, 18 H), 3.43–3.56 (m, 2 H), 3.75–3.89 (m, 2 H), 4.00–4.09 (m, 1 H), 4.14–4.22 (m, 1 H), 4.24–4.32 (m, 4 H), 4.58–4.69 (m, 2 H), 4.75–4.85 (m, 2 H). MS (CI), m/z (%): 85 (36) [DHP $^+$], 118 (19), 499 (11), 516 (9) [$\text{M} + \text{NH}_4 - 2\text{DHP}^+$], 600 (3) [$\text{M} + \text{NH}_4 - \text{DHP}^+$], 685 (100) [$\text{M} + \text{NH}_4^+$]. Anal. Calcd for $\text{C}_{35}\text{H}_{62}\text{O}_8\text{Si}_2$ (667.04): C, 63.02; H, 9.37. Found: C, 62.74; H, 9.56.

4-(tert-Butyldimethylsilanyloxy)-4-[5-[1-(tert-butyl dimethylsilanyloxy)-4-hydroxybut-2-ynyl]-2,2-dimethyl-[1,3]dioxolan-4-yl]but-2-in-1-ol ((1*R*,4*S*)-6 and (1*R*,4*R*)-6). Compound **5** (1.8 g, 2.7 mmol) and $\text{MgBr}_2 \cdot \text{Et}_2\text{O}$, prepared in situ from Mg turnings (0.58 g, 24 mmol) and 1,2-dibromoethane (1.8 mL, 3.93 g, 21 mmol), in dry diethyl ether (30 mL) was stirred at room temp for 24 h. The organic phase was washed with water (three times) and dried over Na_2SO_4 , and the solvent was removed in vacuo. Column chromatography gave two fractions: (1) (1*R*,4*S*)-**6** (0.52 mg, 39%) ($R_f = 0.39$), yellow oil, and (2) (1*R*,4*R*)-**6** (0.47 mg, 35%) ($R_f = 0.24$), colorless soft solid. (1*R*,4*S*)-**6**: $[\alpha]_{\text{D}}^{25} - 18$ (c 0.84 in CH_3CN). IR (neat): $\tilde{\nu} = 3406, 2931, 2896, 2858, 1742, 1253, 1135, 1072, 1026, 977, 940, 838, 779, 670\text{ cm}^{-1}$. $^1\text{H NMR}$: δ 0.13 (s, 3 H), 0.14 (s, 3 H), 0.16 (s, 3 H), 0.17 (s, 3 H), 0.92 (s, 18 H), 1.45 (s, 6 H), 1.91 (s, 2 H), 4.09 (dd, $^3J = 7.3\text{ Hz}$, $^2J = 3.2\text{ Hz}$, 1 H), 4.18 (dd, $^3J = 7.3\text{ Hz}$, $^2J = 4.0\text{ Hz}$, 1 H), 4.29 (d, $^5J = 1.7\text{ Hz}$, 2 H), 4.31 (d, $^5J = 1.7\text{ Hz}$, 2 H), 4.60 (dt, $^3J = 4.0\text{ Hz}$, $^5J = 1.7\text{ Hz}$, 1 H), 4.64 (dt, $^3J = 7.3\text{ Hz}$, $^5J = 1.7\text{ Hz}$, 1 H). $^{13}\text{C NMR}$: δ -5.0 (+), -4.8 (+), -4.6 (+), -4.4 (+), 18.3 (C_{quat}), 18.4 (C_{quat}), 25.8 (+), 25.9 (+), 27.3 (+), 27.6 (+), 51.1 (–), 51.2 (–), 63.6 (+), 64.2 (+), 79.9 (+), 80.5 (+), 84.1 (C_{quat}), 84.2 (C_{quat}), 84.3 (C_{quat}), 84.5 (C_{quat}), 110.6

