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ON THE MECHANISM OF ACTIVATION OF DESIGNED ENEDIYNES WITH SELECTIVE CYTOTOXICITY

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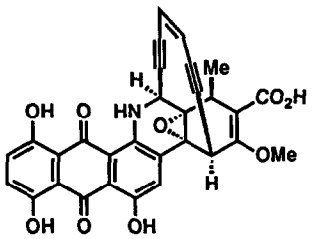
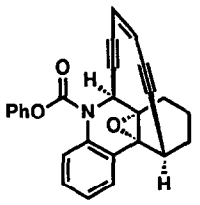
Abstract: *A number of novel enediyne compounds such as* **3** *equipped with a 2-(phenylsulfonyl)ethoxycarbonyl protecting group on the nitrogen atom have demonstrated selective cytotoxicity against a variety of cancer cell lines. Compounds* **4-6** *possessing one or two methyl group(s) at the C2 position of the sulfone residue have been synthesized and tested, showing reduced cytotoxity as compared to* **3**, *suggesting that a β-elimination mechanism as the main initial step for the activation of these cytotoxic agents. The differential in the cytotoxicity of compounds* **4** *and* **5** *with opposite chirality at C2 suggests the possible existence of tumor-associated factors in certain tumor cells that may activate these systems selectively.*

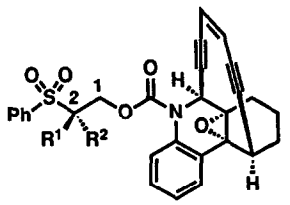
The rapidly growing interest in the chemistry and biology of enediyne anticancer antibiotics[1] has stimulated extensive research activities in both the areas of mechanism of action and synthesis of model systems to mimic the action of the naturally occurring compounds. Dynemicin A (**1**) isolated from the fermentation broth of *Micromonospora chersina* is the most recent addition to this family of natural products.[2] Its unique molecular architecture features a hybrid structure of anthraquinone and enediyne



1: dynemicin A



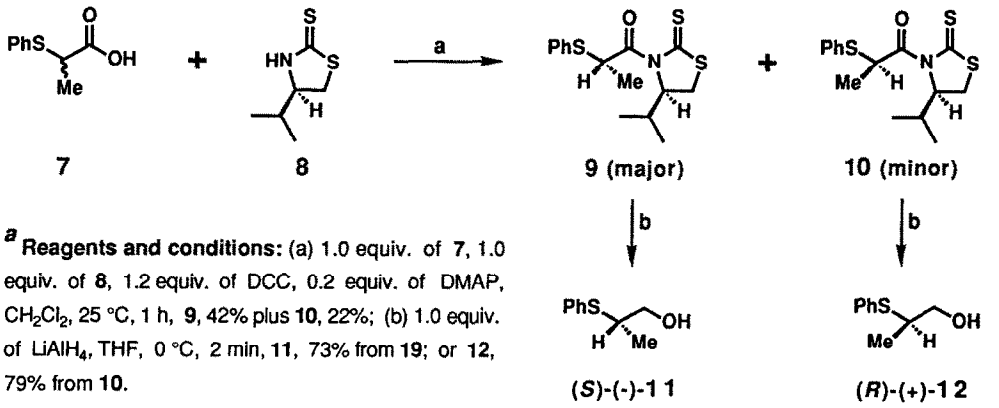
(±)-2



3: $R^1 = R^2 = H$
4: $R^1 = H$, $R^2 = Me$
5: $R^1 = Me$, $R^2 = H$
6: $R^1 = R^2 = Me$

moieties, and a mode of action involving bioreduction followed by Bergman cycloaromatization has been proposed.[3] Reports from these laboratories include the synthesis of model compounds of dynemicin A[4] (such as **2**); the simulation of the dynemicin A reaction cascade,[3c] and biological studies of designed enediynes with various initiators and tethering groups.[5] Among the compounds studied, those bearing a 2-(phenylsulfonyl)ethoxycarbonyl protecting group on the nitrogen atom, such as **3**, demonstrated selective cytotoxicity in cell cultures.[5] Preliminary studies supported a β-elimination[6] of phenyl vinyl sulfone accompanied by release of CO_2 served as the initial steps of activation.[5] In this article we disclose the synthesis and biological activity of compounds **4-6** possessing one or two methyl group(s) at the C2 position of the sulfone residue and provide further support for the β-elimination mechanism and the possibility of the involvement of tumor-associated factors or receptors in the mechanism of activation of these systems.

