Synthesis of the Protein Phosphatase 2A Inhibitor (4*S*,5*S*,6*S*,10*S*,11*S*,12*S*)-Cytostatin**

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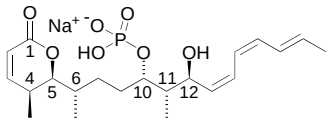
Dedicated to Professor Lutz F. Tietze on the occasion of his 60th birthday

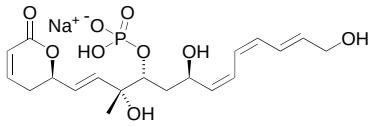
The reversible phosphorylation of proteins is employed by living organisms for the regulation of innumerable cellular processes, and aberrant protein phosphorylation contributes to the development of many human diseases, including cancer and diabetes.[1] Protein kinases (PKs) catalyze protein phosphorylation, whereas protein phosphatases (PPs) are responsible for dephosphorylation. PKs are established targets for drug discovery.[2] However, the development of small-molecule inhibitors of PPs is emerging only very recently[3] as a very rapidly growing area of investigation in clinical biology and medicinal chemistry.[4] Naturally occurring PP inhibitors have been used widely to antagonize PP action in biology experiments.[5] Thus, natural products with PP-inhibitory activity can serve as invaluable starting points in structural space for the development of potent and selective PP inhibitors. This was recently demonstrated by the successful solid-phase synthesis and biological investigation of analogues of the Cdc25 phosphatase inhibitor dysidiolide.[6]

The naturally occurring PP2A inhibitor cytostatin **1** (IC_{50} = 210 nM[7a]), which was isolated from a *Streptomyces* strain by Ishizuka and co-workers,[7b] inhibits the adhesion of B16 melanoma cells to laminin and collagen, displays antimetastatic and cytotoxic activity,[7c] and induces apoptosis of B16 melanoma cells[7d] at submicromolar concentrations. Herein we disclose the synthesis of the 4*S*,5*S*,6*S*,10*S*,11*S*,12*S* isomer of cytostatin.

Ishizuka et al. determined the constitution of cytostatin, but not its relative and absolute configuration.[7e] Therefore, in planning the synthesis, we drew from the structurally related natural products fostriecin **2**[8] and the phoslactomycins **3**.[9] Given that the biosynthesis of these natural products might follow similar pathways and/or use identical starting materials and biocatalysts, the configurations of stereocenters 4, 5, 10, and 12 were chosen by analogy. The configuration of stereocenter 6 was assigned arbitrarily. The unknown absolute configuration of cytostatin prompted us to design the synthesis with a high degree of flexibility to allow rapid and reliable variation of absolute and relative configuration if desired. This was ensured by employing the asymmetric Evans aldol condensation (which gives access to *syn* and *anti* aldol products in both enantiomeric forms[10]), the asymmetric Evans alkylation, and the enantioselective reduction of an acetylenic ketone (for which efficient reagent-controlled processes that give rise to both possible stereoisomers are known).[11]



(4*S*,5*S*,6*S*,10*S*,11*S*,12*S*) –**1**



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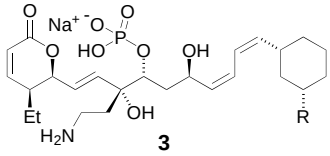

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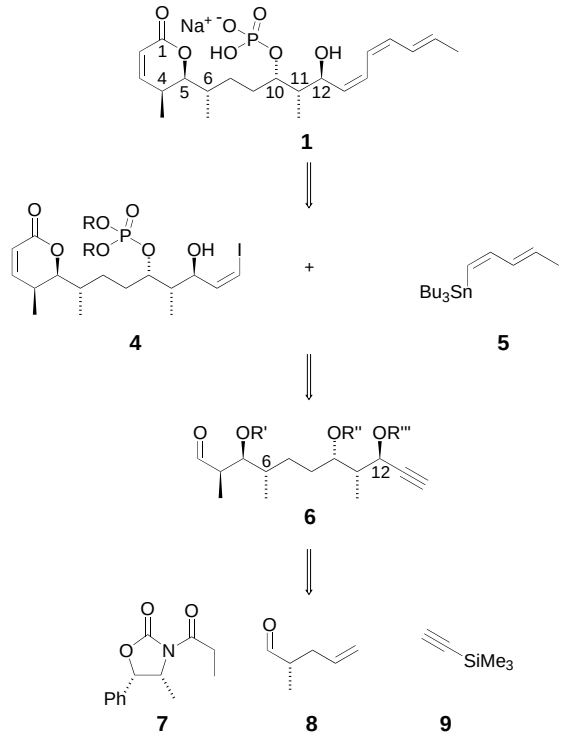
Retrosynthetic analysis of cytostatin (**1**) led to vinyl iodide **4** and dienylstannane **5** (retro Stille coupling). In this strategy, the labile triene unit is generated in a late step of the synthesis, which allows convenient and efficient access to more stable analogues (Scheme 1).[12] The α,β-unsaturated lactone incorporated in **4** was traced back to β-hydroxyaldehyde **6** from which it should be accessible by a Still – Gennari olefination[13] and subsequent lactonization. It was planned to generate both *syn* diols (corresponding to C5/C6 and C10/C11 in the natural product) by means of an asymmetric aldol reaction with *N*-



