Natural Products

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Confirmation of the Stereostructure of (+)-Cytostatin by Fluorous Mixture Synthesis of Four Candidate Stereoisomers**

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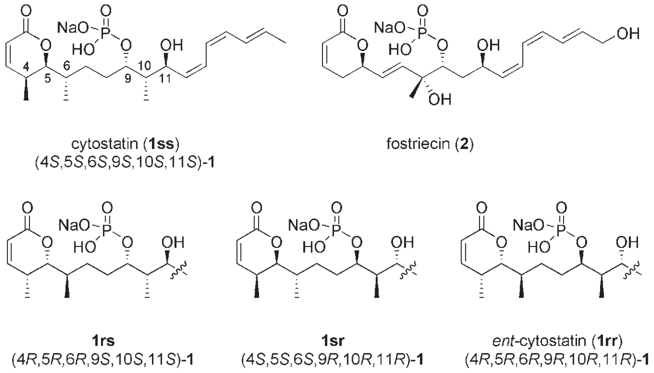
Dedicated to Professor Yoshito Kishi on the occasion of his 70th birthday

Cytostatin (**1**) is a potent and selective inhibitor of protein phosphatase 2A that was isolated from the cultured broth of *Streptomyces sp.* by Ishizuka and co-workers (Scheme 1).[1] It inhibits lung metastasis of melanoma cells in mice and displays potent cytotoxic activity toward leukemia cell lines (inhibitory concentration; $IC_{50} = 42–65$ nM). Ishizuka and co-workers assigned the two-dimensional structure (constitution) of cytostatin (**1**) by analysis of 1D and 2D NMR spectra.



Scheme 1. Candidate structures of cytostatin (**1**) and structure of fostriecin (**2**).

The relative configurations of C4–C6 and C9–C11 of cytostatin were assigned as *syn* at C4/C5 and C9/C10 and as *anti* at C5/C6 and C10/C11 by the research groups of Waldmann and Boger by comparison of key features of the ^{1}H NMR spectra of cytostatin with those of relevant models.[2,3] This approach lowered the number of structure candidates down to four, namely: **1ss**, **1rs**, **1sr**, and **1rr**.[4] Based on an analogy to fostriecin **2**[5] and related compounds (which share three stereocenters with **1**), the research groups of Waldmann and Boger independently synthesized (4*S*,5*S*,6*S*,9*S*,10*S*,11*S*)-**1** (hereafter called **1ss**), and concluded, by comparison of spectroscopic, physical, and biological properties, that this was the natural product.

We have recently commented on the logic of proof and disproof of stereostructures by comparison of synthetic and natural samples.[6] If two or more stereoisomers of a natural product can reasonably be expected to have substantially identical spectra, then a rigorous assignment of the structure should include proof that the candidate isomer matches the natural product and, more importantly, proof that the other isomers do not. Acetogenins, such as the murisolins have very remote groups of stereocenters (10 or more methylene groups apart), so it can be expected that diastereomers might exhibit substantially identical spectra.[6] In contrast, the two groups of stereocenters in cytostatin (**1**) are only insulated by an ethylene group. Will enantiomers **1ss** and **1rr** have different spectra from their diastereomers **1rs** and **1sr**? To answer this question, and thereby to prove which three isomers were not cytostatin, we undertook the fluorous mixture synthesis[7,8] of all four isomers of **1**.

The fluorous mixture synthesis (FMS) strategy to make the four isomers of cytostatin is briefly outlined in Scheme 2. Late introduction of the triene is dictated by its chemical instability, so we followed the strategy of Bialy and Waldmann[2d] by planning to prepare four individual vinyl iodides **3** by coupling with alkenyl stannane **4**.

These four isomers are made over several steps from a single four-compound mixture of fluorous-tagged quasiisomers M-**5**[9] ("quasi" because the compounds have different fluorous tags and are not true isomers[10]). In turn, M-**5** is made by coupling between fluorous-tagged quasiracemic aldehydes M-**6** with quasiracemic keto phosphonates M-**7** by a Horner–Wadsworth–Emmons (HWE) reaction.

