Synthesis and Biological Actions of Optically Active Enediynes Related to Dynemicin A

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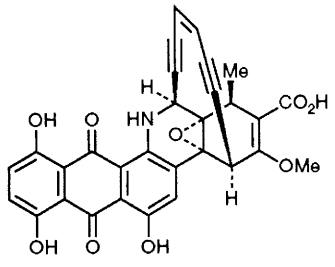
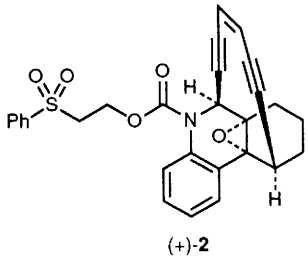
Enantiomerically pure enediynes (+)-**2** and (−)-**2** were synthesized using a chiral resolution method and were shown to exhibit different cytotoxicities against a number of tumour cells.

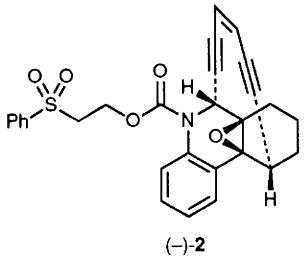
Dynemicin A **1**[1] is a recently discovered antitumour antibiotic of the enediyne class.[2] Its unique molecular architecture, potent antitumour activity and fascinating mechanism of action have stimulated extensive studies during the past few years.[2] Reports from these laboratories included disclosures on the synthesis and biology of a number of novel model systems of this natural product.[3,4] Designed enediyne **2** equipped with a 2-(phenylsulfonyl)ethoxycarbonyl group represents a family of highly potent and selective cytotoxic agents as demonstrated in experiments with a variety of cell lines. Thus, racemic **2** exhibited DNA cleaving properties and an IC_{50} value of 10^{-11} mol dm^{-3} against Molt-4 leukaemia cells.[3e] Owing to this significant observation it was deemed important to prepare pure enantiomers of **2** and test their biological action. In this communication we report the synthesis of enantiomers (+)-**2** and (−)-**2** and their biological action against a number of cell lines.



1: dynemicin A



(+)-**2**



(−)-**2**

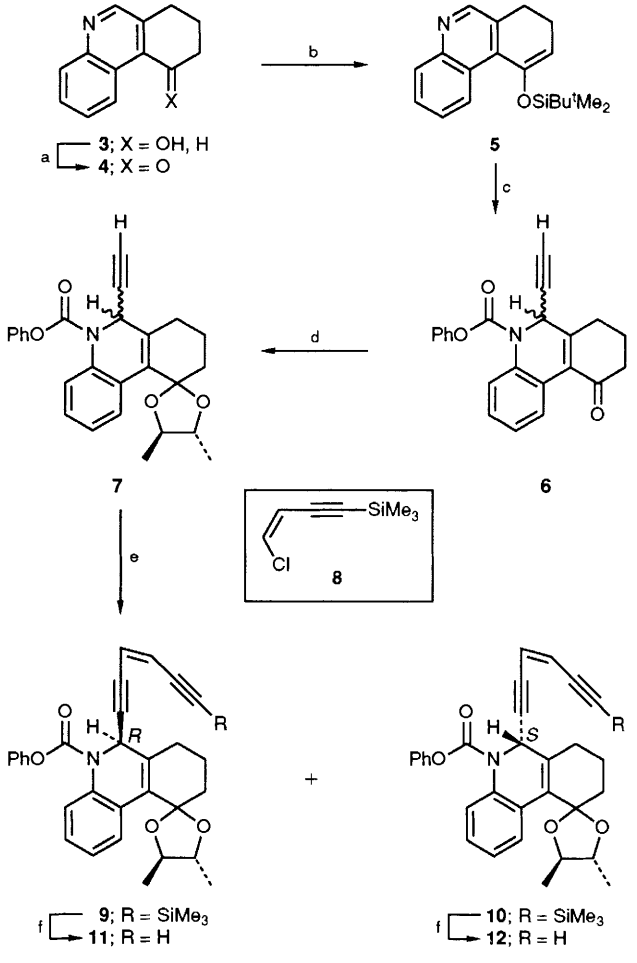
Since both enantiomers of **2** were desired for biological studies, a rapid access to both compounds through a resolution method was sought. Thus, the original synthesis of racemic **2**[3a–e] was significantly modified as outlined in Scheme 1 to produce (+)-**2** and (−)-**2** in pure forms. Hydroxy quinoline **3** was oxidized to ketone **4**† using Jones reagent (98%) and then converted to enol silyl ether **5** in high yield. Sequential treatment of **5** with phenyl chloroformate and ethynylmagnesium bromide afforded, after acidic work-up, acetylenic compound **6** in 91% overall yield. Ketalization of **6** with (2*R*,3*R*)-butane-2,3-diol gave an inseparable mixture of diastereoisomers **7** (*ca.* 1 : 1 by ^{1}H NMR) which was coupled with vinyl chloride **8** under the influence of Pd^0–Cu^I catalysis to afford a 1 : 1 mixture of enediynes **9** and **10** (63% total yield). Flash column chromatography (silica gel, 0.25% ethyl acetate in benzene) led to pure diastereoisomers **9** {R_f = 0.22 (silica gel, 0.25% ethyl acetate in benzene); $[\alpha]_D^{25}$ + 427 (*c* 0.88, benzene)} and **10** {R_f = 0.20 (silica gel, 0.25% ethyl acetate in benzene); $[\alpha]_D^{25}$ − 397 (*c* 0.95, benzene)} in 45 and 42% yield, respectively. Removal of the trimethylsilyl group from **9**

