[5] C. A. Bischoff, E. Fröhlich, *Chem. Ber.* **1907**, *40*, 2779 – 2790.
[6] J. T. Adams, C. R. Hauser, *J. Am. Chem. Soc.* **1944**, *66*, 1220 – 1222; R. Levine, J. A. Conroy, J. T. Adams, C. R. Hauser, *J. Am. Chem. Soc.* **1945**, *67*, 1510 – 1512.
[7] Mononuclear metallacoronates are generated with polyethylene glycol spacered bis-1,3-diketones. See: Y. Kobuke, Y. Satoh, *J. Am. Chem. Soc.* **1992**, *114*, 789 – 790.
[8] For double-stranded intertwined infinite linear silver coordination networks based on bis-monodentate ligands with polyethylene glycol spacer, see: B. Schmaltz, A. Jouaiti, M. W. Hosseini, A. De Cain, *Chem. Commun.* **2001**, 1242 – 1243.
[9] Crystal data for K-**3**: $C_{48}H_{51}Cu_2KO_{16}$, $M_r = 1050.07$; crystal dimensions $0.25 \times 0.15 \times 0.15$ mm^3; monoclinic, space group $C2/c$, $a = 984.0(2)$, $b = 2070.8(4)$, $c = 2475.0(5)$ pm, $\beta = 98.39(3)^\circ$, $V = 4989.0(17)$ Å^3; $Z = 4$; $F(000) = 2192$, $\rho_{calcd} = 1.398$ g cm^{-3}. Diffractometer: Nonius KappaCCD, $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å); $T = 173(2)$ K; graphite monochromator; θ range [°] $2.14 < \theta < 27.50$; section of the reciprocal lattice: $-12 \le h \le 12$, $-26 \le k \le 26$, $-32 \le l \le 32$; of 20042 measured reflections, 5702 were independent and 4339 with $I > 2\sigma(I)$; linear absorption coefficient 1.004 mm^{-1}. The structure was solved by direct methods using SHELXS-97 and refinement with all data (321 parameters) by full-matrix least-squares on F^2 using SHELXL-97;[10] all non-hydrogen atoms were refined anisotropically; $R1 = 0.0607$ for $I > 2\sigma(I)$ and $wR2 = 0.2074$ (all data); largest peak (1.358 e Å^{-3}) and hole (-0.788 e Å^{-3}).[11]
[10] G. M. Sheldrick, C. Krüger, P. Goddard, *Crystallographic Computing 3*, Oxford University Press, Oxford, **1985**, p. 175; G. M. Sheldrick, SHELXS-97, Program for Crystal Structure Solution, Universität Göttingen, **1997**; G. M. Sheldrick, SHELXL-97, Program for Crystal Structure Refinement, Universität Göttingen, **1997**.
[11] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-168265 (**3**, M = K) and CCDC-168266 [(**4**)$_n$]. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
[12] L. Plasseraud, H. Maid, F. Hampel, R. W. Saalfrank, *Chem. Eur. J.* **2001**, *7*, 4007 – 4011; R. W. Saalfrank, H. Maid, F. Hampel, K. Peters, *Eur. J. Inorg. Chem.* **1999**, 1859 – 1867.
