First Total Synthesis of Naturally Occurring (−)-Nitidon and Its Enantiomer

Fabio Bellina,[a] Adriano Carpita,*[a] Luca Mannocci,[a] and Renzo Rossi*[a]

Keywords: Antitumour agents / C−C coupling / Lactones / Natural products / Synthetic methods

The first total synthesis of naturally occurring (–)-nitidon and its enantiomer is reported. The best of the routes investigated for preparation of these enantiomerically pure compounds involves a modification of the Cadiot–Chodkiewicz reaction and the Sharpless asymmetric epoxidation of an (*E*)-2-ene-4,6-diyn-1-ol as key steps and proceeds in five steps and 18% overall yield. Both enantiomers of nitidon and some related 6-(1,3-diyn-1-yl)-2*H*-pyran-2-ones have been found to exhibit significant cytotoxic activity against human cancer cell lines in vitro.

Introduction

Induction of tumour cell differentiation has recently been considered as a new strategy for cancer chemotherapy.[1] Many studies have shown that malignant tumour cells can be induced to undergo terminal differentiation by some inducers,[2] and differentiation-inducing therapy has already been applied to lymphomas, leukaemias and other solid tumours.[2g,3] Several naturally occurring inducers of differentiation have been isolated from fungi, marine organisms and plants. These compounds include bryostatin 1,[2a,2m] trapoxin B,[2d] brefeldin A,[2f] *all-trans*-retinoic acid,[2g] thunberginols A, B and F,[2h] falconensones A and B,[2i] brusatol,[2j] bistratene A,[2k] and racemic *cis*-3′-angeloyl-4′-*O*-acetylkhellactone.[2n] Some of these substances and their analogues and derivatives have attracted considerable interest regarding their chemical synthesis and biochemical mechanism of action.[4−6]

In 1998, Gehrt et al.[7] isolated a new, highly unsaturated pyranone derivative, (−)-**1**, from the basidiomycete *Junghuhnia nitida* and elucidated its structure by spectroscopic methods. Those authors also found that (−)-**1** induces morphological and physiological differentiation of HL-60 and U-937 tumour cell lines at nanomolar concentrations and is also characterized by antibacterial and antifungal activities. Moreover, (−)-**1** was also found to exhibit cytotoxic activity against HL-60 and U-937 cells at 250 ng/mL and against L1210, HeLa S3 and BHK-21 cells from 500 ng/mL.[7] The absolute stereochemistry and the enantiomeric purity of this chiral compound were not determined, however, and its synthesis has thus far not been reported.

Motivated by the biological activities of (−)-**1** and by our interest in the synthesis of naturally occurring compounds possessing antitumour properties,[8] we decided to develop a concise and efficient enantioselective synthesis of this natural product and its enantiomer and examined the possibility of accessing these compounds by a synthetic route involving the Sharpless asymmetric epoxidation[9] of 6-[(*E*)-7-hydroxy-5-heptene-1,3-diyn-1-yl]-2*H*-pyran-2-one (**2a**) as a key step. In fact, through variation of the absolute configuration of the chiral auxiliary, this reaction might allow us to prepare both enantiomers of **1** in high enantiomeric purity and, on the basis of its steric course,[9a] to assign, albeit preliminarily, their absolute configurations. We also felt that 6-chloro-2*H*-pyran-2-one (**3**), which can easily be prepared from commercially available *trans*-glutaconic acid (**4**),[10,11] might be a convenient starting material from which to access **2a**.

Here we report an account on the results of these synthetic studies and of some tests performed to evaluate the cytotoxic activities of the two enantiomers of **1**, of **2a** and of some related 6-(1,3-diynyl)-2*H*-pyran-2-ones synthesized in the course of a study aimed at the development of a new route for the preparation of **2a**.

Results and Discussion

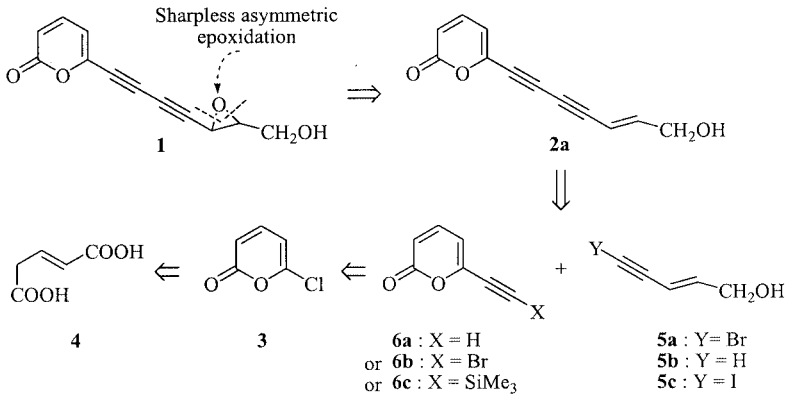
Synthesis of the two Enantiomers of Nitidon

Our synthetic approach to the asymmetric synthesis of the two enantiomers of nitidon (**1**) was envisioned through the retrosynthetic routes shown in Scheme 1.

The (*E*)-enediynol **2a**, the putative direct precursor to both enantiomers of **1**, should be accessible through a Cadiot−Chodkiewicz-type reaction[12] either between (*E*)-5-bromo-2-penten-4-yn-1-ol (**5a**) and 6-ethynyl-2*H*-pyran-2-one (**6a**) or between (*E*)-2-penten-4-yn-1-ol (**5b**) and 6-(bromoethynyl)-2*H*-pyran-2-one (**6b**). Alternatively, compound **2a** should also be available through a CuCl-catalysed cross-coupling reaction between 6-(trimethylsilylethynyl)-

[a] Dipartimento di Chimica e Chimica Industriale, Università di Pisa,
Via Risorgimento n. 35, 56126 Pisa, Italy
Fax: (internat.) +39-050-2219260
E-mail: rossi@dcci.unipi.it

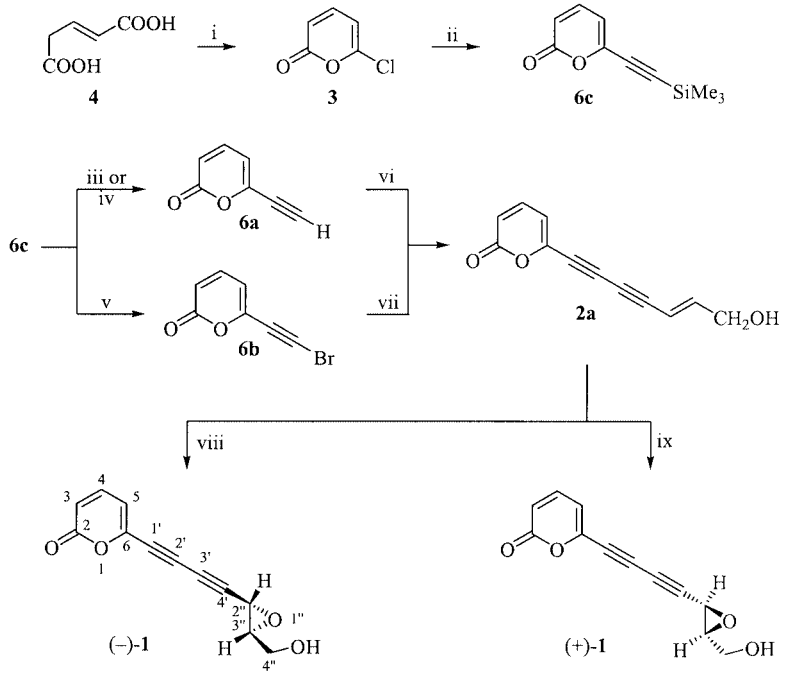
  DOI: 10.1002/ejoc.200400101



Scheme 1. Retrosynthetic routes towards both enantiomers of nitidon (**1**)

2*H*-pyran-2-one (**6c**) and (*E*)-5-iodo-2-penten-4-yn-1-ol (**5c**) by a method similar to that described for the synthesis of unsymmetrical 1,4-diaryl-1,3-butadiynes and aryl(chloro)ethynes.[13] On the other hand, 93% pure **5b** is commercially available or could be synthesized in stereoisomerically pure form from ethyl (*E*)-3-iodopropenoate[14] or as a ca. 9:1 mixture of (*E*) and (*Z*) stereoisomers from sodium acetylide and epichloridrin.[15] Compounds **5a** and **5c** could in turn be easily prepared from **5b** by a standard hydrogen/halogen exchange procedure[16] and compound **6c** could be synthesized in high yield from **3** by a Sonogashira-type reaction.[10] Moreover, compounds **6a** and **6b** should be available from **6c** by a standard protiodesilylation procedure and by use of a literature method for the one-step conversion of trimethylsilylacetylenes into haloacetylenes,[17] respectively.