(C_{quat}). MS (CI), m/z (%): 516.3 (100) [$\text{M} + \text{NH}_4^+$]. HRMS $\text{C}_{25}\text{H}_{47}\text{O}_6\text{Si}_2$ calcd 499.2911, found 499.2915 ± 0.5 ppm. (1*R*,4*R*)-**6**: Mp 116–118 $^\circ\text{C}$. IR (neat): $\tilde{\nu} = 3400, 2956, 2956, 2896, 2886, 2859, 2361, 2342, 1472, 1381, 1254, 1131, 1131, 1075, 1023, 838, 784\text{ cm}^{-1}$. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 0.12 (s, 6 H), 0.16 (s, 6 H), 0.91 (s, 18 H), 1.46 (s, 6 H), 2.35 (s, 2 H), 4.21 (dd, $^3J = 2.3\text{ Hz}$, $^2J = 1.3\text{ Hz}$, 2 H), 4.31 (d, $^5J = 1.7\text{ Hz}$, 2 H), 4.58–4.60 (m, 2 H). $^{13}\text{C NMR}$ (100 MHz, CDCl_3): δ -4.9 (+), -4.4 (+), 18.2 (C_{quat}), 25.8 (+), 27.6 (+), 51.0 (–), 64.1 (+), 80.2 (+), 84.3 (C_{quat}), 84.4 (C_{quat}), 111.0 (C_{quat}). MS (CI), m/z (%): 499 (4) [$\text{M} + \text{H}^+$], 516 (100) [$\text{M} + \text{NH}_4^+$]. Anal. Calcd for $\text{C}_{25}\text{H}_{46}\text{O}_6\text{Si}_2$ (498.81): C, 60.20; H, 9.30. Found C, 60.19; H, 9.47.

4-(tert-Butyldimethylsilanyloxy)-4-[5-[1-(tert-butyl dimethylsilanyloxy)-4-oxobut-2-ynyl]-2,2-dimethyl-[1,3]dioxolan-4-yl]but-2-inal ((1*R*,4*R*)-8). To a solution of (1*R*,4*R*)-**6** (498 mg, 1.00 mmol) in dry CH_2Cl_2 (20 mL) was added Dess–Martin periodinane (1.28 g, 3 mmol) at room temperature, and the suspension was stirred for 17 h. The reaction mixture was filtered, washed with aqueous $\text{Na}_2\text{S}_2\text{O}_5$ (100 mL, 0.4 M), and dried over Na_2SO_4 , and the solvent was removed in vacuo. Column chromatography (petroleum ether/EtOAc, 1:1) gave 425 mg (86%) of (1*R*,4*R*)-**8** ($R_f = 0.87$), as an oil. IR (neat): $\tilde{\nu} = 3054, 2987, 2360, 2411, 1671, 1422, 896, 740\text{ cm}^{-1}$. UV (CH_3CN): λ_{\max} (lg ϵ) = 218 nm (3.867). $^1\text{H NMR}$: δ 0.15 (s, 6 H), 0.20 (s, 6 H), 0.93 (s, 18 H), 1.48 (s, 6 H), 4.28 (dd, $^3J = 2.0\text{ Hz}$, $^2J = 1.2\text{ Hz}$, 2 H), 4.76–4.80 (m, 2 H), 9.26 (s, 2 H). $^{13}\text{C NMR}$: δ -4.9 (+), -4.5 (+), 18.2 (C_{quat}), 25.7 (+), 27.3 (+), 64.0 (+) 79.2 (+), 84.8 (C_{quat}), 93.9 (C_{quat}), 111.7 (C_{quat}), 176.1 (+). MS (CI), m/z (%): 115 (33), 117 (26), 133 (49), 173 (11), 197 (18), 205 (11), 269 (12), 297 (11), 305 (10), 437 (14), 495 (100) [$\text{M} + \text{H}^+$], 296 (37). Anal. Calcd for $\text{C}_{25}\text{H}_{42}\text{O}_6\text{Si}_2$ (494.78): C, 60.69; H, 8.56. Found: C, 59.61; H, 8.54.

2,9-Bis-(tert-butyl dimethylsilanyloxy)-12,12-dimethyl-5,6-dihydroxy-11,13-dioxobicyclo-[8.3.0]trideca-3,7-diyne ((1*R*,4*R*)-9). To dry CH_2Cl_2 (12 mL) were added $\text{VCl}_3 \cdot 3\text{THF}$ (1.37 g, 3.7 mmol) and zinc powder (160 mg, 2.46 mmol) at room temperature, and the mixture was stirred for 30 min and diluted with CH_2Cl_2 (12 mL) and DMF (1.4 mL). Compound (1*R*,4*R*)-**8** (236 mg, 0.48 mmol), dissolved in CH_2Cl_2 (12 mL), was added by syringe pump over 120 min. The mixture was stirred for an additional 4 h and poured into water (30 mL); the organic phase was separated and washed with aqueous Na/K tartrate (40%) and brine. The solvent was removed in vacuo and the crude product purified by column chromatography (petroleum ether/EtOAc, 1:1) to yield 124 mg (52%) of (1*R*,4*R*)-**9** ($R_f = 0.91$), colorless solid. IR (neat): $\tilde{\nu} = 3380, 2931, 2896, 2858, 2251, 1739, 1473, 1372, 1255, 1109, 839, 780\text{ cm}^{-1}$. $^1\text{H NMR}$: δ -0.04–0.26 (m, 12 H), 0.76–1.03 (m, 18 H), 1.39 (m, 6 H), 4.02–4.17 (m, 4 H), 4.21–4.39 (m, 4 H), 4.41–4.64 (m, 2 H). MS (FI/ED), m/z (%): 439 (100) [$\text{M} + (\text{CH}_3)_2\text{CO}^+$], 497 (68) [$\text{M} + \text{H}^+$].