Scheme 1. Retrosynthetic analysis of (4*S*,5*S*,6*S*,10*S*,11*S*,12*S*)-cytostatin.

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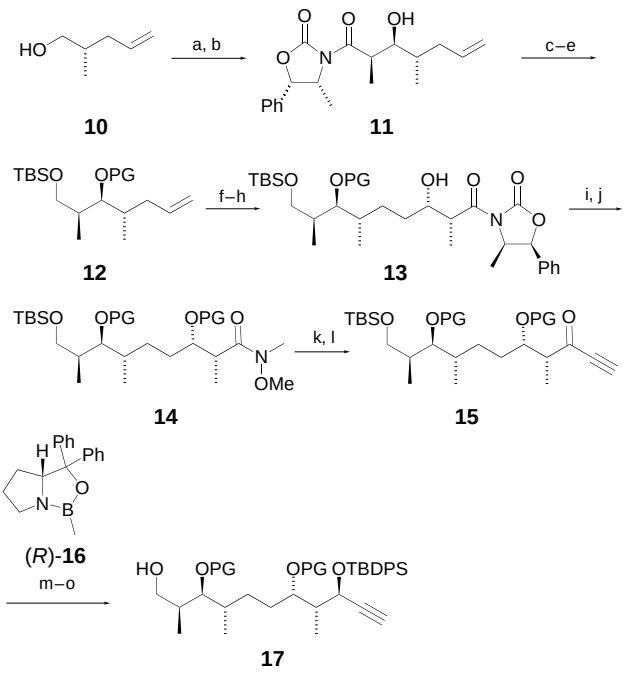
propionyloxazolidinone **7** as nucleophile.[14] The chiral aldehyde **8**, which is accessible by means of an asymmetric alkylation of the same acyloxazolidinone **7**,[15] should serve as electrophile. After conversion of the double bond into an aldehyde group, a second aldol reaction would be carried out. Elaboration of the resulting *N*-acyl group into an alkynyl ketone by using trimethylsilylacetylene (**9**) as nucleophile and subsequent reduction would yield **6**, the precursor to vinyl iodide **4**.

Chiral alcohol **10** was synthesized as described by Evans et al.[15] and subjected to Swern oxidation to yield the corresponding aldehyde. The volatile aldehyde was employed in an asymmetric aldol reaction with the dibutylboron enolate derived from acyloxazolidinone **7** (Scheme 2). Crystalline aldol adduct **11** was obtained in high yield and with very high stereoselectivity. Only traces of undesired diastereomers were detected and were readily separated by crystallization. Protection of the hydroxy group as the methoxymethyl (MOM) ether, reductive removal of the chiral auxiliary with "$LiBH_3OH$"[16] (generated in situ), and masking of the primary alcohol as the *tert*-butyldimethylsilyl (TBS) ether



