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SYNTHESIS AND CYTOTOXICITY OF THE ACYCLIC (E)- AND (Z)-DIENEDIYNE SYSTEMS RELATED TO NEOCARZINOSTATINE CHROMOPHORE

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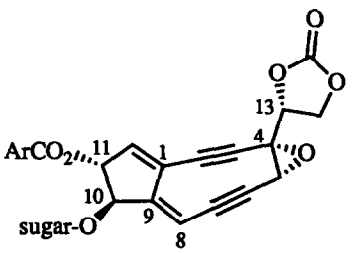
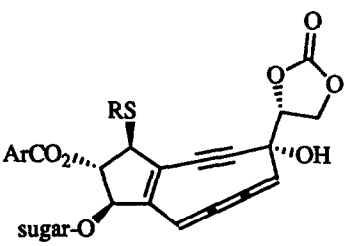
Summary: Synthesis of the title compounds could be achieved by featuring the Pd-catalyzed coupling reaction of stereo-defined (E)-and (Z)-enol triflates with an acetylene derivative. It was found that the acyclic (Z)-dienediyne system obviously exhibits *in vitro* cytotoxicity against P388 murine leukemia stronger than that for the corresponding (E)-isomer.

Neocarzinostatine chromophore **1** (NCS-Chr),[2,3] which is responsible for the antitumor activity of antibiotic neocarzinostatine[4] consisting of apo-protein (apo-NCS) and **1**, has attracted much attention because of its notable bicyclo[7.3.0]dodecadienediyne system[5] and strong DNA-cleaving activity.[6] The proposed mechanism of its DNA-cleaving action is based on generation of a reactive biradical species from the dienediyne system by way of the Bergman-type cyclization of the eneynecumulene **2**.[7] Recently, Myers[8] and Saito[9] independently disclosed generation of a biradical species from simple acyclic eneyneallenes. Their results clearly demonstrated that not only strain energy but also ring system involved in **1** may not be responsible for generating a reactive biradical species.

In connection with the remarkable mechanism of **1** to produce a biradical species, we were interested in the



NCS-Chr **1**



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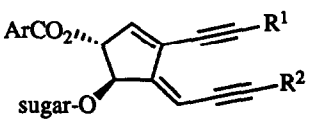

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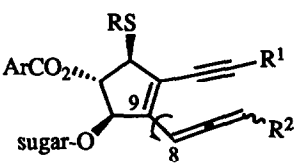

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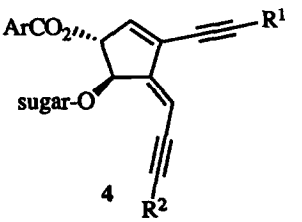

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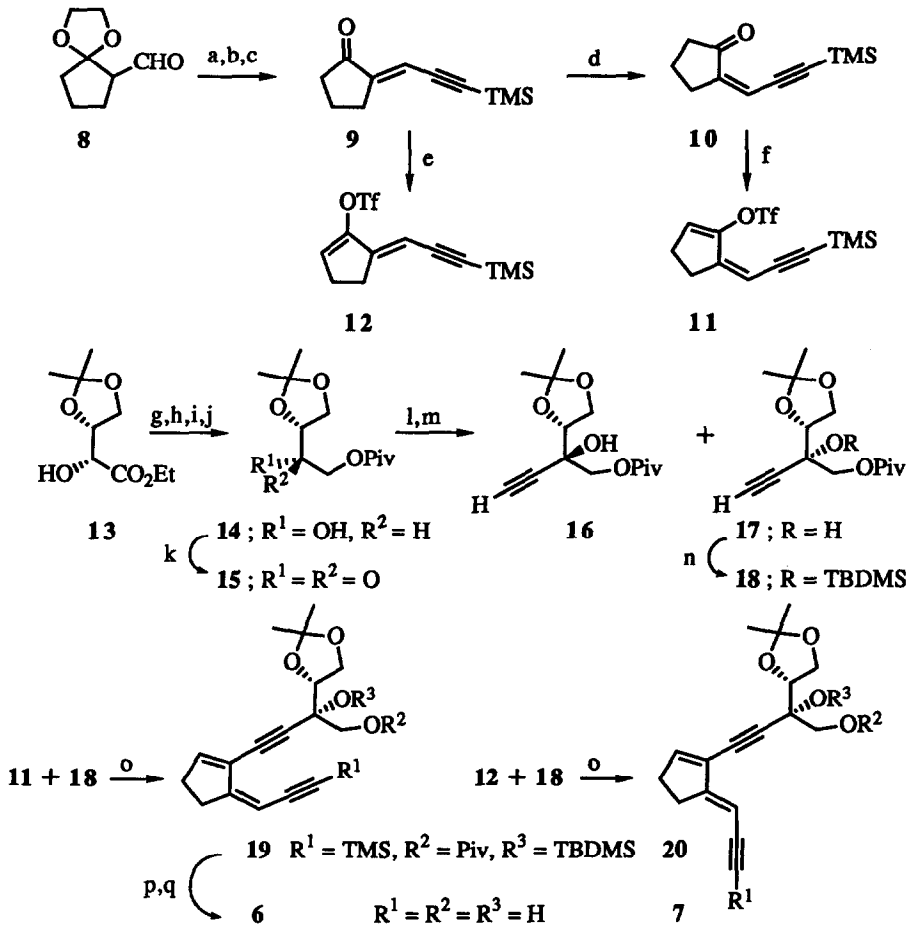

