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# Design, synthesis and biological evaluation of simplified analogues of the RNA polymerase inhibitor etnangien

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ABSTRACT

Novel simplified analogues of the potent RNA polymerase inhibitor etnangien were obtained by total synthesis and evaluated for antibacterial activity against Gram-positive bacteria and one Gram-negative bacterium.



Various strains of the myxobacterium *Sorangium cellulosum* are the natural sources of etnangien (**1**, Fig. 1), a poly-unsaturated 22-membered polyketide macrolide.[1–3] It presents a highly potent antibiotic, which inhibits the growth of Gram-positive bacteria with average $IC_{50}$ values in the submicromolar range and shows only low cytotoxicity against eukaryotic cell lines.[1,2] At the molecular level, it inhibits bacterial RNA polymerase in various strains, an attractive drug target for antibiotic development.[4–6] So far, the rifamycins are the only class of clinically used RNA polymerase inhibitors. However, resistance has been increasingly emerging[7] necessitating the need of structurally novel RNA polymerase inhibitors. Importantly, etnangien shows no cross-resistance to rifampicin, and also retains a certain activity against retroviral DNA polymerase which adds to its attractiveness for further development.[1,2] Preclinical advancement, however, is severely hampered by its notorious instability, which is intrinsically associated with the polyene side chain. This renders the development of stable and more readily available analogues an important research goal.

So far, only one derivative of etnangien has been reported, the methyl ester **2**, which has been shown to retain the biological potential of the parent natural product.[8] The relative and absolute configuration of etnangien has been determined in our group in cooperation with Rolf Müller by extensive high-field NMR-studies, modelling, chemical derivatization and an innovative bioinformatics approach.[8] This assignment was recently confirmed by the first total synthesis of these macrolide antibiotics, accomplished in our group.[9,10]

Herein we describe the synthesis of a first set of carefully selected simplified etnangien analogues, which lack the labile polyene side chain. The antibacterial activity of these compounds against a range of Gram-positive and -negative bacteria relative to etnangien (**1**) and etnangien methyl ester (**2**) is reported.