From the FMS standpoint, the configurations of the stereocenters at C4–C6 (*SSS* or *RRR*) and C9–C11 (*SSS* or *RRR*) are encoded by differing silyl groups. Demixing[11] a late-stage mixture by fluorous HPLC to provide its individual components will be possible because each quasiisomer has a different number of fluorine atoms. The fluorine atoms are

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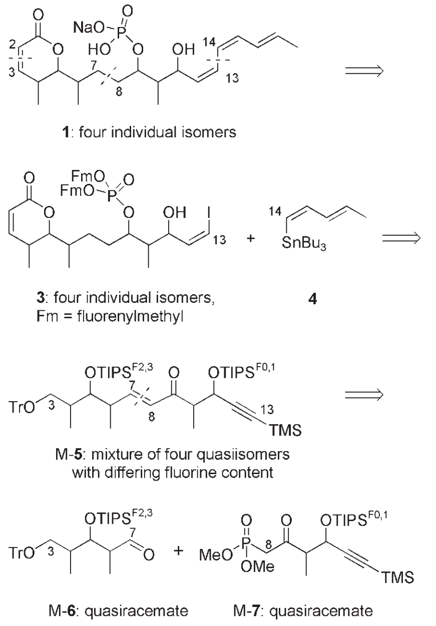
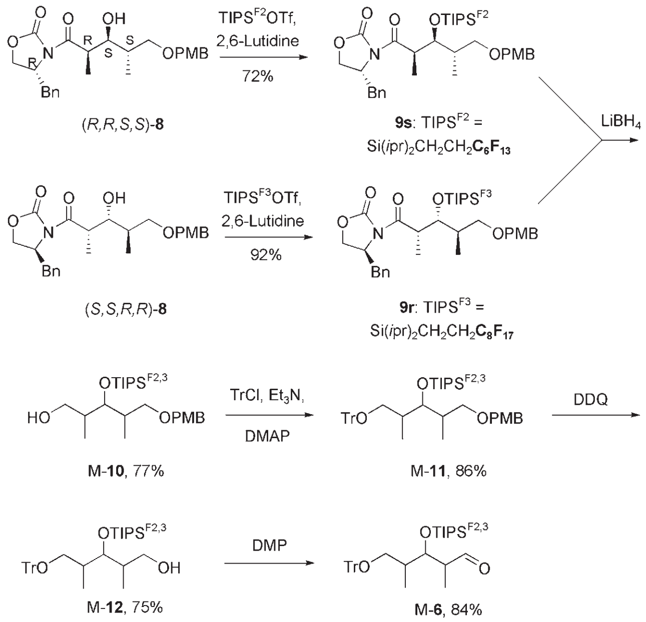
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.



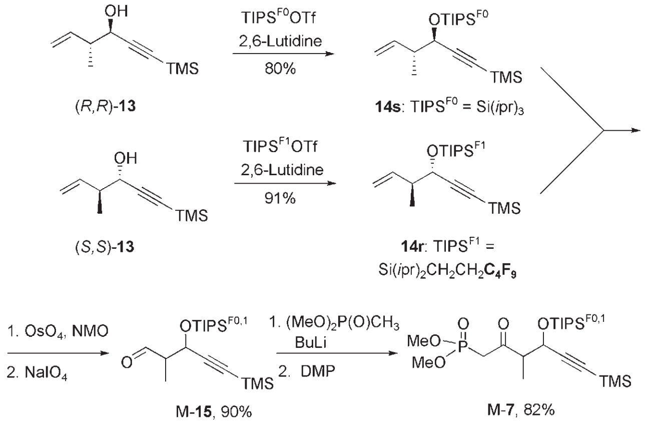
TIPS^{F0-3} groups are silyl groups with differing numbers of fluorine atoms. These groups encode the configurations of the fragments:

TIPS^{F0} = Si(*i*pr)$_3$ (zero fluorine); TIPS^{F1} = Si(*i*pr)$_2$CH$_2$CH$_2$C$_4$F$_9$
TIPS^{F2} = Si(*i*pr)$_2$CH$_2$CH$_2$C$_6$F$_{13}$; TIPS^{F3} = Si(*i*pr)$_2$CH$_2$CH$_2$C$_8$F$_{17}$

Scheme 2. Fluorous mixture synthesis plan.