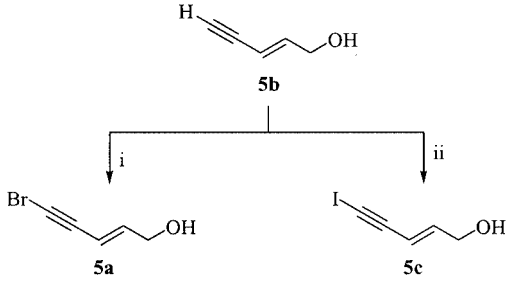
Scheme 2 provides details of the two synthetic routes involving a Cadiot−Chodkiewicz-type reaction used to prepare **2a** and to convert this allylic alcohol into the two enantiomers of nitidon (**1**). In particular, compound **3**, which was prepared in 82% yield from **4** by a modification of the procedure described by Pirkle and Dines,[11] was efficiently converted into the known compound **6c** by a literature procedure.[10] Protiodesilylation of this silylacetylene by treatment with $KF{\cdot}2H_2O$ in DMF[18] allowed us to obtain **6a** in



Scheme 2. Reagents and conditions: (i) PCl_5 (2.0 equiv.), 0 °C, then 100 °C for 10 min, then 0 °C, H_2O, 82%; (ii) ref.[10] 85%; (iii) $KF{\cdot}2H_2O$ (2.0 equiv.), DMF, room temp., 0.5 h, 96%; (iv) $AgNO_3$ (10 mol %), CF_3COOH (1.0 equiv.), H_2O (10 equiv.), acetone, room temp., 18 h, 92%; (v) NBS (1.6 equiv.), H_2O (2.0 equiv.), $AgNO_3$ (20 mol %), acetone, room temp., 3 h, 87%; (vi) **5a** (1.0 equiv.), CuCl (10 mol %), $NH_2OH{\cdot}HCl$ (30 mol %), TMP (2.0 equiv.), DMF, 2 h, 0 °C then 42 h, room temp, 50%; (vii) (*E*)-2-penten-4-yn-1-ol (**5b**) (1.0 equiv.), CuCl (10 mol %), $NH_2OH{\cdot}HCl$ (30 mol %), TMP (2.0 equiv.), DMF, 24 h, 0 °C, 41%; (viii) L-(+)-diethyl tartrate (1.5 equiv.), Ti (O-*i*Pr)$_4$ (1.3 equiv.), CH_2Cl_2, activated molecular sieves (4 Å), *t*BuOOH (3.0 equiv.), −20 °C, 16 h, then 10% aq. tartaric acid, room temp., 71%; (ix) D-(−)-diethyl tartrate (1.5 equiv.), Ti (O-*i*Pr)$_4$ (1.3 equiv.), CH_2Cl_2, activated molecular sieves (4 Å), *t*BuOOH (3.0 equiv.), −20 °C, 16 h, then 10% aq. tartaric acid, room temp., 76%

96% yield. Alternatively, **6a** was also prepared in 92% yield by a modification of the literature procedure involving treatment of a silylacetylene with a molar excess of $AgNO_3$ in a protic solvent and subsequent treatment with a large excess of potassium cyanide.[19] The synthetically useful modification used to prepare **6a**, which does not require the use of potassium cyanide, consists of treatment of **6c** with a catalytic amount of $AgNO_3$ in acetone containing 1 equiv. of trifluoroacetic acid and 10 equiv. of water (Scheme 2).

Bromide **5a**, which we used as the cross-coupling partner for **6a** in the preparation of **2a**, was synthesized in 70% yield from commercially available **5b**, as shown in Scheme 3.



Scheme 3. Reagents and conditions: (i) NBS (1.2 equiv.), $AgNO_3$ (10 mol %), acetone, 0 °C, 2 h, 70%; (ii) NIS (1.2 equiv.), $AgNO_3$ (9.8 mol %), acetone, room temp., 45 min, 67%

Unfortunately, preliminary experiments carried out with the aim of synthesising **2a** through a Cadiot−Chodkiewicz reaction between **5a** and **6a** under standard reaction conditions[12] or by the method employed by Saalfrank et al.[20] for the preparation of 2-methyl-6-trimethylsilyl-3,5-hexadiyn-2-ol, were unsuccessful. In fact, **6a** proved to be highly unstable in the presence of triethylamine, various primary amines or pyrrolidine.

Nevertheless, we finally found that 2,2,6,6-tetramethylpiperidine (TMP) could be used as a base in the reaction between **5a** and **6a** in DMF solution in the presence of hydroxylamine hydrochloride. This modification of the reaction conditions usually employed for the Cadiot−Chodkiewicz reaction allowed us to obtain compound **2a** in 50% yield (Scheme 2). We also tried to prepare **2a** in a higher yield and investigated its preparation by a second route, also based on a Cadiot−Chodkiewicz-type reaction. We found, however, that when **6b**, which was prepared in 87% yield by treatment of **6c** with 1.2 equiv. of *N*-bromosuccinimide (NBS) in acetone in the presence of a catalytic amount of $AgNO_3$, was treated with **5b** in a procedure very similar to that employed for the CuCl-catalysed coupling between **5a** and **6a**, compound **2a** was obtained in 41% yield (Scheme 2). Much more disappointing was the result of a third synthetic approach involving the CuCl-promoted cross-coupling reaction between **6c** and iodide **5c** in 1,3-dimethylimidazolidone (DMI). In fact, we found that treatment of **5c** − which was prepared in 67% yield by treatment of **5b** with *N*-iodosuccinimide (NIS) in the presence of a catalytic amount of $AgNO_3$ (Scheme 3) − with **6c** in DMI at 80 °C for 2 h provided **2a** in 5% yield (Table 1, Entry 1). Unfortunately, compound **2b**, a derivative of **2a**, was also obtained from the CuCl-promoted reaction between **6c** and iodide **7a** in only 8% yield (Table 1 Entry 2).

Despite these unsatisfactory results, the procedure involving the CuCl-promoted reaction between **6c** and 1-halo-1-ynes other than **5c** and **7a** in DMI or DMF at 80 °C allowed us to prepare some analogues of **2a** (i.e., compounds **2c**−**g**) in modest to satisfactory yields (Table 1, Entries 4, 5, 6, 8, 11). We also found that the reactions promoted by 10 mol % CuCl furnished yields similar to those obtained in the reactions promoted by 50 mol % CuCl (e.g., compare Entries 8 and 10 in Table 1), but that these last reactions proceeded in shorter reaction times. Moreover, we observed: (i) that the crude mixtures obtained from reactions involving 1-iodo-1-ynes contained significant amounts of the 1,3-diynes **8**, originating from the homocoupling of these iodides, and alkynes **9** corresponding to these halides, and (ii) that the yields of 6-(1,3-diynyl)-2*H*-pyran-2-ones prepared from 1-chloro- or 1-bromo-1-ynes were lower than those obtained from structurally similar 1-iodo-1-ynes (e.g., compare Entries 3 and 7 in Table 1 with Entries 2 and 6).

It is also interesting to note that it was observed during the development of this method for the synthesis of diynes **2a**−**g** that either CuCl of high purity or a 1.5−3:1 molar ratio between 1-iodo-1-alkynes and **6c** were essential for satisfactory results.

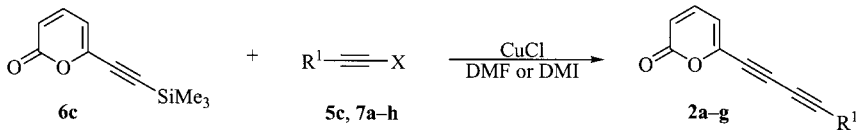
With compound **2a** accessible through Cadiot−Chodkiewicz-type reactions either between **5a** and **6a** or between **5b** and **6b**, we investigated the conversion of this (*E*)-2-ene-4,6-diyn-1-ol into the enantiomers of **1**. Compound **2a** was thus subjected to Sharpless asymmetric epoxidation[9e][9f] with L-(+)-diethyl tartrate as ligand to afford 99% chemically pure (−)-**1** in 71% yield (Scheme 2). This compound, which, after recrystallization from a mixture of hexane and $CHCl_3$ at −23 °C, was estimated to have an enantiomeric excess higher than 99% on the basis of HPLC analyses on a Chiracel OJ column, had physical and spectral properties in satisfactory agreement with those of the natural product,[7] but its specific rotatory power, $[\alpha]_D^{22} = -21.0$ (c = 1.10, $CHCl_3/CH_3OH$, 1:1), was lower than that reported for naturally occurring (−)-**1**, $[\alpha]_D^{22} = -34$ (c = 1.1, $CHCl_3/CH_3OH$, 1:1).[7] Similarly, Sharpless asymmetric epoxidation of **2a** with D-(−)-diethyl tartrate as ligand furnished 98% chemically pure (+)-**1**, which, after recrystallization from a mixture of hexane and $CHCl_3$ at −23 °C, was estimated to be 98% enantiomerically pure on the basis of HPLC analyses on a Chiracel OJ column. This compound had $[\alpha]_D^{22} = +20.0$ (c = 1.10, $CHCl_3/CH_3OH$, 1:1).

It should also be noted that, on the basis of the steric course of the Sharpless asymmetric epoxidation of allylic alcohols,[9a] we were able to assign (−)-**1**, albeit preliminarily, the (2′′*S*,3′′*S*) configuration.