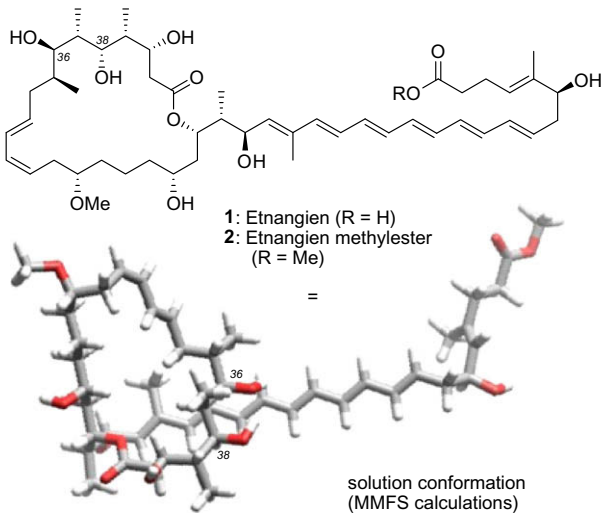


**Figure 1.** Etnangien and its methyl ester: potent macrolide antibiotics.

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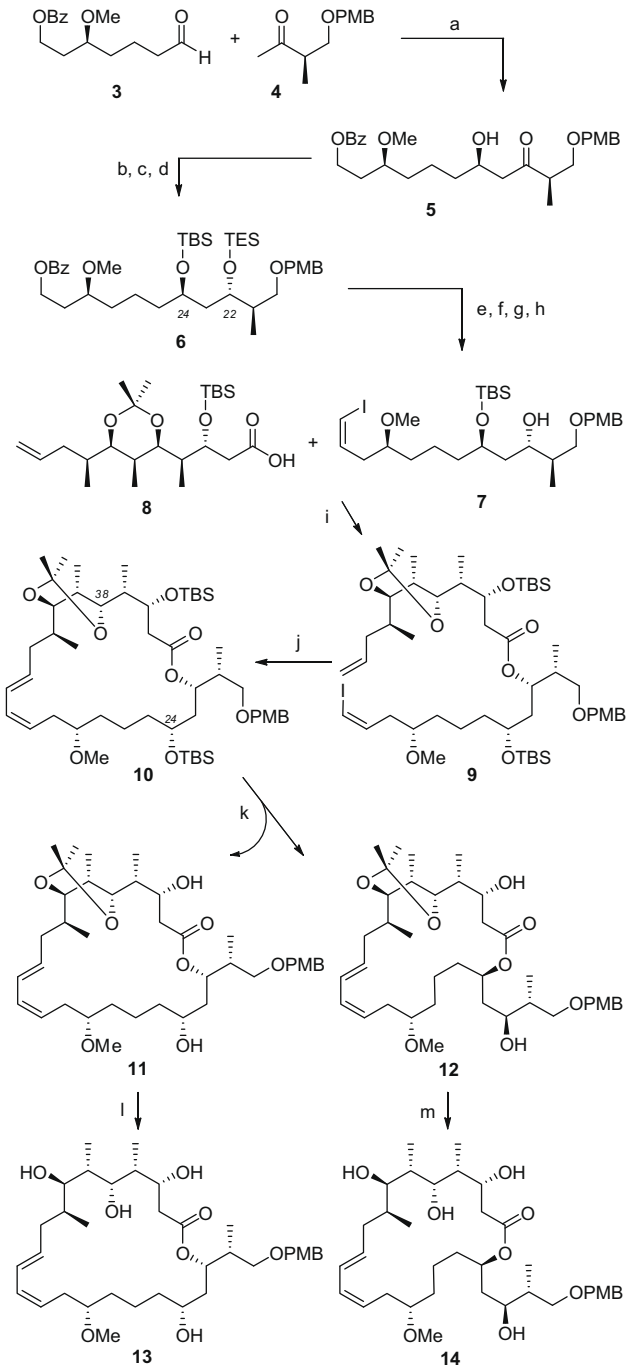
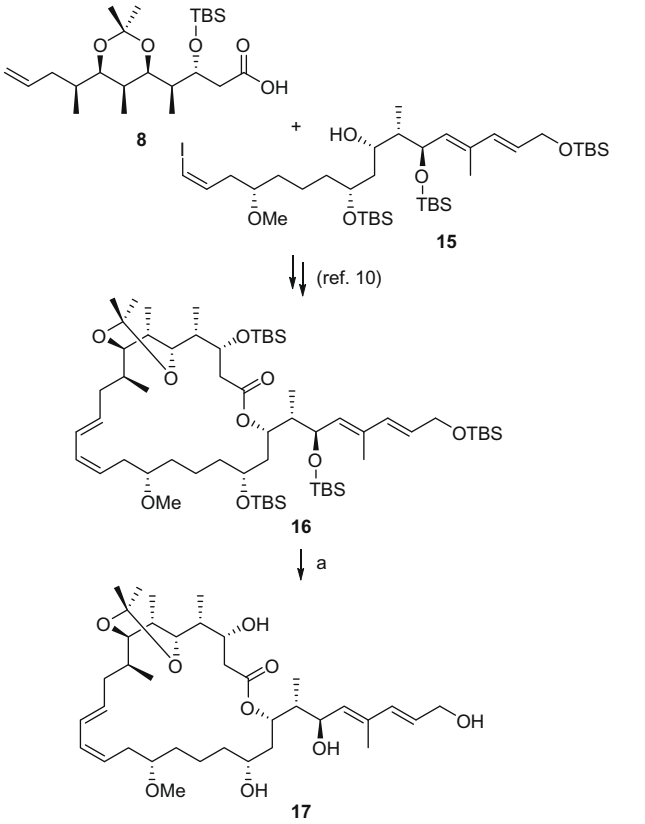
Our starting point for analogue studies were conformational analyses (MMFS, MacroModel 8.5) of etnangien and related structures with simplified side chains, which were carried out both in vacuo and in solution (water).[11] These calculations revealed that the side chain only had a minor impact on the respective 3D-structures. Importantly, in all cases the characteristic hydrogen bond between OH-36 and -38 was correctly predicted, as shown for etnangien in Figure 1. These findings together with lability of the polyene fragment prompted us to evaluate the importance of this polyene portion on biological activity, by preparing and evaluating truncated macrocyclic analogues.