Scheme 3. Synthesis of quasiracemate M-**6**. Bn = benzyl, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DMAP = 4-dimethylaminopyridine, DMP = Dess–Martin periodinane, PMB = *para*-methoxybenzyl, Tf = triflate, TIPS = triisopropylsilyl, Tr = trityl = triphenylmethyl.



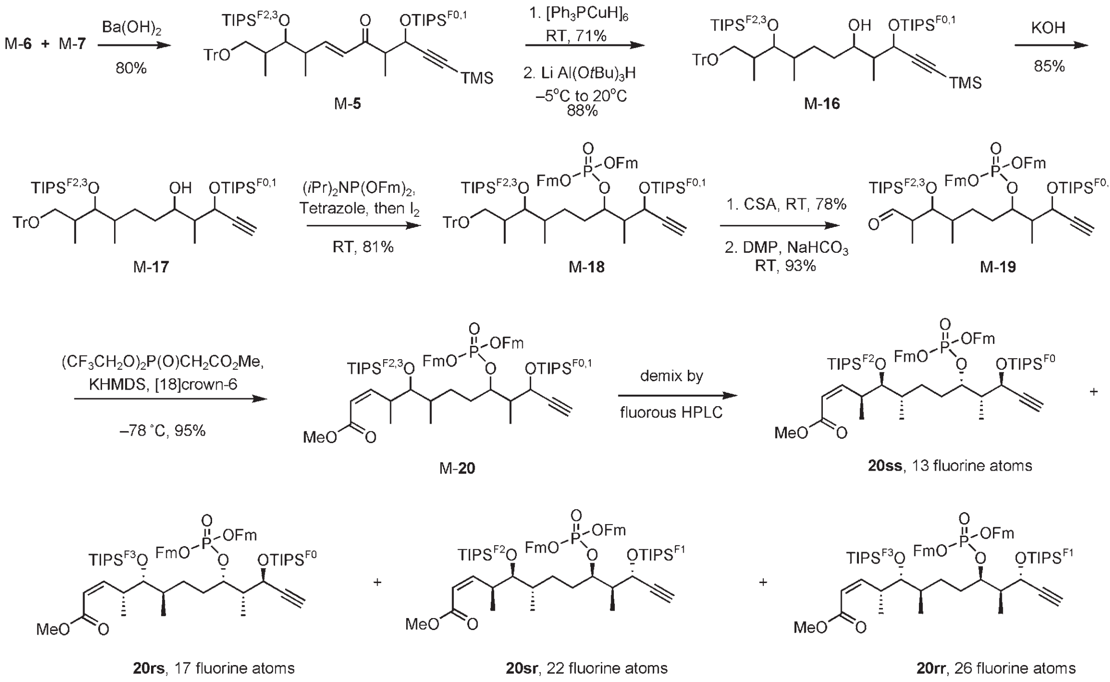
Scheme 4. Synthesis of quasiracemate M-**7**. NMO = 4-methylmorpholine *N*-oxide, TMS = trimethylsilyl.

distributed over two silyl groups, so their approximate additivity upon HPLC demixing is important.[8f,12]

The syntheses of quasiracemates M-**6** and M-**7** are summarized in Schemes 3 and 4, respectively. To make the left-side quasiracemate M-**6** (Scheme 3), readily available Evans aldol[13] adduct (*R*,*R*,*S*,*S*)-**8** and its enantiomer were tagged with different fluorous silyl groups to encode the configurations, and the tagged products **9s** and **9r** were then mixed. Reductive removal of the Evans auxiliary with LiBH$_4$ afforded the primary alcohol M-**10** in 77 % yield, which was subsequently protected with TrCl to give M-**11** in 86 % yield. Selective deprotection of the PMB group with DDQ provided M-**12** in 75 % yield. Subsequent treatment with DMP gave the fluorous quasiracemic mixture of aldehyde M-**6** in 84 % yield.

