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Synthesis and Biological Evaluation of a Bicyclo[7.4.1]enediyne

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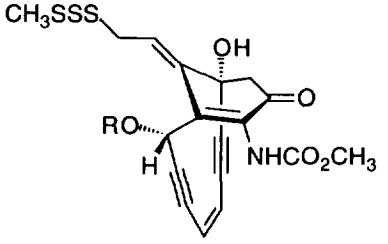
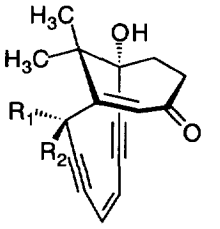
Abstract: The synthesis of bicyclo[7.4.1]enediynes **2a** and **2b** are described. The bicyclo[7.4.1]enediyne **2b** was found to be a poor DNA cleaver but did cause damage to several protein isolates.

Since the discovery of calicheamicin (**1a**)[1] and esperamicin[2] in 1987 an avalanche of reports describing investigations related to the synthesis, biological activity and mode of action of various enediyne containing compounds have appeared.[3] Propelling this research effort has been the identification of structurally novel enediyne antibiotics as well as the potential for developing therapeutically useful agents. The latter objective has required a multidisciplinary approach and has provided insight into the unique mode of action of these antitumor agents. As has often been noted, the cytotoxicity of enediyne antibiotics is associated with their ability to induce DNA strand cleavage by way of chemical activation leading to the generation of a reactive biradical intermediate.



