Antitumor Agents

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Total Synthesis, Configuration, and Biological Evaluation of Anguinomycin C**

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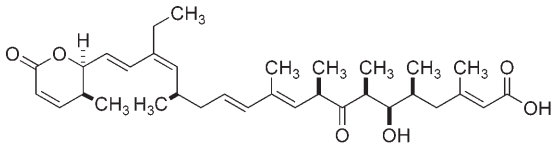
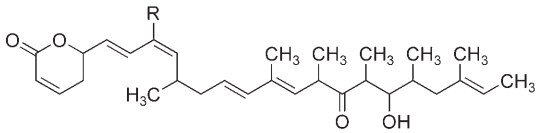
Dedicated to Professor Dieter Seebach on the occasion of his 70th birthday

Natural products provide interesting lead structures for cancer research and thus enable promising chemical approaches.[1] The compound class of the leptomycins is characterized by an extremely potent antitumor activity on cancer cell lines, which has drawn the attention of many synthetic chemists.[2] Prototypic examples such as leptomycin B and callystatin were, however, found to be too toxic to normal cells, leading to their failure in the clinical evaluation.[3] In contrast to these results, two related compounds, anguinomycin C and D, were reported to display selectivity for pRB tumor suppressor inactivated, immortalized cells.[4] These anguinomycins thus cause apoptosis in such tumor cell lines in picomolar concentrations, and, remarkably, induce only growth arrest in normal cells. This astonishing selectivity for tumor cells must reside in the (minimal) structural differences between anguinomycin C and leptomycin B; however, the exact reason is unknown. In addition, the relative and absolute configuration of the six stereogenic centers of anguinomycin could not be assigned. In this communication, we report the total synthesis of anguinomycin C, the determination of the absolute configuration of the six stereogenic centers, and first experiments on its biological mode of action.

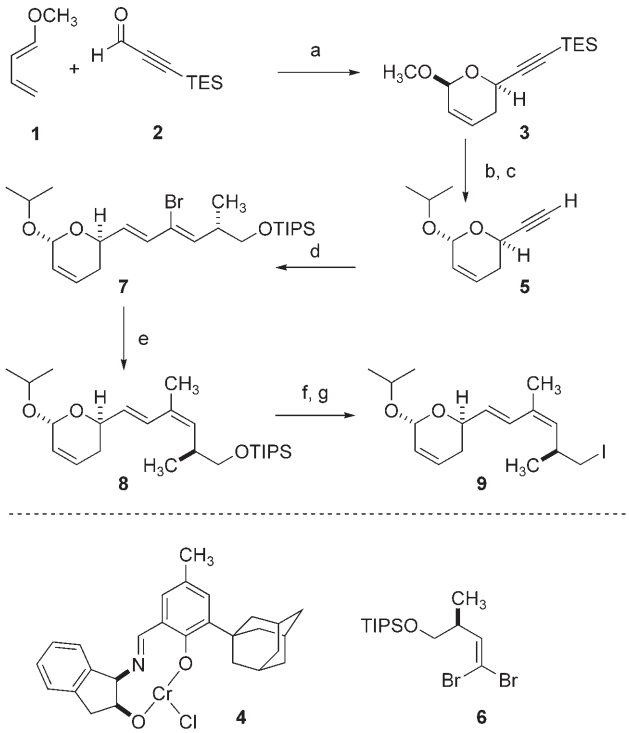


Leptomycin B



Anguinomycin C R = CH_3
Anguinomycin D R = CH_2CH_3

The total synthesis of anguinomycin C began with the preparation of the dihydropyran fragment **3** (Scheme 1). We chose a catalytic, asymmetric hetero-Diels–Alder reaction as a direct approach to this heterocycle. Treatment of commercially available methoxybutadiene (**1**) with the protected propargylic aldehyde **2**[5] in the presence of the Cr^{III} catalyst **4** (developed by Jacobsen and co-workers)[6] resulted in product