Scheme 1[a]

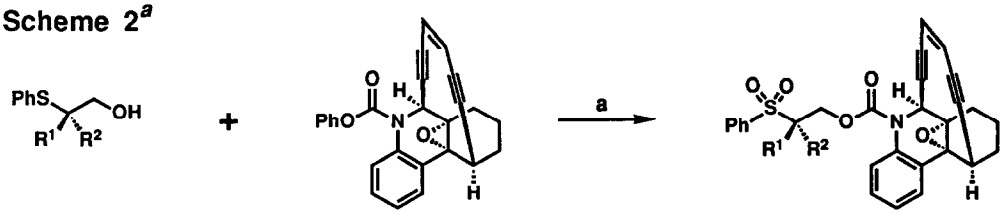


[a] **Reagents and conditions:** (a) 1.0 equiv. of **7**, 1.0 equiv. of **8**, 1.2 equiv. of DCC, 0.2 equiv. of DMAP, CH_2Cl_2, 25 °C, 1 h, **9**, 42% plus **10**, 22%; (b) 1.0 equiv. of $LiAlH_4$, THF, 0 °C, 2 min, **11**, 73% from **19**; or **12**, 79% from **10**.

The requisite optically active 2-phenylthio-1-propanols **11** and **12** were prepared by an asymmetric resolution method based on the chemistry of chiral 1,3-thiazolidine-2-thione **8** (Scheme 1).[7] Coupling of the racemic acid **7**[8] with **8**[9] in the presence of DCC-DMAP afforded chiral imides **9**[10] and **10**[10] which were easily separated by flash column chromatography. Reduction of **9** and **10** with $LiAlH_4$ in THF at 0 °C provided **11**[11] and **12**[11] in 73 and 79% yield, respectively. Scheme 2 depicts the synthesis of compounds **4-6**. Thus, treatment of the known enediyne (±)-**2**[4a,b] with a THF solution of sodium alkoxide prepared from **11** or **12** and NaH, furnished, after oxidation with *m*CPBA, an inseparable diastereomeric mixture of sulfone **4** or **5** in 79% overall yield. The racemic compound **6** was obtained from **13**[12] and (±)-**2** in a similar manner in 66% yield (Scheme 2).

With compounds **4-6** in hand, we first examined their DNA cleaving properties and compared them with the parent system **3** (Figure 1). ΦX174 DNA (50 μM per base pair) was incubated with **3** (1.0 mM) and **4-6** (5.0 mM each) in different buffers at 37 °C for 48 h. As shown in Figure 1, compounds **4** and **5** (lanes 3 and 4) exhibited greatly reduced

Scheme 2[a]



11: R^1 = H, R^2 = Me
12: R^1 = Me, R^2 = H
13: R^1 = R^2 = Me

(±)-**2**

4: R^1 = H, R^2 = Me
5: R^1 = Me, R^2 = H
6: R^1 = R^2 = Me

[a] **Reagents and conditions:** (a) 1.2 equiv. of **11-13**, 1.0 equiv. of **2**, 1.2 equiv. of NaH, THF, 25 °C, 0.5 h; then 2.5 equiv. of *m*CPBA, CH_2Cl_2, 0 °C, 30 min, **4**, 79% from **11**, or **5**, 79% from **12**, or **6**, 66% from **13**.

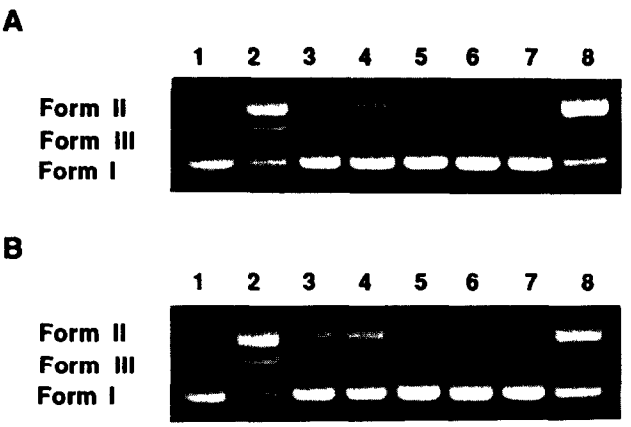


Figure 1. Supercoiled DNA interaction with synthesized enediynes and related compounds. ΦX174 DNA was incubated for 48 h at 37 °C with the indicated compounds [1.0 mM for **3** and 5.0 mM for **4-6**, **14**, **15**, and phenyl vinyl sulfone] in buffers (**A**: 50 mM Tris-HCl, pH 8.5 and **B**: 50 mM Tris-HCl, pH 9.0) and analyzed by electrophoresis (1% agarose gel, ethidium bromide stain). Lane1: DNA control; Lane 2: **3**+DNA; Lane 3: **4**+DNA; Lane 4: **5**+DNA; Lane 5: **6**+DNA; Lane 6: **15**+DNA; Lane 7: **14**+DNA; Lane 8: phenyl vinyl sulfone (**16**)+DNA. Key: Form I, supercoiled DNA; Form II, nicked DNA; Form III, linear DNA.

