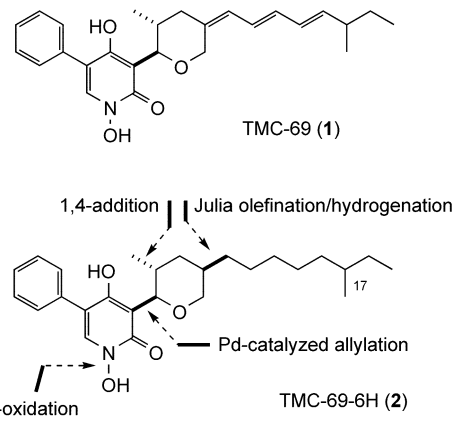
Phosphatase Inhibitors

Total Synthesis and Reassessment of the Phosphatase-Inhibitory Activity of the Antitumor Agent TMC-69-6H**

Alois Fürstner, Fabian Feyen, Heino Prinz, and Herbert Waldmann*

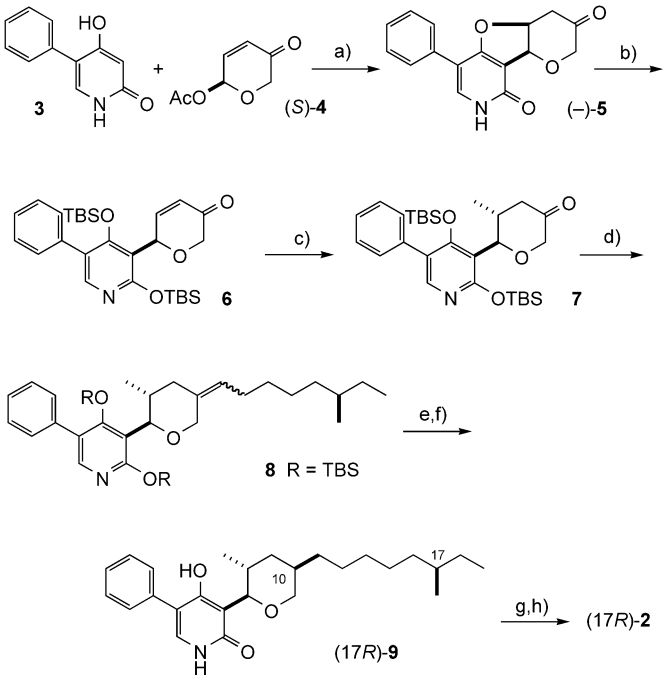
The family of the Cdc25 dual specific protein phosphatases is critically involved in cell-cycle control.[1] Their physiological substrates are cyclin-dependent kinases, which trigger key transitions in the process of eukaryotic cellular division. Therefore the homologous Cdc25 enzymes exert crucial regulatory functions at the crossroads between cellular proliferation, cell cycle arrest, and apoptosis. Their oncogenic properties together with the fact that Cdc25A and -B are overexpressed in many human tumors render these isoenzymes molecular targets of utmost interest in the quest for anticancer drugs.[1–3]

Despite this widely recognized fact, only a rather limited number of small molecules are presently known that might qualify as lead structures in the search for selective inhibitors of Cdc25 phosphatases.[3] One such compound is TMC-69 (**1**), a 2-pyridone derivative isolated from the culture broth of the fungus *Chrysosporium sp.* TC 1068 (Scheme 1).[4] According to the literature, this compound is distinguished by excellent activity (IC_{50} values in the low micromolar range) and by a surprising selectivity for Cdc25A and -B over other phosphatases. Although pyridone **1** is inherently labile (it degrades within 2 weeks at 0 °C), hydrogenation of its triene moiety results in significantly improved stability and even further enhanced potency.[4] Importantly, TMC-69-6H (**2**) thus formed also exhibits a remarkable efficacy against P388 murine leukemia and B16 melanoma in nude mice.[4] Outlined below is the first total synthesis of the 17*R* and 17*S* isomers of **2** in enantiomerically pure form. The absolute stereochemistry at this remote stereogenic center in the natural product **1** and its derivative **2** has not yet been established.[4]



Scheme 1. TMC-69 and TMC-69-6H. Retrosynthetic analysis for **2**.

