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Design, synthesis and biological evaluation of simplified side chains of the macrolide antibiotic etnangien

Mario Altendorfer^a, Herbert Irschik^b, and Dirk Menche^{c,*}

^a Organische Chemie, Ruprecht-Karls-Universität Heidelberg, INF 270, D-69120 Heidelberg, Germany

^b Helmholtz-Zentrum für Infektionsforschung, Research Group Microbial Drugs, Inhoffenstr. 7, D-38124 Braunschweig, Germany

^c Institut für Organische Chemie und Biochemie, Universität Bonn, Gerhard-Domagk-Str. 1, D-53121 Bonn, Germany

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ABSTRACT

Novel simplified side chains of the potent RNA polymerase inhibitor etnangien were designed, synthesized and evaluated for antibacterial activity against Gram-positive bacteria and one Gram-negative bacterium.

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The labile polyketide macrolide antibiotic etnangien (**1**, Figure 1) presents a highly potent antibiotic against bacteria, isolated from various strains of the myxobacterium *Sorangium cellulosum* So ce750 and So ce1045.^{1,2} It is a structurally unique type of a particularly efficient RNA polymerase inhibitor *in vitro* and *in vivo*³ with average IC₅₀ values in the submicromolar scope.³ In detail, it is effective against a broad panel of Gram-positive bacteria, especially those belonging to the actinomycetes.³ Notably, etnangien (**1**) shows no cross-resistance to rifampicine, which presents the only curative useful RNA-polymerase inhibitor so far. This qualifies the bacterial RNA polymerase as one of the rare validated, but underexploited, targets for broad-spectrum antibiotics.⁴ Furthermore, etnangien (**1**) also retains activity against retroviral DNA polymerase and shows low cytotoxicity against mammalian cells, which add to its attractiveness for further investigation.³ The notorious instability, of the side chain embodying a polyunsaturated hexaene subunit demands the development of stable and more readily available analogues to further advance this highly promising macrolide antibiotic.

The full relative and absolute stereochemistry of etnangien (**1**) has been determined in our group in a cooperation with the group of Rolf Müller by a combination of extensive high-field NMR-studies, modeling, chemical derivatization and an

innovative bioinformatics approach.⁵ Furthermore, a first and so far only total synthesis of etnangien (**1**) and its equipotent methyl ester derivative (**2**) have been accomplished in our group.^{6,7} In connection with the total synthesis a few simplified etnangien analogues have been obtained and biologically evaluated against a range of Gram-positive and negative bacteria⁸

Figure 1. Etnangien (**1**) and its methyl ester (**2**).

Herein, we report the synthesis of the first side chain analogues of etnangien and their evaluation for antibacterial activity against Gram-positive bacteria and one Gram-negative bacterium.

Inspired by various polyene antibiotics,⁹ we initiated a synthetic programme developed towards polyene side chain

*F Corresponding author. Tel.: +49-(0)228-732653; fax: +49-(0)228-735813; e-mail: dirk.menche@uni-bonn.de

analogues of etnangien in a rationale to understand the biological importance of this part of the etnangien structure. One focus was placed on the design and synthesis of modified side chains, lacking the notoriously labile polyene subunit. As shown in Scheme 1, our concept relied on a late stage 3-fragment cross coupling strategy, involving a Stille/Suzuki-Miyaura coupling sequence bases on a central bis-metallated reagent **5**. Notably, this sequence avoids a labile precursor molecule by installing the polyene fragment at a very late stage. Also, replacement of the central bis-metallated fragment enables a rapid access to various analogues.

Suzuki-Miyaura coupling¹⁶ applying mild reactions conditions with Pd(dppf)Cl₂ as catalyst and Ba(OH)₂·8H₂O as a base.¹⁷

In total four different types of protected side chains could be prepared demonstrating the general usefulness of our approach. With the highly unsaturated polyenes **10a**, **10b**, **11a** and **11b** in hand, efforts were then directed towards the challenging deprotection of these polyenes. After considerable experimentation, it was found that best results in terms of yield and practicability were obtained with a protocol reported by Kishi,¹⁸ which involves treatment with TBAF and anhydrous work-up procedure. In detail, methyl esters **10a**, **10b**, **11a** and **11b** were treated with 1 M TBAF and stirred at room temperature over night, resulted complete and clean conversion. Finally, after working up with CaCO₃, DOWEX and methanol the desired target compounds **3a**, **3b**, **4a** and **4b** could be isolated in good yields.

