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Solid-Phase Synthesis of Dysidiolide-Derived Protein Phosphatase Inhibitors

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Abstract: Biologically active natural products can be regarded as evolutionary selected and biologically validated starting points in structural space for the development of compound libraries. For libraries designed and synthesized around a given natural product, a higher hit rate and the identification of biologically relevant hits can be expected, justifying a probably higher investment in the development of the corresponding syntheses. This approach requires the development of complex multistep reaction sequences on the solid phase. Employing the protein phosphatase Cdc25 inhibitor dysidiolide as an example, we demonstrate that this goal can be achieved successfully. The reaction sequences developed led to dysidiolide analogues in overall 8−12 linear steps with the longest sequence on the solid support amounting to up to 11 sequential transformations. The desired products were obtained in overall yields ranging from 6% to 27% and in multimilligram amounts starting from 100 mg of resin. The transformations applied include a variety of very different reaction types widely used in organic synthesis (i.e., an asymmetric cycloaddition employing a removable chiral auxiliary, different organometallic transformations, olefination reactions, different oxidation reactions, acidic hydrolyses, and a nucleophilic substitution). Biological investigation of the eight dysidiolide analogues synthesized showed that they inhibit Cdc25C in the low micromolar range with the IC_{50} value varying by a factor of 20 and that they display considerable and differing biological activities in cytotoxicity assays employing different cancer cell lines.

Introduction

The combinatorial synthesis of compound libraries on polymeric supports is at the heart of protein ligand and inhibitor discovery, in particular for research in medicinal chemistry and chemical biology. To achieve high efficiency in this process, powerful strategies for the design of compound libraries are of paramount importance.

A key to the efficient discovery of new ligands and inhibitors for known and, in particular, for newly discovered proteins is to identify compound classes already validated as being biologically relevant and to employ them as starting points in structural space for library development. Libraries designed and synthesized around the basic structure of such compounds should yield modulators of protein activity with high hit rates.

The prerequisite of biological prevalidation is fulfilled by biologically active natural products that can be regarded as chemical entities evolutionarily selected and validated for binding to particular protein domains.[1] Consequently, the underlying structural architectures of such natural products may provide powerful guiding principles for library development.[1−3]

Paramount to the success of this approach is that efficient and reliable methods and multistep sequences for the total synthesis of natural products and analogues thereof on polymeric supports are available. The corresponding transformations must proceed with a degree of selectivity and robustness typical of related classical solution-phase transformations, irrespective of the stringencies and differing demands imposed by the presence of and the anchoring to the polymeric support. However, currently this challenge to organic chemistry is nearly unmet. In a few cases structural variation of natural products via solid-phase methodologies has been achieved,[1,3] mostly in the late steps of the syntheses and via modification of a core structure presynthesized in solution. But the feasibility of natural product and analogue total synthesis in long multistep sequences (e.g., 10 steps and more) on polymeric supports has only been achieved in a single case.[4]

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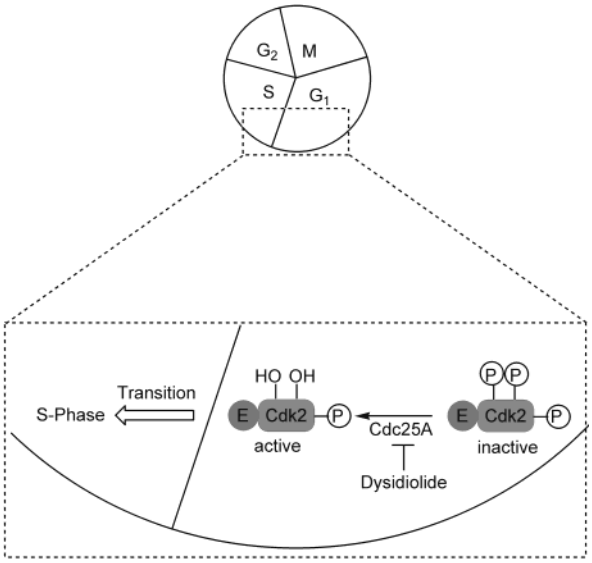

Figure 1. Activation of Cdk2-cyclin E complex by Cdc25A as essential step for G1-S transition.

Here we describe the solid-phase synthesis of a close analogue of the protein phosphatase inhibitor dysidiolide and of a small library of dysidiolide analogues that rapidly yielded compounds with significantly improved biological activity.[5]

The sesquiterpenoid dysidiolide **1** is a naturally occurring inhibitor of the dual-specificity Cdc25 protein phosphatase family that plays a crucial role in the regulation of the cell cycle.[6]

The Cdc25 phosphatases activate cyclin-dependent kinases (Cdks) and thereby initiate progression of cells through different phases of the cell cycle. For instance, Cdc25A activates the triply phosphorylated complex between Cdk2 and cyclin E by dephosphorylation of Cdk2 at Thr 14 and Tyr 15 (Figure 1). Subsequently, the cells can progress beyond the G_1/S checkpoint.[7] Similarly, Cdc25B and Cdc25C are thought to be regulators of the G_2/M transition through dephosphorylation and activation of the Cdk1−cyclin B complex.[8,9]

The crucial roles of the Cdc25 dual specificity phosphatases in cell-cycle regulation led to the notion that these enzymes might be promising targets for the development of new anticancer drugs.[10,11] This prospect spurred studies aiming at the development of selective Cdc25 inhibitors.[12]

Among these, dysidiolide was identified as particularly promising, since it inhibits Cdc25A with an IC_{50} value of 9.4 μM, whereas the phosphatases calcineurin, CD45, and LAR are not inhibited by the natural product.[13] In addition, dysidiolide induces growth arrest of different cancer cell lines and arrest in the G_1 phase of the cell cycle or apoptosis.[13,14]

Because of these biological properties and its unique structure, dysidiolide has been of intense interest to biologists, pharmacologists, and organic chemists,[15] resulting in the completion of five successful total syntheses since its discovery.

Results and Discussion

Planning of the Solid-Phase Synthesis. Dysidiolide is a sesquiterpene with a decalin-type framework that carries a lipophilic side chain terminating in an olefin and a hydrophilic side chain incorporating an alcohol and a γ-hydroxybutenolide.