The readily available pyranone **4**[5] served as a key building block en route to **2** (Scheme 2). Its enone moiety allowed the stereoselective installation of the methyl group by 1,4-addition, followed by attachment of the lateral chain by olefination of the remaining ketone. More importantly, however, the allylic leaving group in **4** opened several options for the introduction of the heteroaromatic ring. Rather than taking recourse to known oxo-carbenium cation chemistry,[6] we decided to rely on palladium-catalyzed C–C bond formations because they allow control of the stereochemical course of the envisaged allylation process more accurately (Scheme 2).



Scheme 2. Synthesis of (17*R*)-**2**: [{(allyl)PdCl}$_2$] (0.5 %), **10** (1.5 %), Et_3N, DMF, 65 %, 96 % *ee*; b) TBSCl, Et_3N, 18 h, 78 %; c) Me_2CuLi, THF, −70 °C; d) LiHMDS, (*R*)-**15**, 69 % (over both steps); e) H_2, Pd/C, EtOH; f) TBAF, THF, 69 % (over two steps); g) $HN(SiMe_3)_2$, TMSCl cat., reflux; h) 1) [(pyridine)MoO_5(hmpa)], CH_2Cl_2; 2) saturated aqueous EDTA-Na, EtOAc, 61 % (over three steps). DMF = *N,N*-dimethylformamide, TBS = *tert*-butyldimethylsilyl, HMDS = hexamethyldisilazide, TBAF = tetrabutylammonium fluoride, TMS = trimethylsilyl, HMPA = hexamethylphosphortriamide, EDTA = ethylenediaminetetraacetate.

Surprisingly, though, palladium-catalyzed transformations of 6-hydroxy-6*H*-pyran-3-one derivatives are scarce and seem to be restricted to reactions with O nucleophiles.[7] To the best

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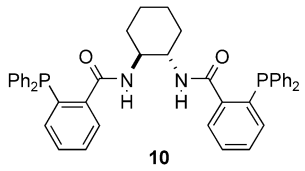
of our knowledge, no Pd-catalyzed *C*-arylation of such a substrate with a phenol derivative has been described previously. Gratifyingly, however, (±)-**4** reacts smoothly and regioselectively at the enol site of 4-hydroxypyridone **3**[8] in the presence of $[Pd(PPh_3)_4]$ catalyst and Et_3N in DMF to give the tricyclic product (±)-**5** in 89% yield. This novel palladium-catalyzed C–C bond formation is accompanied by a spontaneous 1,4-addition of the 4-OH group to the enone entity of the emerging product. Not only are we unaware of any precedence for this transformation, but this result must also be seen in the context of previous reports, which show that 2-pyridones tend to react with allylic substrates at their N atom in the presence of palladium catalysts.[9]

Attempts to perform this reaction enantioselectively with *rac*-**4** as the substrate and the chiral diphosphane **10**[10] as



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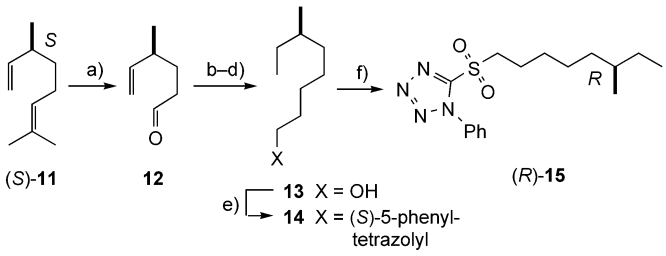
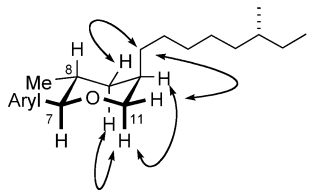
ligand to Pd were unrewarding (~30% *ee*). However, the use of **10** in combination with enantiomerically enriched (*S*)-**4** (81% *ee*), which is easily prepared on a multigram scale by a lipase-catalyzed dynamic resolution,[11] served our purpose very well, and delivered the tricyclic ketone (−)-**5** in good yield and with excellent enantiomeric purity (96% *ee*) (Scheme 2).