† All new compounds exhibited satisfactory spectral and analytical and/or exact mass data. Yields refer to chromatographically and spectroscopically homogeneous materials.

Table 1 Cytotoxicities of enediynes (±)-**2**, (+)-**2** and (−)-**2**

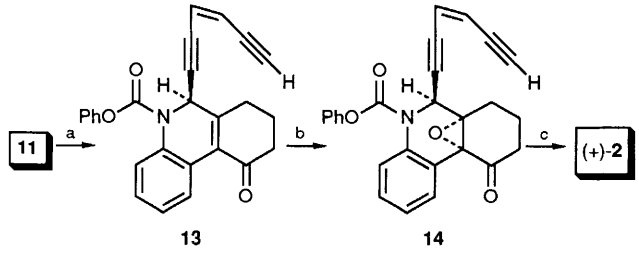
Cell type	Cell line	IC_{50}/mol dm^{-3} (±)-**2**	(+)-**2**	(−)-**2**
Melanoma	SK-Mel-28	6.3×10^{-6}	6.3×10^{-6}	6.3×10^{-6}
Pancreatic carcinoma	Capan-1	1.6×10^{-6}	3.9×10^{-7}	1.6×10^{-6}
Breast carcinoma	MCF-7/ADR[a]	1.6×10^{-6}	7.8×10^{-7}	1.6×10^{-6}
Promyeocytic leukemia	HL-60	3.9×10^{-6}	$>9.8 \times 10^{-8}$	7.8×10^{-7}
T-cell leukemia	Molt-4	1.0×10^{-11}	1.0×10^{-13}	1.0×10^{-7}

[a] Adriamycin resistant cell line.



Scheme 1 *Reagents and conditions*: (a) 1.3 equiv. of Jones reagent, 1.0 equiv. of H_2SO_4, AcOH–acetone (1 : 1), 0 to 25 °C, 30 min, 98%; (b) 1.2 equiv. of Bu^{t}Me$_2$SiOSO$_2$CF$_3$, 1.5 equiv. of Et_3N, CH_2Cl_2, 25 °C, 3 h, 99%; (c) 1.1 equiv. of ethynylmagnesium bromide, 1.1 equiv. of PhOCOCl, tetrahydrofuran (THF), −78 to 25 °C, 1 h; then 10% HCl, 25 °C, 10 min, 92%; (d) 1.5 equiv. of (2*R*,3*R*)-butane-2,3-diol, 0.2 equiv. of *p*-MeC$_6$H$_4$SO$_3$H·H$_2$O, PhH, reflux, 20 h, 95%; (e) 1.5 equiv. of **8**, 0.05 equiv. of Pd(PPh$_3$)$_4$, 0.2 equiv. of CuI, 1.5 equiv. of Bu^{n}NH$_2$, PhH, 25 C, 2 h, 63% (1 : 1 mixture of **9** and **10**); column separation over silica gel, 45% of **9** plus 42% of **10**; (f) 4.0 equiv. of $AgNO_3$, EtOH–THF–H_2O (1 : 1 : 1), 25 °C, 2 h; then 7.0 equiv. of NaCN, 25 °C, 30 min, 81%