[13] R. W. Saalfrank, I. Bernt, F. Hampel, *Chem. Eur. J.* **2001**, *7*, 2770 – 2774.
[14] M. Albrecht, S. Kotila, *Angew. Chem.* **1996**, *108*, 1299 – 1300; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1208 – 1210; D. L. Caulder, K. N. Raymond, *J. Chem. Soc. Dalton Trans.* **1999**, 1185 – 1200; R. W. Saalfrank, V. Seitz, F. W. Heinemann, C. Göbel, R. Herbst-Irmer, *J. Chem. Soc. Dalton Trans.* **2001**, 599 – 603
[15] Crystal data for (**4** · 3 HOMe)$_n$: $C_{51}H_{60}Cs_2Ni_2O_{21}$, $M_r = 1392.24$; crystal dimensions $0.40 \times 0.35 \times 0.35$ mm^3; monoclinic, space group $P2(1)/n$, $a = 1767.67(5)$, $b = 1459.88(2)$, $c = 2271.77(3)$ pm, $\beta = 103.9790(10)^\circ$, $V = 5688.88(13)$ Å^3; $Z = 4$; $F(000) = 2788$, $\rho_{calcd} = 1.626$ g cm^{-3}. Diffractometer: Nonius KappaCCD, $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å); $T = 173(2)$ K; graphite monochromator; θ range [°] $1.32 < \theta < 27.48$; section of the reciprocal lattice: $-22 \le h \le 22$, $-18 \le k \le 14$, $-29 \le l \le 29$; of 19152 measured reflections, 12962 were independent and 9364 with $I > 2\sigma(I)$; linear absorption coefficient 1.997 mm^{-1}. The structure was solved by direct methods using SHELXS-97 and refinement with all data (685 parameters) by full-matrix least-squares on F^2 using SHELXL-97;[10] all non-hydrogen atoms were refined anisotropically; $R1 = 0.0446$ for $I > 2\sigma(I)$ and $wR2 = 0.1396$ (all data); largest peak (1.304 e Å^{-3}) and hole (-1.607 e Å^{-3}).[11]
[16] See: S. Y. Lai, T. W. Lin, Y. H. Chen, C. C. Wang, G. H. Lee, M. H. Yang, M. K. Leung, S. M. Peng, *J. Am. Chem. Soc.* **1999**, *121*, 250 – 251; H. C. Chang, J. T. Li, C. C. Wang, T. W. Lin, H. C. Lee, G. H. Lee, S. M. Peng, *Eur. J. Inorg. Chem.* **1999**, 1243 – 1251.
[17] D. J. Eichorst, D. A. Payne, S. R. Wilson, K. E. Howard, *Inorg. Chem.* **1990**, *29*, 1458 – 1459; R. Fuchs, N. Habermann, P. Klüfers, *Angew. Chem.* **1993**, *105*, 895 – 897; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 852 – 854; S. I. Troyanov, O. Yu. Gorbenko, A. A. Bosak, *Polyhedron* **1999**, *18*, 3505 – 3509.
[18] The microanalytical data deviate from theory due to crystal solvents and are not reported here.