The cyclic ether moiety of **5** acts as a temporary protecting group for the enone which can be released by a retro-Michael reaction upon treatment with oxophilic reagents in the presence of a base.[12] Optimal results were obtained with TBSCl in combination with Et_3N. Exposure of the resulting disilyl ether derivative **6** to Me_2CuLi afforded the somewhat labile *trans*-disubstituted ketone **7**, which was subjected to a Julia-Kocienski olefination reaction[13] with the lithiated sulfone (*R*)-**15** (prepared from (*S*)-citronellene **11** as shown in Scheme 3).[14] HPLC analyses of alkene **8** (*E*/*Z* 1:1) on chiral columns and comparison with the racemic series confirmed that no racemization had occurred during these



Scheme 3. Reagents and conditions: a) O_3, CH_2Cl_2, −78 °C; then Me_2S, 98%; b) $(EtO)_2P(O)CH_2COOMe$, NaH, THF, −78 °C, 87%; c) DIBAL-H, Et_2O, 91%; d) H_2, Pd/C, 88%; e) 1-phenyl-1H-tetrazole-5-thiol, DEAD, PPh_3, THF, 68%; f) $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (0.1 equiv), H_2O_2 (10 equiv), EtOH, 93%. DIBAL-H = diisobutylaluminum hydride, DEAD = diethyl azodicarboxylate.

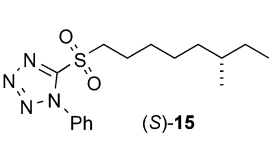
steps (97% *ee*). Subsequent hydrogenation of **8** over Pd/C followed by cleavage of the TBS groups furnished a separable mixture of the desired product **9** and its C10 epimer in 69% yield (over two steps). The axial orientation of the alkyl chain on the tetrahydropyran ring in **9** was evident from an analysis of the pertinent coupling constants and NOESY data (Scheme 4).[15]



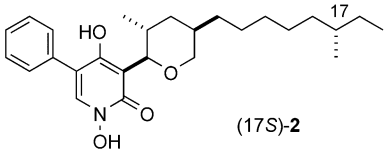
Scheme 4. Schematic representation of compound **9** with characteristic NOESY data. Pertinent coupling constants: $^3J_{H7,H8} = 10.4$ Hz, $^3J_{H10,H11ax} = 2.5$ Hz, $^3J_{H10,H11eq} \leqslant 1$ Hz (TMC-69-6H numbering).

For the final *N*-oxidation to the desired hydroxamic acid derivative,[16] compound **9** was heated at reflux with hexamethyl disilazane and the resulting bis(silyl ether) was treated with the peroxo complex $[(pyridine)MoO_5(hmpa)]$.[17] Aqueous work-up with EDTA to sequester all metal cations then completed the first total synthesis of (17*R*)-TMC-69-6H ((17*R*)-**2**).

As mentioned above, no secured information concerning the absolute stereochemistry of the remote stereogenic center on the lateral chain of compound **2** derived from natural sources was available. Therefore the same sequence of reactions was repeated with ketone (−)-**5** and the antipodal sulfone (*S*)-**15** to give (17*S*)-**2** in similar overall yield. The



(*S*)-**15**



(17*S*)-**2**

diastereomeric compounds (17*R*)-**2** and (17*S*)-**2** thus obtained, however, are virtually indistinguishable by NMR spectroscopy and match the literature data reported for TMC-69-6H very well.[4] Even a direct comparison of their spectra recorded at 600 MHz with those of an authentic sample did not allow the rigorous assignment of the absolute stereochemistry of TMC-69-6H at that site.[18]