2,9-Bis-(tert-butyl dimethylsilanyloxy)-12,12-dimethyl-5,6-(thiocarbonyldioxy)-11,13-dioxo-bicyclo-[8.3.0]trideca-3,7-diyne ((1*R*,4*R*)-11). A mixture of diol (1*R*,4*R*)-**9** (73 mg, 0.15 mmol) and *N,N*-thiocarbonyldiimidazole (**10**) (79 mg, 0.42 mmol) in 20 mL of dry THF was refluxed for 6 h. The solvent was removed in vacuo, and the crude product was purified by column chromatography (petroleum ether/EtOAc, 5:1) yielding 56 mg (71%) of (1*R*,4*R*)-**11** ($R_f = 0.61$), yellow oil. IR (neat): $\tilde{\nu} = 2956, 2858, 2240, 1825, 1727, 1472, 1347, 1168, 983, 844, 781, 670\text{ cm}^{-1}$. UV (CH_3CN): λ_{\max} (lg ϵ) = 237 nm (4.031). $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 0.13 (s, 6 H), 0.14 (s, 3 H), 0.15 (s, 3 H), 0.91 (s, 9 H), 0.92 (s, 9 H), 1.39 (s, 3 H), 1.40 (s, 3 H), 3.99 (dd, $^3J = 9.0\text{ Hz}$, $^2J = 5.4\text{ Hz}$, 1 H), 4.12 (dd, $^3J = 9.0\text{ Hz}$, $^2J = 5.4\text{ Hz}$, 1 H), 4.32 (dd, $^3J = 9.0\text{ Hz}$, $^5J = 2.0\text{ Hz}$, 1 H), 4.37 (d, $^3J = 9.0\text{ Hz}$, 1 H), 5.56 (dd, $^3J = 8.0\text{ Hz}$, $^5J = 2.0\text{ Hz}$, 1 H), 5.58 (d, $^3J = 8.0\text{ Hz}$, 1 H). $^{13}\text{C NMR}$ (100 MHz, CDCl_3): δ -4.8 (+), -4.8 (+), -4.7 (+), 18.3 (C_{quat}), 25.7 (+), 27.6 (+), 66.6 (+), 66.7 (+), 74.6 (+), 74.8 (+), 82.4 (+), 82.6 (+), 93.3 (C_{quat}), 94.3 (C_{quat}), 110.9 (C_{quat}), 188.7 (C_{quat}). MS (CI), m/z (%): 556 (100) [$\text{M} + \text{NH}_4^+$].