Scheme 1. a) Cr catalyst **4** (2.3 mol%), 4-Å molecular sieves, 86%, 96% *ee*; b) *para*-toluenesulfonic acid, iPrOH, 86%; c) TBAF, THF, 95%; d) 1. $[Cp_2ZrHCl]$, THF, 2. $ZnCl_2$, THF, 3. $[Pd(PPh_3)_4]$ (5 mol%), DIBAH (10 mol%), **6**, 81%, d.r. > 97:3; e) $[Pd(PPh_3)_4]$, $(CH_3)_2Zn$, THF, 68%, d.r. > 97:3; f) TBAF, THF, 99%; g) PPh_3, imidazole, I_2, toluene/ether, 75%. TBAF: tetrabutylammonium fluoride, TES: triethylsilyl, THF: tetrahydrofuran, DIBAH: diisobutylaluminum hydride, TIPS: triisopropylsilyl.

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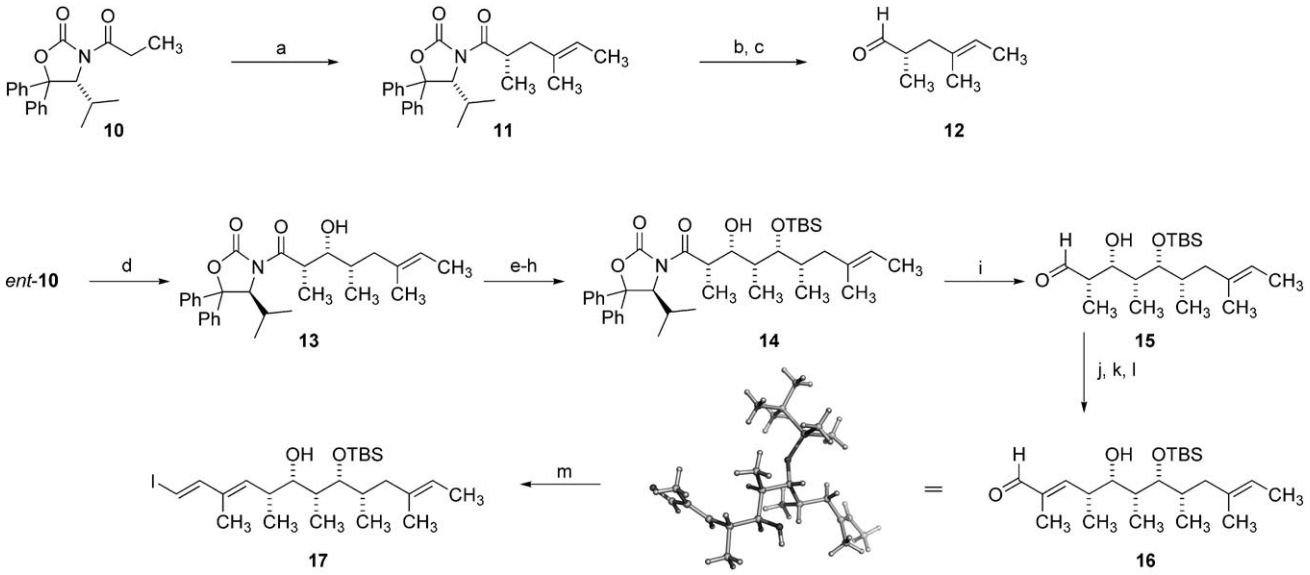
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