Natural Products Are Biologically Validated Starting Points in Structural Space for Compound Library Development: Solid-Phase Synthesis of Dysidiolide-Derived Phosphatase Inhibitors**

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The combinatorial synthesis of compound libraries on polymeric supports is at the heart of protein ligand and inhibitor discovery, in particular in the development of new drugs and chemical tools for the study of biological processes. To achieve high efficiency in this process, powerful and alternative strategies for the design of compound libraries are of paramount importance. Herein, a structure-based approach to this fundamental problem is outlined. The key feature is to employ the structural frameworks of biologically active natural products, which are evolutionarily selected for binding to specific protein domains, as a guiding principle for library development. Furthermore, it is demonstrated that the key synthetic challenge posed by this approach, the multistep solid-phase synthesis of natural products and their analogues, can successfully be met.

Proteins can be regarded as modularly built biomolecules assembled from individual domains as building blocks. Since the total number of all available protein domains appears to be fairly limited,[1] it has to be expected that in newly discovered proteins with widely varying function and activity the same modules (i.e. domains) or close relatives will be found repeatedly in varying combinations and arrangements as structure- and function-determining entities. Thus, 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 biologically validated as being

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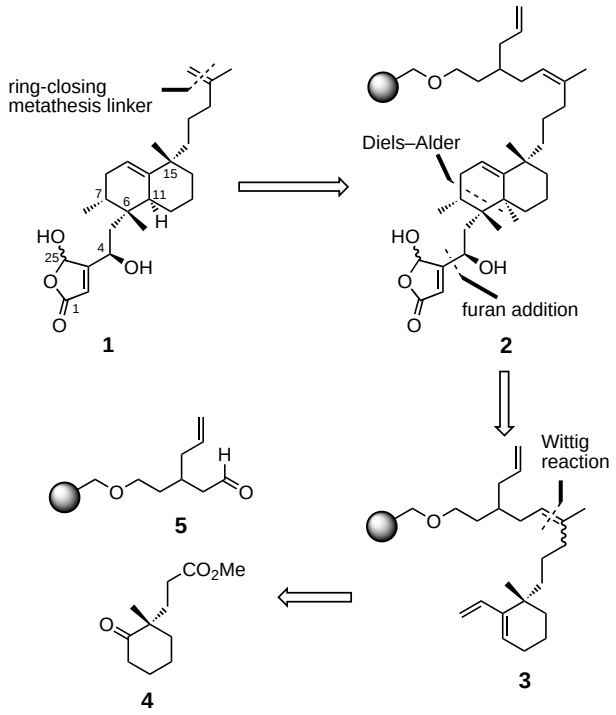
capable of binding to specific protein domains. These compound classes can then be employed as starting points in structural space for library development. Libraries designed and synthesized around the basic structure of such compounds should yield modulators of protein activity with high hit rates. Furthermore they should yield modulators of activity for proteins with differing activity, function and origin which bear the same or very similar domains. Within a group of closely related protein domains usually the individual amino acid sequence and the detailed structure associated with it display substantial variation, which often provides the basis for selective binding and inhibition (e.g. in the ATP-binding sites of protein kinases). Consequently, a central and important aspect of this concept is that it is not necessary to build up a given structure in every detail. Rather, the basic underlying structural framework has to be conserved for the individual library members, and its stereochemistry and functional group pattern have to be varied to achieve selectivity between related proteins.

Biologically active natural products can be regarded as chemical entities that were evolutionarily selected and validated for binding to particular protein domains. Therefore, they are already biologically validated, and the underlying structural architectures of such natural products may provide powerful guiding principles for library development. This structure-based concept differs in its fundamental reasoning from related approaches,[2] which focus on the creation of chemical diversity. However, our concept does not neglect this issue, and builds on the diversity created by Nature itself.

Efficient and reliable methods and multistep sequences for the total synthesis of natural products and their analogues on polymeric supports are paramount to the success of this approach. The corresponding transformations must proceed with a degree of selectivity and robustness typical of related classical transformations in solution, irrespective of the stringencies and differing demands imposed by the presence of and the anchoring to the polymeric support. In a few cases, the structural variation of natural products by means of solid-phase methodologies has been achieved,[3] mostly in the late steps of the syntheses and through the modification of a core-structure that had been pre-synthesized in solution. But the total synthesis of a natural product and its analogues in long multistep sequences (i.e. ten steps and more) on polymeric supports has only been successful in one case.[3, 4] Herein we describe the solid-phase synthesis of analogues of the phosphatase inhibitor dysidiolide, which rapidly yielded compounds with significantly improved biological activity.

The sesquiterpenoid dysidiolide (**1**) is the first naturally occurring inhibitor of the dual-specificity cdc25 protein phosphatase family, which play a crucial role in the regulation of the cell cycle.[5] Because of this property and the resulting antitumor activity, dysidiolide has been of intense interest to chemists, biologists, and pharmacologists.[6] 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 incorporated in **2** was thought to fulfill these requirements (Scheme 1). The γ-hydroxybutenolide moiety should be obtainable by the addition of 3-lithiofuran to a corresponding aldehyde, and subsequent oxidation of the heterocycle with singlet oxygen. The bicyclic core structure of **1** could be generated by means of a Diels–Alder route previously



Scheme 1. Retrosynthetic analysis of dysidiolide and delineation of a solid-phase synthesis strategy with an olefin-metathesis linker.

investigated in our laboratories[7] (**2** $\Rightarrow$ **3**) and successfully employed in the total syntheses of natural dysidiolide in solution.[16b–e] The knowledge gleaned from these studies suggested that this cycloaddition leads primarily 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 from commercially available chiral ketoester **4**. The diene would then be attached to the linker resin **5** by means of a Wittig reaction in a convergent strategy (**3** $\Rightarrow$ **4** + **5**).