As a first target, we decided to remove the side chain completely and target truncated analogue **13**. As shown in Scheme 1, the synthesis was based an Ipc-boron mediated aldol reaction of Roche ester derived methyl ketone **4** with aldehyde **3**,[12] which proceeded with excellent yield and selectivity. Subsequent 1,3-*anti* reduction[13] of the derived β-hydroxy ketone **5** and step-wise selective protection of the less hindered hydroxyl at C-24 as TBS ether and the more congested 22-OH as TES ether gave rise to **6**. Homologation to the southern ring fragment **7** was effected by removal of the benzoate under basic conditions, oxidation of the primary alcohol, Wittig–Stork reaction of the derived aldehyde and selective removal of the TES ether (49% yield, 4 steps). Union with our previously reported poly-propionate fragment **8**[10] relied on a Yamaguchi esterification, followed by a Heck-macrocyclization of derived ester **9**, which proceeded with high yield and stereoselectivity. Final deprotection proved challenging, due to a pronounced tendency of lactone **10** towards translactonizations under basic or acidic conditions. Various degrees of transesterification by attack of OH-24, as well as OH-38 leading to irreversible δ-lactone formation, were observed. Finally, the desired compound **13** was obtained in reliable fashion by a two step-procedure. This involved first removing all TBS-groups with TBAF/AcOH under neutral conditions, giving **11** and **12** as the main products. Secondly, removal of the acetonide was achieved with aq. AcOH, giving the desired macrolactone **13** as well as the contracted congener **14** starting from **11** and **12**, respectively, with acceptable yields.[14]




**Scheme 1.** Synthesis of simplified analogues **13** and **14**. Reagents and conditions: (a) (+)-Ipc$_2$BCl,TEA, Et$_2$O, −78 °C 4.5 h then −20 °C 14 h, 75%, dr = 19:1; (b) Me$_4$NBH(OAc)$_3$, CH$_3$CN/AcOH, −40 °C, 99%, dr > 20:1; (c) TBSCl, imidazole, DMAP, DMF, 0 °C to rt, 2 h, 47%, 79% brsm; (d) TESOTf, 2,6-lutidine, DCM, −78 °C, 30 min; (e) K$_2$CO$_3$, 95% MeOH, rt, 3 h, 77% over 2 steps; (f) Dess–Martin periodinane, NaHCO$_3$, DCM, rt, 40 min, 81%; (g) [Ph$_3$PCH$_2$I]$^+$I$^-$, NaHMDS, THF/HMPA, 20 °C, 1 min then −78 °C, aldehyde, 20 min, 83%, *Z*/*E* = 19:1; (h) PPTS, EtOH, rt, 1 h, 85%; (i) TEA, DMAP, 2,4,6-trichlorobenzoyl chloride, toluene, rt, 30 min; (j) Pd(OAc)$_2$, Bu$_4$NCl, K$_2$CO$_3$, DMF, 60 °C, 90 min, 47%; (k) 2.0 M TBAF and 0.2 M AcOH in DMF, 0 °C, 2 h, 35% for **11** and 53% for **12**; (l) 65% AcOH, rt, 2 h, 41%; (m) 65% AcOH, 0 °C, 2 h, 44%.




**Scheme 2.** Synthesis of analogue **17**. Reagents and conditions: (a) 2.0 M TBAF and 0.2 M AcOH in DMF, 0 °C to rt, 160 min.

**Table 1**
Antimicrobial activity of simplified etnangien analogues (**13**, **14**, **17**) in comparison to etnangien (**1**) and its methyl ester (**2**)

| Test organism | MIC[a] (μg/mL) | | | | |
|---|---|---|---|---|---|
| | **1** | **2** | **13** | **14** | **17** |
| *Staph. aureus* | 1 | 2.5 | >20 | >20 | >20 |
| *Micrococcus luteus* | 0.06 | 0.39 | >20 | >20 | >20 |
| *Escherichia coli* | >80 | >20 | >20 | >20 | >20 |
| *Corynebact. glutamicum* | 0.03 | 0.24 | 10 | 20 | 10 |
| *Mycobact. phlei* | 0.12 | n.d. | 20 | >20 | 20 |
| *Saccharomyces cerevisiae* | >80 | >40 | >20 | >20 | >20 |

[a] Experiments were run in duplicates or triplicates.