Scheme 2. Synthesis of intermediate **17**, which incorporates all stereogenic centers. a) $(COCl)_2$, DMSO, NEt_3, CH_2Cl_2, $-78\,^\circ C \rightarrow RT$; b) **7**, Bu_2BOTf, DIPEA, CH_2Cl_2, $-78\,^\circ C \rightarrow RT$, then H_2O_2, pH 7, $0\,^\circ C \rightarrow RT$, 68% over two steps; c) MOMCl, DIPEA, CH_2Cl_2, room temperature, 97%; d) $LiBH_4$, H_2O, Et_2O, $0\,^\circ C \rightarrow RT$, 78%; e) TBSCl, imidazole, DMF, room temperature, 97%; f) 9-BBN, THF, room temperature, then H_2O_2, NaOH, 83%; g) $(COCl)_2$, DMSO, NEt_3, CH_2Cl_2, $-78\,^\circ C \rightarrow RT$; h) **7**, Bu_2BOTf, DIPEA, CH_2Cl_2, $-78\,^\circ C \rightarrow RT$, then H_2O_2, pH 7, $0\,^\circ C \rightarrow RT$; TBSCl, NEt_3, DMAP, CH_2Cl_2, room temperature, 86% over two steps; i) Me_3Al, $(H_2NMeOMe)^+Cl^-$, THF, $-10\,^\circ C \rightarrow 0\,^\circ C$, 84%; j) MOMCl, DIPEA, CH_2Cl_2, room temperature, 92%; k) **9**, BuLi, $-78\,^\circ C$; THF, $-78\,^\circ C \rightarrow -10\,^\circ C$; l) borax, methanol, H_2O, $-10\,^\circ C \rightarrow RT$, 95% over two steps; m) (*R*)-**16**, $BH_3 \cdot Me_2S$, THF, $-30\,^\circ C$, 96%; n) TBDPSCl, imidazole, DMF, room temperature; o) HF/pyridine, THF, room temperature, 40 min, 88% over two steps. DMSO = dimethyl sulfoxide, Tf = trifluoromethanesulfonyl, DIPEA = diisopropylethylamine, DMF = *N*,*N*-dimethylformamide, 9-BBN = borabicyclo[3.3.1]nonane, DMAP = 4-dimethylaminopyridine, PG = protecting group = MOM = methoxymethyl.

yielded olefin **12** with high overall efficiency. Hydroboration of the double bond by using 9-BBN, oxidative workup, and subsequent Swern oxidation of the resulting primary alcohol delivered the desired aldehyde intermediate. As expected, a second asymmetric *syn* aldol reaction with the boron enolate formed from acyloxazolidinone **7** gave β-hydroxyamide **13** with high overall yield and high stereoselectivity (only one diastereomer could be detected by ^1H NMR spectroscopic analysis). The absolute configuration of aldol adduct **13** was assigned based on the assumption that the aldol reaction proceeds in a reagent-controlled fashion with *syn* selectivity, as described for various other cases.[14] In the course of the aldol reaction the TBS group was cleaved, but the primary alcohol was readily resilylated.

To generate the last stereocenter, an alkynyl ketone was required. This ketone was obtained by conversion of acyloxazolidinone **13** into the fully protected Weinreb amide **14**, which was treated with lithium trimethylsilylacetylide. After removal of the TMS group by treatment with catalytic amounts of borax in methanol, alkynyl ketone **15** was obtained in high overall yield. The stereoselective reduction of the keto group was attempted with different reagents, including Alpine borane[11a] and borane in the presence of a phenylglycine-derived oxazaborolidine.[11e] The highest yields were obtained with borane in the presence of oxazaborolidine (*R*)-**16** (2 equiv).[11c,d] The desired secondary alcohol was formed in nearly quantitative yield, and a second diastereomer could not be detected by means of ^1H NMR spectroscopic analysis. The protection of the propargylic alcohol required a blocking group that is stable under the conditions of the planned Still–Gennari olefination and the subsequent acid-mediated lactonization. Furthermore, it had to survive the removal of the TBS ether. After substantial experimentation, it was found that the *tert*-butyldiphenylsilyl (TBDPS) group fulfils these criteria, and the protecting group pattern was arranged accordingly. Primary alcohol **17** was selectively formed by removal of the TBS group with HF/pyridine.

Alcohol **17** was oxidized to the corresponding aldehyde with DMP,[17] and the carbonyl compound was converted into *Z*-olefin **18** under Still–Gennari conditions (Scheme 3). Subsequently, the MOM protecting groups were removed by treatment with CBr_4 in 2-propanol. Under these conditions (HBr is probably formed in situ),[18] lactone **19** was formed simultaneously.

To develop a reaction sequence that would give flexible access to different phosphorylated analogues of the natural product, it was planned to introduce the phosphate group at this stage of the synthesis. The correct choice of phosphate-protecting group proved to be crucial. Initial experiments with the methoxybenzyl group, which was successfully applied in the synthesis of fostriecin,[8b] were not successful. Similarly, the cyanoethyl ester was not suitable because only one cyanoethyl group could be removed in the final deprotection step without destroying the entire molecule. Finally, the use of the fluorenylmethyl ester[19] allowed the successful cleavage of the protecting group.