For the planned solid-phase synthesis, we intended to attach the olefinic side chain to a robust linker that would provide the terminal alkene structure after cleavage from the solid support in a mild and traceless manner. The new olefin metathesis linker[4,16] incorporated in **2** was thought to fulfill these requirements (Scheme 1). Transition-metal catalyzed olefin metathesis should liberate the desired compound accompanied by formation of a polymer bound cyclopentene (step I, Scheme 1). Attachment to the polymer via an ether bond was thought to provide the required stability during the planned multistep synthesis. The γ-hydroxybutenolide moiety should be obtained by addition of 3-lithiofuran to an aldehyde group and subsequent oxidation of the heterocycle with singlet oxygen (step II, Scheme 1). For the generation of the bicyclic core structure, it was planned to apply the Diels−Alder route (step III, Scheme 1) previously investigated in our laboratories[15f] and successfully employed in total syntheses of natural dysidiolide in solution.[15b−e] The knowledge gleaned from these studies suggested that this cycloaddition primarily leads to the 6-epimer of the dysidiolide framework. However, we chose to accept this deviation from the goal of synthesizing the parent natural product itself, since in the concept delineated above this is not necessarily required to identify new biologically active compounds. It was planned to synthesize the diene for the Diels−Alder reaction in solution starting from commercially available chiral ketoester **8** and attach it to the linker resin **5** carrying an aldehyde group by a Wittig reaction with ylide **6** (step IV) in a convergent strategy (**4** ⟹ **5** + **6**).

Aldehyde resin **5** should be accessible from diol **7**[17] by coupling to Merrifield resin via Williamson ether formation and subsequent oxidation of the remaining primary alcohol to the aldehyde (step V, Scheme 1). The diene unit in **6** was traced back to ketone **8** via addition of a vinyl Grignard to **8** followed by elimination of the resulting tertiary alcohol. Formation of the ylide was envisaged to be feasible by means of a nucleophilic

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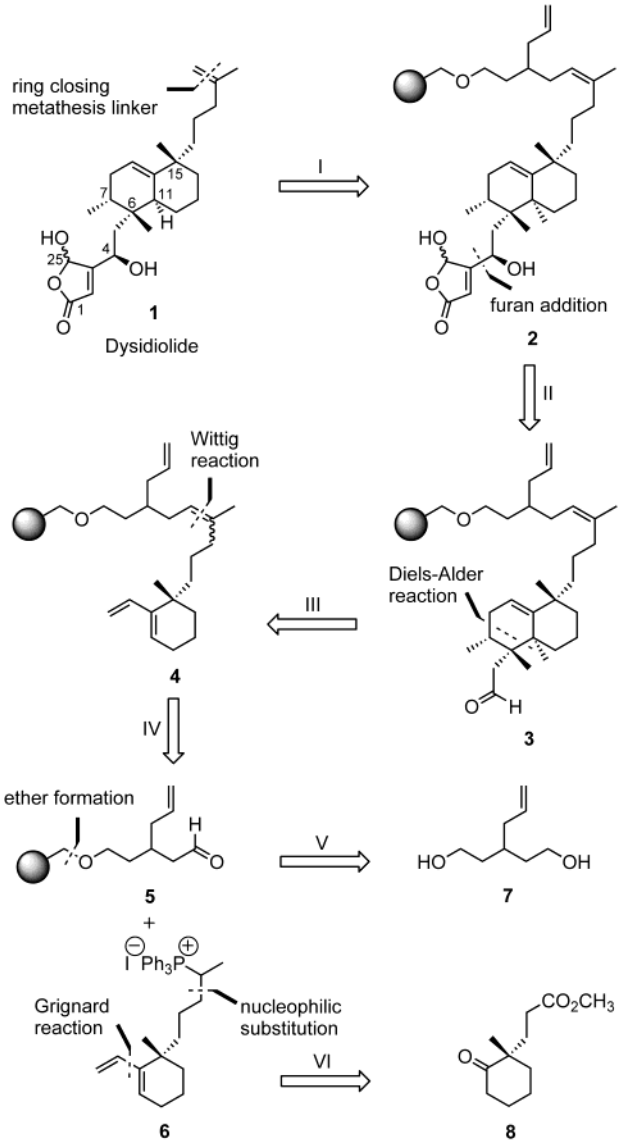
Scheme 1. Retrosynthetic Analysis of Dysidiolide and Delineation of a Solid-Phase Synthesis Strategy Employing an Olefin Metathesis Linker

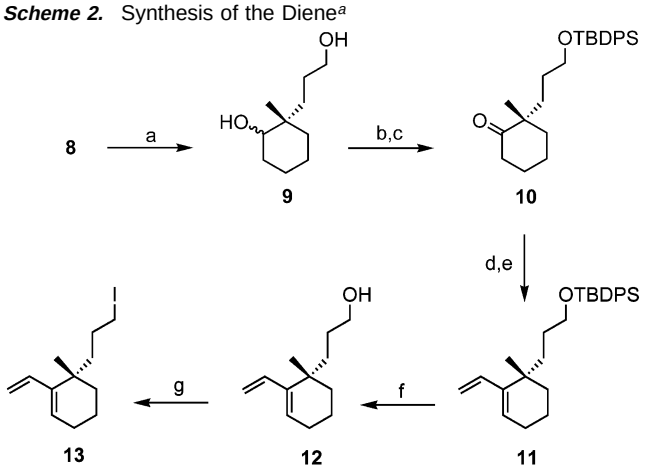


substitution reaction employing an alkyl iodide obtainable from ester **8** (step IV, Scheme 1).

Synthesis of the Diene Building Block. Dienyl iodide **13** was synthesized from commercially available ketoester **8** in seven steps and with an overall yield of 40% (Scheme 2). After reduction of **8** with $LiAlH_4$, the primary hydroxyl group of **9** was selectively masked as TBDPS-ether, and then the secondary alcohol was reoxidized with TPAP/NMO to yield masked ketone **10** in high yield. Vinylmagnesium chloride was added to the carbonyl group, and the resulting tertiary alcohol was converted into diene **11** by Lewis acid mediated elimination of water. The best results were obtained with $CuSO_4$ in benzene or $BF_3{\cdot}OEt_2$ in benzene/THF = 4:1. Then the primary alcohol was liberated again and transformed into the iodide required for the subsequent Wittig reaction by means of iodine, PPh_3, and imidazole.