Scheme 1. Late stage cross coupling strategy of simplified etnangien side chains.

As a first target, we decided to synthesize dienyl iodide **7**. This was available from the commercially available acid **8** in a six step reaction sequence (Scheme 2), involving acid catalyzed esterification, cross metathesis with crotonaldehyde using Hoveyda Grubbs II catalyst, Brown allylation of the terminal allylic aldehyde,¹⁰ TBS protection, another second cross metathesis croton aldehyde and a subsequent Takai homologation.¹¹ This sequence afforded vinyl iodide **7** in high stereoselectivities and good yields (45% yield, 6 steps). Light sensitive dienyl iodide **7** was then immediately connected to the bis-metallated central building block **5** by a Stille coupling.¹² Accordingly, treatment of iodide **7** and diene **5a** with PdCl₂(CH₃CN)₂ in degassed DMF afforded the desired pinacolborane **8a** in good yield. The analogous Stille coupling of stannane **5b** and dienyl iodide **7** under same conditions proceeded in moderate yield generating methyl ester **8b**. To complete the coupling sequence we next focused on the construction of western fragments **6**. The primary alcohol function of **9** was initially protected both as a silyl and a PMB ether. Starting from aldehyde **9a** we advanced towards (*E*)-vinyl iodide **6a** via asymmetric alkyne addition of TMS-acetylene¹³ to aldehyde **9a** as a first key step. After TBS protection of the selectively installed hydroxy group TMS was removed and the terminal alkyne underwent a hydrozirconation reaction¹⁴ followed by iodolysis¹⁵ to obtain bis-TBS ether **6a** (55% yield, 4 steps). Using the same reaction conditions PMB ether **9b** was readily transformed into vinyl iodide **6b** in 47% yield over 4 steps. Subsequently, pinacolboranes **8** and iodides **6** underwent a

Scheme 2. Synthesis of novel simplified etnangien side chains **3** and **4**. Reagents and conditions: (a) H₂SO₄, MeOH, reflux, 3 h, 72%; (b) crotonaldehyde, Hoveyda Grubbs II, DCM, reflux, 2 h, 93%; (c) (-)-(Ipc)₂B(allyl), Et₂O, -100 °C, 90%, 96% *ee*; (d) TBSOTf, 2,6-Lutidine, DCM, -78 °C, 1 h, 99%; (e) crotonaldehyde, Grubbs II, DCM, reflux, 2 h, 86%; (f) CHI₃, CrCl₂, THF/Dioxane (1:6), 0 °C to rt, 87%, *E/Z* 7:1; (g) PdCl₂(CH₃CN)₂, DMF, rt, 12 h, 80% for **8a** and 68% for **8b**; (h) Et₂Zn, TMS-acetylene, toluene, reflux, 1 h then Ti(O-*i*Pr)₄, (*R*)-BINOL, Et₂O, rt, 12h, *d/r* = 8:1 using **9a** and *d/r* = 9:1 using **9b**; (i) TBSOTf, 2,6-Lutidine, DCM, -78 °C, 1 h; (j) K₂CO₃, MeOH, rt, 1 h; (k) ZrCp₂Cl₂, DIBAL-H, THF, 30 min, -78 °C to rt then I₂, -78 °C, 30 min, 55% over 4 steps for **6a** and 47% for **6b**; (l) Pd(dppf)Cl₂, Ba(OH)₂·8H₂O, DMF, rt, 4 h, 49% for **10a**, 47% for **10b**, 77% for **11a** and 83% for **11b**; (m) TBAF, THF, rt, 12 h then CaCO₃,

DOWEX, MeOH, rt, 1 h, 59% for **3a**, 95% for **3b**, 27% for **4a** and 61% for **4b**.

Based on the syntheses of the simplified etnangien side chains reported above, we decided to apply this 3-fragment coupling concept also for the construction of the original side chain **12** of etnangien. Again, we were focusing on avoiding unstable precursors which allow an assembling of the labile hexaene subunit in an efficient late stage cross coupling sequence.