DNA cleaving ability at basic pHs as compared with **3**, while 2,2-dimethyl sulfone **6** (lane 5) lost its DNA cleaving activity. Phenyl isopropenyl sulfone **15**[13] and 2-(phenylsulfonyl)propanol **14**[13] were also tested for controls (lanes 6 and 7), confirming that the DNA cleavage by compounds **4** and **5** was a result of damage caused by benzenoid diradicals generated from the enediyne core **17** (Scheme 3). Since phenyl vinyl sulfone (**16**) is an alkylating agent, it is not surprising to see the Form II DNA formation in these experiments at high concentration (lane 8), but no DNA cleavage was observed at 1.0 mM (data not shown). The increased DNA damage observed for compounds **4** and **5** at higher pH (Figure 1B) supported the notion that stronger basic conditions will facilitate the β-elimination generating the damaging species.

Cytotoxicity assays were then performed with compounds **3-6** against a number of cancer and normal cell lines (Table 1). The reduced potency in cell killing by compounds **4**-

Scheme 3

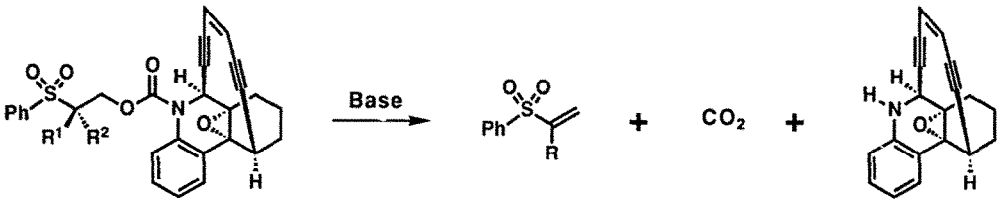


Table 1. Cytotoxicity (IC_{50}) of designed enediynes containing β-sulfone triggers.

cell line	cell type	3	4	5	6
NHDF	normal	6.3×10^{-6}	$<10^{-4}$	$<10^{-4}$	non toxic
CHO	normal	6.3×10^{-6}	$<10^{-4}$	$<10^{-4}$	non toxic
Molt-4	T cell leukemia	10^{-12}	10^{-9}	10^{-6}	10^{-4}
HL-60	promyeocytic leukemia	9.8×10^{-8}	7.8×10^{-7}	1.6×10^{-6}	2.5×10^{-5}
Capan-1	pancreatic carcinoma	7.8×10^{-8}	3.1×10^{-6}	6.3×10^{-6}	5.0×10^{-5}
P-388	mouse leukemia	9.8×10^{-8}	1.6×10^{-6}	1.6×10^{-6}	1.3×10^{-5}
Ovcar-3	ovarian carcinoma	7.8×10^{-7}	3.1×10^{-6}	1.3×10^{-5}	5.0×10^{-5}
HT-29	colon carcinoma	3.9×10^{-7}	7.8×10^{-7}	1.3×10^{-5}	2.5×10^{-5}
UCLA-P3	lung carcinoma	7.8×10^{-7}	3.1×10^{-6}	1.3×10^{-5}	5.0×10^{-5}
MCF-7	breast carcinoma	3.1×10^{-6}	2.5×10^{-5}	$<10^{-4}$	2.5×10^{-5}
H-322	lung carcinoma	3.1×10^{-6}	1.3×10^{-5}	non toxic	5.0×10^{-5}
SK-Mel-28	melanoma	6.3×10^{-5}	5.0×10^{-5}	$<10^{-4}$	$<10^{-4}$

6 again reflected that the C2 methyl group(s) attached next to the sulfone residue hindered the activation of these agents via a β-elimination process. As shown in Table 1, significant differences were obtained with the most sensitive Molt-4 leukemia cell line (10^3- to 10^6-fold less active by attaching a methyl group at the C2 position; 10^8-fold less active by attaching two methyl groups at the same position). The differential in cytotoxicities for compounds **4** and **5** was intriguing in that it suggested the involvement of chiral molecules in the activation of these agents in living cells. The reduced cytotoxicity of compound **4** against normal cell lines while maintaining considerable activity against cancer cell lines is noteworthy in the context of selective therapeutic agents.