and **10** led to enediynes **11** and **12** in high yields. Assignment of absolute stereochemistry in this series was based on X-ray crystallographic analysis of **12**. Transformation of the diastereoisomeric compounds **11** and **12** into the targeted molecules (+)-**2** and (−)-**2** was carried out as illustrated in Scheme 2 for the synthesis of (+)-**2**. Thus, acid hydrolysis of



Scheme 2 *Reagents and conditions*: (a) 0.2 equiv. of *p*-MeC$_6$H$_4$SO$_3$H·H$_2$O, PhH–acetone–H$_2$O (100 : 1 : 1), reflux, 6 h, 85%; (b) 2.0 equiv. of *m*CPBA, aqueous NaHCO$_3$–CH$_2$Cl$_2$ (1 : 1), 25 °C, 1.5 h, **14**, 37% plus 13% of recovered **13**; (c) see ref. 3*a–c*

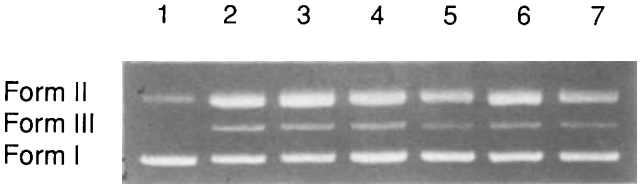


Fig. 1 Supercoiled DNA interaction with synthesized enediynes. ΦX174 DNA was incubated for 24 h at 37 °C in buffer (50 mmol dm^{-3} Tris-HCl, pH 8.5) with compounds (±)-**2**, (+)-**2**, and (−)-**2**. Lane 1: DNA control. Lanes 2–4: (±)-**2**, (+)-**2**, and (−)-**2** (1000 μmol dm^{-3} each). Lanes 5–7: (±)-**2**, (+)-**2**, and (−)-**2** (100 μmol dm^{-3} each). Key: Form I, supercoiled DNA; Form II, nicked DNA; Form III, linear DNA.

ketal **11** afforded enone **13** (85%) which was converted into epoxyketone **14** using chloroperbenzoic acid (*m*CPBA) under basic conditions (43% yield based on 87% conversion). From here on the sequence followed the reported pathway for the racemic series.[3a–c] It is noteworthy that the enantiomer (+)-**2** had the same absolute stereochemistry[5] and sign of optical rotation as dynemicin A {(+)-**2**: $[\alpha]_D^{25}$ +586 (*c* 0.46, benzene); dynemicin A **1**: $[\alpha]_D^{24}$ +270 (*c* 0.01, dimethylformamide)[1]}. Enantiomer (−)-**2** {$[\alpha]_D^{25}$ −562 (*c* 0.50, benzene)} was synthesized from **12** in a similar manner.

As shown in Fig. 1 compounds (±)-**2**, (+)-**2**, and (−)-**2** cleaved ΦX174 supercoiled DNA under basic conditions (pH 8.5) with comparable potencies (at 1000 and 100 μmol dm^{-3} concentrations).[6] More striking were the *in vitro* cytotoxicities of these compounds against tumour cell lines. As exhibited in Table 1 the (+)-enantiomer of **2** exhibited higher potencies against a number of cell lines, particularly the more sensitive Molt-4 leukaemia cells.

The described chemistry renders these enediynes available in their enantiomerically pure forms and provides support for the proposed[5] absolute stereochemistry of dynemicin A. The biological observations point to rather selective interactions of these molecules with their target cells, particularly in the cases of the most sensitive cell types.

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