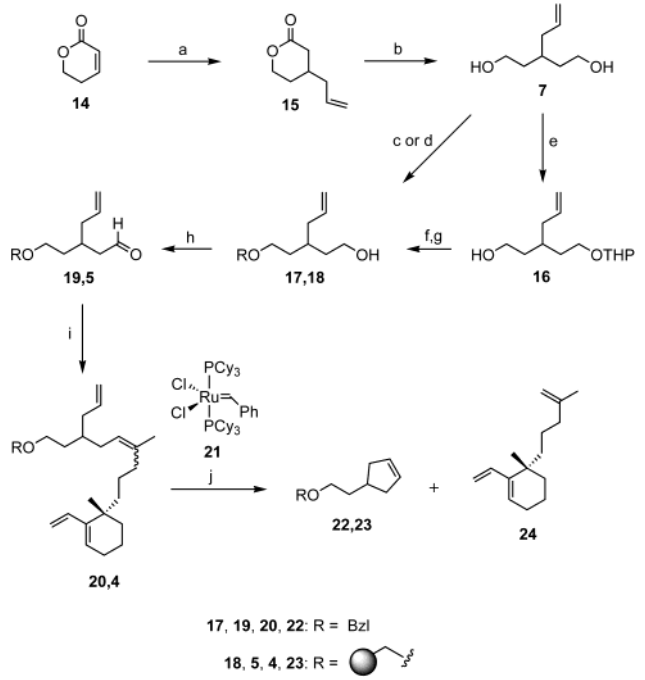
Synthesis and Preliminary Investigation of the Linker Group. The development of the linker group commenced with addition of diallyl zinc (generated from allylmagnesium chloride and $ZnBr_2$) to α,β-unsaturated lactone **14** in the presence of TMSCl as a Lewis acid and anion scavenger. Lactone **15** was

Scheme 2. Synthesis of the Diene[a]



[a] Key: (a) LAH, THF, 0 °C, 30 min, 100%; (b) TBDPS-Cl, DMAP, Et_3N, dichloromethane, r.t., 17 h; (c) TPAP, NMO, MS 4 Å, dichloromethane, r.t., 4 h, 85% (b−c); (d) vinylmagnesium bromide, THF, r.t., 5 h; (e) $CuSO_4{\cdot}5\ H_2O$, benzene, Δ, 40 h, 58% (d−e); (f) $(n\text{Bu})_4NF$ (TBAF), THF, r.t., 3 h, 95%; (g) iodine, imidazole, PPh_3, dichloromethane, r.t., 1 h, 85%.

Scheme 3. Synthesis of the Linker and Attachment to the Resin[a]



[a] Key: (a) allylmagnesium bromide, $ZnBr_2$, TMS-Cl, THF, −78 °C, 6 h, 52%; (b) LAH, THF, r.t., 1 h, 97%; (c) NaH, $(n\text{Bu})_4NI$, benzyl bromide, DMF, r.t., 12 h, 58%; (d) NaH, $(n\text{Bu})_4NI$, Merrifield-Cl resin (1.1 mmol/g), DMF, r.t., 18 h, 59%; (e) dihydropyrane, Dowex 50 Wx2, toluene, r.t., 3 h, 95%; (f) NaH, $(n\text{Bu})_4NI$, Merrifield-Cl resin (1.1 mmol/g), DMF, r.t., 18 h; (g) pyridinium-*p*-toluene sulfonate, EtOH, dichloroethane, Δ, 18 h, 98% (f−g); (h) solution: $(n\text{Pr})_4N\ RuO_4$ (TPAP), NMO, MS4 Å, r.t., 30 min, 83%; solid-phase: *o*-iodoxybenzoic acid (IBX), THF/DMSO = 1:1, r.t., 8 h, 92%; (i) $EtPPh_3I$, *n*BuLi, **13**, THF, r.t., 16−20 h, then *n*BuLi, 0 °C, 1−2 h; (j) $(PCy_3)_2(Cl)_2Ru{=}CHPh$ (**21**) (2 × 10 mol %), dichloromethane, r.t., 16 h.

obtained under optimized conditions from the intermediary formed silylenol ether (identified by GCMS analysis of the crude reaction mixture) by means of acidic hydrolysis in 51% yield (Scheme 3). Application of a Cu-catalyzed addition of the Grignard reagent led to oligomerization of the unsaturated lactone, and also the Sakurai reaction employing allyltrimethylsilane and $TiCl_4$ was not successful.

Reduction of lactone **15** with $LiAlH_4$ yielded diol **7** in multigram amounts. To investigate whether the linker group could be used as planned, diol **7** was converted into monobenzyl ether **17**, and in a second experiment it was attached to Merrifield polystyrene resin (loading 1.1 mmol/g). In the solution-phase reaction formation of the mono- and the bis-benzyl ether was observed. On the solid-phase derivatization of the introduced primary alcohol groups with Fmoc-Cl, cleavage of the Fmoc group with piperidine and UV-spectroscopic determination of the amount of the fluorenylmethyl piperidine formed[18] indicated that only 59% loading was achieved. However, gravimetric determination of the loading indicated quantitative attachment of the linker. Thus, direct alkylation of diol **7** with Merrifield chloride leads to partial cross-linking. This is in marked contrast with observations reported for the attachment of 1,4-butanediol to Merrifield resin, which proceeds with nearly quantitative yield.[19]

To avoid this undesired cross-linking, diol **7** was converted into mono-THP ether **16**, which was then attached to the polymeric support. After cleavage of the THP group with pyridinium-tosylate (PPTS), linker resin **18** was obtained in nearly quantitative yield and with loading levels up to 1 mmol/g. Alcohols **17** and **18** were then oxidized to aldehydes **19** and **5**. Oxidation on the solid support proceeded best with IBX.[20] Use of the Dess−Martin reagent, the SO_3−pyridine complex in DMSO/THF, or Swern oxidation gave inferior results.

For quantitative determination of the formed aldehyde a new assay was developed. A defined amount of the aldehyde resin was treated with a stock solution of dinitrophenylhydrazine (DNPH), leading to partial consumption of the hydrazine by hydrazone formation. DNPH consumption is then readily determined by UV spectrometric quantitation of the remaining DNPH and comparison with the concentration of the stock solution.

Diene **13** was coupled with aldehyde **5** and **19** in a two-step process (Scheme 3). First, the diene was treated with the ylide formed from ethyltriphenylphosphonium iodide and *n*-butyllithium to form phosphonium salt **6**. Deprotonation of this intermediate with a second equivalent of base and the subsequent Wittig reaction with **5** and **19** yielded alkenes **4** and **20**. Soluble olefin **20** was obtained in 90% yield as an *E*/*Z* mixture, and the IR spectrum of resin **4** did not show the strong C=O bond characteristic of the linker anymore, thus indicating a high degree of conversion.