In summary, enediynes of dynemicin A type possessing a triggering device on the nitrogen atom and C2 substituents on the triggering device have been synthesized and exhibited interesting biological properties. Specifically, the substituent effects at the C2 position of the sulfone residue on the biological behavior of these systems strongly suggest a β-elimination step in their mechanism of activation. The cancer cell selectivity of these sulfone-bearing enediynes may arise from the different rates by which different cells activate them. The results obtained in this study on structure-activity relationships may

serve as the basis for further advances in drug design and development.

Acknowledgment. This work was financially supported by the National Institutes of Health (USA) and The Scripps Research Institute.

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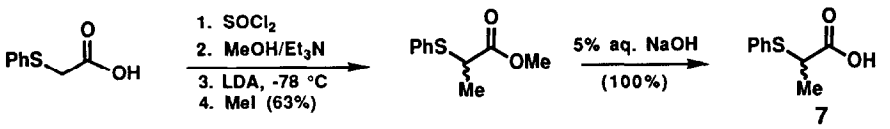
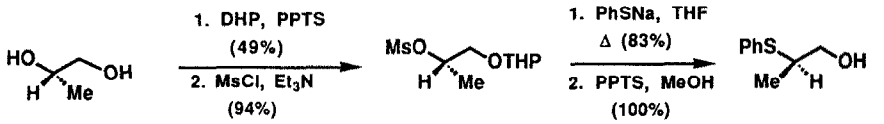
7. Fujita, E.; Nagao, Y. in "*Advances in Heterocyclic Chemistry*" **1989**, *45*, 1-36.
8. The racemic acid **7** was prepared from phenylthioacetic acid as follows:



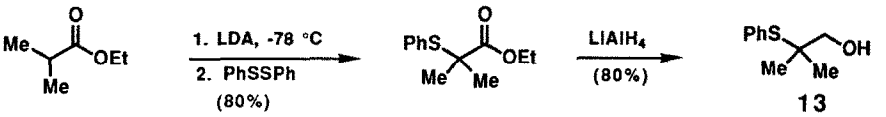
7

9. 4(*R*)-Isopropyl-1,3-thiazolidine-2-thione [4(*R*)-IPTT] **8** was prepared according to known procedure: Nagao, Y.; Hagiwara, Y.; Kumagai, T.; Ochiai, M.; Inoue, T.; Hashimoto, K.; Fujita, E. *J. Org. Chem.* **1986**, *51*, 2391.
10. Major diastereomer **9**: $[\alpha]_D^{25}$ -565.0° (*c* 0.1, EtOH), R_f = 0.41 (silica, 10% ethyl ether in petroleum ether); minor diastereomer **10**: $[\alpha]_D^{25}$ -268.0° (*c* 0.1, EtOH), R_f = 0.27 (silica, 10% ethyl ether in petroleum ether). The 2:1 ratio of **9**:**10** may be explained by epimerization of one of the distereomers under the reaction conditions or by the differential in formation of the imide bond between the two enantiomers of **7** and **8**.
11. (*S*)-**11**: $[\alpha]_D^{25}$ -10.3° (*c* 0.6, EtOH); (*R*)-**12**: $[\alpha]_D^{25}$ +9.9° (*c* 0.87, EtOH). The assignment of absolute stereochemistry was made by an independent synthesis of (*R*)-**12**:



(*S*)-(+)-1,2-propanediol (*R*)-(+)-**12**

Compounds **11** and **12** are reported in the literature, but with no optical rotation data: Mathieu-Pelta, I.; Evans, Jr. S. A. *J. Org. Chem.* **1992**, *57*, 3409. Yura, T.; Iwasawa, N.; Clark, R.; Mukaiyama, T. *Chem. Lett.* **1986**, 1809.
12. Compound **13** was synthesized from ethyl isobutyrate as follows:



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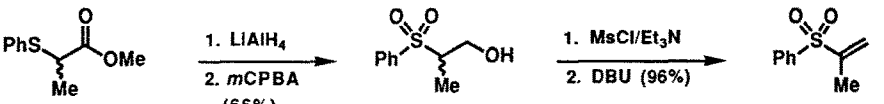
13. 2-(Phenylsulfonyl)propanol **14** and phenyl isopropenyl sulfone **15** were prepared from methyl 2-(phenylthio)propionate as follows:



14 **15**