2,9-Bis-(*tert*-butyldimethylsilyloxy)-12,12-dimethyl-11,13-dioxo-bicyclo-[8.3.0]trideca-5-en-3,7-diyne ((1*R*,4*R*)-13**).** To a solution of (1*R*,4*R*)-**11** (40 mg, 74.2 μ mol) in 4 mL of dry dioxane was added 1,3-dimethyl-2-phenyl-1,3,2-diazaphospholidine (**12**, 96 μ L, 101 mg, 520 μ mol), and the reaction mixture was stirred for 4 h at 40 °C. The solvent was removed in vacuo and column chromatography (petroleum ether/EtOAc, 5:1) was performed on the crude product. Yield: 14 mg (41%) of (1*R*,4*R*)-**13** (R_f = 0.95), colorless oil. $[\alpha]_D^{25}$ = -79 (c 0.67 in CH₃CN). IR (neat): $\tilde{\nu}$ = 2928, 2856, 1824, 1731, 1463, 1378, 1332, 1259, 1109, 1018, 840, 780, 752 cm⁻¹. UV (CH₃CN): λ_{\max} ($\lg \epsilon$) = 271 nm (4.021). ¹H NMR (400 MHz, CDCl₃): δ 0.14 (s, 6 H), 0.15 (s, 6 H), 0.91–0.99 (m, 18 H), 1.42 (s, 6 H), 4.10–4.15 (m, 2 H), 4.41–4.46 (m, 2 H), 5.94 (s, 2 H). ¹³C NMR (100 MHz, CDCl₃): δ -4.7 (+), -4.7 (+), 18.4 (C_{quat}), 25.8 (+), 28.0 (+), 67.2 (+), 84.5 (+), 86.7 (C_{quat}), 100.3 (C_{quat}), 110.5 (C_{quat}), 123.4 (+). MS (EI, 70 eV), m/z (%): 73 (100), 75 (24) [Me₂-SiOH⁺], 347 (88) [M⁺ - Me₂SiC₄H₉], 348 (25), 462 (8) [M⁺]. HRMS C₂₅H₄₂O₄Si₂ calcd 462.2621, found 462.2621 \pm 1.5 ppm.

2,3-O-Isopropylidencyclodec-7-en-5,9-diyne-1,2,3,4-tetraol ((1*R*,4*R*)-14**).** To a solution of (1*R*,4*R*)-**13** (110 mg, 0.24 mmol) in 20 mL of THF was added TBAF (1.1 mL, 1.1 mmol, 1 M solution in THF), and the mixture was stirred for 90 min at 0 °C, diluted with diethyl ether, washed with brine (three times), and dried over Na₂SO₄. The solvent was removed in vacuo, and column chromatography (petroleum ether/EtOAc, 1:1) gave 16 mg (29%) of (1*R*,4*R*)-**14** (R_f = 0.32), colorless oil. IR (neat): $\tilde{\nu}$ = 3996, 3429, 3428, 2884, 1807, 1730, 1639, 1467, 1354, 1280, 1114, 947, 843 cm⁻¹. UV (CH₃CN): λ_{\max} ($\lg \epsilon$) = 269 nm (3.926). ¹H NMR (400 MHz, CD₃CN): δ 1.41 (s, 6 H), 3.67 (dd, ³ J = 4.6 Hz, 2 H), 4.00–4.05 (m, 2 H), 4.44–4.50 (m,

2 H), 6.05 (s, 2 H). ¹³C NMR (100 MHz, CD₃CN): δ 28.0 (C12, C13), 66.7 (C1, C4), 85.2 (C2, C3), 87.6 (C6, C9), 100.5 (C5, C10), 111.7 (C11), 124.6 (C7, C8). MS (CI), m/z (%): 234 (28) [M⁺ - H₂O], 252 (100) [M + NH₄⁺]. HRMS C₁₃H₁₅O₄ calcd 235.0970, found 235.0969 \pm 1.5 ppm.

General Procedure for Cleavage of the Isopropylidene Protecting Group. Eneidyne (15 μ mol) to be deprotected was dissolved in 1 mL of EtSH and 1 mL of concentrated TFA. The reaction mixture was stirred for 1 h at room temperature; the solvents were removed in vacuo at 0 °C, and the reaction products were used without further purification.

Acknowledgment. We thank the Fonds der Chemischen Industrie, the Deutsche Forschungsgemeinschaft (GRK 760 "Medicinal Chemistry"), and the DAAD (International Quality Network Medicinal Chemistry) for support of this research.

Supporting Information Available: Structure of compound (1*R*,4*R*)-**6** in crystalline form; experimental procedures for the preparation of compound (1*R*,4*S*)-**13**; kinetic analyses of the thermolysis of (1*R*,4*R*)-**16** and (1*R*,4*R*)-**15**; in vitro cytotoxicity cell assay; copies of proton and carbon NMR spectra of compounds **3**, (1*R*,4*S*)-**6**, (1*R*,4*R*)-**6**, (1*R*,4*S*)-**8**, (1*R*,4*R*)-**13**, (1*R*,4*S*)-**13**, and (1*R*,4*R*)-**14**; and copies of proton NMR spectra of compounds **4**, **5**, (1*R*,4*S*)-**9**, (1*R*,4*R*)-**9**, (1*R*,4*S*)-**11**, and (1*R*,4*R*)-**11**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

JO035250N