Biological Results

Compounds (−)-**1**, (+)-**1**, **2a**, **2c**, **2e**, **2f** and **2g** were selected by the US National Cancer Institute (NCI) for evaluation in an in vitro preclinical antitumour screening program for primary anticancer assay against three human tu-

Table 1. Synthesis of **2a** and related 6-(1,3-diynyl)-2*H*-pyran-2-ones through CuCl-catalysed reactions between **6c** and 1-halo-1-alkynes



Entry[a]	1-halo-1-alkyne			5c−7/6c	Solvent	Reaction time	Product	
	5 or 7	R^1	X	Molar ratio		(h)	2	Yield (%)
1	**5c**	(*E*)-CH=CH−CH_2OH	I	1.5	DMI	2	**2a**	5
2	**7a**	(*E*)-CH=CH−CH_2OTBDMS	I	3.0	DMI[b]	6	**2b**	8
3	**7b**	(*E*)-CH=CH−CH_2OTBDMS	Br	3.0	DMI[b]	23	**2b**	–
4	**7c**	$(CH_2)_3$OAc	I	3.0	DMF	22	**2c**	30
5	**7d**	$(CH_2)_3$OTBDMS	I	3.0	DMI	22	**2d**	20
6	**7e**	1-cyclohexenyl	I	3.0	DMI	20	**2e**	51
7[c]	**7f**	1-cyclohexenyl	Cl	1.5	DMI	23	**2e**	23
8	**7g**	*n*-C_4H_9	I	1.5	DMF	14	**2f**	54
9[d]	**7g**	*n*-C_4H_9	I	1.5	DMF	21	**2f**	25[e]
10[f]	**7g**	*n*-C_4H_9	I	2.0	DMF	34	**2f**	50
11	**7h**	*n*-C_6H_{13}	I	1.5	DMI	16	**2g**	50

[a] Unless otherwise reported, the reactions were performed at 80 °C in the presence of 50 mol % 99.99% chemically pure CuCl. [b] This reaction was performed in the presence of 2.0 equiv. of nBu_3N. [c] This reaction was performed at 105 °C. [d] This reaction was performed in the presence of 10 mol % 97% chemically pure CuCl. [e] The crude reaction mixture obtained after hydrolysis with an aqueous NH_4Cl solution was found to be contaminated with unchanged **7g** and a significant amount of **6c**. [f] This reaction was performed in the presence of 10 mol % 99.99% chemically pure CuCl.

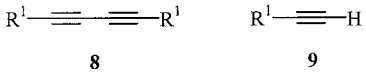


Figure 1. Chemical structures of compounds **8** and **9**

mour cell lines: MCF-7 (breast), SF-268 (CNS) and NCI-H460 (lung). All tested compounds proved to be active, as they reduced the growth of any one of the cell lines to 32% or less, but only (−)-**1**, (+)-**1**, **2c**, **2e**, **2f** and **2g** were significantly cytotoxic against all three cell lines (Table 2, Entries 1, 2, 4, 5, 6 and 7). Interestingly, the cytotoxicity of compound (−)-**1** was not significantly different from that of (+)-**1**.

Table 2. Primary anticancer assay for compounds (−)-**1**, (+)-**1**, **2a**, **2c** and **2e**−**g**

Entry	Compound	Percentage of growth inhibition			Activity
		MCF-7	SF-268	NCI-H460	
1	(−)-**1**	0	0	0	active
2	(+)-**1**	1	0	0	active
3	**2a**	8	53	0	active
4	**2c**	2	5	0	active
5	**2e**	1	14	0	active
6	**2f**	2	3	1	active
7	**2g**	2	2	1	active

Compounds **2c**, **2e**, **2f** and **2g** were further evaluated for potential anticancer activity in the US NCI's human tumour cell-line screen involving the full panel of 60 cell lines over a 5-log dose range.[21] The log GI_{50} values obtained with selected cell lines, along with the mean graph-midpoint (MGM MD) values, are summarized in Table 3. The data summarized in this table indicate indisputable cytotoxicity in compounds **2e** and **2g**, which showed MGM MD values of −5.85 and −5.66, respectively. These data also show that the SK-MEL-5 melanoma cell line was very sensitive to compound **2g** and that **2e** was very active against the NCI-H226 (lung), HCT-116 (colon) and LOX-IMVI (melanoma)cell lines. It should also be mentioned that compounds **2e** and **2g** are currently under review by Biological Evaluation Committee of the US NCI.

Conclusion

We have described the first total synthesis of both enantiomers of naturally occurring nitidon (**1**). These compounds have been prepared in very high enantiomeric purity by asymmetric epoxidation of enediynol **2a**, which was conveniently synthesized by two different routes. Each of these routes involved the use of a modification of the classical procedure for the Cadiot−Chodkiewicz reaction. The synthesis of (−)-**1** by the shortest of these routes proceeds in five steps from commercially available *trans*-glutaconic acid and (*E*)-2-penten-4-yn-1-ol, in 18% overall yield. We have also shown that, although the yield of **2a** from a CuCl-promoted reaction between silylacetylene **6c** and (*E*)-5-iodo-2-penten-4-yn-1-ol was very low, similar CuCl-promoted reactions between **6c** and some 1-iodo-1-alkynes pro-

Table 3. Cytotoxicities of compounds **2c** and **2e−g**

	log molar drug concentration required for 50% growth inhibition										
	Leukemia CCRF-CEM	Lung NCI-H226	Lung NCI-H23	Colon HCT-116	Melanoma LOX-IMVI	Melanoma SK-MEL-5	Kidney 786−0	Prostate DU-145	Breast MDA-MB-435	MGM	MD
2c	−	−4.68	−4.80	−4.84	−4.80	−4.79	−4.72	−4.93	−4.86	−4.77	
2e	−6.77	−5.61	−7.30	−7.13	−7.08	>−4.00	−6.77	−5.65	−6.81	−5.85	
2f	−4.81	−4.79	−4.74	−4.83	−4.79	−4.84	−4.78	−4.66	−4.71	−4.71	
2g	−6.76	−6.24	−6.37	−6.19	−6.80	<−8.00	−6.43	−5.74	−6.07	−5.66	

vide analogues of **2a** in modest to satisfactory yields. Interestingly, both enantiomers of nitidon, compound **2a** and other 6-(1,3-diynyl)-2*H*-pyran-2-ones were found to be active in the NCI three-cell-line, one-dose primary anticancer assay. Moreover, two of these diynes exhibited significant cytotoxicities in the NCI 60-cell-line panel and one of these compounds proved to be very active against the SK-MEL-15 melanoma cell line.

Experimental Section

General Remarks: Melting points and boiling points were uncorrected. Pre-coated Merck 60 F_{254} aluminium silica gel sheets were used for TLC analyses. GLC analyses were performed on a Dani GC 1000 instrument with a PTV injector, which was equipped with a Dani DDS 1000 data station. Two types of capillary columns were used: an Alltech AT-35 bonded FSOT column (30 m × 0.25 mm i.d.) and an Alltech AT-1 bonded FSOT column (30 m × 0.25 mm i.d). Purifications by MPLC on silica gel (Merck 60 silica gel, particle size 0.015−0.040 mm) were performed on a Büchi B-680 system with a Knauer K-2400 differential refractometer as detector. GLC/EI-MS analyses were performed with a Q-mass 910 spectrometer interfaced with a Perkin−Elmer 8500 gas chromatograph. The MS spectrum of compound (−)-**1** was recorded with a Perkin−Elmer SCIEX API III triple quadrupole mass spectrometer by the atmospheric pressure photoionization (APPI) technique by a tandem mass spectrometry approach. HPLC analyses were performed on a Waters system with a 1525 LC pump and a 2996 photodiode array detector and with a Perkin−Elmer system with a 410 LC pump and a 735A UV/Vis detector. IR spectra were recorded with a Perkin−Elmer 1725 FT-IR spectrophotometer. NMR spectra were recorded with a Varian Gemini 200 MHz and Varian Gemini 300 MHz spectrometers with TMS as the internal standard. Measurements of optical activity were performed with a Perkin−Elmer 142 spectropolarimeter in 1-dm tubes. All reactions involving air- and water-sensitive materials were performed in flame-dried glassware under argon by standard syringe, cannula and septa techniques. The following compounds were prepared by published procedures: $[PdCl_2(PPh_3)_2]$,[22] 1-iodo-1-hexyne (**7g**),[23] 1-iodo-1-octyne (**7h**),[24] 5-acetoxy-1-pentyne,[25] 5-(*tert*-butyldimethylsilyloxy)-1-pentyne,[26] and (*E*)-5-(*tert*-butyldimethylsilyloxy)-3-penten-1-yne.[27] The cytotoxic activities of compounds (−)-**1**, (+)-**1**, **2a**, **2c**, **2e**, **2f** and **2g** were evaluated in vitro against the US National Cancer Institute (NCI) three-cell-lines panel consisting of MCF-7 (breast), SF-268 (CNS) and NCI-H460 (lung). In this procedure, each cell line was inoculated and pre-incubated on a microtiter plate. Test agents were then added at a single concentration (1.00×10^{-4} M) and the culture was incubated for 48 h. End-point determinations were made with sulforhodamine B. Compounds **2c**, **2e**, **2f** and **2g** were further evaluated for potential anticancer activity in the US NCI's human tumour cell-line screen involving the full panel of 60 cell lines over a 5-log dose range.[21] For each compound, dose response curves were measured with five different drug concentrations and the log GI_{50} values (GI_{50} being the molar drug concentration required for half growth inhibition) obtained with the cell lines were tabulated, along with the mean graph-midpoint (MGM MD) values. The MGM MD is based on a calculation of the average log GI_{50} for all of the cell lines tested, in which GI_{50} values below and above the test range (10^{-4}−10^{-8}) are taken as the minimum (10^{-8}) and maximum (10^{-4}) drug concentration used in the screening test.