Our recently reported route[10] to **16** from acid **8** and vinylic iodide **15** opened the possibility of also elaborating a more closely related analogue (see Scheme 2). Selective deprotection proved again challenging, even more so due to the lability of the liberated terminal homoallylic alcohol, which was prone to various decomposition pathways. As before, TBS-deprotection could only be effectuated in a practical and reliable fashion by use of buffered TBAF solution, giving the desired analogue with acceptable yields.[15]

The potent antibiotic activity of etnangien based on RNA-polymerase inhibition prompted us to likewise analyze the foregoing analogues for their antimicrobial potential. Table 1 summarizes their inhibitory activities against different microorganisms, in direct comparison to etnangien (**1**) and its methyl ester (**2**). As expected, bacteria belonging to the Corynebacterineae, such as *Nocardia corallina* and some *Mycobacteria*, were particularly sensitive to **1** and **2**, while yeast and Gram-negative *Escherichia coli* proved to be rather resistant. Possibly, this may be correlated with the Gram-negative nature of the producing myxobacterium.[1,2] In agreement with these data, the novel analogues showed likewise no antimicrobial activity against Gram-negative *E. coli* and the yeast *Saccharomyces cerevisiae*. However, a slight activity against *Corynebact. glutamicum* and *Mycobact. phlei*. was observed for **13** and **17**. In both cases, the inhibition was 40–300-fold smaller as compared to **1** and **2**, which suggests that the side side-chain is part of the pharmacophoric region. No activity was observed for analogue **14** bearing a contracted macrocycle, indicating that the authentic ring size also is of importance for biological potency.

To further evaluate these data, the stability of etnangien (**1**) and its methyl ester (**2**) was studied. Firstly, the stability of the compounds under various pH-values in solution was evaluated. While considerable degrees of decomposition were observed for **1** and **2** at very low (pH >3) and high pH-values (pH >10), both compounds were stable under netural conditions and can be conveniently stored at pH 7 in dark vessels in solution. Furthermore, the integrity of etnangien methyl ester was evaluated under assay-type conditions, by HPLC–MS analysis, revealing no signs of conversion to the parent natural product, demonstrating the observed potency of **2** was not caused by intermediate conversion to the parent natural product by ester cleavage. Furthermore, only very low degrees of isomerisation of **2** were detected (<5%) even after prolonged times (48 h). These results demonstrate that it is indeed possible to modify and stabilize the etnangien structure, yet still retain activity.

In conclusion, based on molecular modeling and stabilization considerations, we have developed a first series of simplified analogues of the etnangiens with simplified side-chains and/or contracted macrolactone ring. The synthesis of these compounds was enabled in a convergent manner by late stage-diversification using different southern ring fragments. During preparation of these compounds a strong tendency of these macrolides towards translactonization processes under basic or adidic conditions was observed. Truncation of the side chain leads to significant loss of activity, which suggests this must be part of the pharmacophore region and the complete loss of activity for a contracted macrocyclic analogue (**14**) indicates a significance of the authentic core for biological potency. In total, these results will be helpful in designing further SAR-studies and the development of potent but more stable as well as simplified analogues, to further advance the development of these macrolide antibiotics.

