Synthesis and cellular characterization of the detransformation agent, (–)-depudecin

Junichi Shimada, Ho Jeong Kwon, Masaya Sawamura and Stuart L Schreiber*

Howard Hughes Medical Institute, Department of Chemistry, Harvard University, Cambridge, MA 02138, USA

Background: (–)-Depudecin is a fungal metabolite that reverts the rounded phenotype of NIH3T3 fibroblasts transformed with v-*ras* and v-*src* oncogenes to the flat phenotype of the nontransformed parental cells. The mechanism of action of this detransformation agent is unknown. Although depudecin appears to be an excellent molecule for probing signaling pathways that regulate changes in the cytoskeletal architecture, reagents based on depudecin are not available as it has not yet been successfully synthesized. We therefore set out to synthesize (–)-depudecin.

Results: An asymmetric synthesis of (–)-depudecin has been developed. A cell staining assay has been used to reveal the ability of synthetic depudecin, but not several structural variants, to induce a flattened morphology in v-Ha-*ras*-transformed NIH3T3 cells. This assay also shows that depudecin induces an intricate network of actin stress fibers in these cells and in MG63 osteosarcoma cells and reveals the essential role of the epoxide and hydroxyl moieties in depudecin. Cycloheximide and actinomycin D inhibited the ability of depudecin to induce a morphological change, suggesting that both mRNA synthesis and *de novo* protein synthesis are required for depudecin-mediated suppression of the transformed phenotypes in *ras*-transformed cells.

Conclusions: The synthetic procedure provides access to (–)-depudecin and could be readily modified to produce depudecin-related reagents for the identification of depudecin's cellular target(s). This target appears to be involved in the regulation of the assembly of the actin microfilament component of the cytoskeleton in mammalian cells.

Chemistry & Biology August 1995, **2**:517–525

Key words: actin stress fibers, depudecin, detransformation, signal transduction, total synthesis

Introduction

Natural products have been used to study the cellular functions of proteins in several ways. By identifying the cellular receptors for these compounds, new proteins have been discovered with functions linked to the effects the natural products have on cells [1,2]. When the receptors have been identified, and when it has been established that their binding interactions are specific, the natural products have been used to reveal whether their targets are involved in specific cellular processes [3]. Mutations in the receptors that prevent natural-product binding, but that do not otherwise inhibit the actions of the receptors, have proven to be especially useful. Here, the role of residues and domains in the mutant receptors can be examined by performing assays in the presence of the natural product and the mutant receptors. In this context, the natural product can be viewed as a chemical equivalent to a conditional loss-of-function allele in that it conditionally inhibits the wild-type, endogenous receptor ([4] and Brown, E.J., Beal, P.J., Chen, J., Shin, T.B. & S.L.S., unpublished data).

A fascinating class of natural products with detransforming properties may be especially illuminating as probes of cellular signaling pathways. Examples of such compounds include trapoxin A [5], herbimycin A [6], radicicol [7], and azatyrosine [8]. These compounds are able to revert the morphological changes seen following transformation of cells in culture with either v-*sis*, v-*src*, or v-*ras* oncogenes. Although some of these agents have been shown to interact with the oncogene products directly, others act indirectly and many of their effects are still poorly understood. The molecular characterization of their actions in cells promises to illuminate these oncogene-mediated signaling pathways.

Another example of this class of molecules is (–)-depudecin (compound **1**, Fig. 1), whose mechanism of detransformation action is completely obscure [9,10]. Depudecin was discovered in the culture broth of the fungus *Alternaria brassicicola* using an assay for the morphological detransformation of NIH3T3 cells doubly transfected with v-*ras* and v-*src* oncogenes [9,10]. An X-ray crystallographic study of a depudecin derivative revealed its unprecedented structure, which consists of a highly oxidized 11-carbon chain containing two epoxides conjugated through a trans-disubstituted olefin. The purified compound was reported to induce the flat phenotype at concentrations lower than that required to inhibit proliferation of the same cells. On this basis, we selected depudecin as a molecule to probe signaling pathways that regulate the cytoskeletal architecture. As depudecin has not been synthesized previously and is not easily obtained otherwise, we initiated our studies by developing a total synthesis of (–)-depudecin. Using synthetic molecules including (–)-depudecin, we also initiated studies of the depudecin-sensitive step in transformed cells. Simple modifications of the synthesis should provide access to depudecin-based reagents useful for the identification of

*Corresponding author.

interacting proteins and for the further dissection of the depudecin-sensitive signaling pathway.

Results and discussion

Our synthetic strategy to generate the sensitive bis-*trans*-epoxide moiety of compound **1** uses a one-pot procedure for the stereoselective conversion of syn-vicinal diols into *trans*-epoxides developed by Sharpless and others [11–14]. The synthesis of tetraol **19**, a key intermediate that contains the entire depudecin skeleton, is summarized in Figs 1 and 2. Methylenation of a D-threose derivative **2** [15], *tert*-butyl dimethylsilyl (TBDMS) deprotection with tetrabutyl ammonium fluoride (TBAF), and subsequent Swern oxidation gave aldehyde **5** [16]. To achieve a one carbon homologation, compound **5** was reacted with 2-trimethylsilylthiazole to afford *anti*-alcohol **6**, which was then converted into the desired *syn* diastereomer **8** through an oxidation–reduction sequence [17–19]. The *syn* isomer was obtained selectively (4:1) using $NaBH_4$; the more bulky reducing agent, K-Selectride, failed to improve the selectivity. The initial formation of isomer **6** is consistent with previous demonstrations that 2-trimethylsilylthiazole reacts with α,β-dialkoxy aldehydes in a non-chelation manner to give the *anti*-alcohol [17]. Furthermore, the relative chemical shifts of newly formed hydroxyl-stereocenters in compounds **6** and **8** support the assigned stereochemistry. The methine protons of these centers in *anti*-isomers usually have chemical shifts downfield of those in *syn*-isomers [17]. The corresponding chemical shifts of compounds **6** and **8** are δ5.27 and 4.97 respectively. After TBDMS protection of the hydroxyl group, the aldehyde **10** (80 % isomeric purity) was released by cleavage of the thiazole ring [20] using standard procedures involving N-methylation, reduction with $NaBH_4$ and copper-assisted hydrolysis (Fig. 1).

Ketophosphonate **12**, which was synthesized [21] from ester **11** [22], was condensed with aldehyde **10** to yield enone **13** in 88 % yield. Sodium borohydride reduction in the presence of cerium chloride [23] yielded the desired non-chelation product **14** in 96 % yield. The reduction proceeded in about 90 % diastereoselectivity as determined by ^1H NMR. Deprotection of the silyl group followed by flash chromatography afforded diol **15** in isomerically pure form. ^1H NMR coupling constants ($J_{C4H-C5H}$, $J_{C6H-C7H}$) between the olefinic protons (C5H and C6H) of **15** and their adjacent methine protons (C4H and C7H) are similar in value, suggesting that the assigned stereochemistry of **15** is correct (pseudo-C2 symmetry). To elaborate tetraol **19** from **15**, we planned to synthesize a bis-cyclic carbonate **17** as a key intermediate. Treatment of diol **15** with 2,2,2-trichloroethyl chloroformate in the presence of 2,6-lutidine, followed by treatment with anhydrous methanolic hydrochloride for 6 h gave the bis-carbonate derivative **17** in 44 % yield. Compound **17** isomerizes to an inseparable mixture of five- and six-membered bis-carbonates, even in neutral media (MeOH solution, ~15 mM, 30 % isomerization at 23 °C, 16 h). Isolation of the labile intermediate **17** was facilitated by the reactivity of the 2,2,2-trichloroethyl carbonate relative to other carbonates examined, that is, methyl carbonate and *p*-nitrophenyl carbonate, and by the increased stability of **17** in acidic media. Treatment of compound **17** with a large excess of dimethyl sulfide in the presence of benzoyl peroxide [24] gave bis-methylthiomethyl-protected carbonate **18** in 34 % yield. Basic hydrolysis of **18** gave the tetraol **19** (Fig. 2).