3 in high yield (86%) and enantioselectivity (96% *ee*). The observed diastereoselectivity of only ca. 5:1 was a consequence of epimerization under the reaction conditions. This low selectivity was not a problem, as the acetal **3** (as a mixture of diastereoisomers) was transformed in the presence of acid in iPrOH[2o] to the thermodynamically more stable, configurationally homogenous product **5**[2k] (after deprotection). This alkyne was hydrozirconated by using Schwartz's reagent and then transmetalated in situ to give the vinyl zinc species. Subsequent Negishi cross-coupling with the readily available dibromide **6**[7] gave the trisubstituted vinyl bromide **7** in 81% yield. Interestingly, the addition of small amounts of DIBAH consistently resulted in higher yields. However, compound **7** displayed the wrong configuration of the trisubstituted olefin; as a result a stereoinversion was required in this synthesis. Negishi and co-workers recently reported that similarly substituted haloalkenes undergo cross-coupling under inversion (and not under retention).[8] Therefore, vinyl bromide **7** was allowed to react under Pd catalysis with dimethylzinc, and we observed in the NMR spectrum a clean inversion at the double bond to the *cis* compound **8** (68% yield, d.r. > 97:3). This is even more remarkable, as the reversal of reagents, that is, first reaction of dibromide **6** with dimethylzinc followed by the dihydropyran derivative starting from **5**, led to low yields and a mixture of isomers. The mechanism of this stereoinversion in the Negishi cross-coupling reaction remains unknown; in the literature σ-bound Pd–allenyl species were postulated as intermediates.[8] In the context of our research, the clean inversion of **7** to **8** was of great use. Removal of the terminal protecting group and transformation of the hydroxy group to the iodide **9** was carried out under standard conditions.

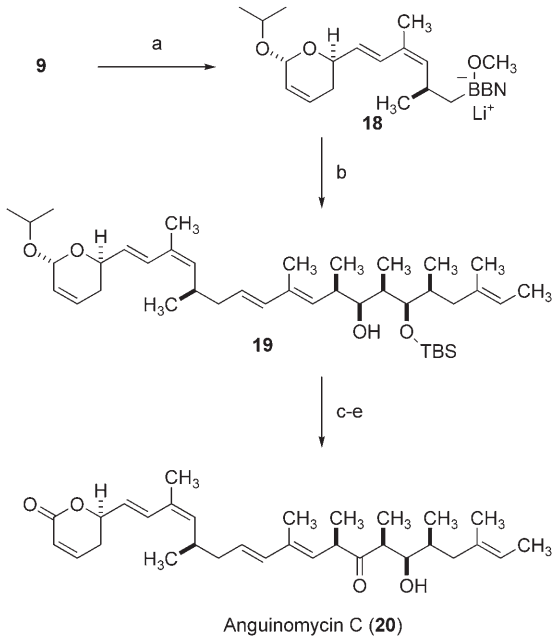
We chose an Evans aldol strategy for the synthesis of the second fragment,[9] but opted for the DIOZ auxiliary (4-isopropyl-5,5-diphenyloxazolidin-2-one), which was developed by Seebach and Hintermann.[10] This chiral oxazolidinone impressively demonstrated its usefulness in the synthesis of discodermolide by chemists at Novartis.[11] The benefits, including higher selectivity and crystallinity of the intermediates (with the drawback of increased molecular weight), were also of great use in the reactions described in this communication. An enantioselective alkylation of the Li enolate of **10**[10] with tigloyl bromide[12] gave **11** in high yield (92%) and excellent selectivity (d.r. > 97:3, Scheme 2). Cleavage (and recycling) of the chiral auxiliary by $LiAlH_4$ followed by a Swern oxidation gave aldehyde **12**[13] in 98% yield over two steps. This chiral aldehyde was then transformed in a boron-mediated aldol reaction using *ent*-**10** to give the *syn*-aldol **13**. The selectivity of this reaction (87:13 for the desired isomer, separable by flash chromatography) is less than perfect but comparable to reactions of similar substrates in the literature.[2c] Transformation of compound **13** to the Weinreb amide was easily accomplished using $Al(CH_3)_3$, as were subsequent TBS protection and reduction by DIBAH to the aldehyde (85% over three steps). Another boron-mediated aldol reaction gave hydroxy amide **14** featuring an all-*syn* configuration with excellent stereoselectivity (d.r. > 97:3). The direct reduction of the auxiliary-bound imide **14** to the aldehyde **15** was possible by LAH in toluene; this surprising reaction exemplifies the strengths of the DIOZ auxiliary. Wittig reaction gave the α,β-unsaturated ester, which was transformed through reduction and subsequent oxidation to the α,β-unsaturated aldehyde **16**. X-ray crystallographic analysis of **16** (m.p. 75–77 °C) allowed for the unambiguous determination of the configuration of the newly formed stereogenic centers. The transformation to the vinyl iodide **17** following Takai[14] was possible in excellent yield and stereoselectivity.