The decisive ring-closing metathesis reaction, including a triply substituted double bond, proceeded rapidly and in high yield if soluble diene **20** and the Grubbs catalyst[21a] **21** (10 mol %) were employed (Scheme 3). Within 1 h conversion was complete (GCMS control), and cyclopentene **22** and triene **24** were isolated in 99% and 81% yield, respectively.

In contrast to these findings, the desired metathesis product **24** was obtained under the same conditions from resin-bound intermediate **4** in only 40% yield (for coupling to the support and release of the metathesis product). Longer reaction times, elevated temperature, or use of 2×10 mol % of the catalyst resulted in only a moderate increase of yield to 60%. Also performing the reaction in an ethylene atmosphere[22] or with additional styrene[23] to release product from the intermediary formed ruthenium carbene complex did not result in higher yield. In addition, the metathesis reaction was performed with the new Grubbs catalyst (benzylidene-(1,3-dimesityl-4,5-dihydroimidazol-2-ylidene)-(tricyclohexylphosphin)-ruthenium dichloride) that is well-known to have a higher reactivity, especially for highly substituted double bonds.[21b] However, the yield was even lower (38%) as compared to that of the reaction with **21** under the same conditions.

We speculated that these results might be due to partial cross-linking of neighboring allylic side chains of the linker by cross-metathesis on the solid support preventing release of the target compound. To investigate this possibility and to identify possible dimers formed by cross-metathesis on the resin, the synthesis was repeated employing a double linker that would allow release of the dimers under mild conditions. To this end, an acid-sensitive tetrahydropyranyl linker was introduced (i.e., diol **7** was selectively masked as mono-TBDPS ether) and coupled to Ellman's dihydropyran resin[24] as shown in Scheme 4.

After removal of the TBDPS group from **26** the synthesis sequence described above was carried out, yielding resin **29**. Loading of this polymer with diene was determined by the release of alcohol **30** from a resin sample under acidic conditions. Surprisingly, subjection of intermediate **29** to the ring-closing metathesis conditions described above resulted in the release of triene **24** in 82% yield (i.e., with nearly the same result observed for the corresponding reaction in solution (see above)). Upon subsequent treatment of the resin with PPTS in dichloroethane−ethanol, cyclopentene **32** was isolated in 84% yield. In addition, several intermediates that had not been converted completely were identified; however, products arising from undesired cross-linking by competing metathesis between neighboring linker groups could not be detected. These findings demonstrate that cross-linking most probably is not the reason for the observed yields of 40−60% recorded in the metathesis employing resin **4**. This conclusion is further supported by the result that the yield does not increase if a resin with a lower loading level is used.

Model Synthesis in Solution. To explore whether the planned synthesis sequence for the construction of the dysidiolide framework is feasible, it was investigated in solution employing achiral model diene **33** (Scheme 5). To this end, instead of ethylaluminum dichloride, which had proven very useful in our model studies of the central cycloaddition,[15f] TMS-triflate introduced by Danishefsky et al. for related transformations[15c]

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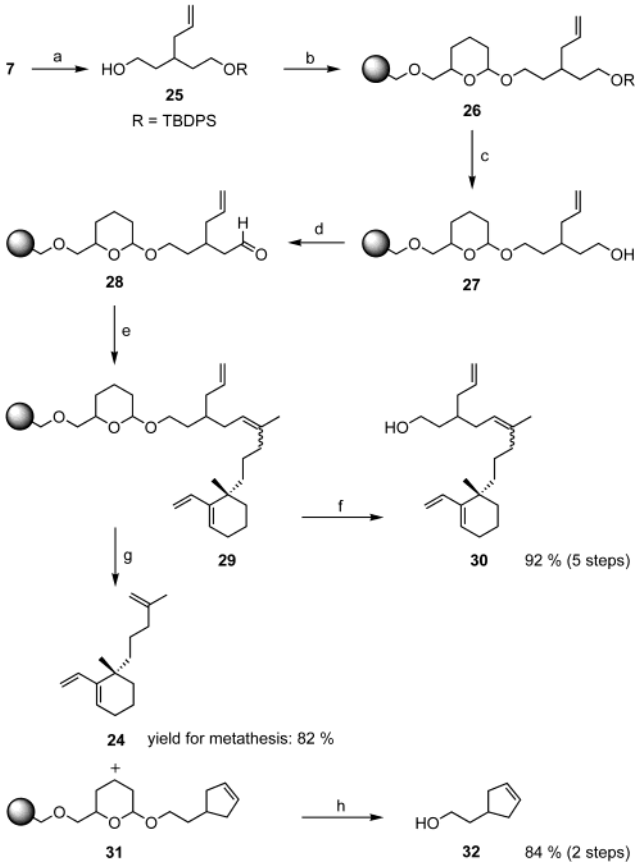
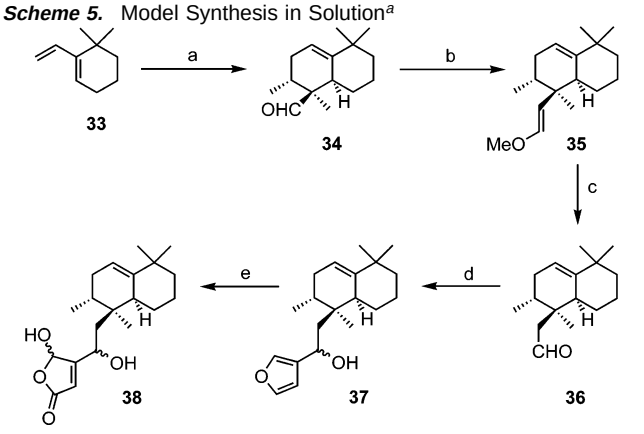
Scheme 4. Synthesis on Dihydropyran-Resin[a]



[a] Key: (a) TBDPS-Cl, Et_3N, DMAP, dichloromethane, r.t., 20 h, 39%; (b) dihydropyran-resin (1 mmol/g), pyridinium-*p*-toluene sulfonate, dichloroethane, 80 °C, 20 h; (c) $(n\text{Bu})_4\text{NF}$ (TBAF), THF, r.t., 19 h; (d) *o*-iodoxybenzoic acid (IBX), THF/DMSO = 1:1, r.t., 12 h; (e) $EtPPh_3I$, *n*BuLi, **13**, THF, r.t., 12 h, then *n*BuLi, 0 °C, 2h; (f) pyridinium-*p*-toluene sulfonate, dichloroethane:ethanol = 1:1, 80 °C, 20 h, 92% (b−f); (g) $(PCy_3)_2(Cl)_2Ru{=}CHPh$ (**21**) (2 × 10 mol %), dichloromethane, r.t., 16 h, 82%; (h) pyridinium-*p*-toluene sulfonate, dichloroethane:ethanol = 1:1, 80 °C, 20 h, 84% (g−h).