Starting from dihydropyranone **6**, linker **8** was synthesized in the reaction with diallylzinc (generated in situ) in the presence of trimethylsilyl chloride (TMSCl), subsequent reduction of lactone **7** with lithium aluminum hydride (LAH), and monoprotection of the resulting diol (Scheme 2 A). The protection of one hydroxy group with dihydropyran was necessary to prevent crosslinking in the subsequent attachment of the linker to the Merrifield resin by ether formation. After deprotection and subsequent oxidation of the hydroxy group, the active linker resin **5** was obtained in high yield (90 %, 3 steps in solid phase) with loadings of up to 1.1 mmol g^{-1}.

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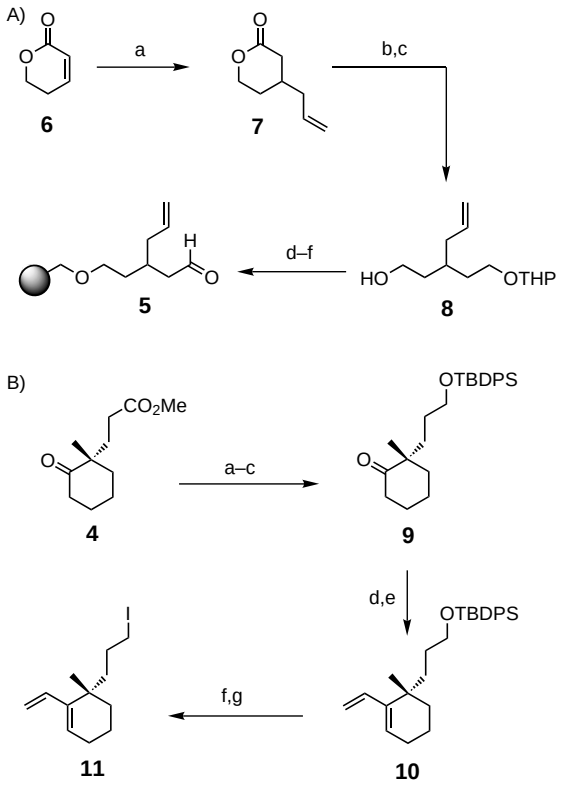
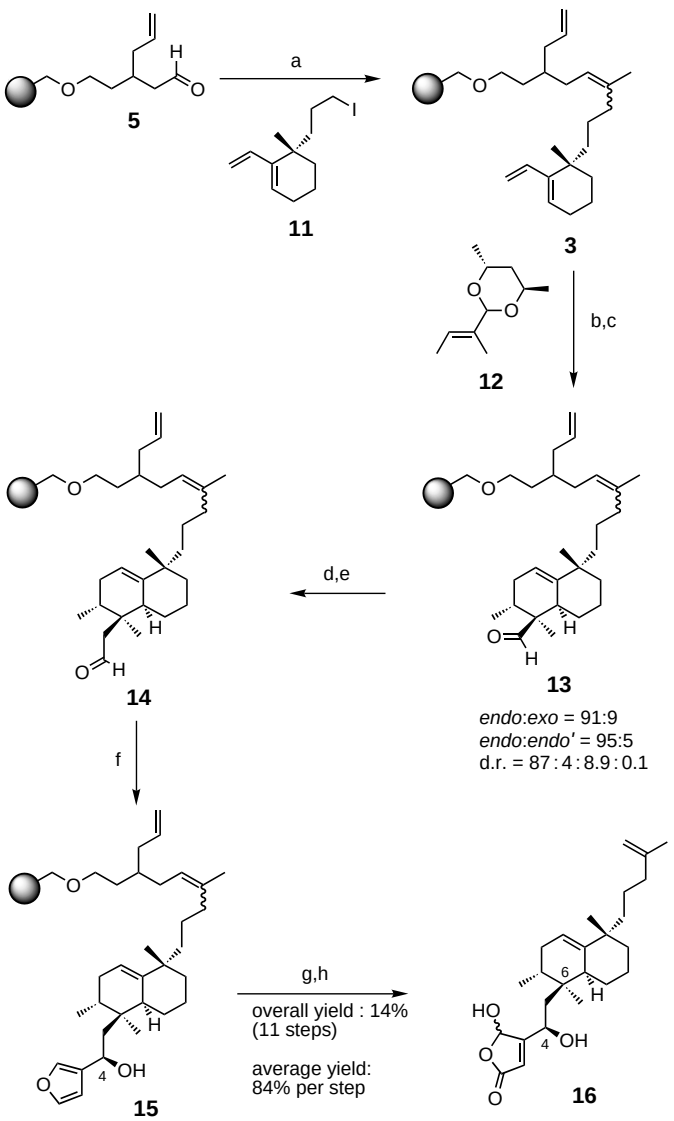