Treatment of compound **19** with trimethylorthoacetate in the presence of catalytic pyridinium *para*-toluenesulfonate (PPTS) followed by trimethylsilyl chloride in a triethylamine buffer [14] gave diacetoxy dichloride **20** exclusively. The crude product **20** was then subjected to base-mediated saponification resulting in spontaneous cyclization to furnish bis-*trans* epoxide **21** in 74 % overall

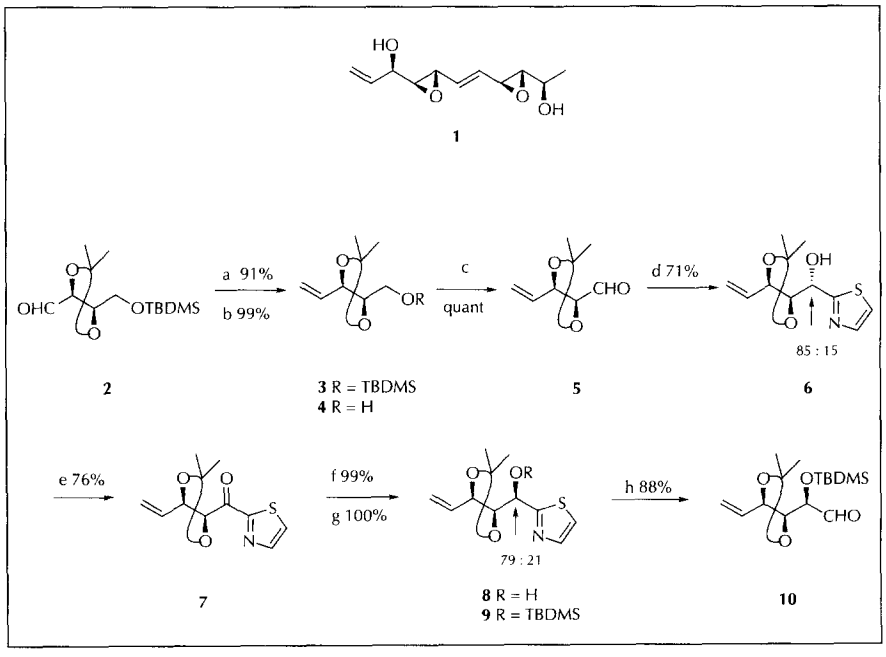

Fig. 1. Synthesis of the aldehyde **10**, representing the left part of depudecin **1**. Reagents and conditions: (a) $Ph_3P^+MeI^-$, *tert*-BuOK, 23 °C; (b) TBAF, tetrahydrofuran (THF), 23 °C; (c) Swern oxidation; (d) 2-trimethylsilylthiazole, THF, 23 °C, then TBAF; (e) Swern oxidation; (f) $NaBH_4$, MeOH, –78 °C → –20 °C; (g) TBDMSCl, imidazole, dimethyl formamide (DMF), 70 °C; (h) MeI, MeCN, reflux; $NaBH_4$; then CuO, $CuCl_2$, MeCN–H_2O.

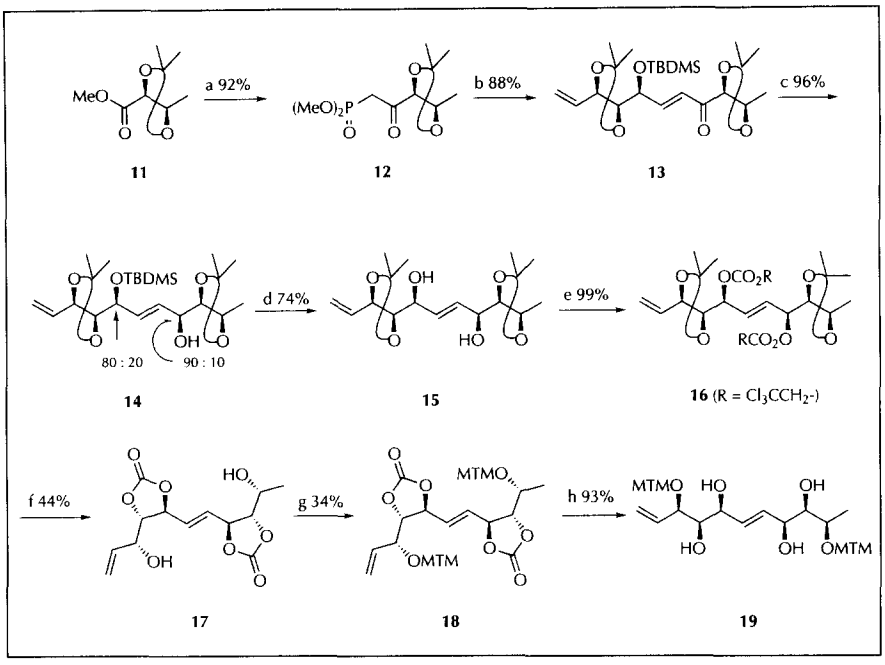

Fig. 2. Synthesis of tetraol **19**, a key intermediate that contains the complete depudecin 1 framework. Reagents and conditions: (a) $LiCH_2P(O)(OMe)_2$, THF, 0 °C; (b) NaH, THF, 23 °C, then compound **10**, 23 °C; (c) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, –78 °C → –20 °C; (d) TBAF, then separation of stereoisomers; (e) $ClCO_2CH_2CCl_3$, 2,6-lutidine, CH_2Cl_2, 23 °C; (f) HCl (anhyd.), MeOH, 23 °C, 6 h; (g) Me_2S, benzoyl peroxide (BPO), MeCN, 0 °C; (h) 1 M LiOH, THF, 23 °C.

yield. All three operations were carried out in one reaction vessel without isolation of any intermediates. Treatment of compound **21** with ten equivalents of mercuric chloride in the presence of excess calcium carbonate gave mono-protected epoxide **22** in 73 % yield. By using a larger excess of mercuric chloride, depudecin **1** was isolated in 52 % yield (Fig. 3). The structure of synthetic depudecin was identical to that of natural depudecin, which was kindly provided by Drs Shigeru Matsutani, Hitoshi Arita, and Toshiyuki Kamigauchi of Shionogi Research Laboratories, as judged by comparison of optical rotation ($[\alpha]^{23}_D$ -31° (*c* = 0.080, $CHCl_3$), lit. [9]: $[\alpha]^{23}_D$ -35.8° (*c* = 0.52, $CHCl_3$)), infrared, ^1H, and ^{13}C NMR spectra. We therefore conclude that the first total synthesis of (–)-depudecin has been achieved. The synthesis proceeds in 22 total steps and in 1.4 % overall yield. The one-pot stereoselective conversion of *syn*-vicinal diols into *trans*-epoxides efficiently constructs the two epoxide moieties simultaneously, and should prove valuable in the synthesis of depudecin-related molecules that may facilitate the isolation and characterization of the cellular target(s) of depudecin.

The synthetic studies described above addressed our first challenge — to access samples of depudecin. We are now attempting to modify the synthetic pathway to produce radiolabeled and immobilized derivatives to facilitate the purification of proteins that interact with depudecin. To identify structural elements that might be modified without a loss in activity, and to develop an experimental system to investigate the depudecin-sensitive signaling pathway, we analyzed the effect of synthetic depudecin and its derivatives on NIH3T3 cells transformed with v-Ha-*ras*. These and other cells transformed by oncogenes acquire abnormal phenotypes resulting from constitutively activated pathways important in the control of cell growth, shape, and mobility. Examples of such abnormal phenotypes are cell growth without contact inhibition, round or spindle cell shape, loss of actin stress fibers, and anchorage-independent

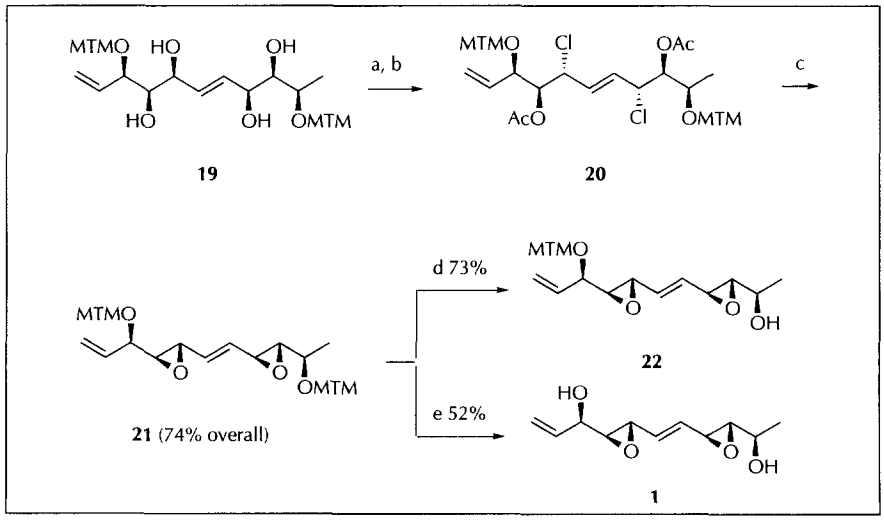

Fig. 3. Synthesis of (–)-depudecin **1** by a procedure that efficiently constructs the two epoxide moieties simultaneously. Reagents and conditions: (a) $MeC(OMe)_3$, cat. PPTS, 23 °C; (b) trimethylsilyl chloride (TMSCl), triethylamine (TEA), 23 °C; (c) K_2CO_3, MeOH, 23 °C; (d) 10 eq. $HgCl_2$–$CaCO_3$, MeCN–H_2O, 23 °C, 3.5 h; (e) 50 eq. $HgCl_2$–$CaCO_3$, MeCN–H_2O, 23 °C, 3.5 h.