To make the right-side quasiracemate M-**7** (Scheme 4) isomerically pure enantiomers (*R*,*R*)-**13** and (*S*,*S*)-**13** (prepared by Brown–Ramachandran allylboration)[14] were tagged to encode configuration information, and the tagged quasienantiomers **14s** and **14r** were then mixed. The mixture of M-**14** was treated with OsO$_4$ and NMO in *t*BuOH/H$_2$O to provide a mixture of diols, which was directly treated with NaIO$_4$ to afford M-**15** in 90 % yield over two steps. Treatment of aldehyde M-**15** with the lithium anion of (MeO)$_2$P(O)CH$_3$, followed by oxidation with DMP, afforded the fluorous quasiracemic mixture of keto phosphonate M-**7** in 82 % yield over two steps.

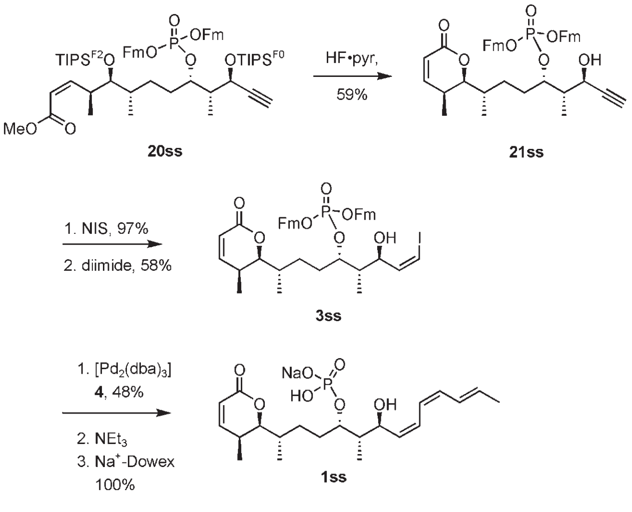
The fragment coupling and subsequent seven-step synthesis were carried out with mixtures of four quasiisomers (Scheme 5). A HWE reaction between M-**6** and M-**7** provided the enone M-**5** in 80 % yield, then 1,4-reduction using Stryker reagent[15] gave a saturated ketone. Reduction of this ketone with LiAl(*t*BuO)$_3$H gave M-**16** with a *syn* relationship between C9 and C10, wherein the configuration of the C9 stereocenters was controlled by the C10 stereocenters (substrate control). Removal of the terminal TMS group from the alkyne with KOH gave M-**17**, and installation of Fm-protected phosphonate with a phosphoramidite (*i*Pr)$_2$NP-(OFm)$_2$ provided M-**18**. Selective cleavage of the trityl group with CSA followed by oxidation using DMP afforded M-**19**. The mixture of four aldehydes M-**19** was subjected to Still–Gennari olefination[16] to give α,β-unsaturated esters M-**20** in 95 % yield. Just prior to removal of the fluorous tags, the mixture M-**20** was demixed into four individual quasiisomers



Scheme 5. Fluorous mixture synthesis and demixing. CSA = camphorsulfonic acid, Fm = fluorenylmethyl, KHMDS = potassium 1,1,1,3,3,3-hexamethyldisilazane.

(**20ss**, **20sr**, **20rs**, **20rr**) by preparative fluorous HPLC. As expected, the quasiisomers were well separated and eluted in order of increasing fluorine content.