6-Chloro-2*H*-pyran-2-one (3): Phosphorus pentachloride (32.05 g, 153.7 mmol) was added portionwise to *trans*-glutaconic acid (**4**; techn. 90%, 10.0 g, 76.9 mmol) cooled to 0 °C. The mixture was heated to 100 °C for 10 min, and then cooled to 0 °C, poured into water (100 mL) and extracted with CH_2Cl_2 (3 × 80 mL). The organic extract was washed with cold aqueous $NaHCO_3$ solution (10%, 3 × 60 mL), dried and concentrated under reduced pressure. The residue was fractionally distilled to give **3** (8.22 g, 82%) as a colourless solid; m.p. 27 °C (m.p.[11] 27 °C); b.p. 73 °C/2.5 mbar. EI-MS: *m*/*z* (%) = 132 (13) [M^+], 130 (28) [M^+], 104 (5), 95 (100), 73 (6), 66 (6), 39 (59). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 6.24 (dd, *J* = 9.0, 1.0 Hz, 1 H), 6.27 (dd, *J* = 7.0, 1.0 Hz, 1 H), 7.37 (dd, *J* = 9.0, 7.0 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 104.1, 112.1, 144.2, 149.7, 160.1 ppm.

6-(Trimethylsilylethynyl)-2*H*-pyran-2-one (6c): A deaerated solution of **3** (1.40 g, 10.7 mmol) in benzene (10 mL) and trimethylsilylacetylene (2.11 g, 21.5 mmol) were sequentially added to a suspension of $[PdCl_2(PPh_3)_2]$ (376 mg, 0.540 mmol) and CuI (306 mg, 1.60 mmol) in benzene (20 mL), which was stirred under argon at 0 °C. A solution of Et_3N (4.48 mL, 32.2 mmol) in benzene (20 mL) was then added dropwise over 1.5 h and the resulting mixture was stirred under argon at room temperature for 21 h. It was then poured into a saturated aqueous NH_4Cl solution (200 mL), and the mixture was stirred open to the atmosphere until the aqueous phase became deep blue. The mixture was extracted with diethyl ether (4 × 50 mL), dried and concentrated under reduced pressure. The residue was purified by MPLC on silica gel, with a mixture of petroleum ether and EtOAc (86:14) as eluent, to give chemically pure **6c** (1.75 g, 85%) as a colourless solid; m.p. 63−65 °C. EI-MS: *m*/*z* (%) = 192 (38) [M^+], 177 (58), 149 (100), 121 (24), 95 (20), 75 (14), 67 (13). IR (KBr disk): $\tilde{\nu}$ = 2161, 1723, 1619, 1611, 1540, 1090, 844 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.25 (s, 9 H), 6.33 (dd, *J* = 9.5, 1.0 Hz, 1 H), 6.40 (dd, *J* = 7.0, 1.0 Hz, 1 H), 7.28 (dd, *J* = 9.5, 7.0 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = −0.70 ppm (3 C), 95.5, 102.8, 110.2, 117.1, 142.6, 144.3,

160.8 ppm. $C_{10}H_{12}O_2Si$ (192.29): calcd. C 62.46, H 6.29; found C 62.38, H 6.24.

6-Ethynyl-2*H*-pyran-2-one (6a): This compound was prepared from **6c** by two different procedures (Methods A and B).

Method A: $KF{\cdot}2H_2O$ (565 mg, 6.00 mmol) was added to a solution of **6c** (577 mg, 3.00 mmol) in DMF (6 mL) and the mixture was stirred at room temperature for 30 min, at which point the reaction was complete by GLC analysis. The reaction mixture was poured into HCl (5%, 20 mL) and extracted with EtOAc (6 × 10 mL). The organic extract was washed with water (3 × 20 mL), dried and concentrated under reduced pressure. The residue was purified by MPLC on silica gel, with a mixture of CH_2Cl_2, hexane and diethyl ether (75:22:3) as eluent, to give **6a** (345 mg, 96%) as a colourless solid; m.p. 85−86 °C. EI-MS: *m*/*z* (%) = 121 (4) [M^+ + 1], 120 (49) [M^+], 93 (7), 92 (100), 74 (7), 63 (38), 53 (40). IR (KBr disk): $\tilde{\nu}$ = 2114, 1751, 1728, 1615, 1538, 1094, 806 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 3.45 (s, 1 H), 6.40 (d, *J* = 9.6 Hz, 1 H), 6.47 (d, *J* = 6.6 Hz, 1 H), 7.30 (dd, *J* = 9.6, 6.6 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 75.4, 83.5, 111.0, 117.9, 142.4, 143.8, 160.6 ppm. $C_7H_4O_2$ (120.11): calcd. C 70.00, H 3.36; found C 69.85, H 3.29.

Method B: $AgNO_3$ (398 mg, 2.34 mmol), water (4.22 g, 234 mmol) and trifluoroacetic acid (2.67 g, 23.4 mmol) were sequentially added to a solution of **6c** (4.50 g, 23.4 mmol) in acetone (210 mL) and the mixture was stirred in the dark at room temperature for 18 h, at which point the reaction was complete by GLC analysis. The reaction mixture was poured into a saturated NaCl solution (500 mL) and extracted with EtOAc (6 × 80 mL). The organic extract was washed with a saturated NaCl solution (4 × 30 mL), dried and concentrated under reduced pressure. The residue was purified by MPLC on silica gel, with a mixture of CH_2Cl_2, hexane and diethyl ether (75:22:3) as eluent, to give **6a** (2.60 g, 92%) as a colourless solid; m.p. 85−86 °C.

6-(Bromoethynyl)-2*H*-pyran-2-one (6b): A solution of compound **6c** (1.49 g, 7.74 mmol) in acetone (21 mL), $AgNO_3$ (263 mg, 1.55 mmol) and water (279 mg, 15.5 mmol) were sequentially added to a solution of *N*-bromosuccinimide (2.20 g, 12.4 mmol) in acetone (21 mL). The resulting mixture was stirred in the dark at room temperature for 3 h, at which point the reaction was complete by GLC analysis. The reaction mixture was then poured into cold water (200 mL) and extracted with EtOAc (4 × 60 mL). The organic extract was washed with water (3 × 25 mL), dried and concentrated under reduced pressure. The residue was purified by MPLC on silica gel, with a mixture of petroleum ether and EtOAc (75:25) as eluent, to give **6b** (1.34 g, 87%) as a colourless solid: m.p. 111−113 °C. EI-MS: *m*/*z* (%) = 200 (46) [M^+], 198 (48) [M^+], 172 (75), 170 (77), 133 (18), 91 (7), 63 (100). IR (KBr disk): $\tilde{\nu}$ = 2192, 1731, 1619, 1539, 1097, 983, 802 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 6.39 (d, *J* = 9.3 Hz, 1 H), 6.40 (d, *J* = 6.6 Hz, 1 H), 7.27 (dd, *J* = 9.3, 6.6 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 59.6, 72.9, 110.7, 117.8, 142.4, 144.2, 160.6 ppm. $C_7H_3BrO_2$ (199.00): calcd. C 42.25, H 1.52; found C 42.15, H 1.39.

(*E*)-5-Bromo-2-penten-4-yn-1-ol (5a): A solution of 93% chemically pure (*E*)-2-penten-4-yn-1-ol (**5b**; 2.00 g, 24.4 mmol, obtained by fractional distillation of commercially available **5b**) in acetone (80 mL) was added to a solution of *N*-bromosuccinimide (5.20 g, 29.2 mmol) in acetone (85 mL), cooled to 0 °C. $AgNO_3$ (414 mg, 2.44 mmol) was added, and the mixture was stirred at 0 °C in the dark for 2 h. The reaction mixture was poured into cold water (350 mL) and extracted with EtOAc (6 × 60 mL). The crude product obtained after conventional workup was purified by MPLC on silica gel, with a mixture of CH_2Cl_2 and diethyl ether (95:5) as eluent, to give compound **5a** (3.03 g). A GLC analysis showed that this compound was contaminated with ca. 8% of the volatile impurity initially present in **5b**. This bromide was therefore purified by stirring at 0.01 mbar for 12 h at room temperature to give 99% chemically pure **5a** (2.74 g, 70%) as a colourless solid; m.p. 34−36 °C (m.p[28] 37−38 °C). EI-MS: *m*/*z* (%) = 162 (4) [M^+], 161 (6), 160 (4) [M^+], 131 (10), 119 (10), 116 (13), 81 (100). IR (KBr disk): $\tilde{\nu}$ = 3295, 2211, 1416, 1088, 1003, 951, 907 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 2.04 (s, 1 H), 4.21 (dd, *J* = 4.8, 2.0 Hz, 2 H), 5.74 (dt, *J* = 15.8, 2.0 Hz, 1 H), 6.32 (dt, *J* = 15.8, 4.8 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 50.0, 62.5, 78.0, 109.6, 143.4 ppm. C_5H_5BrO (161.00): calcd. C 37.30, H 3.13; found C 37.24, H 3.05.

(*E*)-5-Iodo-2-penten-4-yn-1-ol (5c): A solution of 93% chemically pure (*E*)-2-penten-4-yn-1-ol (**5b**; 739 mg, 9.00 mmol) in acetone (15 mL) and $AgNO_3$ (150 mg, 0.882 mmol) were sequentially added to a solution of *N*-iodosuccinimide (2.36 g, 10.5 mmol), and the mixture was stirred in the dark at room temperature for 45 min. The crude product obtained after conventional workup was purified by MPLC on silica gel, with a mixture of CH_2Cl_2 and diethyl ether (93:7) as eluent, to give 99% chemically pure **5c** (1.25 g, 67%) as a pale grey solid; m.p. 61−63 °C. EI-MS: *m*/*z* (%) = 208 (24) [M^+], 180 (37), 164 (39), 127 (60), 81 (75), 63 (14), 53 (100). ^1H NMR (200 MHz, $CDCl_3$): δ = 1.69 (s, 1 H), 4.25 (dd, *J* = 4.9, 1.8 Hz, 2 H), 5.89 (dt, *J* = 16.0, 1.8 Hz, 1 H), 6.32 (dt, *J* = 16.0, 4.9 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 6.4, 62.5, 92.0, 110.3, 144.0 ppm. C_5H_5IO (208.00): calcd. C 28.87, H 2.42; found C 28.77, H 2.29.