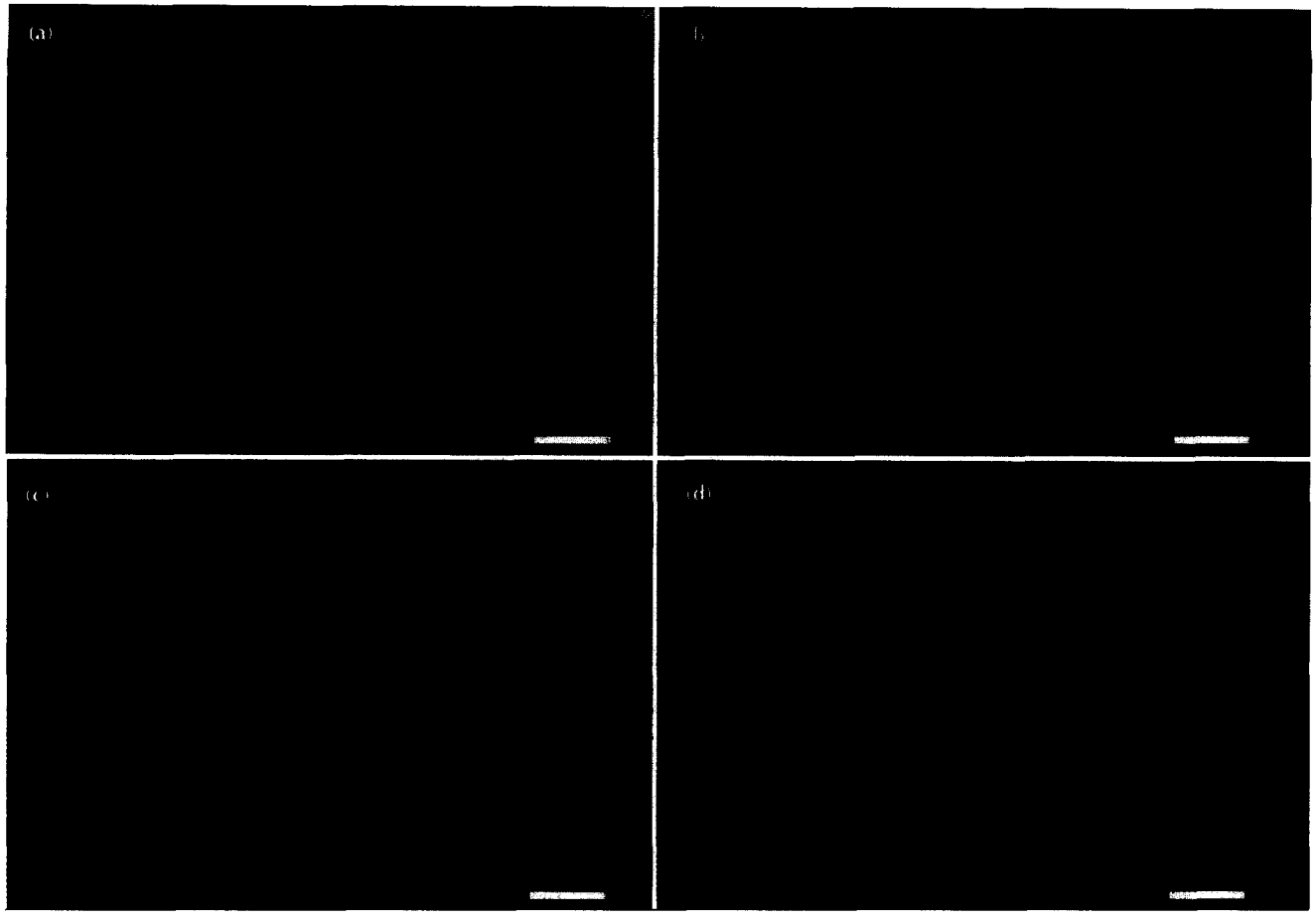

Fig. 4. Restoration of actin stress fibers in v-Ha-*ras*-transformed NIH3T3 and MG63 cells by treatment with synthetic (–)-depudecin. Actin stress fibers and nuclei in cells grown on slide cover slips were stained with both FITC-phalloidin (green) and propidium iodide (red). **(a)** Control v-Ha-*ras*-transformed NIH3T3 cells; **(b)** v-Ha-*ras*-transformed NIH3T3 cells treated with 4.7 μM synthetic (–)-depudecin; **(c)** Control MG63 cells; **(d)** MG63 cells treated with 4.7 μM synthetic (–)-depudecin. (Bar = 10 μm)

growth [25,26]. Since depudecin has been shown to alter the shape of oncogene-transformed cells, it probably affects other processes related to cellular transformation. We therefore selected a cell-staining assay that would provide insight into the effects of synthetic depudecin and its structural variants on the cytoskeleton as well as on the shape of cells.

Double staining for actin stress fibers and for nuclei using fluorescein isothiocyanate (FITC)-labeled phalloidin and propidium iodide, respectively, showed that the v-Ha-*ras*-transformed cells have a spindle-like morphology and that they lack the network of actin stress fibers (Fig. 4a) that are present in the parent NIH3T3 cell line (data not shown). Following incubation with synthetic depudecin (4.7 μM) for 24 h at 37 °C, the morphology of the transformed cells reverted to a flattened shape, similar to that of the parent cell line NIH3T3, and the actin stress fibers reappeared (Fig. 4b). There was no significant change in the shape of nuclei (Fig. 4). In contrast to synthetic depudecin, the mono-methylthiomethyl-protected depudecin **22** showed only a weak reversion activity even at 10 times higher concentration (47 μM, data not shown), and the bis-methylthiomethyl-protected depudecin **21** and bis-protected tetraol **19** had no activity at the same concentration (47 μM, data not shown). These results suggest that the epoxide and hydroxy groups are essential for the detransforming activity of depudecin. The possibility that the actions of depudecin are antagonized by analogs that do not exhibit activity was also examined. Treatment of cells with ~20-fold molar excess of each inactive compound relative to depudecin did not result in antagonism by any of the inactive analogs (Table 1). This implies that the inactive compounds do not competitively bind to or modify the site on the target protein(s) that mediates the effects of depudecin.

To obtain an understanding of the mechanism underlying morphological reversion by depudecin, we examined the effects of several cell-permeable inhibitors on depudecin-mediated reversion of v-Ha-*ras*-transformed NIH3T3 cells (Table 1). Simultaneous addition of actinomycin D (an inhibitor of RNA synthesis) and cycloheximide (an inhibitor of protein synthesis) with synthetic depudecin abolished both the morphological change and formation of bundles of actin microfilaments. On the other hand, morphological change by synthetic depudecin was not interrupted by hydroxyurea

Table 1. Actin stress fiber restoration by synthetic depudecin (compound **1**) and its structural variants in v-Ha-*ras*-transformed NIH3T3 cells.

	Activity					
Treatment	**0.47**	**4.7**	**9.4**	**23.5**	**47**	**100 (μM)**
Compound						
1	–[a]	+	+	++	+++	ND
22	–	–	–	–	±	+
21	–	–	–	–	–	–
19	–	–	–	–	–	–
1[b] + **21** (100 μM)				+		
1 + **19** (100 μM)				+		
1 + Hydroxyurea (1 mM)				+		
1 + Actinomycin D (1 μg ml^{-1})				–		
1 + Cycloheximide (1 mM)				–		
1 + FK506 (100 nM)				+		
1 + Rapamycin (10 nM)				++		
1 + Wortmannin (10 nM)				++		
1 + Staurosporine (20 nM)				+		
1 + Sodium vanadate (1 mM)				+		

[a]Actin stress fiber restoration activity: –, no effect; ±, weak; +, moderate; ++, strong; +++, very strong; ND, not determined.
[b]4.7 μM depudecin was used throughout for inhibition assays.