Scheme 3. Late stage cross coupling strategy of the original etnangien side chain.

The synthesis of the identical etnangien side chain **12** could again be realized in a similar way to the above reported construction of simplified side chain derivatives **3** and **4**, demonstrating again the usefulness of this approach. Starting from aldehyde **9b** asymmetric addition¹³ of propyne afforded the desired propargylic alcohol **16** in an anti Felkin-Anh preference (*dr* = 12:1). After TBS protection of the installed secondary hydroxyl group, the triple bond was transformed selectively into a stannylated olefin via Palladium-catalyzed hydrostannylation.¹⁹ Finally, metal/halogen exchange²⁰ afforded vinyl iodide **14** in 27% over 4 steps. Subsequent Stille coupling¹² of western fragment **14** with stannane **13**⁹ gave pentaene **17** in good yields considering the lability of this compound (67%). Next, the eastern fragment **15** was obtained in reliable fashion by a 12 step procedure starting from commercially available alcohol **18** (6.0% over 12 steps).⁶ As illustrated in Scheme 4, pinacolborane **17** and eastern fragment **15** underwent a Suzuki-Miyaura reaction^{16,17} to obtain bis-TBS ether **19** in high yield (83%). Global deprotection using the established Kishi protocol¹⁸ afforded the desired methyl ester **12** in 56% yield.²¹

The potent antimicrobial activity of etnangien motivated us to likewise analyse the developed and more readily available side chain analogues for their antibiotic potential. Table 1 presents their inhibitory qualities against several microorganisms, in direct comparison to the authentic natural product etnangien (**1**) and its methyl ester derivative (**2**). As previously reported, bacteria belonging to the suborder of Corynebacterineae, such as *Nocardia coralline* and some *Mycobacteria*, were particularly

sensitive to (**1**) and (**2**).

Scheme 4. Synthesis of the original etnangien side chain **11**. Reagents and conditions: (a) Et₂Zn, propyne, toluene, reflux, 1 h then Ti(O-*i*Pr)₄, (*R*)-BINOL, Et₂O, rt, 12h, 60%, *dr* = 12:1; (b) TBSOTf, 2,6-Lutidine, DCM, -78 °C, 1 h, 96%; (c) Pd(Ph)₃Cl₂, *n*-Bu₃SnH, THF, rt, 30 min, 68%; (d) I₂, DCM, 0 °C, 15 min, 98%; (e) PdCl₂(CH₃CN)₂, DMF, rt, 12 h, 67%; (f) Pd(dppf)Cl₂, Ba(OH)₂·8H₂O, DMF, rt, 4 h, 83%; (g) TBAF, THF, rt, 12 h then CaCO₃, DOWEX, MeOH, rt, 1 h, 58%.

However, yeast and Gram-negative *Escherichia coli* proved to be rather resistant. Presumably, this may be arising from the Gram-negative character of myxobacteria.^{1,3} In agreement with these data for the parent natural product **1** and its methyl ester **2**, the side chain analogues **3a**, **3b**, **4a**, **4b** and **12** likewise showed no activity against Gram-negative *E. Coli* and the yeast *Saccharomyces cerevisiae*. However, a certain activity of triol **3b** was observed against *Bacillus subtilis*, with a similar potency as the parent natural product **1** and its methyl ester derivative **2**. Also a certain inhibition against *Corynebacterium mediolanum* was observed for **3b**. All other analogues, including the original side chain **12**, showed no or very low degrees of activities, suggesting a critical importance of the macrocyclic part for full biological potency.

Table 1. Antibiotic activity of etnangien analogues (**3**, **4**, **12**) in comparison to etnangien (**1**) and its methyl ester (**2**)²²

Test organism	MIC ^a (µg/mL)						
	1	2	3a	3b	4a	4b	12
<i>Staphylococcus aureus</i>	1	2.5	>80	80 ^b	>80	>80	>40
<i>Bacillus subtilis</i>	10	20	>80	20	>80	>80	>40
<i>C. glutanicum</i>	0.03	0.24	>80	80 ^b	80 ^b	>80	>40
<i>C. mediolanum</i>	0.06	n. d.	>80	40	>80	>80	>40
<i>Mycobacterium phlei</i>	0.12	n. d.	>40	>40	>40	>40	>40
<i>Micrococcus luteus</i>	0.39	0.06	>80	>80	>80	>80	>40

<i>Escherichia coli</i>	>80	>20	>80	>80	>80	>80	>40
<i>S. cerevisiae</i>	>80	>40	>80	>80	>80	>80	>40

^a Experiments were run in duplicates or triplicates.