(*E*)-6-(7-Hydroxy-5-heptene-1,3-diynyl)-2*H*-pyran-2-one (2a): This compound was prepared by three different procedures (Methods A, B and C).

Method A: A deaerated solution of **5a** (1.61 g, 10.0 mmol) in DMF (10 mL) was added to a mixture of 99.99% chemically pure CuCl (99.0 mg, 1.00 mmol), hydroxylamine hydrochloride (208 mg, 3.00 mmol) and **6a** (1.20 g, 10.0 mmol) in deaerated DMF (23 mL), and the mixture was stirred under argon at 0 °C. 2,2,6,6-Tetramethylpiperidine (2.83 g, 20.0 mmol) was then added over 5 min. After stirring for 2 h at 0 °C, the mixture was warmed to room temperature, stirred at this temperature for 42 h and subsequently poured into HCl (5%, 150 mL). The mixture was extracted with EtOAc (6 × 50 mL) and the organic extract was washed with brine until neutrality (5 × 50 mL) and dried. It was then concentrated under reduced pressure, and the residue was purified by MPLC on silica gel, with a mixture of CH_2Cl_2 and diethyl ether (85:15) as eluent, to give **2a** (1.00 g, 50%) as a pale orange solid; m.p. 120−121 °C. MS (Tandem Mass Spectrometry on the 201 [M^+ + 1] ion), *m*/*z* (%) = 201 (19), 133 (23), 127 (30), 115 (36), 105 (30), 91 (31), 77 (100). IR (KBr disk): $\tilde{\nu}$ = 3417, 2198, 1747, 1619, 1090, 948, 803 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 1.79 (br. s, 1 H), 4.30 (dd, *J* = 4.4, 2.2 Hz, 2 H), 5.93 (dt, *J* = 16.2, 2.2 Hz, 1 H), 6.37 (dd, *J* = 9.6, 0.7 Hz, 1 H), 6.47 (dd, *J* = 6.6, 0.7 Hz, 1 H), 6.55 (dt, *J* = 16.2, 4.4 Hz, 1 H), 7.29 (dd, *J* = 9.6, 6.6 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 62.5, 71.9, 73.0, 80.1, 84.7, 107.5, 111.9, 118.0, 142.5 144.1, 148.3, 160.7 ppm. $C_{12}H_8O_3$ (200.19): calcd. C 72.00, H 4.03; found C 71.89, H 3.97. Compound **2a** had chemical and stereoisomeric purity higher than 99.5%.

Method B: A deaerated solution of **5b** (164 mg, 2.01 mmol) in DMF (2.5 mL) and a deaerated solution of bromide **6b** (400 mg, 2.01 mmol) in DMF (2 mL) were sequentially added to a mixture of 99.99% chemically pure CuCl (19.9 mg, 0.201 mmol) and hy-

droxylamine hydrochloride (41.9 mg, 0.603 mmol) in DMF (2 mL), and the mixture was stirred under argon at 0 °C. 2,2,6,6-Tetramethylpiperidine (568 mg, 4.02 mmol) was added over 5 min, and the resulting mixture was stirred at 0 °C for 24 h and worked up as described for the preparation of **2a** by Method A. The crude product obtained was purified by MPLC on silica gel to give 99% pure **2a** (165 mg, 41%) as a pale orange solid. The physical and spectral properties of this compound were in agreement with those of **2a** prepared by Method A.

Method C: Compound **6c** (500 mg, 2.60 mmol) and biphenyl (internal standard, 120 mg, 0.780 mmol) were added to a deaerated mixture of 99.99% chemically pure CuCl (129 mg, 1.30 mmol), dry DMI (20 mL) and iodide **5c** (811 mg, 3.90 mmol), and the mixture was stirred under argon at 80 °C for 2 h. After this period of time, the degree of conversion remained unchanged. The mixture was therefore cooled to room temperature, poured into a saturated aqueous NH_4Cl solution (100 mL) and stirred open to the atmosphere until the aqueous phase became blue. It was then extracted with EtOAc (5 × 30 mL), and the organic extract was washed with brine (2 × 10 mL), dried and concentrated under reduced pressure. The residue was purified by MPLC on silica gel to give **2a** (26 mg, 5%) (Table 1, Entry 1). The physical and spectral properties of this chemically pure compound, which had a stereoisomeric purity higher than 98%, were in agreement with those of **2a** prepared by Methods A and B.

(*E*)-5-(*tert*-Butyldimethylsilyloxy)-1-iodo-3-penten-1-yne (7a): A solution of (*E*)-5-(*tert*-butyldimethylsilyloxy)-3-penten-1-yne (2.06 g, 10.5 mmol) in acetone (20 mL) and $AgNO_3$ (175 mg, 1.03 mmol) were added sequentially to a solution of *N*-iodosuccinimide (2.76 g, 12.3 mmol) in acetone (50 mL), and the mixture was stirred in the dark at room temperature for 1 h. The crude product obtained after conventional workup was purified by MPLC on silica gel, with a mixture of petroleum ether and benzene (95:5) as eluent, to give **7a** (2.83 g, 84%) as a pale yellow liquid. EI-MS: *m*/*z* (%) = 322 (3) [M^+], 265 (66), 209 (72), 191 (33), 185 (100), 138 (48), 73 (21). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.08 (s, 6 H), 0.92 (s, 9 H), 4.26 (dd, *J* = 4.0, 2.2 Hz, 2 H), 5.90 (dt, *J* = 16.0, 2.2 Hz, 1 H), 6.27 (dt, *J* = 16.0, 4.0 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = −5.4 (2 C), 5.3, 18.3, 25.8 (3 C), 62.5, 92.4, 108.8, 144.7 ppm. $C_{11}H_{19}IOSi$ (322.26): calcd. C 41.00, H 5.94; found C 40.83, H 5.79.

(*E*)-1-Bromo-5-(*tert*-butyldimethylsilyloxy)-3-penten-1-yne (7b): A solution of (*E*)-5-(*tert*-butyldimethylsilyloxy)-3-penten-1-yne (2.06 g, 10.5 mmol) in acetone (20 mL) and $AgNO_3$ (173 mg, 1.02 mmol) were added sequentially to a solution of *N*-bromosuccinimide (2.12 g, 11.9 mmol) in acetone (50 mL), and the mixture was stirred in the dark at room temperature for 1 h. The crude product obtained after conventional workup was purified by MPLC on silica gel, with hexane as eluent, to give **7b** (2.27 g) as a pale yellow liquid. This liquid was then fractionally distilled to give chemically pure **7b** (1.97 g, 70%): b.p. 75 °C/0.3 mbar. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.08 (s, 6 H), 0.92 (s, 9 H), 4.23 (dd, *J* = 4.0, 2.2 Hz, 2 H), 5.75 (dt, *J* = 15.8, 2.2 Hz, 1 H), 6.29 (dt, *J* = 15.8, 4.0 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = −5.4 (2 C), 18.3, 25.8 (3 C), 49.1, 62.6, 78.4, 108.1, 144.1 ppm. $C_{11}H_{19}BrOSi$ (275.26): calcd. C 48.00, H 6.96; found C 47.86, H 6.89.

5-Acetoxy-1-iodo-1-pentyne (7c): Morpholine (27.0 mL, 309 mmol) was added to a solution of iodine (30.1 g, 119 mmol) in benzene (125 mL), heated under argon at 45 °C. After the mixture had been stirred for 45 min, a solution of 5-acetoxy-1-pentyne (5.00 g, 39.6 mmol) in benzene (25 mL) was added, and the system was stirred at 45 °C for 3 h. It was then cooled to room temperature, poured into an aqueous $Na_2S_2O_3$ solution (10%, 150 mL) and extracted with diethyl ether (4 × 50 mL). The organic extract was washed with brine (50 mL), dried and concentrated under reduced pressure. The residue was fractionally distilled to give **7c** (5.89 g, 60% yield) as a light yellow liquid; b.p. 75 °C/0.3 mbar. EI-MS: *m*/*z* (%) = 237 (14), 192 (100), 168 (15), 165 (24), 127 (19), 125 (36), 65 (31). IR (film): $\tilde{\nu}$ = 2186, 1738, 1433, 1389, 1366, 1246, 1044 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.85 (pseudo-quint, *J* = 6.5 Hz, 2 H), 2.06 (s, 3 H), 2.47 (t, *J* = 7.0 Hz, 2 H), 4.14 (t, *J* = 6.0 Hz, 2 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 17.5, 20.4, 27.4, 62.8, 68.9, 92.8, 170.7 ppm. $C_7H_9IO_2$ (252.05): calcd. C 33.36, H 3.59; found C 33.45, H 3.55.