a) LiC≡CTMS, THF, -78°C b) PPTS, acetone, H_2O, reflux c) MsCl, Et_3N, CH_2Cl_2, 67% (3 steps) d) hv (254nm) acetone, 36% (the recovery of **9**, 55%) e) Tf_2O, 2,6-di-^{t}Bu-4-MePy, CH_2Cl_2, 80% f) LDA, Tf_2NPh, THF, 80% g) EVE, PPTS, CH_2Cl_2 h) LAH, ether i) PivCl, Py j) PPTS, MeOH, 79% (4 steps) k) PDC, 4A-MS, CH_2Cl_2, 86% l) LiC≡CTMS, TMEDA, THF, -78°C m) TBAF, THF, 60% (**16**) and 32% (**17**) (2 steps) n) TBDMSOTf, 2,6-Lu, CH_2Cl_2, 59% o) $Pd(PPh_3)_4$, CuI, Et_2NH, DMF, 73% (**19**), 86% (**20**) p) DIBAL-H, CH_2Cl_2 q) TBAF, THF, 61% (**6**) (2 steps), 71% (**7**) (2 steps)

difference of cytotoxicity between the acyclic (E)-and (Z)-dienediyne systems related to **1** (e.g. **3** and **4**). Since **3** and **4** can produce the same intermediate (e.g. **5**) after the initial activation proposed for **1**, they might show comparable cytotoxicity to each other.

We wish to report the synthesis and cytotoxicity of acyclic (Z)-and (E)-dienediyne systems, **6** and **7**, corresponding to **3** and **4**, respectively.[10] Contrary to our expectation, since **6** was found to exhibit stronger

cytotoxicity than **7**, it appeared that the stereochemistry of the C_8-C_9 double bond (the NCS-Chr numbering) may play an important role for the cytotoxicity of acyclic analogues of **1**.

The key to the successful preparation of both **6** and **7** was anticipated to be the Pd-catalyzed coupling reaction of the stereo-defined (Z)-and (E)-enol triflates, **11** and **12**, with the optically active acetylene **18** which involves the correct absolute configuration of **1** proposed by Myers.[7b] Towards this end, the syntheses of **11**, **12** and **18** were first attempted.

Thus, addition of lithium trimethylsilylacetylide to the readily available aldehyde **8**[11] provided the propagylic alcohol as a diastereomeric mixture, which without separation was converted to the (E)-ketoeneyne **9** by sequential hydrolysis and dehydration. Irradiation of **9** smoothly effected photo-induced isomerization of the exocyclic olefinic bond, affording the (Z)-ketoeneyne **10** along with recovered **9** (55% recovery yield). Successive treatments of **10** with LDA and N-phenyltrifluorosulufonimide[12] readily produced the (Z)-enol triflate **11**, while **9** could be derived to the (E)-enol triflate **12** by a combined use of triflic anhydride and 2,6-di-*tert*-butyl-4-methylpyridine.[13] The 400MHz ^{1}H-NMR spectra clearly established that more than 95% of stereochemical integrities were kept at the stages of **11** and **12**.[14]

The optically active acetylene **18** with 4(S)- and 13(R)-configuration (the NCS-Chr numbering) could be prepared in a straight forward manner. Thus, the pivaloate **14** obtained from the hydroxy ester **13**[15] in 4 steps was oxidized with PDC to give the ketone **15**.[16] Addition of lithium trimethylsilylacetylide to **15** followed by desilylation and separation by a silica gel column produced the diastereomeric acetylenes **16** and **17** in 60 and 32% yields, respectively.[17] The single crystal X-ray analysis of **17**[18] confirmed the stereochemistries of both isomers as depicted. The tertiary alcohol of **17** was protected in a form of the TBDMS ether to give the acetylene **18**.[19]

The key coupling reaction of **11** and **18** proceeded smoothly, giving rise to the (Z)-dienediyne **19**. Stepwise deprotections of **19** furnished **6**[20] as a fairly unstable solid. By employing the same reaction sequences, **7**[21] could be produced from **12** and **18** as a solid by way of the protected (E)-dienediyne **20**. The acyclic (Z)- and (E)-dienediyne systems (**6** and **7**) were next subjected to *in vitro* cytotoxicity assay against P388 murine leukemia. The IC_{50} values of 3.1×10^{-2}mM (8.9μg/ml) (adriamycin: IC_{50} 3×10^{-6}mM) and $>10^{-1}$mM (adriamycin: IC_{50} 1×10^{-6}mM) were recorded for **6** and **7**, respectively.[22] Correcting the cytotoxicity of adriamycin used as a reference compound, the cytotoxicity of **6** was found to be at least 10 times stronger than that of **7**. It is noteworthy that the difference of cytotoxicity of **6** and **7** was caused only by their stereochemistries at the C_8-C_9 double bond although the cytotoxicity of **6** was relatively weak.