calicheamicin (R = sugar) **1a**
calicheamicinone (R = H) **1b**

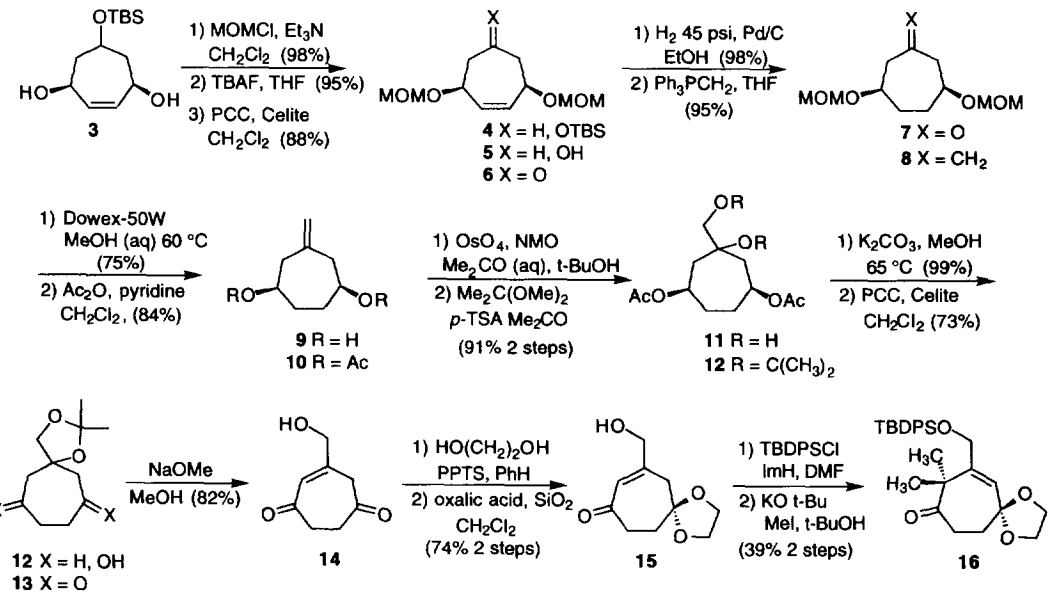


2a R_1 = OH; R_2 = H
2b R_1 = H; R_2 = OH

A great deal of effort has been directed towards synthesizing unnatural enediynes with enhanced DNA cleaving potency and/or selectivity.[4] However, increased DNA cleaving potency does not always correlate with increased cytotoxicity.[4g] Two approaches which have meet with varying degrees of success have been to device unique triggering mechanisms[4a-e] and to conjugate the biradical precursor to a DNA recognition element.[4f,g] We reasoned that expansion of the bicyclic[7.3.1]enediyne core structure common to calicheamicinone (**1b**) to a bicyclo[7.4.1]enediyne structure (cf. **2**) may lead to new opportunities for triggering biradical formation and/or altered DNA binding selectivity. During the course of our investigations Maier and Langenbacher reported the synthesis of a bicyclo[7.4.1]enediyne which spontaneously underwent a Bergman cyclization at room temperature.[5] Also included in this report were calculations suggesting the barrier to cyclization to be dependent upon the hybridization of atoms within the ring system. Herein we describe the synthesis and biological activity of bicyclo[7.4.1]enediyne **2**.

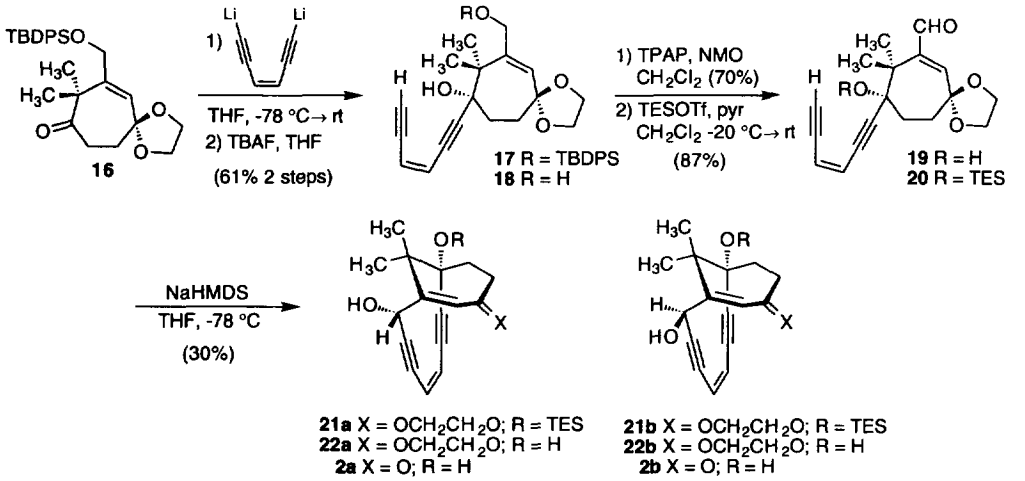
Our starting material for the synthesis of **2** is cycloheptendiol **3** available in four steps from tropone.[6,7] First, the hydroxyl groups of diol **3** were protected as the corresponding MOM ethers. Removal of the unique TBS group and oxidation of the resulting secondary alcohol afforded ketone **6** in excellent overall yield. Hydrogenation of **6** followed by methylenation of the keto group then provided alkene **8** (93%). Our synthetic scheme required at this point replacement of the MOM ether protecting groups with acetyl groups (**8**→**10**). Next, treatment of diacetate **10** with catalytic osmium tetroxide followed by acetonide formation afforded **11** in 91% yield.[8] Removal of the acetyl groups followed by PCC oxidation generated diketone **13** which on exposure to methanolic sodium methoxide gave cycloheptendione **14** (82%). Bis-ketalization of diketone **14** followed by acid hydrolysis led to the production of mono-ketal **15**. Protection of the allylic hydroxyl group followed by methylation afforded ketone **16** appropriately functionalized for the installation of the enediyne component.