5-(*tert*-Butyldimethylsilyloxy)-1-iodo-1-pentyne (7d): A solution of methyllithium in diethyl ether (1.8 M, 14.0 mL, 25.2 mmol) was added dropwise to a solution of 5-(*tert*-butyldimethylsilyloxy)-1-pentyne (5.0 g, 25.2 mmol) in THF (35 mL), stirred under argon at −70 °C. The resulting solution was warmed up to room temperature, and was then cooled to −40 °C and treated with iodine (6.40 g, 25.2 mmol). The mixture was stirred under argon for 15 min at −40 °C, warmed to room temperature and worked up as usually. The crude product was purified by MPLC on silica gel, with petroleum ether as eluent, to give **7d** (7.12 g, 87%) as a colourless liquid. EI-MS: *m*/*z* (%) = 267 (96), 239 (25), 187 (22), 185 (100), 139 (17), 109 (13), 75 (24). IR (film): $\tilde{\nu}$ = 2189, 1471, 1255, 1107, 957, 835, 776 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.03 (s, 6 H), 0.87 (s, 9 H), 1.68 (pseudo-quint, *J* = 6.5 Hz, 2 H), 2.43 (t, *J* = 7.0 Hz, 2 H), 3.65 (t, *J* = 6.0 Hz, 2 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = −5.3 (2 C), 17.3, 18.3, 25.9 (3 C), 31.5, 61.3, 69.7, 94.2 ppm. $C_{11}H_{21}IOSi$ (324.28): calcd. C 40.74, N 6.53; found C 40.65, H 6.39.

1-(Iodoethynyl)cyclohexene (7e): This compound was synthesized in 48% yield from 1-ethynylcyclohexene by a procedure very similar to that used for the preparation of **7d**. Compound **7e**, which was obtained as an orange liquid, had a b.p. of 109 °C/16 mbar. EI-MS: *m*/*z* (%) = 232 (100) [M^+], 204 (12), 127 (28), 105 (63), 103 (50), 79 (73), 77 (97). IR (film): $\tilde{\nu}$ = 2152, 1444, 1434, 1346, 918, 841, 798 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.52−1.67 (m, 4 H), 2.04−2.16 (m, 4 H), 6.11 (pseudo-quint, *J* = 2.0 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 21.3, 22.2, 25.5, 28.9, 74.3, 96.1, 121.2, 136.9 ppm. C_8H_9I (232.06): calcd. C 41.41, H 3.91; found C 41.43, H 3.69.

1-(Chloroethynyl)cyclohexene (7f): A solution of methyllithium in diethyl ether (1.8 M, 34 mL, 61.2 mmol) was added dropwise to a solution of 1-ethynylcyclohexene (6.23 g, 58.7 mmol) in THF (150 mL), which was stirred under nitrogen at −78 °C. The resulting solution was warmed to −25 °C and treated with *N*-chlorosuccinimide (9.0 g, 67.5 mmol), and the mixture was stirred for 2 h at −25 °C. It was then warmed to room temperature, poured into cold HCl (3 N, 100 mL) and extracted with pentane (3 × 50 mL). The organic extract was washed with NaOH (3 N, 2 × 30 mL) and brine (30 mL), dried and concentrated. The residue was fractionally distilled to give **7f** (4.61 g, 56%) as a colourless liquid: b.p. 67−69 °C (b.p.[29] 51 °C/3 Torr). EI-MS: *m*/*z* (%) = 142 (15) [M^+], 140 (46) [M^+], 125 (16), 105 (67), 103 (29), 77 (85), 51 (100). IR (film): $\tilde{\nu}$ = 2205, 1447, 1436, 1136, 919, 842, 707 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.55−1.70 (m, 4 H), 2.00−2.25 (m, 4 H), 6.12 (pseudo-quint, *J* = 2.0 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 21.4, 22.2, 25.6, 28.9, 64.9, 71.1, 119.7, 135.9 ppm.

Synthesis of 6-(1,3-Diynyl)-2*H*-pyran-2-ones 2b−g through CuCl-Promoted Reactions between 6-(Trimethylsilylethynyl)-2*H*-pyran-2-

one (6c) and 1-Halo-1-alkynes 7a−g: A 1-halo-1-alkyne **7** (7.80−15.60 mmol), compound **6c** (1.00 g, 5.20 mmol) and biphenyl (internal standard, 160 mg, 1.04 mmol), were added at room temperature to a deaerated mixture of 99.9% chemically pure CuCl (257 mg, 2.60 mmol, 50 mol %) and dry DMF or DMI (30 mL). The mixture was stirred at 80 °C under argon until the degree of conversion into the desired cross-coupled product remained unchanged. It was then cooled to room temperature and poured into a saturated aqueous NH_4Cl solution (200 mL), and the mixture was stirred open to the atmosphere until the aqueous phase became blue. It was then extracted with EtOAc (4 × 60 mL), and the organic extract was washed with brine (2 × 20 mL), dried and concentrated under reduced pressure. The residue was purified by MPLC on silica gel. This general procedure was used for the preparation of compounds **2b**, **2c**, **2d**, **2e**, **2f** and **2g** (Table 1, Entries 2, 3, 4, 5, 6 and 9, respectively).

(*E*)-6-[7-(*tert*-Butyldimethylsilyloxy)-5-heptene-1,3-diynyl]-2*H*-pyran-2-one (2b): The crude product obtained from the CuCl-promoted reaction between **6c** and **7a** in DMI (Table 1, Entry 2) was purified by MPLC on silica gel, with a mixture of petroleum ether and EtOAc (80:20) as eluent, to give **2b** (130 mg, 8%) as a pale brown solid; m.p. 75−79 °C. EI-MS: *m*/*z* (%) = 314 (21) [M^+], 285 (37), 257 (54), 241 (34), 155 (41), 775(70), 73 (100). IR (KBr disk): $\tilde{\nu}$ = 2201, 1747, 1534, 1119, 1084, 836, 800 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 0.08 (s, 6 H), 0.92 (s, 9 H), 4.30 (dd, *J* = 3.8, 2.4 Hz, 2 H), 5.93 (dt, *J* = 15.8, 2.4 Hz, 1 H), 6.38 (d, *J* = 9.6 Hz, 1 H), 6.47 (d, *J* = 6.6 Hz, 1 H), 6.53 (dt, *J* = 15.8, 3.8 Hz, 1 H), 7.28 (dd, *J* = 9.6, 6.6 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = −5.4 (2 C), 18.4, 25.8 (3 C), 62.7, 71.8, 72.8, 80.1, 85.2, 106.3, 111.8, 117.9, 142.4, 144.1, 148.9, 160.6 ppm. $C_{18}H_{22}O_3Si$ (314.46): calcd. C 68.75, H 7.05; found C 68.62, H 6.90. Compound **2b** had chemical and stereoisomeric purity higher than 94%.

6-(7-Acetoxy-1,3-heptadiynyl)-2*H*-pyran-2-one (2c): The crude product obtained from the CuCl-promoted reaction between **6c** and **7c** in DMI (Table 1, Entry 4) was purified by MPLC on silica gel, with hexane/EtOAc (70:30) as eluent, to give **2c** (381 mg, 30%) as a colourless liquid. EI-MS: *m*/*z* (%) = 244 (64) [M^+], 184 (100), 156 (44), 128 (43), 115 (10), 102 (24), 75 (9). IR (film): $\tilde{\nu}$ = 2234, 1740, 1617, 1537, 1242, 1083, 801 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 1.93 (pseudo-quint, *J* = 6.5 Hz, 1 H), 2.07 (s, 3 H), 2.52 (t, *J* = 7.0 Hz, 2 H), 4.16 (t, *J* = 6.0 Hz, 2 H), 6.37 (dd, *J* = 9.5, 1.0 Hz, 1 H), 6.49 (dd, *J* = 6.5, 1.0 Hz, 1 H), 7.32 (dd, *J* = 9.5, 6.5 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 16.3, 20.6, 26.7, 62.4, 64.4, 65.9, 79.9, 88.3, 111.7, 117.6, 142.4, 143.6, 160.3, 170.5 ppm. $C_{14}H_{12}O_4$ (244.25): calcd. C 68.84, H 4.95; found C 69.01, H 4.80.

6-[7-(*tert*-Butyldimethylsilyloxy)-1,3-heptadiynyl]-2*H*-pyran-2-one (2d): The crude product obtained from the CuCl-promoted reaction between **6c** and **7d** in DMI (Table 1, Entry 5) was purified by MPLC on silica gel, with a mixture of hexane and EtOAc (80:20) as eluent, to give a liquid mixture of **2d** and **6c** (516 mg) in a 62:48 molar ratio. Some properties of **2d**, which was thus obtained in ca. 20% yield, are as follows. EI-MS: *m*/*z* (%) = 259 (55), 217 (100), 173 (9), 161 (24), 128 (7), 95 (7), 75 (25). ^1H NMR (200 MHz, $CDCl_3$): δ = 0.06 (s, 6 H), 0.89 (s, 9 H), 1.78 (pseudo-quint, *J* = 6.5 Hz, 2 H), 2.49 (t, *J* = 7.0 Hz, 2 H), 3.69 (t, *J* = 6.0 Hz, 2 H), 6.36 (dd, *J* = 9.0, 1.0 Hz, 1 H), 6.44 (dd, *J* = 6.5, 1.0 Hz, 1 H), 7.27 (dd, *J* = 9.0, 6.5 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = −5.4 (2 C), 16.2, 18.3, 25.9 (3 C), 30.8, 61.1, 64.1, 65.7, 80.6, 89.9, 111.6, 117.7, 142.4, 144.1, 160.6 ppm.