Since this excellent match leaves no doubt about the integrity of the synthetic materials, we were surprised to find that their phosphatase inhibitory activity *deviates significantly* from the reported profile both in terms of potency and selectivity. In contrast to what has been reported, the compounds prepared by total synthesis as well as an *authentic* sample of **2** all turned out to be only rather weak inhibitors of Cdc25A ($IC_{50} > 30$ μM) in our assay. Instead, they exhibit promising activities against the tyrosine protein phosphatase PTB1B, the dual specific phosphatase VHR, and the serine/

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Table 1: IC_{50} values [μM] of authentic and synthetic TMC-69-6H and its immediate precursors against different phosphatases.[a]

Compound	Cdc25A	PTP1B	VHR	PP1
authentic **2**	⩾50	3.2±1.6	7.0±3.5	6±3
(17*R*)-**2**	45±23	4±2.0	6±3	8.5±4.5
(17*S*)-**2**	32±16	3.5±1.7	5.5±3	8±4
(17*S*)-**9**	⩾50	11±5.5	9±5	30±5
(17*S*)-**8b**[b]	⩾50	23±11	11±5.5	32±16

[a] The enzymatic activity was determined by hydrolysis of *para*-nitrophenyl phosphate in standard buffers for PTP1B,[22a] VHR,[22b] and PP1.[22b] [b] The isomer with an *E* configuration between the CH_2O group of the tetrahydropyran and C11 was tested.

threonine phosphatase PP1 (Table 1). PTP1B is a key negative regulator of insulin-receptor activity, and PTP1B-inhibitors are expected to enhance insulin sensitivity and act as effective therapeutics for the treatment of Type II diabetes, insulin resistance, and obesity. The vaccinia VH1-related phosphatase VHR is a physiological regulator of extracellular regulated kinases of the MAP (mitogen-activated protein) kinase family and influences signaling through the MAP kinase pathway. PP1 is a major eukaryotic phosphatase that regulates diverse cellular processes such as signal transduction, cell-cycle progression, protein synthesis, muscle contraction, carbohydrate metabolism, and transcription.

These phosphatases have been subject to intense research activities aimed at the development of inhibitors for biological studies and drug development.[19–22] Thus, PP1 inhibitors are expected to be potent anticancer drugs and have been used clinically for the treatment of cancers.[21] In particular, PTB1B is currently a major target of medicinal chemistry research in the pharmaceutical industry.[19]

Notably, TMC-69-6H incorporates a structural framework not present in any of the PTP1B and PP1 inhibitors developed so far. Thus, it provides a unique and unprecedented lead structure for the development of a new series of selective phosphatase inhibitors. Its proven activity in cellular assays (see above) renders such research activities particularly promising. Our preliminary results also seem to indicate that the presence of the *N*-OH group in the heterocycle, though not strictly required for phosphatase inhibition, enhances the potency of such compounds (compare, for instance, the IC_{50} values determined for (17*S*)-**2** with the data for the corresponding amide (17*S*)-**9**).

Although we are unable to reconcile these findings with the literature reports concerning the physiological activity of TMC-69-6H,[4] the excellent reproducibility of our results, the internal control against authentic **2**, the consistency within the individual series, and the established validity of our Cdc25 assay[3b] leave no room for interpretation. These data also make clear that *N*-hydroxy-2-pyridone derivatives constitute a promising new class of selective phosphatase inhibitors and certainly deserve further studies to establish pertinent structure–activity relationships and assess their potency in more detail.[23,24] Synthesis-driven studies along these lines are underway and will be reported in due course.

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[18] Unfortunately, the optical rotations did also not allow us to make a final decision. The optical rotation reported for TMC-69-6H is $[\alpha]^{25} = +143.4$ ($c = 0.48$, MeOH);[4] the optical rotation of the authentic sample of **2** made available to us, however, was lower than this reported value. When corrected for the residual water in the sample, we record an $[\alpha]^{20}$ value of $\approx +99$ ($c = 0.18$, MeOH). The data of our analytically pure synthetic compounds are as follows: (17*R*)-**2**: $[\alpha]^{20} = +102.3$ ($c = 0.60$, MeOH); (17*S*)-**2**: $[\alpha]^{20} = +88.6$ ($c = 0.50$, MeOH).

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