## Acknowledgements

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14. All new compounds had spectroscopic data in full support of the assigned structures. $^1$H NMR spectroscopic data:
Compound **13**: $^1$H NMR (600 MHz, acetone-$d_6$) $\delta$ = ppm 0.97 (d, $J$ = 7.0 Hz, 3H), 0.97 (d, $J$ = 6.8 Hz, 3H), 1.00 (d, $J$ = 6.8 Hz, 3H), 1.03 (d, $J$ = 6.6 Hz, 3H), 1.40–1.60 (m, 6H), 1.64 (m, 3H), 1.83 (m, 1H), 1.87 (m, 3H), 2.25 (m, 1H), 2.31 (m, 1H), 2.36 (m, 2H), 2.46 (m, 1H), 3.23 (m, 1H), 3.28 (s, 3H), 3.32 (dd, $J$ = 9.3, 6.2 Hz, 1H), 3.43 (dd, $J$ = 9.2, 5.6 Hz, 1H), 3.44 (m, 1H), 3.52 (m, 1H), 3.60 (m, 2H), 3.78 (s, 3H), 4.16 (m, 1H), 4.40 (s, 2H), 5.23 (m, 1H), 5.40 (ddd, $J$ = 10.4, 7.9, 7.9 Hz, 1H), 5.77 (m, 1H), 6.08 (*pseudo*-t, $J$ = 11.5 Hz, 1H), 6.40 (dd, $J$ = 14.3, 10.6 Hz, 1H), 6.90 (dd, $J$ = 8.6, 2.4 Hz, 2H), 7.27 (dd, $J$ = 8.8, 2.2 Hz, 2H); ESI-HRMS calcd for $C_{36}H_{58}NaO_9$ [M+Ma]$^+$: 657.3979, found: 657.3984. Compound **14**: $^1$H NMR (600 MHz, acetone-$d_6$) $\delta$ = 0.81 (d, $J$ = 7.0 Hz, 3H), 0.91 (d, $J$ = 7.0 Hz, 3H), 0.98 (d, $J$ = 6.9 Hz, 3H), 1.10 (d, $J$ = 6.8 Hz, 3H), 1.47–1.64 (m, 7H), 1.74 (m, 2H), 1.83 (m, 2H), 2.13 (m, 1H), 2.19 (m, 2H), 2.34 (dd, $J$ = 13.9, 10.8 Hz, 1H), 2.39 (m, 1H), 2.47 (m, 1H), 2.48 (dd, $J$ = 13.9, 2.6 Hz, 1H), 3.14 (m, 1H), 3.26 (s, 3H), 3.40 (dd, $J$ = 9.1, 6.1 Hz, 1H), 3.51 (dd, $J$ = 9.1, 6.1 Hz, 1H), 3.60 (m, 1H), 3.63 (dd, $J$ = 10.5, 2.3 Hz, 1H), 3.79 (s, 3H), 3.81 (dd, $J$ = 9.4, 2.3 Hz, 1H), 3.97 (m, 1H), 4.42 (s, 2H), 5.29 (tt, $J$ = 9.9, 2.8 Hz, 1H), 5.41 (ddd, $J$ = 10.4, 7.5, 7.5 Hz, 1H), 5.75 (ddd, $J$ = 15.4, 9.2, 6.0 Hz, 1H), 5.98 (*pseudo*-t, $J$ = 10.8 Hz, 1H), 6.34 (dd, $J$ = 14.5, 10.7 Hz, 1H), 6.91 (d, $J$ = 8.7 Hz, 2H), 7.27 (d, $J$ = 8.8 Hz, 2H); ESI-HRMS calcd for $C_{36}H_{58}NaO_9$ [M+Ma]$^+$: 657.3979. found: 657.3986. Compound **17**: $^1$H NMR (600 MHz, acetone-$d_6$) $\delta$ = 0.83 (d, $J$ = 7.1 Hz, 3H), 0.89 (d, $J$ = 7.0 Hz, 3H), 0.94 (d, $J$ = 6.6 Hz, 6H), 1.31 (s, 3H), 1.39 (s, 3H), 1.30–1.46 (m, 6H), 1.63 (m, 1H), 1.66 (m, 3H), 1.78 (s, 3H), 1.81 (m, 1H), 1.90 (m, 1H), 2.01 (m, 1H), 2.24 (m, 1H), 2.30 (m, 1H), 2.36 (dd, $J$ = 15.5, 10.1 Hz, 1H), 2.43 (dd, $J$ = 15.5, 1.2 Hz, 1H), 2.48 (m, 1H), 3.23 (m, 1H), 3.28 (s, 3H), 3.50 (d, $J$ = 10.2 Hz, 1H), 3.58 (m, 1H), 3.61 (d, $J$ = 4.6 Hz, 1H), 3.64 (dd, $J$ = 9.2, 1.4 Hz, 1H), 3.83 (d, $J$ = 4.44 Hz, 1H), 3.99 (d, $J$ = 3.7 Hz, 1H), 4.13 (t, $J$ = 5.5 Hz, 1H), 4.15 (m, 1H), 4.37 (ddd, $J$ = 9.0, 8.3, 4.6 Hz, 1H), 5.40 (ddd, $J$ = 9.9, 8.3, 8.3 Hz, 1H), 5.44 (d, $J$ = 9.5 Hz, 1H), 5.44 (m, 1H), 5.77 (m, 1H), 5.80 (dt, $J$ = 15.6, 5.6 Hz, 1H), 6.09 (dd, $J$ = 10.8, 10.8 Hz, 1H), 6.27 (d, $J$ = 15.6 Hz, 1H), 6.40 (dd, $J$ = 15.2, 10.8 Hz, 1H); LC–ESIMS calcd for $C_{37}H_{62}NaO_9$ [M+Na]$^+$: 673.4. found: 673.5.
15. Removal of the acetonide could not be effected without extensive decomposition.