6-[4-(1-Cyclohexenyl)-1,3-butadiynyl]-2*H*-pyran-2-one (2e): The crude product obtained from the CuCl-promoted reaction between **6c** and **7e** in DMI (Table 1, Entry 6) was purified by MPLC on silica gel, with a mixture of hexane and EtOAc (85:15) as eluent, to give chemically pure **2e** (594 mg, 51%) as a pale yellow solid; m.p. 97−100 °C. EI-MS: *m*/*z* (%) = 224 (100) [M^+], 196 (33), 167 (24), 152 (41), 139 (22), 101 (14), 87 (15). IR (KBr disk): $\tilde{\nu}$ = 2200, 1737, 1614, 1531, 1089, 917, 801 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 1.50−1.75 (m, 4 H), 2.10−2.25 (m, 4 H), 6.36 (dd, *J* = 9.5, 1.0 Hz, 1 H), 6.42 (pseudo-quint, *J* = 2.0 Hz, 1 H), 6.46 (dd, *J* = 6.5, 1.0 Hz, 1 H), 7.30 (dd, *J* = 9.5, 6.5 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 20.9, 21.8, 25.9, 28.1, 70.1, 71.4, 80.4, 88.4, 111.5, 117.5, 118.8, 141.5, 142.4, 144.0, 160.5 ppm. $C_{15}H_{12}O_2$ (224.26): calcd. C 80.33, H 5.39; found C 80.16, H 5.25.

6-(1,3-Octadiynyl)-2*H*-pyran-2-one (2f): The crude product obtained from the CuCl-promoted reaction between **6c** and **7g** in DMF (Table 1, Entry 8) was purified by MPLC on silica gel, with a mixture of hexane and EtOAc (82:18) as eluent, to give chemically pure **2f** (562 mg, 54%) as a colourless liquid. EI-MS: *m*/*z* (%) = 200 (100) [M^+], 157 (19), 144 (28), 130 (38), 129 (52), 128 (48), 75 (35). IR (film): $\tilde{\nu}$ = 2233, 1740, 1617, 1536, 1321, 1081, 801 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 0.93 (t, *J* = 7.0 Hz, 3 H), 1.35−1.65 (m, 4 H), 2.40 (t, *J* = 7.0 Hz, 2 H), 6.36 (dd, *J* = 9.5, 6.5 Hz, 1 H), 6.45 (dd, *J* = 6.5, 1.0 Hz, 1 H), 7.29 (dd, *J* = 9.5, 6.5 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 13.4, 19.3, 21.8, 29.8, 63.9, 65.7, 80.6, 90.3, 111.5, 117.6, 142.4, 144.0, 160.6 ppm. $C_{13}H_{12}O_2$ (200.24): calcd. C 77.98, H 6.04; found C 78.02, H 6.11.

6-(1,3-Decadiynyl)-2*H*-pyran-2-one (2g): The crude product obtained from the CuCl-promoted reaction between **6c** and **7h** in DMI (Table 1, Entry 11) was purified by MPLC on silica gel, with a mixture of hexane and EtOAc (85:15) as eluent, to give chemically pure **2g** (594 mg, 50%) as a colourless liquid. EI-MS: *m*/*z* (%) = 228 (93) [M^+], 157 (62), 128 (100), 115 (88), 102 (89), 95 (61), 75 (95). IR (film): $\tilde{\nu}$ = 2232, 1743, 1616, 1537, 1322, 1081, 801 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 0.95 (t, *J* = 7.0 Hz, 3 H), 1.25−1.75 (m, 8 H), 2.40 (t, *J* = 7.0 Hz, 2 H), 6.36 (dd, *J* = 9.5, 1.0 Hz, 1 H), 6.45 (dd, *J* = 6.5, 1.0 Hz, 1 H), 7.29 (dd, *J* = 9.5, 6.5 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 13.4, 19.3, 21.8, 22.7, 23.5, 29.8, 63.9, 65.7, 80.6, 90.3, 111.5, 117.6, 142.4, 144.0, 160.6 ppm. $C_{15}H_{16}O_2$ (228.29): calcd. C 78.92, H 7.06; found C 78.86, H 7.01.

(−)-Nitidon [(−)-(1)]: Titanium(IV) isopropoxide (739 mg, 2.60 mmol) was slowly added to a mixture of L-(+)-diethyl tartrate (619 mg, 3.00 mmol) and powdered activated molecular sieves (4 Å, 1.00 g) in CH_2Cl_2 (16 mL), cooled to −20 °C. The mixture was allowed to stand at −20 °C for 1 h, and a suspension of compound **2a** (400 mg, 2.00 mmol) in CH_2Cl_2 (6 mL) was added. The reaction mixture was stirred at −20 °C for an additional 20 min, and a decane solution of *tert*-butyl hydroperoxide (5.5 M, 1.09 mL, 6.00 mmol) was added over 5 min. The reaction mixture was maintained at −20 °C for an additional 16 h, quenched with 10% aqueous tartaric acid (35 mL) and stirred at the same temperature for 30 min and at room temperature for 1 h. It was then extracted with CH_2Cl_2 (6 × 20 mL), and the organic extract was dried and concentrated under reduced pressure. The residue was purified by MPLC on silica gel, with a mixture of EtOAc and hexane (70:30) as eluent, to give (−)-**1** (305 mg, 71%) as a colourless solid; m.p. 111−114 °C (m.p.[7] 115−117 °C). $[\alpha]_D^{22}$ = −19.0 (*c* = 1.10, $CHCl_3$/CH_3OH, 1:1) {$[\alpha]_D^{22}$ = −34 (*c* = 1.1, $CHCl_3$/CH_3OH, 1:1)}.[7] HPLC and TLC analyses showed that this compound was contaminated with a small amount of L-(+)-diethyl tartrate. The enantio-

meric excess of (−)-**1** was estimated as 95% by HPLC analysis [column: Chiracel OJ; solvent: 2-propanol/hexane (30:70); flow rate: 0.8 mL/min]; t_R = 20.7 min. It was recrystallized from a mixture of hexane and $CHCl_3$ at −23 °C. The compound (−)-**1** obtained had a m.p. of 114−115 °C. $[\alpha]_D^{22}$ = −21.0 (*c* = 1.10, $CHCl_3$/CH_3OH, 1:1). MS (Tandem Mass Spectrometry on the 217 [M^+ + 1] ion), *m*/*z* (%) = 217 (1), 157 (11), 129 (100), 119 (10), 103 (12), 101 (40), 83 (14). IR (KBr disk): $\tilde{\nu}$ = 3471, 2228, 1702, 1614, 1537, 1094, 846 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.79 (br. s, 1 H), 3.43 (pseudo-t, *J* = 3.0, 2.1 Hz, 1 H), 3.63 (d, *J* = 2.1 Hz, 1 H), 3.79 (dd, *J* = 13.2, 3.0 Hz, 1 H), 3.99 (dd, *J* = 13.2, 2.1 Hz, 1 H), 6.42 (dd, *J* = 9.6, 0.6 Hz, 1 H), 6.51 (dd, *J* = 6.6, 0.6 Hz, 1 H), 7.29 (dd, *J* = 9.6, 6.6 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 42.2, 59.6, 60.3, 67.3, 68.9, 78.9, 83.3, 112.6, 118.7, 142.3, 143.4, 160.4 ppm. These NMR spectroscopic data were in satisfactory agreement with those of the natural product.[7] Compound (−)-**1**, which had an enantiomeric excess higher than 99%, proved to be homogeneous by TLC and HPLC analyses.

(+)-Nitidon [(+)-(1)]: This compound was synthesized by asymmetric epoxidation of **2a** (430 mg, 2.15 mmol) by a procedure very similar to that described for its enantiomer, but with D-(−)-diethyl tartrate as chiral ligand. The compound (+)-**1** obtained (354 mg, 76%) had a m.p. of 115−116 °C. $[\alpha]_D^{22}$ = +20.0 (*c* = 1.10, $CHCl_3$/CH_3OH, 1:1). Its spectral properties were in very good agreement with those of (−)-**1** synthesized from **2a**. The enantiomeric excess of (+)-**1** recrystallized from a mixture of hexane and $CHCl_3$ at −23 °C was estimated to be 98% by HPLC analysis [column: Chiracel OJ; solvent: 2-propanol/hexane (30:70); flow rate: 0.8 mL/min]; t_R = 18.8 min. The enantiomeric excess of compound (+)-**1** before recrystallization was 94%.

Acknowledgments

This work was supported by the MIUR (Rome) and the University of Pisa. We are grateful to Professor Piero Salvadori for the use of the Perkin−Elmer Sciex Api III triple quadrupole mass spectrometer. We wish also to thank Mr. Piergiorgio Vergamini for recording IR spectra, Dr. Angela Cuzzola for recording the mass spectra by tandem mass spectrometry and Prof. Rita Menicagli for some HPLC analyses.

[1] [1a] Ø. Bruserud, B. T. Gjertsen, T.-S. Huang, *The Oncologist* **2000**, *5*, 454−462. [1b] V. M. Richon, S. E. Emiliani, E. Verdin, Y. Webb, R. Breslow, R. A. Rifkind, P. A. Marks, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 3003−3007. [1c] G.-L. Ouyang, Q.-F. Li, X.-X. Peng, Q.-R. Liu, S.-G. Hong, *World Gastroenterol.* **2002**, *8*, 1053−1058. [1d] P. A. Marks, V. M. Richon, R. A. Rifkind, *Int. J. Hematol.* **1996**, *63*, 1−17. [1e] M. Yoshida, S. Horinouchi, T. Beppu, *Bioassays* **1995**, *17*, 423−430.