(an inhibitor of DNA synthesis) [27], FK506 (an inhibitor of calcineurin) [28], staurosporine (a general inhibitor of protein kinases) [29] or sodium vanadate (a general inhibitor of protein phosphatases) [30]. These results indicate that the induced transcription of a gene or genes followed by *de novo* protein synthesis is required for the reversion activity of synthetic depudecin. Depudecin may therefore interact with or block the function of a target protein that suppresses the expression of anti-Ras or anti-tumor activity. Interestingly, rapamycin (an inhibitor of the FKBP–rapamycin-associated protein (FRAP)) [31] and wortmannin (an inhibitor of phosphatidyl inositol 3-kinase (PI3K)) [32] apparently enhance the flat reversion activity of synthetic depudecin. This also implies that the target molecule of depudecin does not participate in the PI3K and FRAP-mediated $p70^{S6}$ kinase signaling pathway, and that the cooperative interruption of different growth signaling pathways may result in a synergistic effect on detransformation.

We also analyzed the effect of synthetic depudecin on the morphology of a human osteosarcoma cell line (MG63), and found that it induced well organized actin stress fibers, as seen in a comparison to non-treated cells (Fig. 4c,d). This cell line is derived from a tumor, and therefore was not transformed by the laboratory introduction of a specific oncogene, as is the case for the v-Ha-*ras*-transformed NIH3T3 cells described above. Sugita *et al.* have reported that natural depudecin induces reversion of the flat phenotype of cells transformed with the oncogene *raf* [10]. The protein product of *raf*, Raf-1, acts as a downstream mediator of the Ras signal [33]. Together with our data, the results suggest that depudecin may interact with a target protein common to multiple cell types and that its ability to induce stress fibers does not require transformation with *ras*. Recent studies of growth-factor-mediated actin stress fiber formation in Swiss 3T3 cells suggest that whereas signaling through Ras is not required, signaling through the related small GTPases Rac and Rho is essential [34]. Sequential elements of the pathway include growth factor receptors, PI3K, Rac, arachidonic acid formation and conversion to leukotrienes, leukotriene-dependent Rho activation, and stress fiber formation. The identification of the depudecin-binding protein(s) and others whose activities are modulated by depudecin should advance the findings in this work and promote our understanding of the molecular mechanism of this signaling pathway.

Significance

The detransformation agent depudecin appears to be a valuable probe of signaling pathways regulating the formation of actin stress fibers. It is not easily obtained, however, and many experiments aimed at investigating the depudecin-sensitive signaling pathways will require synthetic derivatives of depudecin. Using a total of 22 synthetic transformations, we have achieved a total synthesis of the natural (–)-form of depudecin that provides ample quantities of the compound for mechanistic investigations and that should be easily modified to prepare derivatives useful in identifying the protein receptor(s) of depudecin. We report a cell staining assay that reveals the ability of synthetic depudecin not only to alter the spindle-shaped morphology of v-Ha-*ras*-transformed NIH3T3 cells to a flattened shape, but also to induce an intricate actin stress fiber network in these cells and in MG63 osteosarcoma cells. Depudecin-mediated stress fiber formation appears to require the induced transcription of genes and subsequent *de novo* protein synthesis. These findings create a framework for studies that will illuminate the growth factor-mediated signaling pathways leading to stress fiber formation.

Materials and methods

Synthetic conditions

Melting points were measured with a Mel-Temp apparatus and are uncorrected. Infrared spectra (IR) were recorded using a Nicolet 5PC FT-IR spectrometer (γ max in cm^{-1}). Samples were prepared as thin films by evaporation onto a salt plate (NaCl) or as solutions in noted solvents using a NaCl solution cell. ^{1}H NMR spectra were recorded on either a Bruker AM-500 (500 MHz) or AM-400 (400 MHz) spectrometer as noted at ambient temperature. Data were reported as follows: chemical shifts in parts per million (ppm) using residual protio solvent as internal standard (7.26 for $CDCl_3$) on the δ scale, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet), coupling constant(s) in Hz, and integration. ^{13}C NMR spectra were recorded on a Brucker AM-400 (100 MHz) spectrometer and were reported

in ppm using solvent resonance as internal standard (77.0 for $CDCl_3$). All ^{13}C spectra were determined with complete proton decoupling. Mass spectra were obtained using either a JEOL AX-505 or SX-102. Optical rotations were measured using a Perkin-Elmer 241 polarimeter at 23 °C using a sodium lamp (589 nm) and are reported in degrees with concentration in units of 10 mg ml^{-1}.

Cells and cell culture conditions

v-Ha-*ras*-transformed NIH3T3 cells were kindly supplied by R.L. Erikson of the Department of Molecular and Cellular Biology, Harvard University, and were cultured at 37 °C in a humidified atmosphere of 5 % CO_2 in Dulbecco's modified minimal Eagle's medium supplemented with 10 % fetal calf serum (FCS). MG63 cells were cultured in RPMI 1640 + 10 % FCS using the same culture conditions used with v-Ha-*ras*-transformed NIH3T3 cells.

Double staining of actin stress fibers and nuclei with FITC-phalloidin and propidium iodide

For observation of actin stress fibers and nuclei by fluorescence microscopy, cells were stained with both FITC-phalloidin and propidium iodide according to the method of Hall *et al.* [35]. Briefly, asynchronous cells were cultured on a slide cover slip for 18 h. After treatment with depudecin or related molecules for an additional 24 h, cells were fixed with 3.7 % paraformaldehyde in phosphate-buffered saline (PBS) for 20 min at room temperature. Following 3x washing with PBS, cells were treated with 0.1 % Triton X-100 in PBS for 5 min to increase cell permeability. Next, the cells were treated with FITC-phalloidin (2.5 μg ml^{-1}, Sigma) for 20 min and, after 3x washing with PBS, the cells were mounted on a slide with antifader (1,4-diazabicyclo[2.2.2]octane, Aldrich) containing propidium iodide (0.1 μg ml^{-1} in 50 % glycerol). Cells were inspected by conventional fluorescence microscopy (Leica, Germany).

Selected experimental procedures for the synthesis of depudecin and synthetic intermediates follow (a complete listing of experimental procedures and spectroscopic data are available from the authors upon request).

*Synthesis of (2R, 3S, 5E, 7S, 8S, 9R)-2,3:8,9-O-bis-isopropylidene-7-*tert*-butyldimethylsiloxyundeca-5,10-dien-4-one (compound **13**)*