The remaining steps were carried out on each isomer individually; Scheme 6 illustrates these steps for the preparation of **1ss**. Two silyl groups in **20ss** were removed with HF·pyr to provide lactone **21ss** in 59% yield. As prescribed by Bialy and Waldmann,[2d] iodination of the triple bond with NIS in the presence of a catalytic amount of AgNO$_3$ afforded an iodoalkyne, which was reduced to the *Z*-iodoalkene **3ss** with diimide. The Stille coupling was performed with the stannane **4** and [Pd$_2$(dba)$_3$] to provide the (12*Z*,14*Z*,16*E*)-triene, which was carefully purified by preparative HPLC. Cleavage of the fluorenylmethyl group under basic conditions followed by ion-exchange using Dowex provided cytostatin stereoisomer **1ss**. Likewise, the other three candidate isomers (**1rs**, **1sr**, and **1rr**; see Scheme 1) were made by the same sequence of reactions starting from the appropriate demixed product **20**.



Scheme 6. Final steps on individual isomers illustrated in the "**ss**" series. NIS = *N*-iodosuccinimide, pyr = pyridine.

We then compared the four synthetic samples of **1** with each other and with a natural sample to prove which three samples were not the natural products. The measurement of optical rotations provided what proved to be misleading information. The optical rotation of **1ss** ($[\alpha]_D^{20} = +45$; $c = 0.08$, MeOH) agreed well with data from the research groups of Waldmann and Boger,[2] while the rotation of its diastereomer **1sr** was $[\alpha]_D^{25} = +29$ ($c = 0.09$, MeOH). The rotation value of **1sr** is in better agreement with that reported for an aged[17] natural product sample (+20; $c = 0.27$, MeOH).[2d] These values could be taken as evidence that cytostatin is **1sr**. However, subsequent experiments disproved this assignment.

Comparisons of high-field ^{1}H NMR spectra (500 MHz) of the five samples gave unexpected results. The ^{1}H NMR spectra were very similar, but none of the five spectra exactly matched another one (see the Supporting Information for copies of the spectra). Since two pairs of synthetic samples are enantiomers and one of the four synthetic samples has to be the natural product, the tiny differences in the chemical shift that were observed between identical compounds or enantiomers must arise as a consequence of concentration or pH differences between the samples. Despite this complication, we still identified two reliable differences in the spectra of the two diastereomers. Surprisingly, the two protons that have clearly different chemical shifts are not attached to any of the stereocenters, but are instead the diastereotopic protons at C8. The H8 and H8′ resonances of **1ss** matched those of the natural sample, while the resonances of **1sr** were different.

Finally, simple TLC experiments on standard silica gel conclusively proved that cytostatin was not **1sr**. Cospotting of the natural sample with synthetic samples showed that it was inseparable from **1ss**. In contrast, **1sr** was slightly faster moving on the plate and separated from both the natural sample of cytostatin and the sample of **1ss**. These experiments prove that **1sr** is not cytostatin, and accordingly its enantiomer **1rs** cannot be cytostatin either. The large differences in the size and magnitude of the optical rotation prove that **1rr** is not cytostatin. No information was obtained to prove that **1ss** is not cytostatin and, accordingly, we concur with the research groups of Waldmann and Boger that it is.

In summary, we have synthesized four closely related candidate isomers for cytostatin (**1**) by using the technique of fluorous mixture synthesis. With the isomers in hand, we proved by a combination of NMR, TLC, and optical rotation experiments that three of the isomers were not cytostatin, and accordingly we confirmed structure **1ss** for this important natural product. Rigorous proof of the stereostructure of natural products by disproof of all the candidate stereoisomers but one is today not common because traditional "one at a time" approaches to stereoisomer synthesis are too long and tedious. The emerging techniques of fluorous mixture synthesis offer a respite from this tedium by providing additional stereoisomers without a proportional increase in effort.

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[4] In the candidate isomers for **1**, the two groups of stereocenters either have all *S* or all *R* absolute configurations. The pair of small letters "s/r" after the numbers abbreviate the configurations of the three centers at C4–C6 (left letter) and C9–C11 (right letter). As a result of Cahn–Ingold–Prelog priority changes, synthetic intermediates may have different configurations from the natural product.

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[9] The prefix "M" stands for a mixture of two or four quasiisomers. Numbers without prefixes are individual compounds.

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[17] Waldman and Boger have both commented that the rotation of the natural sample may not be accurate because of its modest level of purity; see Ref. [2].