Scheme 5. Model Synthesis in Solution[a]



[a] Key: (a) tiglic aldehyde, TMSOTf, dichloromethane, −100 °C, 1 h, 85%; (b) Ph_3PCH_2OMeBr, $NaNH_2$, HMDS, r.t., 1 h, 89%; (c) pyridinium-*p*-toluene sulfonate, THF/H_2O = 10:1, Δ, 18 h, 91%; (d) 3-bromofuran, *n*BuLi, THF, −78 °C, 90 min, 94%; (e) bengal rose, O_2, $Et(i\text{Pr})_2N$, *hν*, dichloromethane, −78 °C, 2 h, then r.t., 10 min, 73%.

was investigated. Thereby, the formation of insoluble aluminum salts during workup that would not be easily removable from the resin is prevented . In the presence of 0.1 equiv of TMSOTf, diene **33** was converted to Diels−Alder adduct **34** at −100 °C within 1 h. In the presence of $EtAlCl_2$, 2 h at −20 °C were required for completion of the reaction. In addition, the endo/exo selectivity was raised from 91:9 to 96:4 at the lower temperature.

Next the carbon chain of carbonyl compound **34** was elongated by the Wittig reaction to give enol ether **35**, which was hydrolyzed under acidic conditions. The latter step turned out to be crucial because even under weakly acidic conditions (10% acetic acid) an undesired rearrangement of the carbon skeleton dominated over the hydrolysis. Finally, use of pyridinium tosylate in refluxing THF/H_2O gave aldehyde **36** in high yield.

The furan ring was introduced by addition of 3-lithiofuran to the aldehyde, which proceeded with a selectivity of 2:1 and in 94% yield. Final oxidation of the furan to γ-hydroxybutenolide **38** was achieved by means of in situ generated singlet oxygen in the presence of diisopropylethylamine.[15a,c−e] Thus, the planned reaction sequence was established successfully, resulting in the identification of reagents and reaction conditions that should be applicable on the solid phase as well.

Solid-Phase Synthesis of 6-*epi*-Dysidiolide. For the solid-phase synthesis of 6-*epi*-dysidiolide, polymer-bound diene **4** initially was subjected to the Diels−Alder reaction with tiglic aldehyde **42** in the presence of TMS-triflate (Scheme 6). To achieve complete conversion within a reasonable time the reaction temperature had to be raised to −30 °C, and under these conditions cycloadduct **41** was obtained as a mixture of four isomers that were formed in the ratio of endo/endo′/exo/exo′ = 67:16:16:1, with the desired endo isomer predominating. The isomer ratios were determined by ^{1}H NMR spectroscopy after release of the cycloadducts from the solid support by olefin metathesis and comparison with literature data.[15d,f] Thus, the cycloaddition proceeded with an endo/exo ratio of 83:17, and the two endo isomers were formed in a 81:19 ratio.

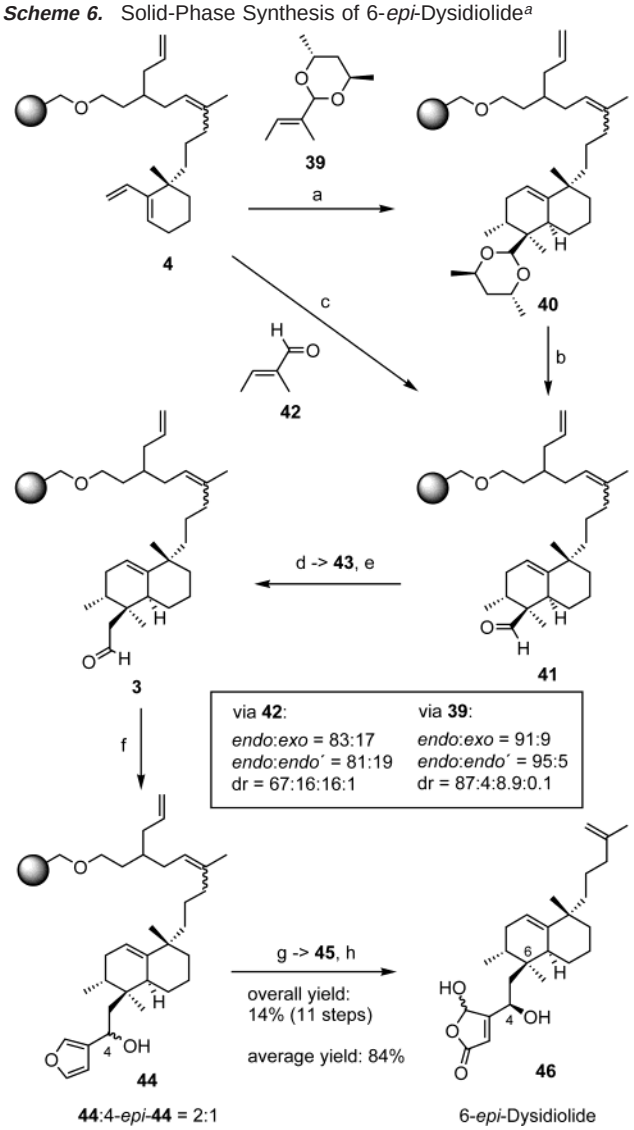
To increase the stereoselectivity and to achieve a more efficient stereochemical steering of the cycloaddition, tiglic aldehyde was converted into quasi-C_2-symmetric chiral acetal **39** derived from (*R*,*R*)-2,4-pentanediol.[25] This more reactive chiral dienophile underwent the asymmetric Diels−Alder reaction already at −78 °C, and after hydrolytic removal of the chiral auxiliary group, aldehyde **41** was obtained as a mixture of four isomers formed in the ratio endo/endo′/exo/exo′ = 87:4:9:0.1. Thus the endo/exo ratio was increased to 91:9, the two endo isomers were formed in the ratio 95:5, and the amount of the main isomer was raised from 67% to 87%. In particular, the formation of the second endo isomer was largely suppressed.