The phosphate was formed by phosphitylation and subsequent oxidation. After selective cleavage of the TBDPS ether, the desired alkyne **20** was obtained in high yield. Conversion

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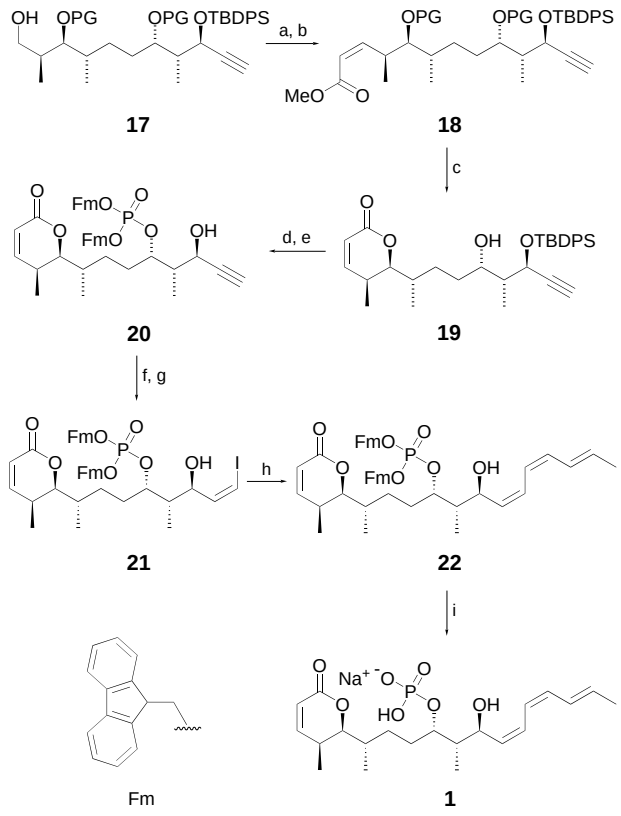

Scheme 3. Synthesis of **1**. a) DMP, CH_2Cl_2, $NaHCO_3$, 93%; b) $(CF_3CH_2O)_2P(O)CH_2CO_2Me$, [18]crown-6, KHMDS, THF, −78°C, 92%; c) CBr_4, 2-propanol, 82°C, 83%; d) $(FmO)_2PNiPr_2$, tetrazole, CH_2Cl_2/CH_3CN, 0°C→RT, then I_2, pyridine, H_2O, THF; 95%; e) HF/pyridine, THF, room temperature, 28 h, 82%; f) NIS, $AgNO_3$, DMF, room temperature, quant.; g) $K^+{}^-OOC{-}N{=}N{-}COO^-K^+$, HOAc, 2-propanol/dioxane, 63%; h) **5**, $[PdCl_2(CH_3CN)_2]$ (cat.), DMF/THF, 62%; i) NEt_3:CH_3CN 1:5, room temperature, then Na^+-Dowex resin, 85%. DMP = Dess—Martin periodinane, HMDS = hexamethyldisilazane, NIS *N*-iodosuccinimide; PG = MOM.

of **20** into vinyl iodide (*Z*)-**21** proved to be problematic. After substantial experimentation, **21** was obtained by conversion of the alkyne into the corresponding alkynyl iodide in the presence of silver nitrate followed by reduction of the triple bond with diimide.[20] All attempts to reduce the triple bond with different reagents gave undesired side reactions, in particular, the reduction of the α,β-unsaturated lactone and ring opening. However, when diimide was generated in situ in 2-propanol, these side reactions were largely suppressed and vinyl iodide (*Z*)-**21** was obtained in 63% yield (Scheme 3). This advanced intermediate was then subjected to Stille coupling with *Z*,*E*-dienylstannane **5** with $[PdCl_2(CH_3CN)_2]$ as catalyst and without additional phosphane.[12] Under these conditions, sensitive (*Z*,*Z*,*E*)-**22** was formed in a satisfactory yield (62%). Dienylstannane **5** was synthesized from crotonaldehyde: the latter was converted into the dibromoolefin with CBr_4 and PPh_3 treated with *n*BuLi followed by Bu_3SnCl to give an enyne stannane, and subsequently subjected to hydrozirconation (not shown). Finally, the phosphate was unmasked. Upon treatment of phosphoric acid triester **22** with excess triethylamine, both fluorenylmethyl groups were cleaved in a β-elimination reaction, and the 4*S*,5*S*,6*S*,10*S*,11*S*,12*S* isomer of cytostatin **1** was isolated in 85% yield.

Synthetic **1** displays a specific rotation of $[\alpha]_D^{20} = +50$ (c = 0.114 in $[D_4]$MeOH). Unfortunately, this value can not be employed to ascertain the absolute configuration of the natural product because Ishizuka and co-workers did not report the specific rotation of the isolated sample.[7e]

The phosphatase-inhibiting activity of synthetic **1** was investigated with *p*-nitrophenyl phosphate as substrate.[7d] Isomer **1** inhibited PP2A (IC_{50} = 33 nM). This value is one order of magnitude lower than that of the natural product. This finding suggests that our assumption about the absolute configuration of the natural product may be correct, at least for most of the stereogenic centers.