To a suspension of NaH (60 % in mineral oil, 115 mg, 2.89 mmol) in THF (3 ml) was added dropwise a solution of the phosphate **12** (885 mg, 3.32 mmol) in THF (3 ml) at 0 °C. After stirring for 5 min at ambient temperature, to the mixture was added a solution of the aldehyde **10** (666 mg, 2.22 mmol) in THF (3 ml). The reaction was stirred for additional 2 h at ambient temperature, quenched with water (5 ml) and extracted into Et_2O. The combined organic extracts were washed with brine, dried over $MgSO_4$ and concentrated. Purification by flash chromatography (eluent: 6.25 % ethyl acetate:hexane) gave 861 mg (88 %) of compound **13** (isomeric mixture) as a colorless oil: IR (thin film) : 2988, 2957, 2934, 2888, 2859, 1696, 1630, 1381, 1372, 1254, 1171, 1125, 1100, 1069, 986, 837 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.12 (dd, 1x4/5 H, J = 4.1, 15.7 Hz, C_6–H), 6.99 (dd, 1x1/5 H, J = 4.2, 15.7 Hz, C_6–H), 6.89 (dd, 1x4/5 H, J = 1.8, 15.7 Hz, C_5–H), 6.85 (dd, 1x1/5 H, J = 1.8, 15.7 Hz, C_5–H), 5.81 (ddd, 1x4/5 H, J = 6.5, 10.4, 17.0 Hz, C_{10}–H), 5.76 (ddd, 1x1/5 H, J = 6.4, 10.4, 17.0 Hz, C_{10}–H), 5.34 (td, 1x4/5 H, J = 1.3, 17.0 Hz, C_{11}–H), 5.30 (td, 1x1/5 H, J = 1.4, 17.0 Hz, C_{11}–H), 5.21 (td, 1x4/5 H, J = 1.1, 10.4 Hz, C_{11}–H), 5.18 (td, 1x1/5 H, J = 1.1, 10.4 Hz, C_{11}–H), 4.58 (brdt, 1x4/5 H, J = 1.8, 4.4 Hz, C_7–H), 4.56 (dt, 1x1/5 H, J = 1.8, 4.1 Hz, C_7–H), 4.44 (brdd, 1x1/5 H, J = 6.4, 7.7 Hz, C_9–H), 4.26 (brdd, 1x4/5 H, J = 6.5, 7.9 Hz, C_9–H), 4.11–4.01 (m, 2 H, C_2–H, C_3–H), 3.79 (dd, 1x4/5 H, J = 4.9, 7.9 Hz, C_8–H), 3.75 (dd, 1x1/5 H, J = 4.1, 7.7 Hz, C_8–H), 1.46, 1.43, 1.42, 1.39 (s, 3 H, acetonide), 1.40 (d, 3 H, J = 6.0 Hz, C_1–H), 0.94 (s, 9x1/5 H, *tert*-BuSi), 0.92 (s, 9x4/5 H, *tert*–BuSi), 0.11 (s, 3x1/5 H, CH_3Si), 0.08 (s, 3x4/5 H, CH_3Si), 0.06 (s, 3x4/5 H, CH_3Si), 0.04 (s, 3x1/5 H, CH_3Si); ^{13}C NMR (100 MHz; $CDCl_3$) δ 197.6, 147.8 (minor), 147.1 (major), 136.1 (minor), 135.9 (major), 124.9 (major), 124.4 (minor), 117.9, 110.2 (major), 110.0 (minor), 109.5, 86.0 (major), 85.91 (minor), 82.8, 78.3 (major), 77.8 (minor), 74.6 (major), 74.4 (minor), 71.8 (minor), 71.7 (major), 27.2 (major), 27.2 (minor), 27.0 (major), 26.9 (minor), 26.8 (major), 26.2 (minor), 26.2 (major), 25.9 (minor), 25.7, 18.5 (major), 18.4 (minor), –4.6 (minor), –4.8 (major); high resolution mass spectrometry (HRMS) (CI NH_3) *m/e* calc'd for $C_{23}H_{44}NO_6Si$ 458.2939 $[(M+NH_4)^+]$, found 458.2938.

*Synthesis of (2R, 3S, 4S, 5E, 7S, 8S, 9R)-2,3:8,9-O-bis-isopropylidene-7-*tert*-butyldimethylsiloxy-undeca-5,10-dien-4-ol (compound **14**)*

To a stirred solution of compound **13** (200 mg, 0.454 mmol, 8:2 isomeric mixture) and $CeCl_3 \cdot H_2O$ (846 mg, 2.27 mmol) in MeOH (6.5 ml) was added $NaBH_4$ (51 mg, 1.36 mmol) at –78 °C. The reaction was stirred for 20 min at –78 °C, then slowly warmed to –10 °C over 1 h and quenched with water (20 ml). The product was extracted into Et_2O. The combined organic extracts were washed with brine, dried over $MgSO_4$ and concentrated. Purification by flash chromatography (eluent: 20 % ethyl acetate:hexane) gave 193 mg (96 %) of compound **14** (about 75 % isomeric purity) as a colorless oil: IR (thin film) 3500 (br), 2986, 2934, 2859, 1379, 1372, 1252, 1217, 1173, 1123, 1092, 1067, 837 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.91 (dd, 1 H, J = 5.5, 15.6 Hz, C_5–H), 5.83 (ddd, 1 H, J = 6.8, 10.4, 17.2 Hz, C_{10}–H), 5.75 (dd, 1 H, J = 7.3, 15.6 Hz, C_6–H), 5.33 (td, 1 H, J = 0.8, 17.2 Hz, C_{11}–H), 5.19 (td, 1 H, J = 0.8, 10.4 Hz, C_{11}–H), 4.37–4.33 (m, 2 H, C_7–H, C_9–H), 4.11 (brq, 1 H, J = 5.5 Hz, C_4–H), 4.03 (dq, 1 H, J = 6.0, 8.1 Hz, C_2–H), 3.70 (dd, 1 H, J = 4.4, 8.0 Hz, C_8–H), 3.50 (dd, 1 H, J = 4.5, 8.1 Hz, C_3–H), 2.28 (d, J = 6.2 Hz), 1.40 (s, 6 H, acetonide), 1.40 (s, 3 H, acetonide), 1.39 (s, 3 H, acetonide), 1.28 (d, 3 H, J = 6.0 Hz, C_1–H), 0.89 (s, 9 H, *tert*-BuSi), 0.06 (s, 3 H, CH_3Si), 0.05 (s, 3 H, CH_3Si); ^{13}C NMR (100 MHz; $CDCl_3$) δ 136.3, 131.6, 130.6, 117.7, 109.2, 108.6, 85.1, 83.7, 78.1, 73.3, 71.9, 71.5, 27.5, 27.1, 27.0, 27.0, 25.9, 18.3, –4.4, –4.7; HRMS (CI NH_3) *m/e* calc'd for $C_{23}H_{46}NO_6Si$ 460.3096 $[(M+NH_4)^+]$, found 460.3099.

*Synthesis of (4S, 5S)-4-[(E)-2-[(4R, 5R)-5-[(1R)-1-hydroxyethyl]-2-oxo-1,3-dioxolan-4-yl]ethenyl]-5-[(1S)-1-hydroxy-2-propenyl]-1,3-dioxolan-2-one (compound **17**)*

To a solution of compound **16** (227 mg, 0.334 mmol) in MeOH (4.6 ml) was added methanolic hydrogen chloride solution (2.3 ml, ~15 % w/w) at 0 °C. The reaction was stirred for 6 h at ambient temperature and concentrated. The residue was subjected to flash chromatography (eluent 72.5 % ethyl acetate:hexane) to yield compound **17** (44.2 mg, 44 %) as a colorless oil: $[\alpha]^{23}_D$ –109.0° (c = 1.74, CH_3OH); IR (thin film) 3443 (br), 2982, 1788, 1375, 1183, 1067, 978, 860 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.09 (m, 2 H, C_5–H, C_6–H), 5.89 (ddd, 1 H, J = 6.1, 10.6, 17.0 Hz, C_{10}–H), 5.44 (td, 1 H, J = 1.2, 17.0 Hz, C_{11}–H), 5.31

(td, 1 H, J = 1.1, 10.6 Hz, C_{11}–H), 5.16–5.11 (m, 2 H, C_4–H, C_7–H), 4.47 (dd, 1 H, J = 3.6, 5.8 Hz, C_8–H), 4.35 (dd, 1 H, J = 2.8, 6.1 Hz, C_3–H), 4.29–4.27 (m, 1 H, C_9–H), 3.87 (dq, 1 H, J = 2.8, 6.5 Hz, C_2–H), 1.26 (d, 3 H, J = 6.5 Hz, C_1–H); ^{13}C NMR (100 MHz; CD_3OD) δ 156.3, 156.1, 136.4, 131.7, 131.7, 118.8, 85.7, 84.4, 79.0, 78.6, 72.4, 67.0, 18.8; HRMS (CI NH_3) *m/e* calc'd for $C_{13}H_{20}NO_8$ 318.1189 $[(M+NH_4)^+]$, found 318.1199.