Scheme 2. A) Synthesis of the linker and attachment to the resin. Reagents and conditions: a) allylmagnesium bromide, $ZnBr_2$, TMSCl, THF, −78 °C, 6 h, 52 %; b) LAH, THF, RT, 1 h, 97 %; c) 3,4-dihydropyran, Dowex 50WX2, toluene, RT, 3 h, 95 %; d) NaH, nBu_4NI, Merrifield-Cl resin (1.1 mmol g^{-1}), DMF, RT, 18 h; e) pyridinium-*p*-toluene sulfonate, EtOH, dichloroethane, Δ, 18 h; f) *o*-iodoxybenzoic acid, THF/DMSO (1:1), RT, 8 h; 90 % (three steps). B) Synthesis of the diene: a) LAH, THF, 0 °C, 30 min, 100 %; b) TBDPSCl, dimethylaminopyridine, Et_3N, dichloromethane, RT, 17 h, 80 %; c) PCC, dichloromethane, RT, 4 h, 94 %; d) vinylmagnesium bromide, THF, RT, 5 h; e) $BF_3 \cdot OEt_2$, benzene/THF (4:1), Δ, 43 % (d–e); f) nBu_4NF, THF, RT, 3 h, 95 %; g) iodine, imidazole, PPh_3, dichloromethane, RT, 1 h, 85 %.



Scheme 3. Solid-phase synthesis of **16**. Reagents and conditions: a) $EtPPh_3I$, *n*BuLi, **11**, THF, RT, 16 h, then *n*BuLi, 0 °C, 2 h; b) trimethylsilyltrifluoromethanesulfonate, dichloromethane, −78 °C, 7 h; c) *p*-toluenesulfonic acid, acetone, dichloroethane, H_2O, Δ, 20 h; d) Ph_3PCH_2OMeCl, KO*t*Bu, THF, RT, 4 h; e) pyridinium *p*-toluene sulfonate, THF, H_2O (1 %), Δ, 16 h; f) 3-bromofuran, *n*BuLi, THF, −78 °C, 5 h; g) O_2, Et*i*Pr$_2$N, rose bengal, *hν*, dichloromethane, −78 °C, 5 h, then RT, 10 min; h) $(PCy_3)_2Cl_2$-Ru=CHPh (2 × 10 Mol %), dichloromethane, RT, 16 h.

The diene was synthesized in solution starting from commercially available chiral ketoester **4**. After reduction with LAH to form the diol, the primary hydroxy group was protected with *tert*-butyldiphenylsilyl chloride (TBDPSCl) and the secondary alcohol was oxidized by using pyridinium chlorochromate (PCC) to obtain ketone **9** in high yield (Scheme 2B). Vinylmagnesium chloride was added to the carbonyl group and the resulting tertiary alcohol was converted into diene **10** by elimination of water under Lewis acid catalysis. After cleavage of the protecting group, the alcohol was finally transformed into alkyl iodide **11**, the precursor for the subsequent Wittig reaction.