Thus, we have succeeded in developing an efficient synthetic scheme to the acyclic (Z)-and (E)-dienediynes such as **6** and **7** and in disclosing that the stereochemistry of the C_8-C_9 double bond may play an important role for the cytotoxicity of acyclic analogues of **1**. From the viewpoint to explore prominent anticancer agents, one of the goals in the synthesis and testing of analogues of **1** is the separation of antitumor activity from extreme chemical instability since a stable NCS-Chr analogue may be utilized without the risks inherent in clinical uses of the peptide derived from microorganisms. Taking into account these aspects, our findings may have values for designing various structural types of NCS-Chr analogues.

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14. The structure of **11** and **12** could be rigorously established by comparing their ^{1}H-NMR spectra.
    11: ^{1}H-NMR($CDCl_3$) δ = 0.18(s, 9H), 2.53(m, 2H), 2.72(m, 2H), 5.49(m, 1H), 6.23(m, 1H).
    12: ^{1}H-NMR($CDCl_3$) δ = 0.20(s, 9H), 2.60(m, 2H), 2.81(m, 2H), 5.52(m, 1H), 6.17(m, 1H).
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16. This sample showed $[\alpha]_D^{20}$ +91.8° (C=0.82, $CHCl_3$). The synthesis of ent-**15** has been reported although its optical purity was not specified. Marco, J. L. *J. Chem. Research (S)* **1988**, 276.
17. The observed ratio of **16** to **17** which was the best among those so far recorded, was obtained by employing TMEDA (1 eq.) as an additive. Direct conversion of **15** into **17** by using lithium acetylide resulted in the predominant formation of undesired **16**.
18. This sample showed $[\alpha]_D^{20}$ +18.0° (C=1.03, $CHCl_3$). The optical purity of **17** was determined to be more than 98%e.e. by the 400MHz ^{1}H-NMR spectra of (R)- and (S)-MTPA esters derived from **17** by sequential reductive removal of the pivaloyl group and acylations with (R)- and (S)-MTPA chloride.
19. The isomer of **18** in which pivaloyl and TBDMS groups were transposed was obtained in 32% yield.
20. $[\alpha]_D^{20}$ +16.2° (C=0.38, MeOH). ^{1}H-NMR($CDCl_3$) δ = 1.37, 1.48(s×2, 3H×2), 2.24(dd, 1H, J=9.0, 5.3Hz, C_5-OH), 2.54(m, 2H, C_{11}-CH_2), 2.58(m, 2H, C_{10}-CH_2), 2.93(s, 1H, C_4-OH), 3.15(s, 1H, C_6-CH), 3.72(dd, 1H, J=11.3, 8.9Hz, C_5-CH), 3.86(dd, 1H, J=11.4, 5.1Hz, C_5-CH), 4.16(dd, 1H, J=8.4, 7.1Hz), 4.19(dd, 1H, J=8.5, 5.7Hz), 4.23(dd, 1H, J=7.1, 5.7Hz), 5.45(m, 1H, C_8-CH), 6.67(m, 1H, C_{12}-CH). HRMS calculated for $C_{16}H_{17}O_4$ [$(M\text{-}Me)^+$]; 273.1125, found; 273.1133.
21. $[\alpha]_D^{20}$ +46.3° (C=0.19, MeOH). ^{1}H-NMR($CDCl_3$) δ = 1.37, 1.47(s×2, 3H×2), 2.14(dd, 1H, J=7.7, 6.0Hz, C_5-OH), 2.61(m, 2H, C_{11}-CH_2), 2.73(m, 2H, C_{10}-CH_2), 2.83(s, 1H, C_4-OH), 3.22(s, 1H, C_6-CH), 3.79(dd, 1H, J=11.4, 7.7Hz, C_5-CH), 3.84(dd, 1H, J=11.4, 5.9Hz, C_5-CH), 4.14(dd, 1H, J=8.6, 6.8Hz), 4.17(dd, 1H, J=8.6, 6.0Hz), 4.24(t, 1H, J=6.4Hz), 5.63(m, 1H, C_8-CH), 6.57(m, 1H, C_{12}-CH). HRMS calculated for $C_{17}H_{20}O_4$ (M^+); 288.1360, found; 288.1373.

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