*Synthesis of (4S, 5S)-4-[(E)-2-[(4R, 5R)-5-[(1R)-1-(methylthiomethoxy)ethyl]-2-oxo-1,3-dioxolan-4-yl]-ethenyl]-5-[(1S)-1-methylthiomethoxy-2-propenyl]-1,3-di-oxolan-2-one (compound **18**)*

To a solution of diol **17** (38 mg, 0.127 mmol) in MeCN (1.3 ml) at 0 °C was added Me_2S (0.223 ml, 3.04 mmol) followed by the portionwise addition of benzoyl peroxide (368 mg, 1.52 mmol) over a 5 min period. After stirring for 3.5 h at 0 °C, the mixture was quenched by the addition of saturated aqueous $NaHCO_3$ solution and extracted with $CHCl_3$ (2 ml) 3x. The combined organic extracts were successively washed with 5 M aqueous $Na_2S_2O_3$ solution and brine, dried over Na_2SO_4 and concentrated. Purification by flash chromatography (eluent: 35 % ethyl acetate:hexane) gave 17.8 mg (34 %) of **18** as a colorless oil: $[\alpha]^{23}_D$ –252° (c = 1.36, $CHCl_3$); IR (thin film) 2922, 1796, 1051 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.98 (m, 2 H, C_5–H, C_6–H), 5.78 (ddd, 1 H, J = 7.4, 10.4, 17.7 Hz, C_{10}–H), 5.72 (d, 1 H, J = 10.4 Hz, C_{11}–H), 5.48 (td, 1 H, J = 1.0, 17.7 Hz, C_{11}–H), 5.09 (ddd, 1 H, J = 1.3, 2.7, 5.5 Hz, C_4–H), 5.06 (ddd, J = 1.3, 2.5, 4.9 Hz, C_7–H), 4.80 (d, 1 H, J = 11.9 Hz, –OC<u>H</u>$_2$SCH$_3$), 4.79 (d, 1 H, J = 12.0 Hz, –OC<u>H</u>$_2$SCH$_3$), 4.59 (d, 1 H, J = 12.0 Hz, –OC<u>H</u>$_2$SCH$_3$), 4.53 (d, 1 H, J = 11.9 Hz, –OC<u>H</u>$_2$SCH$_3$), 4.40–4.35 (m, 2 H, C_8–H, C_9–H), 4.31 (dd, 1 H, J = 3.0, 5.5 Hz, C_3–H), 3.94 (dq, 1 H, J = 3.0, 6.5 Hz, C_2–H), 2.170 (s, 3 H, SCH_3), 2.166 (s, 3 H, SCH_3), 1.28 (d, 3 H, J = 6.5 Hz, C_1–H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 153.8, 153.7, 130.9, 129.3, 129.2, 123.1, 82.7, 81.6, 76.5, 76.3, 75.5, 73.7, 73.1, 70.3, 14.4 (X2), 14.36; HRMS (FAB, *m*–nitrobenzylalcohol, added NaI) *m/e* calc'd for $C_{17}H_{24}O_8S_2Na$ 443.0804 $[(M+Na)^+]$, found 443.0797.

*Synthesis of (2R, 3S, 4S, 5E, 7S, 8S, 9R)-2,9-di(methylthio-methoxy)undeca-5,10-dien-3,4,7,8-tetraol (compound **19**)*

To a solution of compound **18** (27.1 mg, 0.0644 mmol) in THF (4 ml) was added 0.38 ml (0.38 mmol) of aqueous 1 M LiOH solution at ambient temperature. After stirring for 1.5 h at ambient temperature, the reaction was quenched by the addition of phosphate buffer (pH 7.0) and extracted into $CHCl_3$–2-PrOH (5:1). The combined extracts were dried over Na_2SO_4 and concentrated. Purification by flash chromatography (eluent: 5 % MeOH/$CHCl_3$) gave compound **19** (22.1 mg, 93 %) as slightly yellowish needles: melting point 99–100 °C; $[\alpha]^{23}_D$ –202° (c = 0.990, $CHCl_3$); IR (thin film) 3420 (br), 2978, 2920, 1429, 1300, 1053, 978 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.91 (m, 2 H, C_5–H, C_6–H), 5.78 (ddd, 1 H, J = 8.0, 11.6, 18.7 Hz, C_{10}–H), 5.40 (td, 1 H, J = 1.0, 11.6 Hz, C_{11}–H), 5.38 (ddd, 1 H, J = 0.9, 1.3, 18.7 Hz, C_{11}–H), 4.76 (d, 2 H, J = 11.7 Hz, –OC<u>H</u>$_2$SCH$_3$(X2)), 4.60 (d, 1 H, J = 11.7 Hz, –OC<u>H</u>$_2$SCH$_3$), 4.54 (d, 1 H, J = 11.7 Hz, –OC<u>H</u>$_2$SCH$_3$), 4.28 (dd, 1 H, J = 5.2, 8.0 Hz, C_9–H), 4.26–4.22 (m, 2 H, C_4–H, C_7–H), 3.94 (dq, 1 H, J = 4.2, 6.3 Hz, C_2–H), 3.50 (m, 1 H, C_8–H), 3.35 (m, 1 H, C_3–H), 2.82 (d, 1 H, J = 4.3 Hz, OH), 2.74 (d, 1 H, J = 4.8 Hz, OH), 2.71 (d, 1 H, J = 5.4 Hz, OH), 2.68 (d, 1 H, J = 5.9 Hz, OH), 2.18 (s, 3 H, SCH_3), 2.17 (s, 3 H, SCH_3), 1.24 (d, 3 H, J = 6.3 Hz, C_1–H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 134.0, 132.1, 131.9, 121.0, 78.5, 77.1, 75.8, 73.4 (X2), 72.8, 72.2, 71.6, 15.8 (X2), 14.5; HRMS (FAB, *m*-nitrobenzylalcohol, added NaI) *m/e* calc'd for $C_{15}H_{28}O_6S_2Na$ 391.1225 $[(M+Na)^+]$, found 391.1224.

*Synthesis of (2R, 3S, 4S, 5E, 7S, 8S, 9R)-2,9-di(methyl-thiomethoxy)-3,4,7,8-diepoxyundeca-5,10-diene (compound **21**)*

A solution of pyridinium *p*-toluenesulfonate (0.02 M in CH_2Cl_2, 82.0 ml, 1.63 mmol) was added to a solution of the tetraol **19** (15.0 mg, 40.8 mmol) and trimethylorthoacetate (156 ml, 1.22 mmol) in CH_2Cl_2 (1 ml) at ambient temperature. After 25 min, the volatiles were evaporated and residual MeOH was removed under high vacuum (0.1 mm Hg) for 2 min. After addition of triethylamine (0.02 M in CH_2Cl_2, 0.41 ml, 8.2 mmol) and CH_2Cl_2 (1 ml), the solution was cooled to –30 °C and chlorotrimethylsilane (0.5 M in CH_2Cl_2, 0.82 ml, 0.41 mmol) was added dropwise. The reaction was stirred for 1.5 h at 0 °C then for 2 h at ambient temperature. The solution was evaporated *in vacuo* to yield the crude diacetoxy dichloride **20**. Physical data of **20** (obtained from a similar experiment as colorless needles by flash chromatography (eluent: 20 % ethyl acetate:hexane)): IR (thin film) 2978, 1750, 1431, 1372, 1223, 1034, 978 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.94–5.84 (m, 2 H, C_5–H, C_6–H), 5.60 (ddd, 1 H, J = 7.5, 9.7, 17.8 Hz, C_{10}–H), 5.40 (td, 1 H, J = 1.1, 17.8 Hz, C_{11}–H), 5.40 (td, 1 H, J = 1.1, 9.7 Hz, C_{11}–H), 5.22 (dd, 1 H, J = 4.7, 7.0 Hz, C_8–H), 5.12 (dd, 1 H, J = 3.7, 7.8 Hz, C_3–H), 4.77 (d, 1 H, J = 11.7 Hz, –OC<u>H</u>$_2$SCH$_3$), 4.76 (d, 1 H, J = 11.7 Hz, –OC<u>H</u>$_2$SCH$_3$), 4.65 (d, 1 H, J = 11.7 Hz, –OC<u>H</u>$_2$SCH$_3$), 4.60 (m, 1 H, C_7–H), 4.59 (m, 1 H, C_4–H), 4.54 (dd, 1 H, J = 4.7, 7.5 Hz, C_9–H), 4.52 (d, 1 H, J = 11.7 Hz, –OC<u>H</u>$_2$SCH$_3$), 3.66 (dq, 1 H, J = 3.7, 6.4 Hz, C_2–H), 2.18 (s, 3 H, SCH_3), 2.17 (s, 3 H, SCH_3), 2.13 (s, 3 H, OAc), 2.10 (s, 3 H, OAc), 1.14 (d, 3 H, J = 6.4 Hz, C_1–H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 169.8, 132.7, 131.6, 131.2, 121.6, 77.3, 75.9, 74.8, 73.5, 72.6, 70.1, 57.32, 57.27, 20.92, 20.87, 15.7, 14.2, 14.1; HRMS (CI NH_3) *m/e* calc'd for $C_{19}H_{34}NO_6S_2Cl_2$ 506.1207 $[(M+NH_4)^+]$, found 506.1225. Potassium carbonate (22.5 mg, 0.163 mmol) was added in one portion to the solution of the crude diacetoxy dichloride 20 in MeOH (1 ml) and the suspension stirred vigorously for 2.5 h. The mixture was filtered, the filtrate evaporated *in vacuo* and the residue purified by flash chromatography (eluent: 20 % ethyl acetate:hexane) to yield compound **21** (10.0 mg, 74 %) as a slightly yellowish oil: $[\alpha]^{23}_D$ –28° (c = 0.55, $CHCl_3$); IR (thin film) 2978, 2921, 1433, 1300, 1049, 963, 887 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) d 5.75 (ddd, 1 H, J = 7.0, 10.5, 17.3 Hz, C_{10}–H), 5.66 (m, 2 H, C_5–H, C_6–H), 5.36 (td, 1 H, J = 1.3, 17.3 Hz, C_{11}–H), 5.33 (td, 1 H, J = 1.2, 10.5 Hz, C_{11}–H), 4.78 (d, 1 H, J = 11.5 Hz, C_9–OC<u>H</u>$_2$SCH$_3$), 4.76 (d, 1 H, J = 11.5 Hz, C_2-OC<u>H</u>$_2$SCH$_3$), 4.72 (d, 1 H, J = 11.5 Hz, C_2-OC<u>H</u>$_2$SCH$_3$), 4.62 (d, 1 H, J = 11.5 Hz, C_9–OC<u>H</u>$_2$SCH$_3$), 4.11 (dd, 1 H, J = 5.9, 7.0 Hz, C_9–H), 3.66 (quin, 1 H, J = 6.5 Hz, C_2–H), 3.29 (dt, 1 H, J = 2.2, 4.4 Hz, C_7–H), 3.19 (dt, 1 H, J = 2.2, 4.5 Hz, C_4–H), 3.01 (dd, 1 H, J = 2.2, 5.9 Hz, C_8–H), 2.92 (dd, 1 H, J = 2.2, 6.5 Hz, C_3–H), 2.15 (s, 6 H, SCH_3), 1.54 (d, 3 H, J = 6.5 Hz, C_1–H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 133.2, 132.1, 131.8, 119.7, 76.6, 73.5, 72.8, 72.5, 63.2, 61.6, 54.2, 53.9, 17.0, 13.9, 13.8; HRMS (FAB, *m*-nitrobenzylalcohol, added NaI) *m/e* calc'd for $C_{15}H_{24}O_4S_2Na$ 355.1015 $[(M+Na)^+]$, found 355.1010.