In a one-pot two-step procedure, alkyl iodide **11** was treated with the ylide that was obtained by the deprotonation of ethyltriphenylphosphonium iodide to form a secondary phosphonium salt in a nucleophilic attack. This resulting phosphonium salt was then deprotonated with a second equivalent of base, and the aldehyde resin **5** was finally added to yield diene resin **3** (Scheme 3). Subsequently, **3** was treated with the chiral unsaturated acetal **12**[8] at −78 °C in a Lewis acid catalyzed asymmetric Diels–Alder reaction to generate the bicyclic core structure of the target molecule with high diastereoselectivity (*endo*/*exo* 91:9; ratio of *endo* isomers 95:5; *endo*/*endo'*/*exo*/*exo'* 87:4:8.9:0.1; the values were determined by means of ^{1}H NMR spectroscopic analysis after hydrolysis of the acetals and release of the resulting aldehydes from the solid support by olefin metathesis). Structure and configuration were determined by comparison with literature data.[6d, 7]

After hydrolysis of the acetal, the carbon chain of aldehyde **13** was elongated by means of a Wittig reaction with methoxymethyltriphenylphosphonium chloride and subsequent hydrolysis of the resulting ether to yield aldehyde **14**. Nucleophilic addition of 3-lithiofuran to the aldehyde resulted in a 2:1 mixture of the epimeric alcohols **15** (determined by means of GC-MS). The furan unit was oxidized with singlet

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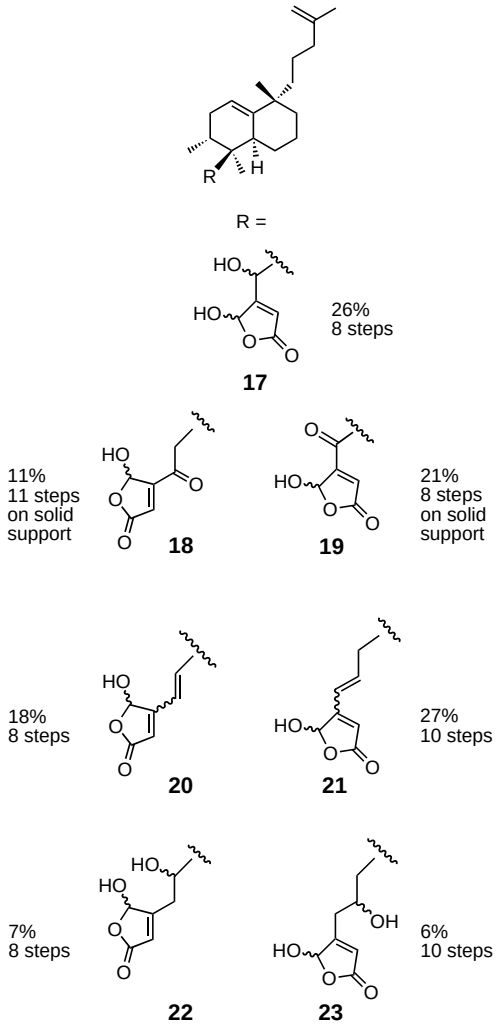
oxygen in the presence of Hünig's base to form the γ-hydroxybutenolide moiety. Finally, the products were released from the polymeric carrier under very mild conditions in an olefin-metathesis reaction mediated by Grubbs catalyst (Scheme 3).[9]

6-*epi*-Dysidiolide and four other diastereomers could be purified by flash chromatography and separated by semi-preparative HPLC on SiO_2 (98% *n*-hexane/2% 2-propanol). The analytical data (^{1}H NMR, ^{1}H,^{1}H-COSY, IR, HR-MS) of the new analogues were in full agreement with the structure, and similar to the data reported for the natural product. The synthesis sequence from the attachment of **8** to the solid phase to the release of **16** into solution proceeds through a total of eleven chemical steps and makes the desired natural product analogue available with an overall yield of 14%, that is, with an average yield of 84% per step. By using this procedure, 48 mg of the desired product can be obtained from one gram of resin with a loading of 1.1 mmol g^{-1}.

Based on the developed synthesis strategy, a small library of seven analogues was synthesized from the intermediate aldehydes **13** and **14**. Thus analogue **17** (Scheme 4) with a shorter carbon chain was synthesized by addition of 3-lithiofuran to aldehyde **13** followed by oxidation of the furan ring.



Scheme 4. Dysidiolide analogues obtained in solid-phase syntheses.