Having both fragments at hand, we chose to merge them using a procedure developed by Marshall et al.[2g,s]



Scheme 2. a) LDA, THF then tigloyl bromide, 92%, d.r. > 97:3; b) LAH, ether, quant. c) Swern oxidation, 99%; d) Bu_2BOTf, Et_3N, CH_2Cl_2, then **12**, 77%, d.r. 87:13; e) $CH_3ONHCH_3{\cdot}HCl$, $Al(CH_3)_3$, CH_2Cl_2, 86%; f) TBSOTf, 2,6-lutidine, 99%; g) DIBAH, quant.; h) *ent*-**10**, Bu_2BOTf, Et_3N, CH_2Cl_2, then aldehyde, 61%, d.r. > 97:3; i) $LiAlH_4$, toluene, 83%; j) (carbethoxyethylidene)triphenylphosphorane, toluene, 99%, k) DIBAH, THF, 93%; l) MnO_2, CH_2Cl_2 86%; m) $CrCl_2$, CHI_3, THF, quant., d.r. > 97:3.

(Scheme 3). Primary alkyl iodide **9** was reacted with 9-methoxy-BBN and *t*BuLi and then treated with iodide **17** using [Pd(dppf)Cl_2] as the catalyst (5 mol%) in a DMF/water mixture in the presence of base. The reaction proceeded smoothly in spite of the complexity of the substrates,[15] and



Scheme 3. a) 9-Methoxy-BBN, *t*BuLi, ether, THF then b) **17**, [Pd(dppf)Cl_2] (5 mol%), $AsPh_3$ (15 mol%), Cs_2CO_3, DMF/H_2O, 80%; c) pyridinium *para*-toluenesulfonic acid, acetone/H_2O, 95%; d) 1. DMP, CH_2Cl_2, 2. MnO_2, CH_2Cl_2, 47%; e) HF·pyridine, pyridine, THF, 86%. BBN: Borabicyclononane, dppf: $Ph_2PC_5H_4FeC_5H_4PPh_2$, DMP: Dess–Martin periodinane.

the product **19** featuring the complete carbon skeleton of anguinomycin C was isolated in 80% yield. The synthesis was then completed first by acid-catalyzed cleavage of the acetal (PPTS, acetone/water, 95%) and then by a two-step oxidation sequence (DMP, then MnO_2, 47% over two steps). The last protecting group was removed using HF in buffered pyridine, and synthetic anguinomycin C (**20**) was obtained after purification by semipreparative HPLC (86% yield). The spectroscopic data of synthetic anguinomycin C (IR, MS, ^1H and ^{13}C NMR, HSQC spectrum) are identical to the published values of the natural product,[16] and the optical rotation ($[\alpha]_D = -101$ ($c = 6.4 \times 10^{-5}$, CH_3OH)) matches its literature value ($[\alpha]_D = -128$ ($c = 0.5$, CH_3OH)). These spectroscopic data establish the absolute configuration of anguinomycin C (**20**) as shown in Scheme 3 as (5*R*,10*R*,16*R*,18*S*,19*R*,20*S*).

Even though anguinomycin C is structurally closely related to leptomycin B, these compounds show differences in their biological activity. Whereas anguinomycin C is toxic to immortalized, pRB-inactivated cells in picomolar concentration, it causes only growth arrest of normal cells.[4] LMB, in contrast, does not show such a selectivity and is toxic to both immortalized and normal cells.