Notably, the hydrolysis of the acetal of **40** required optimization of the reaction conditions. As mentioned above, a weak acid had to be used to prevent undesired rearrangement of the carbon skeleton. The use of toluenesulfonic acid met this demand, whereas TFA was too strong. Also, the choice of the solvent was crucial. Thus, in THF/H_2O, hydrolysis could not be affected. Application of acetone to remove the acetal in a transacetalization in the presence of water proceeded slowly and was incomplete, since the resin does not swell sufficiently in this reaction medium. Only after addition of dichloroethane to guarantee appropriate resin swelling was the aldehyde liberated.

By analogy to the model solution-phase synthesis, it was planned to elongate the carbon chain of aldehyde **41** by means of a two-step sequence including a Wittig reaction and hydroly-

(25) Sammakia, T.; Berliner, M. A. *J. Org. Chem.* **1994**, *59*, 6890−6891.

Scheme 6. Solid-Phase Synthesis of 6-*epi*-Dysidiolide[a]



[a] Key: (a) TMSOTf, dichloromethane, −78 °C, 7 h; (b) *p*-toluenesulfonic acid, acetone, dichloroethane, H_2O, Δ, 20 h; (c) TMSOTf, dichloromethane, −30 °C, 2 h; (d) Ph_3PCH_2OMeCl, KO*t*Bu, THF, r.t., 4 h, →**43**; (e) pyridinium-*p*-toluene sulfonate, THF, 1% H_2O, Δ, 16 h; (f) 3-bromofuran, *n*BuLi, THF, −78 °C, 5 h; (g) bengal rose, O_2, Et(*i*Pr)$_2$N, *hν*, dichloromethane, −78 °C, 5 h, then r.t., 10 min, →**45**; (h) $(PCy_3)_2(Cl)_2Ru$═CHPh (**21**) (2 × 10 mol %), dichloromethane, r.t., 16 h.

sis of the enol ether formed therein. In contrast to the reaction in solution phase, olefination with a mixture of methoxymethyl triphenylphosphonium bromide and sodium amide was only incomplete on the solid phase, as judged by Fourier transform IR of the resin. However, clean and complete conversion to the *Z*/*E* enol ether **43** was achieved by employing the corresponding phosphonium chloride and KO*t*Bu as base, as judged by GCMS after cleavage from a resin sample by means of olefin metathesis.

Acid-mediated hydrolysis of enol ether **43** to aldehyde **3** on the solid phase turned out to be tricky. The reaction conditions that had been developed in the course of the model reaction in solution (see above) could not be employed in the heterogeneous reaction. Rather, even after 24 h reaction time the use of PPTS in H_2O/THF 1:10 led only to little conversion. After substantial experimentation and after various alternative reaction conditions had been explored unsuccessfully, the original conditions were reinvestigated. It was finally observed that the THF/H_2O/PPTS mixture formed a two-phase system with the resin being in the organic phase and most of the acid presumably dissolved in the aqueous phase. Thereby, the concentration of acid in the resin obviously is lowered, resulting in only very slow hydrolysis. Consequently, the amount of water in the reaction mixture was reduced to 1%, resulting in the formation of a homogeneous solution in which the desired hydrolysis proceeded rapidly and without any undesired rearrangement reaction.

This observation in our opinion is worth noting, since it demonstrates that reaction conditions preoptimized in solution cannot necessarily be used on a resin, and this not for chemical but rather for unexpected reasons that are not related to the reaction under investigation. It also demonstrates that optimization and execution of multistep synthesis in an automated fashion may not be as straightforward as often claimed.

Introduction of the furan and its oxidative elaboration to the γ-hydroxybutenolide were carried out as developed in the model reaction sequence. Nucleophilic addition of furyllithium to a resin-bound aldehyde was performed twice to increase the yield. Single addition of the organometallic reagent to enolizable aldehyde **3** did not result in complete conversion to **44**. Secondary alcohol **44** was formed as a 2:1 mixture of diastereomers whose configuration was not determined. Subsequently, furan **44** was oxidized to butenolide **45** with singlet oxygen in the presence of Hünig's base.

Finally, release of the synthesis products from the polymeric carrier was achieved as planned in a traceless manner and under very mild conditions by performing an olefin metathesis reaction with 10 mol % of Grubbs catalyst twice for 8 h each time. The product mixture was readily purified by simple flash chromatography and by filtering through a C18 reverse-phase separation cartridge. 6-*epi*-Dysidiolide **46** and additional diastereomers were formed in a total of 11 steps on the solid phase with an overall yield of 14% (i.e., with an average yield of 84% per step) based on the Merrifield resin. By using this procedure 48 mg of the desired product can be obtained from 1 g of resin with a loading of 1.1 mmol/g.

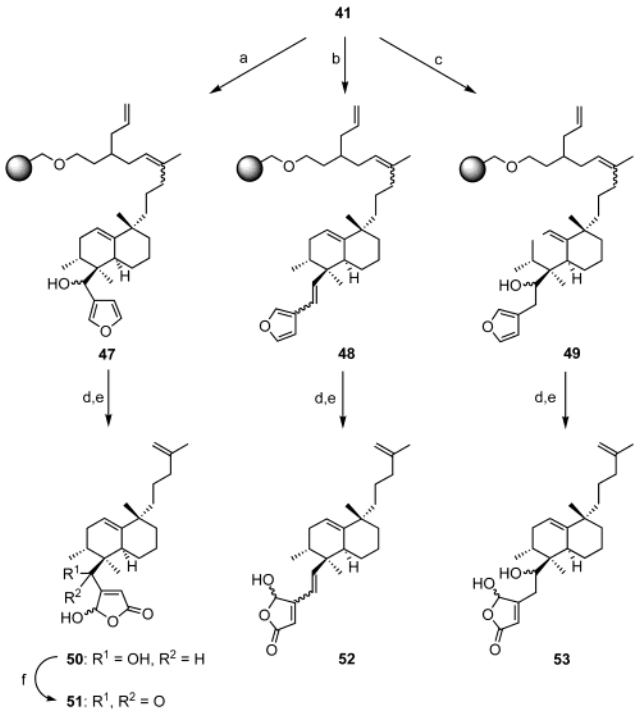
The analytical data recorded for the dysidiolides are in good agreement with the structures of the compounds and similar to the data recorded for the natural product. 6-*epi*-Dysidiolide and its 4-epimer were formed as the main products. In addition, three further isomers were identified in small amounts. By means of semipreparative HPLC on SiO_2 the five diastereomers could be separated.