Scheme 1



The addition of bislithioenediyne (Scheme 2) to substrates possessing the equivalent of a bis-electrophile has proven to be among the most useful approaches to assembling the calicheamicin as well as other enediyne core structures.[9] We also chose to take advantage of this strategy in the present construct. In the event, addition of **16** to a solution of bislithioenediyne in THF, produced adduct **17** in 65% yield.[9a] Desilylation (TBAF, THF) of **17** followed by oxidation produced aldehyde **19**. Next, in preparation for the intramolecular acetylide addition, the tertiary propargyl alcohol was silylated to afford silyl ether **20**. Addition of **20** to a solution of NaHMDS at -78 °C followed by an acidic quench produced an approximate 1:1 mixture of epimeric alcohols **21a** and **21b**. Alcohols **21a** and **21b** were separated by flash chromatography and individually deprotected to afford **2a** and **2b** in 47 and 40% yield, respectively.[10]

Scheme 2



The bicyclo[7.4.1]enediyne **2b** was evaluated for its ability to induce DNA cleavage and interact with protein isolates in vitro. As summarized in Table 1, **2b** was found to be relatively ineffective in cleaving DNA, both in the presence and absence of added thiol. Furthermore, **2b** was weakly cytotoxic against human colon carcinoma cells. For comparison purposes, the corresponding activity of BMS-46108 is also shown in Table 1.[11] The reaction of **2b** with histones H1 and H4, with HCT-116 cell extract and calf brain microtubulin was also examined. These studies found **2b** to agglomerate tubulin, cell membrane extracts and histone H4 in a manner similar to that previously observed for BMS-46108 but to a lesser extent at 1 mg/mL drug concentration.[11] Finally, unlike BMS-46108, **2b** did not damage histone H1.

Table 1. Summary of Biological Data

Compound	IC_{50} (HCT-116)	pM2 DNA[a] Cleavage
2b	18 μg/mL	with β-SH * no β-SH *
BMS -46108	0.02 μg/mL (1.0×10^{-7} M)	with β-SH ** no β-SH *

[a]X = Drug concentration which cleaves 50% of the supercoiled form of pM2 DNA. If $1 \times 10^{-6} < X < 1 \times 10^{-5}$ M *** then good DNA cutter. If $1 \times 10^{-5} < X < 1 \times 10^{-4}$ M ** then O.K. DNA cutter. If $1 \times 10^{-3} < X$* then poor DNA cutter.

In summary, we have found that enediyne **2b** exhibited poor DNA cleavage properties in the presence of added thiol but induced the formation of various protein agglomerates. The synthesis of new bicyclo[7.4.1]enediynes equipped with triggering devices is currently under investigation.[12]

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References and Notes

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7. The structure assigned to each new compound was in accord with its ^{1}H and ^{13}C NMR (200 and 50 MHz respectively) spectra.
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10. **2a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.94 (s, 1H), 5.89 (d, J = 9.6 Hz, 1H), 5.82 (dd, J = 9.6, 1.5 Hz, 1H), 5.25 (d, J = 3.2 Hz), 2.78 (m, 1H), 2.61 (m, 1H), 2.29 (m, 2H), 2.16 (br s, 1H), 2.13 (d, J = 4.7 Hz, 1H), 1.85 (s, 3H), 1.48 (s, 3H).
2b: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.38 (s, 1H), 5.90 (dd, J = 9.6, 0.8 Hz, 1H), 5.84 (dd, J = 9.6, 0.8 Hz, 1H), 5.28 (d, J = 3.9 Hz), 2.94 (dt, J = 13.5, 4.7 Hz, 1H), 2.54 (m, 1H), 2.43 (td, J = 14.0, 3.2 Hz, 1H), 2.17 (td, J = 10.1, 5.0 Hz, 1H), 2.10 (s, 1H), 2.07 (d, J = 4.7 Hz, 1H), 1.57 (s, 3H), 1.49 (s, 3H).
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12. To date, we have been unable to induce **2b** to undergo a Bergman type cycloaromatization. For example, exposure of **2b** to one equivalent of methyl thioglycolate in a 1:1 mixture of THF and pH 7.4 phosphate buffer led to no reaction at room temperature while temperature elevation (50 °C) resulted in decomposition. Currently we are investigating methods of intramolecular nucleophilic activation of **2b**.

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