Leptomycin B is a specific inhibitor of the protein CRM1.[17,18] CRM1 belongs to the karyopherin protein family, whose members mediate the majority of protein transport between the nucleus and the cytoplasm (for a review see Ref. [19]). CRM1 is the main export factor for proteins out of the cell nucleus. The protein substrates of CRM1 contain a short signal sequence, nuclear export signal (NES), which mediates the specific interaction with CRM1. The export activity of CRM1 can be selectively inhibited by leptomycin, which covalently binds to a cysteine in the substrate-binding domain of CRM1, thereby blocking the interaction between CRM1 and the NES of the cargo.[20]

In order to test if anguinomycin C also inhibits the CRM1-dependent export of proteins from the cell nucleus, we analyzed how treatment of cells with this compound affects the intracellular localization of the human protein Rio2. Rio2 is a cytoplasmic protein kinase, which is exported from the nucleus in a CRM1-dependent manner.[21] Inhibition of the CRM1 export pathway leads to accumulation of Rio2 in the cell nucleus.

We incubated HeLa cells with different concentrations of either leptomycin B or anguinomycin C for 90 min and then fixed the cells with paraformaldehyde. The localization of Rio2 was then determined by indirect immunofluorescence using specific antibodies directed to human Rio2. Both anguinomycin C and leptomycin B caused a strong accumulation of Rio2 in the nucleus, whereas in untreated control cells, Rio2 was localized in the cytoplasm as expected (Figure 1). This demonstrates that anguinomycin C, like

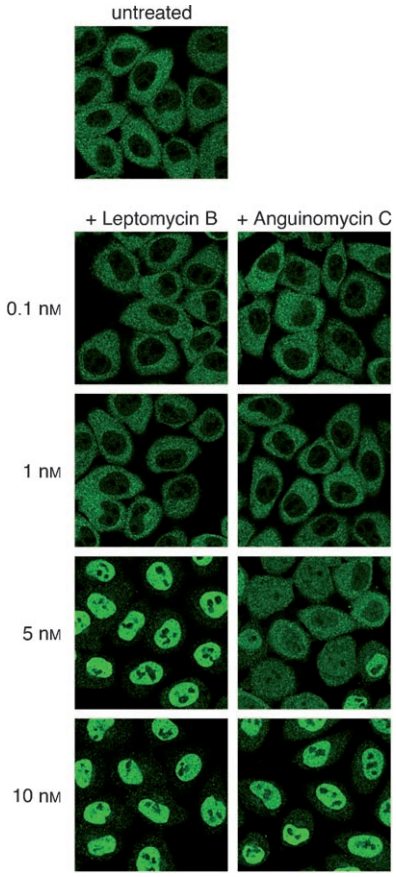


Figure 1. Anguinomycin C inhibits CRM1-dependent nuclear export of Rio2 in HeLa cells. CRM1: Chromosome maintenance region 1.

leptomycin B, is a potent inhibitor of CRM1-dependent protein export from the nucleus. The effect of anguinomycin C treatment was only slightly weaker than for leptomycin B; a complete nuclear accumulation of Rio2 was reached at 10 nM anguinomycin C and at 5 nM leptomycin B. Together, these data show that anguinomycin C is an efficient inhibitor of the CRM1-dependent protein export pathway.

We report in this communication the first total synthesis of the antitumor polyketide anguinomycin C (**20**). Remarkable transformations in this synthesis include: 1) A Cr-catalyzed, enantioselective hetero-Diels–Alder reaction for a quick access to the dihydropyran fragment, 2) a Negishi reaction under stereoinversion for the synthesis of the trisubstituted double bond, and 3) utilization of the chiral DIOZ auxiliary which enabled, for example, the direct reduction of imide **14** to the aldehyde **15**. This convergent route allowed for the definite establishment of the absolute configuration of anguinomycin C (**20**). In addition, we demonstrated that anguinomycin C inhibits the CRM1-mediated export of proteins from the cell nucleus. The consequences of this experimental evidence for the reported selectivity of anguinomycin C for pRB-inactivated cells[4] are currently under investigation in our laboratories.

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