The ketones **18** and **19** were obtained by oxidation of secondary alcohols **16** and **17**, respectively, with *o*-iodoxybenzoic acid, after compounds **16** and **17** were released from the polymeric support. The olefinic analogues **20** and **21** were synthesized by Wittig reaction of the aldehydes **13** and **14**, respectively, with the ylide obtained from furylmethyltriphenylphosphonium bromide and subsequent oxidation with singlet oxygen. Derivatives **22** and **23** were obtained by addition of furylmethylmagnesium bromide to the carbonyl group, followed by oxidation of the aromatic ring. Compounds **17**–**23** were obtained in overall yields of 6–27%.

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

To determine if the solid-state synthesis delivered biologically active natural product analogues with a high frequency, we investigated compounds **16**–**23** as inhibitors of the protein phosphatase cdc25C and in cellular cytotoxicity assays. From the cdc25 phosphatase family, the cdc25C protein was chosen because 6-*epi*-dysidiolide **16** has previously been investigated as an inhibitor of cdc25A and cdc25B,[10] thereby allowing for comparison of data.

The results obtained in the phosphatase assays (Table 1) demonstrate that all dysidiolide analogues inhibit cdc25C in the low micromolar range with IC_{50} values varying by a factor of 20. The IC_{50} of 5.1 μM determined for inhibition of cdc25C

Table 1. Results of the inhibition of cdc25C and the cytotoxicity tests performed on the colon cancer cell lines SW480 and HCT116, the prostrate cell line PC3, and the breast cell line MDA-MB231.

Compound	cdc25C[a] IC_{50} [μM]	SW480[b] IC_{50} [μM]	HCT116[c] IC_{50} [μM]	PC3[c] IC_{50} [μM]	MDA-MB231[c] IC_{50} [μM]
16	5.1	4	1.2	1	1.6
17	16	1			
18	0.8	>33	15	>20	>10
19	1.5	20	11	13	>10
20	6.8	4			
21	2.4	2			
22	6.1	>33			
23	9	>33			

[a] For the phosphatase assay, the compound (5 μL) was dissolved in DMSO (100%) and added to a solution of recombinant cdc25C (0.2 μg) in assay buffer (85 μL; 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 three 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 which is detected at 570 nm. [c] The assays were performed by using the CytoTox 96 Cytotoxicity Assay Kit from Promega Corporation, USA. 3000 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 three days. Cells were lysed, and the cellular lactate dehydrogenase activity was measured which quantitatively reflects the number of cells.

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by 6-*epi*-dysidiolide **16** is considerably lower than the values recorded for the inhibition of cdc25A (13 μM) and cdc25B (18 μM). Furthermore, the most active compound in this enzyme-assay, ketone **18**, exhibited an IC_{50} value in the high nanomolar range (800 nM) and was 6.4 times more active than **16**. These results indicate that dysidiolide analogues and their derivatives can differentiate selectively between different types of phosphatases and conceivably among the three cdc25 family members. The data also indicate that 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 **16** by an unsaturated three-carbon unit (see **21**) or introduction of a keto group (see **18** and **19**) leads to more potent cdc25C inhibitors.

The synthetic dysidiolide analogues also displayed considerable and differing biological activity in a cytotoxicity assay[11] of 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 **17** with a shortened carbon chain was the most active compound, whereas inhibitors **18** and **19** which had shown the lowest IC_{50} values and compounds **22** and **23** in which the alcohol is positioned differently between the hydroxybutenolide and the core structure of dysidiolide were considerably less active. This trend also became apparent when ketones **18** and **19** as well as *epi*-dysidiolide **16** were subjected to cytotoxicity assays of the colon cell line HCT116, the prostate cancer cell line PC3, and the breast cancer cell line MDA-MB231. Ketones **18** and **19** again were substantially less active than *epi*-dysidiolide **16**, which inhibits cell proliferation in all three cases with IC_{50} values in the very low micromolar range (Table 1).

Thus, the data indicate that the small library of natural product analogues already contains potent compounds with significantly different biological activities 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.

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.

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[1] Yu. I. Wolf, N. V. Grishin, E. V. Koonin, *J. Mol. Biol.* **2000**, *299*, 897–905.

[2] S. L. Schreiber, *Science* **2000**, *287*, 1964–1969.