[2] [2a] A. M. Jalava, J. Heikkila, G. Akerlind, G. R. Pettit, K. E. O. Akerman, *Cancer Res.* **1990**, *50*, 3422−3428. [2b] J. Kruh, *Mol. Cell. Biochem.* **1982**, *42*, 65−82. [2c] M. Yoshida, M. Kijima, M. Akita, T. Beppu, *J. Biol. Chem.* **1990**, *265*, 17174−17179. [2d] M. Kijima, M. Yoshida, K. Sugita, S. Horinouchi, T. Beppu, *J. Biol. Chem.* **1993**, *268*, 22429−22435. [2e] H. Nojiri, H. Hori, S. Nojima, *Glyconjugate J.* **1995**, *12*, 459. [2f] J.-W. Zhu, H. Hori, H. Nojiri, T. Tsukuda, Z. Taira, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 139−144. [2g] D. Simoni, R. Rondanin, R. Baruchello, M. Roberti, M. Rossi, S. Grimaudo, N. D'Alessandro, F. P. Invidiata, M. Tolomeo, *Pure Appl. Chem.* **2001**, *73*, 1437−1444. [2h] K. Umehara, M. Matsumoto, N. Nakamura, T. Miyase, M. Kuroyanagi, H. Noguchi, *Chem. Phar. Bull.* **2000**, *48*, 566−567. [2i] N. Takahashi, A. Iwahori, K.-i. Kawai, T. Fukui, *Arch. Biochem. Biophys.* **1998**, *360*, 113−120. [2j] E. Mata−Greenwood, J. F. Daeuble, P. A. Grieco, J. Dou, J. D. McChesney, R. G. Mehta, A. D. Kinghorn, J. M. Pezzuto, *J. Nat. Prod.* **2001**, *64*, 1509−1513. [2k] D. Watters, B. Garrone, J. Coomer, W. E. Johnson, G. Brown, P. Parson, *Biochem. Pharmacol.* **1998**, *55*, 1691−1699. [2l] M. Yoshida, S. Nomura, T. Beppu, *Cancer Res.* **1987**, *47*, 3688−3691. [2m] H. Nojiri, H. Manya, H. Isono, H. Yamana, S. Nojima, *FEBS Lett.* **1999**, *453*, 140−144. [2n] J.-X. Zhang, W.-F. Fong, J. Y.-C. Wu, M. Yang, H.-Y. Cheung, *Planta Med.* **2003**, *69*, 223−229.

[3] [3a] H. P. Koeffler, *Blood* **1983**, *62*, 709−721. [3b] T. R. Breitman, *Methods Enzymol.* **1990**, *190*, 118−130. [3c] A. Thibault, D. Samid, M. R. Cooper, W. D. Figg, A. C. Thompkins, N. Patronas, D. J. Headlee, D. R. Kohler, D. J. Venzon, C. E. Myers, *Cancer* **1995**, *75*, 2932−2938. [3d] P. Fenaux, C. Chomienne, L. Degas, *Semin. Hematol.* **2001**, *38*, 13−25.

[4] For studies on the relationship between structure and activity of retinoids, see: [4a] See ref.[2g] and references cited therein [4b] M. I. Dawson, P. D. Hobbs, in *The Retinoids. Biology, Chemistry and Medicine* (Eds.: M. B. Sporn, A. B. Roberts, D. S. Goodman), 2nd ed., Raven Press: New York, **1994**, pp. 5−178. [4c]M. P. Otero, A. Torrado, Y. Pazos, F. Sussman, A. R. de Lera, *J. Org. Chem.* **2002**, *67*, 5876−5882.

[5] For the synthesis and the evaluation of the bioactivity of brefeldin A derivatives, see: J.-W. Zhu, H. Nagasawa, F. Nagura, S. B. Mohamad, Y. Uto, K. Ohkura, H. Hori, *Bioorg. Med. Chem.* **2000**, *8*, 455−463.

[6] For the synthesis and the evaluation of the bioactivity of analogues of trapoxin B, see: M. Jung, K. Hoffmann, G. Brosch, P. Loidl, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1655−1658.

[7] A. Gehrt, G. Erkel, T. Anke, O. Sterner, *Z. Naturforsch., Teil C* **1998**, *53*, 89−92.

[8] [8a] F. Bellina, C. Anselmi, S. Viel, L. Mannina, R. Rossi, *Tetrahedron* **2001**, *57*, 9997−10007. [8b] R. Rossi, A. Carpita, F. Bellina, P. Stabile, L. Mannina, *Tetrahedron* **2003**, *59*, 2067−2081. [8c] F. Bellina, C. Anselmi, F. Martina, R. Rossi, *Eur. J. Org. Chem.* **2003**, 2290−2302.

[9] [9a] T. Katsuki, K. B. Sharpless, *J. Am. Chem. Soc.* **1980**, *102*, 5974−5976. [9b] T. Katsuki, V. Martin, *Org. React.* **1996**, *48*, 1−300. [9c] A. Pfenniger, *Synthesis* **1986**, 89−116. [9d] R. A. Johnson, K. B. Sharpless, in *Catalytic Asymmetric Synthesis* (Ed.: I. Ojima), VCH: New York, **1993**, pp. 103−158. [9e]Y. Gao, R. M. Hanson, J. M. Klunder, S. Y. Ko, H. Masamune, K. B. Sharpless, *J. Am. Chem. Soc.* **1987**, *109*, 5765−5780. [9f] A. G. Myers, R. Glatthar, M. Hammond, P. M. Harrington, E. Y. Kuo, J. Liang, S. E. Schaus, Y. Wu, J.-N. Xiang, *J. Am. Chem. Soc.* **2002**, *124*, 5380−5401.

[10] M. Biagetti, F. Bellina, A. Carpita, R. Rossi, *Tetrahedron Lett.* **2003**, *44*, 607−610.

[11] W. H. Pirkle, M. Dines, *J. Am. Chem. Soc.* **1968**, *90*, 2318−2323.

[12] [12a] P. Cadiot, W. Chodkiewicz, in *Chemistry of Acetylenes* (Ed.: H. G. Viehe), Marcel Dekker, New York, **1969**, pp. 597−647. [12b] W. Chodkiewicz, *Ann. Chim. (Paris)* **1957**, *2*, 819−869. [12c] G. Eglinton, W. McCrae, *Adv. Org. Chem.* **1963**, *4*, 225−328. [12d] P. Siemsen, R. C. Livingston, F. Diederich, *Angew. Chem. Int. Ed.* **2000**, *39*, 2632−2657.

[13] Y. Nishihara, K. Ikegashira, K. Hirabayashi, J. Ando, A. Mori, T. Hiyama, *J. Org. Chem.* **2000**, *65*, 1780−1787.

[14] R. Rossi, F. Bellina, M. Biagetti, L. Mannina, *Tetrahedron Lett.* **1998**, *39*, 7799−7802.

[15] K. Siegel, R. Brückner, *Chem. Eur. J.* **1998**, *4*, 1116−1122.

[16] H. Hofmeister, K. Annen, H. Laurent, R. Wiechert, *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 727−729.

[17] [17a] T. Nishikawa, S. Shibuya, S. Hosokawa, M. Isobe, *Synlett* **1994**, 485−486. [17b] R. Shen, C. T. Lin, J. A. Porco Jr., *J. Am. Chem. Soc.* **2002**, *124*, 5650−5651.

[18] E. J. Corey, G. W. J. Fleet, M. Kato, *Tetrahedron Lett.* **1973**, *14*, 3963−3966.

[19] [19a] H. M. Schmidt, J. F. Arens, *Recl. Trav. Chim. Pays-Bas*

1967, *86*, 1138−1142. [19b] K. C. Nicolaou, C. A. Veale, S. E. Webber, H. Katerinipoulos, *J. Am. Chem. Soc.* **1985**, *107*, 7515−7518. [19c] N. E. Schore, M. J. Knudsen, *J. Org. Chem.* **1987**, *52*, 569−580.
[20] R. W. Saalfrank, M. Haubner, C. Deutscher, W. Bauer, *Eur. J. Org. Chem.* **1999**, 2367−2372.
[21] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. X. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo, M. Boyd, *J. Natl. Cancer Inst.* **1991**, *83*, 757−766.
[22] J. R. Doyle, P. E. Slade, M. B. Jonassen, *Inorg. Synth.* **1960**, *6*, 216−219.
[23] H. A. Dieck, R. F. Heck, *J. Org. Chem.* **1975**, *40*, 1083−1090.
[24] J. Barluenga, J. M. González, M. A. Rodríguez, P. J. Campos, G. Asensio, *Synthesis* **1987**, 661−662.
[25] J. D. White, T. S. Kim, M. Nambu, *J. Am. Chem. Soc.* **1997**, *119*, 103−111.
[26] D. J. Yoo, E. Y. Kim, M. Oelgemöller, S. C. Shim, *Heterocycles* **2001**, *54*, 1049−1055.
[27] J. P. Marino, M. S. McClure, D. P. Holub, J. V. Comasseto, F. C. Tucci, *J. Am. Chem. Soc.* **2002**, *124*, 1664−1668.
[28] R. B. Bew, *J. Chem. Soc., C* **1966**, 135−139.
[29] Murray, R. E, *Synth. Commun.* **1980**, *10*, 345−349.

Received February 16, 2004