*Synthesis of (2R, 3S, 4S, 5E, 7S, 8S, 9R)-9-methyl-thiomethoxy-3,4,7,8-diepoxyundeca-5,10-dien-2-ol (compound **22**)*

To a suspension of compound **21** (4.5 mg, 14 mmol) and $CaCO_3$ (18.0 mg, 0.180 mmol) in MeCN–H_2O (4:1, 1.6 ml)

was added portionwise $HgCl_2$ (32.6 mg, 0.120 mmol) at ambient temperature. The reaction was stirred for 5.5 h, diluted with 5M NH_4OAc (1 ml) and filtered through a pad of celite. The filtrate was extracted with $CHCl_3$ 3x. The combined extracts were dried over Na_2SO_4 and concentrated. Purification by flash chromatography (eluent: 50 % ethyl acetate:hexane) gave compound **22** (2.7 mg, 73 %) as a colorless oil: $[\alpha]^{23}_D$ –79° (c = 0.10, $CHCl_3$); IR (thin film) 3445, 2974, 2923, 1431, 1260, 1144, 1046, 965, 889 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.76 (ddd, 1 H, J = 6.7, 10.2, 17.3 Hz, C_{10}–H), 5.68 (m, 2 H, C_5–H, C_6–H), 5.37 (td, 1 H, J = 1.4 , 17.3 Hz, C_{11}–H), 5.33 (td, 1 H, J = 1.3, 10.2 Hz, C_{11}–H), 4.79 (d, 1 H, J = 11.5 Hz, C_9–OCH_2SCH_3), 4.63 (d, 1 H, J = 11.5 Hz, C_9–OCH_2SCH_3), 4.11 (dd, 1 H, J = 5.9, 6.8 Hz, C_9–H), 3.73 (dq, 1 H, J = 4.6, 6.4 Hz, C_2–H), 3.37 (ddd, 1 H, J = 2.2, 3.6, 5.5 Hz, C_7–H), 3.30 (ddd, 1 H, J = 2.2, 3.6, 5.6 Hz, C_4–H), 3.01 (dd, 1 H, J = 2.2, 5.5 Hz, C_8–H), 2.90 (dd, 1 H, J = 2.2, 4.6 Hz, C_3–H), 2.16 (s, 3 H, SCH_3), 1.79 (d, 1 H, J = 6.3 Hz, –OH), 1.30 (d, 3 H, J = 6.5 Hz, C_1–H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 133.2, 131.9, 131.8, 119.7, 72.8 (X2), 67.0, 64.1, 61.7, 55.4, 54.3, 20.1, 13.8; HRMS (CI NH_3) m/e calc'd for $C_{13}H_{21}O_4S$ 273.1161 [$(M+H)^+$], found 273.1173.

Synthesis of (2R, 3S, 4S, 5E, 7S, 8S, 9R)-3,4,7,8-diepoxyundeca-5,10-dien-2,9-diol ((–)-depudecin) (compound 1)

To a suspension of compound **21** (3.9 mg, 12 mmol) and $CaCO_3$ (88 mg, 0.88 mmol) in MeCN-H_2O (4:1, 1.6 ml) was added portionwise $HgCl_2$ (159 mg, 0.59 mmol) at ambient temperature. The reaction was stirred for 2.5 h, diluted with 5M NH_4OAc (1 ml) and filtered through a pad of celite. The filtrate was extracted with $CHCl_3$ three times. The combined extracts were dried over Na_2SO_4 and concentrated. Purification by flash chromatography (eluent: 3 % MeOH/$CHCl_3$) gave compound **1** (1.6 mg, 52 %) as a colorless oil: $[\alpha]^{23}_D$ –31° (c = 0.080, $CHCl_3$) (lit.[9] $[\alpha]^{23}_D$ –35.8° (c = 0.52, $CHCl_3$)); IR ($CHCl_3$) 3592, 2990, 1605, 1455, 1381, 965, 893 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.93 (ddd, 1 H, J = 5.5, 10.6, 17.3 Hz, C_{10}–H), 5.70 (m, 2 H, C_5–H, C_6–H), 5.39 (td, 1 H, J = 1.4 , 17.3 Hz, C_{11}–H), 5.27 (td, 1 H, J = 1.4, 10.6 Hz, C_{11}–H), 4.13 (m, 1 H, C_9–H), 3.74 (dq(br), 1 H, J = 4.5, 6.5 Hz, C_2–H), 3.42 (ddd, 1 H, J = 2.2, 3.5, 5.6 Hz, C_7–H), 3.38 (ddd, 1 H, J = 2.2, 3.5, 5.6 Hz, C_4–H), 3.01 (dd, 1 H, J = 2.2, 4.3 Hz, C_8–H), 2.90 (dd, 1 H, J = 2.2, 4.5 Hz, C_3–H), 1.94 (d, 1 H, J = 6.3 Hz, –OH), 1.80 (d, 1 H, J = 6.0 Hz, –OH), 1.30 (d, 3 H, J = 6.5 Hz, C_1–H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 136.2, 132.1, 131.6, 117.2, 71.6, 67.0, 64.2, 62.5, 55.4, 55.0, 20.1; HRMS (CI NH_3) m/e calc'd for $C_{11}H_{20}NO_4$ 230.1392 [$(M+NH_4)^+$], found 230.1390.

Acknowledgements: We thank Dr Jon Collins for his help with the early stages of this work and Dr Andrew Tyler of the Harvard Mass Spectrometry Facility for providing mass spectra. We acknowledge the NIH BRS Shared Instrumentation Grant Program (1 S10 RR0174801) and NSF (CHE88-14019) for providing NMR facilities. J.S. is a visiting scientist from Kyowa Hakko Kogyo Co., Ltd, and M.S. was supported by a Fellowship from the Yamada Science Foundation, Osaka, Japan. H.J.K. is a postdoctoral research associate and S.L.S. is an Investigator at the Howard Hughes Medical Institute.