Solid-Phase Synthesis of Analogues. To demonstrate the robustness and reliability of the methodology described above, we embarked on the solid-phase synthesis of dysidiolide analogues that differ from the natural product in chain length and substitution pattern.

The solid-phase syntheses of the dysidiolide analogues commenced with the decisive and crucial Diels−Alder reaction between chiral polymer-bound diene **4** and tiglic aldehyde **42** leading to immobilized aldehydes **41** and **3** (see above). Use of achiral aldehyde **42** instead of chiral acetal **39** ultimately will open up the opportunity to form and biologically investigate more diastereomers of the corresponding products either as pure compounds or initially as product mixtures.

Dysidiolide analogues **50** and **51** with a shortened carbon chain were obtained via addition of 3-lithiofuran to aldehyde

Scheme 7. Solid-Phase Synthesis of Dysidiolide Analogues **50**−**53**[a]



[a] Key: (a) 3-bromofuran, *n*BuLi, THF, −78 °C, 5 h; (b) 3-furylmethyltriphenylphosphonium bromide, KO*t*Bu, THF, 60 °C, 30 h; (c) 3-bromomethylfuran, Mg, THF, r.t. to 50 °C, 20 h; (d) bengal rose, O_2, Et(*i*Pr$_2$)N, *hν*, dichloromethane, −78 °C, 5 h, then r.t., 10 min; (e) $(PCy_3)_2Cl_2Ru{=}CHPh$ (**21**) (2 × 10 mol %), dichloromethane, r.t., 16 h; (f) IBX, DMSO, r.t., 16 h, 80%.

41. After oxidative elaboration of intermediary formed furyl alcohol **47** with singlet oxygen and subsequent cleavage from the solid support by means of olefin metathesis, compound **50** was obtained in an overall yield of 26% for a total of eight steps on the solid phase (Scheme 7).

Subsequent oxidation of the secondary alcohol with *o*-iodoxybenzoic acid (IBX) gave the corresponding ketone **51**. Alternatively, oxidation of the secondary alcohol was carried out on the solid phase; however, in this case the desired carbonyl compound could only be isolated in trace amounts after release from the solid support. In both cases oxidation of the hydroxybutenolide to the anhydride was not observed.

Application of the same solid-phase reaction sequence to polymer-bound aldehyde **3** delivered 6-*epi*-dysidiolide **46** as expected. Subsequent oxidation of the secondary alcohol yielded ketone **56** in 75% yield (Scheme 8). In this case oxidation of the alcohol on the solid phase prior to olefin metathesis also gave inferior results.

In a second series of experiments dysidiolide analogues **52** and **57**, with an olefinic bridge between the anellated ring systems and the hydroxybutenolide, were synthesized. To this end, polymer-bound aldehydes **41** and **3** were treated with the ylide obtained from 3-furylmethyltriphenylphosphonium bromide[26] by deprotonation with KO*t*Bu in THF at room temperature (r.t.). However, in both cases the Wittig reactions leading to immobilized olefins **48** and **54** only proceeded satisfactorily after the temperature was raised to 50 °C. By analogy to the

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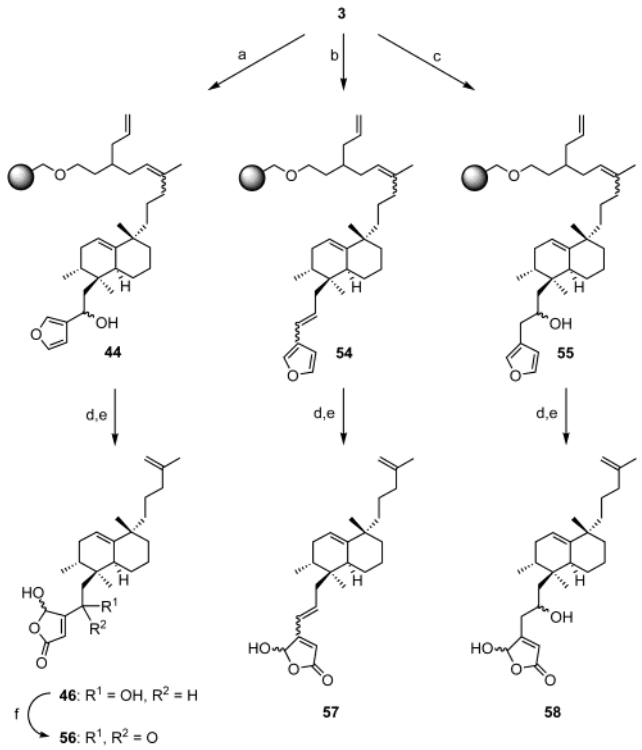
Scheme 8. Solid-Phase Synthesis of Dysidiolide Analogues **46** and **56**−**58**[a]



[a] Key: (a) 3-bromofuran, *n*BuLi, THF, −78 °C, 5 h; (b) 3-furylmethyltriphenylphosphonium bromide, KO*t*Bu, THF, 60 °C, 30 h; (c) 3-bromomethylfuran, Mg, THF, r.t. to 50 °C, 20 h; (d) bengal rose, O_2, Et(*i*Pr)$_2$N, *hν*, dichloromethane, −78 °C, 5 h, then r.t., 10 min; (e) $(PCy_3)_2Cl_2Ru{=}CHPh$ (**21**) (2 × 10 mol %), dichloromethane, r.t., 16 h; (f) IBX, DMSO, r.t., 16 h, 75%.

synthesis described above, the furan rings were oxidized with singlet oxygen, and the olefinic dysidiolide analogues **52** and **57** were released from the resin by means of olefin metathesis in overall yields of 18% for eight steps and 27% for 10 steps, respectively.

Finally, derivative **53** with the secondary alcohol in a different position than in dysidiolide and chain-elongated analogue **58** were synthesized employing a Grignard reaction as the key step. The Grignard reagent was prepared freshly from 3-bromomethyl furan[27] and magnesium in THF and was used immediately for the reactions with aldehyde resins **41** and **3** to yield addition products **49** and **55**. Notably, the Grignard reactions proceeded only with low degrees of conversion that could not be improved by employing up to 5 equiv of reagent, higher temperature (up to 50 °C), or longer reaction times (18 h). Thus, dysidiolide analogues **53** and **58** finally were obtained in overall yields of only 7% for eight steps and 6% for 10 steps on the solid phase, respectively.