^b Incomplete inhibition.

In conclusion, we developed a highly concise first synthesis of side chain analogues of the etnangiens enabling a first biological evaluation of this structural part of these potent antibiotics. The synthesis of these compounds was enabled by a highly modular and convergent 3-fragment coupling strategy. Most side chain analogues demonstrated little or no antibacterial potency, suggesting that the macrocyclic core has to be present for full biological potency. Together with previous data⁷ these results show that *both* the macrocyclic core and the side chain are critical parts of the pharmacophore. However, a certain degree of activity was also observed for simplified side chain analogue **3b**, suggesting that the parent natural structure may still be simplified with retention of activity.

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- All final compounds reported in the paper were chromatographically purified and characterized by NMR and HRMS. Characterization data for compound **3a**: ¹H NMR (500.132 MHz, CDCl₃): δ = 0.85 (d, *J* = 6.9 Hz, 2 H), 1.65 (br. s., 3 H), 1.82 (ddt, *J* = 14.5 Hz, *J* = 10.8 Hz, *J* = 7.3 Hz, 1 H), 2.34 (m, 10 H), 3.65 (m, 1 H), 3.68 (s, 3 H), 3.75 (dd, *J* = 10.8 Hz, *J* = 3.4 Hz, 1 H), 4.05 (t, *J* = 7.7 Hz, 1 H), 4.13 (dt, *J* = 11.9 Hz, *J* = 5.8 Hz, 1 H), 5.63 (m, 6 H), 6.12 (m, 6 H); ¹³C NMR (125.76 MHz, CDCl₃): δ = 13.6, 27.4, 32.4, 32.4, 33.6, 40.5, 41.0, 51.6, 67.6, 71.8, 78.6, 128.8, 129.7, 129.8, 130.6, 130.9, 131.9, 132.3, 132.3, 133.4, 133.9, 133.9, 134.9, 173.4; HR-MS (ESI): found *m/z* = 427.2459 (C₂₄H₃₆O₃Na), calculated *m/z* = 427.2455. Compound **12**: ¹H NMR (600.130 MHz, CDCl₃): δ = 0.83 (d, *J* = 7.0 Hz, 3 H), 1.62 (br. s., 1 H), 1.70 (s, 3 H), 1.82 (s, 3 H), 1.94 (m, 1 H), 2.33 (m, 4 H), 2.45 (m, 2 H), 3.41 (br. s., 1 H), 3.46 (t, *J* = 8.5 Hz, 1 H), 3.59 (dd, *J* = 9.2 Hz, *J* = 4.0 Hz, 1 H), 3.67 (s, 3 H), 3.81 (s, 3 H), 4.41 (m, 2 H), 4.47 (s, 2 H), 5.22 (d, *J* = 8.6 Hz, 1 H), 5.48 (d, *J* = 8.9 Hz, 1 H), 5.68 (m, 1 H), 6.02 (m, 1 H), 6.25 (m, 8 H), 6.89 (d, *J* = 8.4 Hz, 2 H), 7.26 (d, *J* = 8.4 Hz, 2 H); ¹³C NMR (150.90 MHz, CDCl₃): δ = 13.0, 13.4, 16.7, 32.5, 34.3, 39.2, 41.2, 51.6, 55.3, 68.0, 72.8, 73.1, 74.5, 113.8, 127.6, 128.7, 129.4, 129.8, 130.0, 132.0, 132.7, 132.9, 133.0, 133.1, 133.4, 133.8, 133.8, 136.1, 137.1, 137.4, 159.3, 173.6; HR-MS (ESI): found *m/z* = 573.3189 (C₃₄H₄₆O₃Na), calculated *m/z* = 573.3187.
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