In conclusion we have developed an asymmetric synthesis of the 4*S*,5*S*,6*S*,10*S*,11*S*,12*S* of the PP2A inhibitor cytostatin. The synthesis is stereochemically flexible and employs only reagent-controlled transformations, thus allowing access to each desired isomer of cytostatin in a reliable and efficient manner. This successful synthesis now opens up new opportunities for the development of new tools for biological studies and of new cancer drugs.

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Modular Building Blocks for Amino Acid Recognition in Peptides

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The selective recognition of short peptide sequences is a key concept in many natural regulatory processes. Thus, the natural antibiotic vancomycin binds tightly to the C-terminal D-Ala-D-Ala fragment in peptides, which is used for the construction for bacterial cell walls.[1] Numerous cell – cell recognition events rely on the recognition of the specific Arg-Gly-Asp sequence.[2]

The first attempts to mimic Nature's potent peptide hosts with artificial structures, confined the conformational freedom of a host molecule by creating a cleft (Rebek and co-workers), a macrocycle (Still and co-workers), or even a cavity (Still and co-workers); the specific binding sites were often taken from (non)natural amino acids.[3] Several groups have created receptor molecules for important secondary structures found in peptides and proteins. Thus, α-helical[4] and β-sheet[5] portions of polypeptides can be recognized by synthetic ligands with a complementary hydrogen-bond-donor and acceptor pattern. In recent years, considerable progress has been achieved with a combinatorial approach.[6]

Various site-specific proteases, such as thrombin, trypsin, and many others provide a shallow groove for the efficient recognition of the backbone of the peptide to be cleaved, in its extended conformation. This array is combined with a specific binding pocket for the side chains of the target amino acid, and thus defines the cleavage site. We asked ourselves if such a rational design could also be used for artificial peptide recognition. Is it possible to create a set of modules, each of which recognizes a certain amino acid in a peptidic environment or, even better, a short peptide sequence?

We recently reported the stabilization of small peptides in their β-sheet conformation by external ligands.[7] N-acylated 3-aminopyrazoles were shown to interact with every hydrogen-bond-donor (D) and acceptor (A) available at the top face of a dipeptide. Two consecutive amino acids can be clamped together with one of these heterocycles, which causes the formation of three almost-linear hydrogen bonds in a DAD sequence (Figure 1). This recognition process simultaneously fixes the peptide in the thermodynamically favorable β-sheet conformation. Thus, small soluble models of this important secondary structure have been prepared.

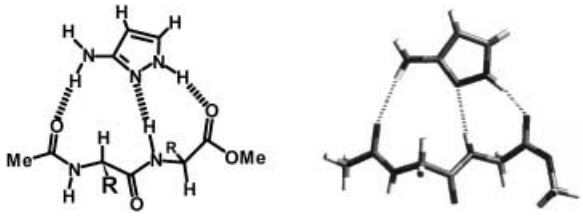

Figure 1. β-Sheet stabilization with aminopyrazoles—three-point binding of the top face of a dipeptide by the DAD binding sites of the aminopyrazole; left: Lewis structure; right: results of molecular-mechanics calculation (Cerius², Molecular Simulations, Dreiding 2.21).

In a β sheet, the N–H and C=O bonds of the backbone point up and down, whereas the amino acid residues (R) are extended horizontally away from the peptide in a well-defined geometry. This preorganized arrangement was our starting point for a new modular concept of peptide receptors: If it were possible to attach to the aminopyrazole a rigid U-shaped substituent with a properly placed binding site at its tip, this could reach down to the side chain of the respective amino acid and lead to an additional, specific noncovalent interaction. With an interchangeable tip, various binding sites could be introduced into the basic peptide receptor, which would lead to a modular set of building blocks, selective for the typical classes of amino acids. To our knowledge, no example of such a rationally designed set of peptide receptors exists to date.

For the U-shaped spacer, we chose the framework of Kemp's triacid, because it is exceptionally rigid and its chemistry is well developed.[8] Two acid groups were designated to carry the monoacylated 3,5-diaminopyrazole unit by way of an imide functionality, while the third could be coupled to an aniline derivative with the correct binding site in the *m*-position. Scheme 1 shows the general structure of the building blocks: both the imide and the neighboring aminopyrazole unit are in the same plane, locked together by an intramolecular hydrogen bond.

During the synthesis, care must be taken to chemoselectively address the three amino functionalities of 3,5-diaminopyrazole, without an extensive use of protecting groups. We begin with imide formation between mono(trifluoroacetylated) diaminopyrazole **1** and Kemp's acid anhydride (Scheme 2). The imide intermediate **2** must be *N*-Boc pro-

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