The syntheses of the dysidiolide analogues detailed above proceed in reaction sequences of overall 8−11 linear steps with the longest sequence on the solid support amounting up to 10 sequential transformations. The overall yields obtained in these unoptimized multistep sequences range from 6% to 27%. Typically, the individual reaction sequences were completed within 7−10 days, and in all cases the desired products were obtained after simple flash chromatography in multimilligram

(27) Mateos, A. F.; Lopez-Barba, A. M. *J. Org. Chem.* **1995**, *60*, 3580−3585.

Table 1. Results of the Inhibition of Cdc25C and the Cytotoxicity Tests Performed on the Colon Cancer Cell Lines SW480 and HCT116, the Prostate Cell Line PC3, and the Breast Cell Line MDA-MB231

cpd	Cdc25C[a] IC_{50} (μM)	SW480[b] IC_{50} (μM)	HCT116[c] IC_{50} (μM)	PC3[c] IC_{50} (μM)	MDA-MB231[c] (μM)
46	5.1	4	1.2	1	1.6
50	16	1			
51	1.5	20	11	13	>10
56	0.8	>33	15	>20	>10
52	6.8	4			
57	2.4	2			
53	6.1	>33			
58	9	>33			
38	>50	8			

[a] For the phosphatase assay, 5 μL of compound dissolved in 100% DMSO was added to a solution of 0.2 μg of recombinant Cdc25C in 85 μL of assay buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM DTT, 1 mM EDTA, and 10% DMSO). After incubation for 30 min at 30 °C, the substrate fluorescein diphosphate (FDP) was added to give a final concentration of 1 μM. Plates were read after a reaction time of 30 min at 485/535 nm (ex/em). *o*-Vanadate (IC_{50} = 0.1 μM) was used as reference compound. [b] The cells were incubated with the compounds at concentrations between 1.2 and 100 μM for 3 days. The viability of the cells was measured with MTT, which is a slightly yellow tetrazolium salt. Living cells reduce the tetrazolium moiety by a mitochondrial dehydrogenase to a dark violet dye that is detected at 570 nm. [c] The assays were performed using the CytoTox 96 cytotoxicity assay kit from Promega Corporation, USA. Three thousand cells were plated per well in a 96-well flat-bottomed plate, incubated with the compounds at concentrations ranging from 0.033 μM to 10 μM for 3 days. Cells were lysed, and the cellular lactate dehydrogenase activity was measured, which quantitatively reflects the number of cells.

amounts starting from only 100 mg of resin (i.e., in sufficient quantity and in sufficient purity for subsequent biological evaluation).

These results demonstrate that the multistep total synthesis of natural products and close analogues thereof on the solid phase is feasible. The transformations applied in the synthesis described above include a variety of very different reaction types widely used in organic synthesis (i.e., an asymmetric cycloaddition employing a removable chiral auxiliary, different organometallic transformations, olefination reactions, different oxidation reactions, acidic hydrolyses of acetals and enol ethers, and a nucleophilic substitution).

To determine if the solid-phase synthesis delivered biologically active natural product analogues with a high frequency, we investigated the dysidiolide analogues as inhibitors for the protein phosphatase Cdc25C and in cellular cytotoxicity assays. From the Cdc25 phosphatase family the Cdc25C protein was chosen, since 6-*epi*-dysidiolide **46** has previously been investigated as an inhibitor of Cdc25A and Cdc25B,[28] thereby allowing for comparison of data.

The results obtained in the phosphatase assay shown in Table 1 demonstrate that all dysidiolide analogues synthesized on solid support inhibit Cdc25C in the low micromolar range with the IC_{50} value varying by a factor of 20. Only analogue **38** that was synthesized in solution showed a weak inhibition (>50 μM). The IC_{50} of 5.1 μM determined for inhibition of Cdc25C by 6-*epi*-dysidiolide **46** is considerably lower than the values recorded for the inhibition of Cdc25A (13 μM) and Cdc25B (18 μM) by this compound. In addition, the most active compound in this enzyme assay, ketone **56**, exhibited an IC_{50} value in the high nanomolar range (800 nM) and was 6.4-fold more active than 6-*epi*-dysidiolide **46**. These results indicate that selectivity between different types of phosphatases and conceivably among the three Cdc25 family members may be achieved by means of dysidiolide analogues and compounds derived therefrom. The data also indicate that a substantial variation of the precise structural details of the natural product itself is tolerated and leads to inhibitors with significantly enhanced potency. Thus, replacement of the hydroxyethyl bridge between the annelated core ring system and the hydroxybutenolide present in compound **46** by an unsaturated three carbon unit (see **57**) or introduction of a keto group (see **51** and **56**) leads to more potent Cdc25C inhibitors. The synthetic dysidiolide analogues also displayed considerable and differing biological activity in a cytotoxicity assay[29] employing the colon cancer cell line SW480 (Table 1).

Four of the eight compounds investigated showed IC_{50} values in the very low micromolar range and pronounced antitumor activity. In this cellular assay alcohol **50** with a shortened carbon chain was the most active compound, whereas inhibitors **51** and **56** had shown the lowest IC_{50} values and compounds **53** and **58**, in which the alcohol is positioned differently between the hydroxybutenolide and the core structure of dysidiolide, were significantly less active.

This trend also became apparent when ketones **51** and **56** as well as 6-*epi*-dysidiolide (**46**) were subjected to cytotoxicity assays on the colon cell line HCT116, the prostate cancer cell line PC3, and the breast cancer cell line MDA-MB231. As shown in Table 1 ketones **51** and **56** again are substantially less active than 6-*epi*-dysidiolide (**46**), which inhibits cell proliferation in all three cases with IC_{50} values in the very low micromolar range.

Thus, the data indicate that the small library of natural product analogues already contains potent compounds with significantly differing biological activity both in vitro and in vivo. The observation that the order of IC_{50} values determined in an enzyme assay does not necessarily parallel the outcome of cellular assays is not uncommon.

Conclusion

In conclusion we have demonstrated that the synthesis of natural product derived libraries in long multistep sequences executed on a polymeric support and employing a variety of widely differing synthetic transformations is feasible and that it can deliver potent biologically active compounds with high frequency. Given this power of organic synthesis, the combinatorial synthesis and variation of natural products lies ahead.

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Supporting Information Available: Experimental procedures for the synthesis and spectroscopic data of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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