[3] For an up-to-date review, see: D. G. Hall, S. Manku, F. Wang, *J. Comb. Chem.* **2001**, *3*, 125–150.

[4] a) K. C. Nicolaou, N. Winssinger, J. Pastor, S. Ninkovic, F. Sarabia, Y. He, D. Vourloumis, Z. Yang, T. Li, P. Giannakakou, E. Hamel, *Nature* **1997**, *387*, 268–272; b) K. C. Nicolaou, D. Vourloumis, T. Li, J. Pastor, N. Winssinger, Y. He, S. Ninkovic, F. Sarabia, H. Vallberg, F. Roschangar, N. P. King, M. R. V. Finlay, P. Giannakakou, P. Verdier-pinard, E. Hamel, *Angew. Chem.* **1997**, *109*, 2181–2187; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2097–2103.

[5] G. Draetta, J. Eckstein, *Biochim. Biophys. Acta* **1997**, *1332*, M53–M63.

[6] a) E. J. Corey, B. E. Roberts, *J. Am. Chem. Soc.* **1997**, *119*, 12425–12431; b) S. R. Magnuson, L. Sepp-Lorenzino, N. Rosen, S. J. Danishefsky, *J. Am. Chem. Soc.* **1998**, *120*, 1615–1616; c) J. Boukouvalas, Y.-X. Cheng, J. Robichaud, *J. Org. Chem.* **1998**, *63*, 228–229; d) M. Takahashi, K. Dodo, Y. Hashimoto, R. Shirai, *Tetrahedron Lett.* **2000**, *41*, 2111–2114; e) M. Jung, N. Nishimura, *Org. Lett.* **2001**, *3*, 2113–2115; f) E. Piers, S. Caillé, G. Chen, *Org. Lett.* **2000**, *2*, 2483–2486; g) D. Demeke, C. J. Forsyth, *Org. Lett.* **2000**, *2*, 3177–3179; h) H. Miyaoka, Y. Kajiwara, Y. Yamada, *Tetrahedron Lett.* **2000**, *41*, 911–914; i) J. W. Eckstein, *Invest. New Drugs* **2000**, *18*, 149–156.

[7] D. Brohm, H. Waldmann, *Tetrahedron Lett.* **1998**, *39*, 3995–3998.

[8] T. Sammakia, M. A. Berliner, *J. Org. Chem.* **1994**, *59*, 6890–6891.

[9] P. Schwab, M. B. France, J. W. Ziller, R. H. Grubbs, *Angew. Chem.* **1995**, *107*, 2197–2181; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2039–2041.

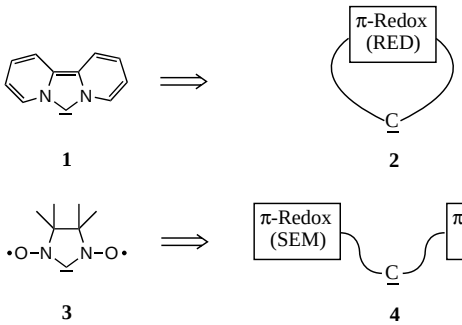
[10] M. Takahashi, K. Dodo, Y. Sugimoto, Y. Aoyagi, Y. Yamada, Y. Hashimoto, R. Shirai, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2571–2574.

[11] T. Mosman, *J. Immunol. Methods* **1983**, *65*, 55–63.

Dinitroxide Carbenes, A New Class of Carbenes with Autoumpolung Character: Preparation in Solution and Stabilization in Transition Metal Complexes**

Robert Weiss* and Norbert Kraut

With the synthesis of **1** we recently reported the prototype of a carbene with autoumpolung character.[1] As the general structure **2** shows, in such a system a singlet carbene center is conjugated with the termini of a two-step π-redox system in the reduced state (RED).[2] The electrons in this reservoir can be used in the sense of a redox umpolung to respond to electronic requirements of the coordination partner at the carbene center.[3] To extend this concept we report here the first synthesis of the dinitroxide carbene **3** in solution and its stabilization in transition metal complexes.



1 **2**

3 **4**

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