References

1. Rosen, M.K. & Schreiber, S.L. (1992). Natural products as probes of celluar function: studies of immunophilins. *Angew. Chem. Int. Ed. Engl.* **31**, 384–400.
2. Schreiber, S.L. (1992). Using the principles of organic chemistry to explore cell biology. *Chem. Eng. News* **70**, 22–32.
3. Luan, S., Li, W., Rusnak, F., Assmann, S.L. & Schreiber, S.L. (1993). Immunosuppressants implicate protein phosphatase regulation of K^+ channels in guard cells. *Proc. Natl. Acad. Sci. USA* **90**, 2202–2206.
4. Zheng, X.-F., Fiorentino, D., Chen, J., Crabtree, G.R. & Schreiber, S.L. (1995). TOR kinase domains are required for two distinct functions, only one of which is inhibited by rapamycin. *Cell* **82**, 121–130.
5. Itazaki, H., *et al.*, & Nakagawa, Y. (1990). Isolation and structural elucidation of new cyclotetrapeptides, trapoxins A and B, having detransformation activities as antitumor agents. *J. Antibiot.* **43**, 1524–1532.
6. Murakami, Y., Mizuno, S., Hori, M. & Uehara, Y. (1988). Reversal of transformed phenotypes by herbimycin A in *src* oncogene expressed rat fibroblasts. *Cancer Res.* **48**, 1587–1590.
7. Kwon, H.J., Yoshida, M., Fukui, Y., Horinouchi, S. & Beppu, T. (1992). Potent and specific inhibition of $p60^{v\text{-}src}$ protein kinase both *in vivo* and *in vitro* by radicicol. *Cancer Res.* **52**, 6926–6930.
8. Krzyzosiak, W.J., Okada, N.S., Teshima, H., Nakajima, K. & Nishimura, S. (1992). Isolation of genes specifically expressed in flat revertant cells derived from activated *ras*-transformed NIH3T3 cells by treatment with azatyrosine. *Proc. Natl. Acad. Sci. USA* **89**, 4879–4883.
9. Matsumoto, M., *et al.*, & Yoshida, T. (1992). Depudecin: a novel compound inducing the flat phenotype of NIH3T3 cells doubly transformed by *ras*- and *src*-oncogene, produced by *Alternaria brassicicola*. *J. Antibiot.* **45**, 879–885.
10. Sugita, K., Yoshida, H., Matsumoto, M. & Matsutani, S. (1992). A novel compound, depudecin, induces production of transformation to the flat phenotype of NIH3T3 cells transformed by *ras*-oncogene. *Biochem. Biophys. Res. Commun.* **182**, 379–387.
11. Russell, A.F., Greenberg, S. & Moffatt, J.G. (1973). Reactions of 2-acyloxyisobutyryl halides with nucleosides. II. Reactions of adenosine. *J. Am. Chem. Soc.* **95**, 4025–4030.
12. Robins, M.J., Hansske, F., Low, N.H. & Park, J.I. (1984). A mild conversion of vicinal diols to alkenes. Efficient transformation of ribonucleosides into 2'-ene and 2',3'-dideoxynucleosides. *Tetrahedron Lett.* **25**, 367–370.
13. Sunay, U., Mootoo, D., Molino, B. & Fraser-Reid, B. (1986). A ready route from vicinal *cis*-diols to epoxides. *Tetrahedron Lett.* **27**, 4697–4700.
14. Kolb, H.C. & Sharpless, K.B. (1992). A simplified procedure for the stereospecific transformation of 1,2-diols into epoxides. *Tetrahedron* **48**, 10515–10530.
15. Iida, H., Yamazaki, N. & Kibayashi, C. (1987). Total synthesis of (+)-nojirimycin and (+)-1-deoxynojirimycin. *J. Org. Chem.* **52**, 3337–3342.
16. Saunders, R.M. & Ballou, C.E. (1965). Synthesis and characterization of D-*threo*-2,3-dihydroxy-4-pentenal. *J. Org. Chem.* **30**, 3219–3220.
17. Dondoni, A., Fantin, G., Fogagnolo, M., Medici, A. & Pedrini, P. (1989). Iterative, stereoselective homologation of chiral polyalkoxy aldehydes employing 2-(trimethylsilyl)thiazole as a formyl anion equivalent. The thiazole route to higher carbohydrates. *J. Org. Chem.* **54**, 693–702.
18. Dondoni, A., Fantin, G., Fogagnolo, M., Medici, A. & Pedrini, P. (1989). Hydroxy group inversion in thiazole polyols by an oxidation-reduction sequence. An entry to *syn* 1,2-diol fragments in masked carbohydrates. *J. Org. Chem.* **54**, 702–706.
19. Dondoni, A., Orduna, J. & Merino, P. (1992). Construction of all *O*-alkoxy D-tetrose and D-pentose stereoisomers from 2,3-*O*-isopropylidene-D-glyceraldehyde using 2-(trimethylsilyl)-thiazole as a formyl anion equivalent. *Synthesis*, 201–210.
20. Dondoni, A., Marra, A. & Perrone, D. (1993). Efficacious modification of the procedure for the aldehyde release from 2-substituted thiazoles. *J. Org. Chem.* **58**, 275–277.
21. Yamanoi, T., Akiyama, T., Ishida, E., Abe, H., Amemiya, M. & Inazu, T. (1989). Horner–Wittig reaction of dimethyl 2,3-*O*-isopropylidene-D-glycerolmethylphosphonate and its application to the formal synthesis of D-*erythro*-C_{18}-sphingosine. *Chem. Lett.*, 335–336.
22. Servi, S. (1985). 2,2,2-Trimethyl-1,3-dioxolane-4-carboxaldehyde as a chiral synthon: synthesis of the two enantiomers of methyl 2,3,6-trideoxy-α-L-*threo*-hex-2-enopyranoside, key intermediate in the synthesis of daunosamine, and of (+)- and (–)-rhodinose. *J. Org. Chem.* **50**, 5865–5867.
23. Gemal, A.L. & Luche, J.-L. (1981). Lanthanoids in organic synthesis. 6. The reduction of α-enone by sodium borohydride in the presence of lanthanoid chlorides: synthetic and mechanistic aspects. *J. Am. Chem. Soc.* **103**, 5454–5459.
24. Medina, J.C., Salomon, M. & Kyler, K.S. (1988). A mild method for the conversion of alcohols to methylthiomethyl ethers. *Tetrahedron Lett.* **29**, 3773–3776.
25. Barbacid, M. (1987). *Ras* genes. *Annu. Rev. Biochem.* **56**, 779–827.

26. Jove, R. & Hanafusa, H. (1987). Cell transformation by the viral *src* oncogene. *Annu. Rev. Cell Biol.* **3**, 31–56.
27. Yarbro, J.W., Kennedy, B.J. & Barnum, C.P. (1965). Hydroxyurea inhibition of DNA synthesis in ascites tumor. *Proc. Natl. Acad. Sci. USA* **53**, 1033–1035.
28. Liu, J., Farmer, J.D., Jr., Lane, W.S., Friedman, J., Weissman, I. & Schreiber, S.L. (1991). Calcineurin is a common target of cyclophilin–cyclosporin A and FKBP–FK506 complexes. *Cell* **66**, 807–815.
29. Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. & Tomita, F. (1986). Staurosporine, a potent inhibitor of phospholipid/Ca^{++}-dependent protein kinase. *Biochem. Biophys. Res. Commun.* **135**, 397–402.
30. Wong, T.W. & Goldberg, A.R. (1983). *In vitro* phosphorylation of angiotensin analogs by tyrosyl protein kinases. *J. Biol. Chem.* **258**, 1022–1025.
31. Brown, E.J., *et al.*, & Schreiber, S.L. (1994). A mammalian protein targeted by G1-arresting rapamycin–receptor complex. *Nature* **369**, 756–758.
32. Yano, H., *et al.*, & Matsuda, Y. (1993). Inhibition of histamine secretion by wortmannin through the blockade of phosphatidylinositol 3-kinase in RBL-2H3 cells. *J. Biol. Chem.* **268**, 25846–25856.
33. Vojtek, A.B., Hollenberg, S.M. & Cooper, J.A. (1993). Mammalian Ras interacts directly with the serine/threonine kinase Raf. *Cell* **74**, 205–214.
34. Peppelenbosch, M.P., *et al.*, & Bos, J.L. (1995). Rac mediates growth factor-induced arachidonic acid release. *Cell* **81**, 849–856.
35. Hall, M.D., Flickinger, K.S., Cultolo, M., Zardi, L. & Culp, L.A. (1988). Adhesion of human dermal reticular fibroblasts on complementary fragments of fibronectin: aging *in vivo* and *in vitro*. *Exp. Cell Res.* **179**, 115–136.

Received: **28 Jun 1995**. Accepted: **10 Jul 1995**.